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# Relationships of *Conoclinium*, a Recently Diverged Genus (Asteraceae, Eupatorieae) and Description of a New Species from Western Mexico.

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**Abstract**: Molecular phylogenetic studies were conducted to clarify the phylogenetic placement of *Conoclinium* within Eupatorieae and to analyze interspecific relationships in the genus. Analysis of a six gene data set placed *Conoclinium* sister to *Ageratum* and closely related to *Fleischmannia* and *Paneroa*, a result that is consistent with the overall appearance and the phytochemistry of these genera but not with their current subtribal placement. Results of an analysis of noncoding DNA, including the chloroplast *trnH-psbA* spacer and the nuclear ITS and ETS regions, was consistent with the close relationship of *Conoclinium* to *Ageratum*, *Fleischmannia*, and *Paneroa*, and showed *Conoclinium* to be monophyletic. Within *Conoclinium*, the results of the phylogenetic analyses were slightly at variance with current classification, and it is suggested that *C. dichotomum* (populations from peninsular Florida previously included within *C. coelestinum*) and *C. oligolepis* (formerly *C. betonicifolium* var. *integrifolium*) be recognized as distinct species. The low overall sequence variability suggests that *Conoclinium* is a relatively recent arrival in eastern North America. *Conoclinium mayfieldii* is not monophyletic and the Sierra Madre Occidental populations of the species are herein recognized as a new species, *C. gonzaleziae*. Neotypes for *C. dichotomum* and *C. oligolepis* are designated.

**Keywords:** *Conoclinium*, Eupatorieae, Fleischmanniinae, Asteraceae, *trnH-psbA*, ITS, ETS, molecular phylogeny

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Although it has been more than three decades since King and Robinson (1987) published the seminal volume that summarized their studies to dismantle *Eupatorium* L., and even longer since the effort was initiated (e.g. King and Robinson 1970a), the implications of their work for other fields such as ecology, biogeography, and speciation have only started to be explored. Their treatment lessens the emphasis on the use of a pappus of capillary bristles as a primary character that had been used to define the mega-genus *Eupatorium*, but the frequency with which the pappus type might be subject to change has not been scrutinized in a phylogenetic context. Similarly, there have been few studies that attempt detailed phylogenetic analysis of genera newly segregated from *Eupatorium*. Recent phylogenetic studies have focused on long-accepted genera, such as *Stevia* (Soejima et al., 2017) or *Brickellia* (Schilling et al., 2015), or have taken a broader synthetic approach sampling across multiple genera of the tribe Eupatorieae (Tippery et al., 2012; Rivera et al., 2016). Given the large size of the tribe and its widespread geographic distribution within the New World, studies of genera segregated from *Eupatorium* hold promise to reveal new insights that would have applications beyond the simple understanding of the systematics of the tribe.

*Conoclinium* (Fig. 1) is a small genus centered in northern Mexico that extends broadly across eastern North America as far north as Canada (King and Robinson, 1970b; Patterson, 1994; Patterson and Nesom, 2006; Wooten and Clewell, 1971). It is most easily separated from *Eupatorium*, with which it is often submerged (e.g. Cronquist,

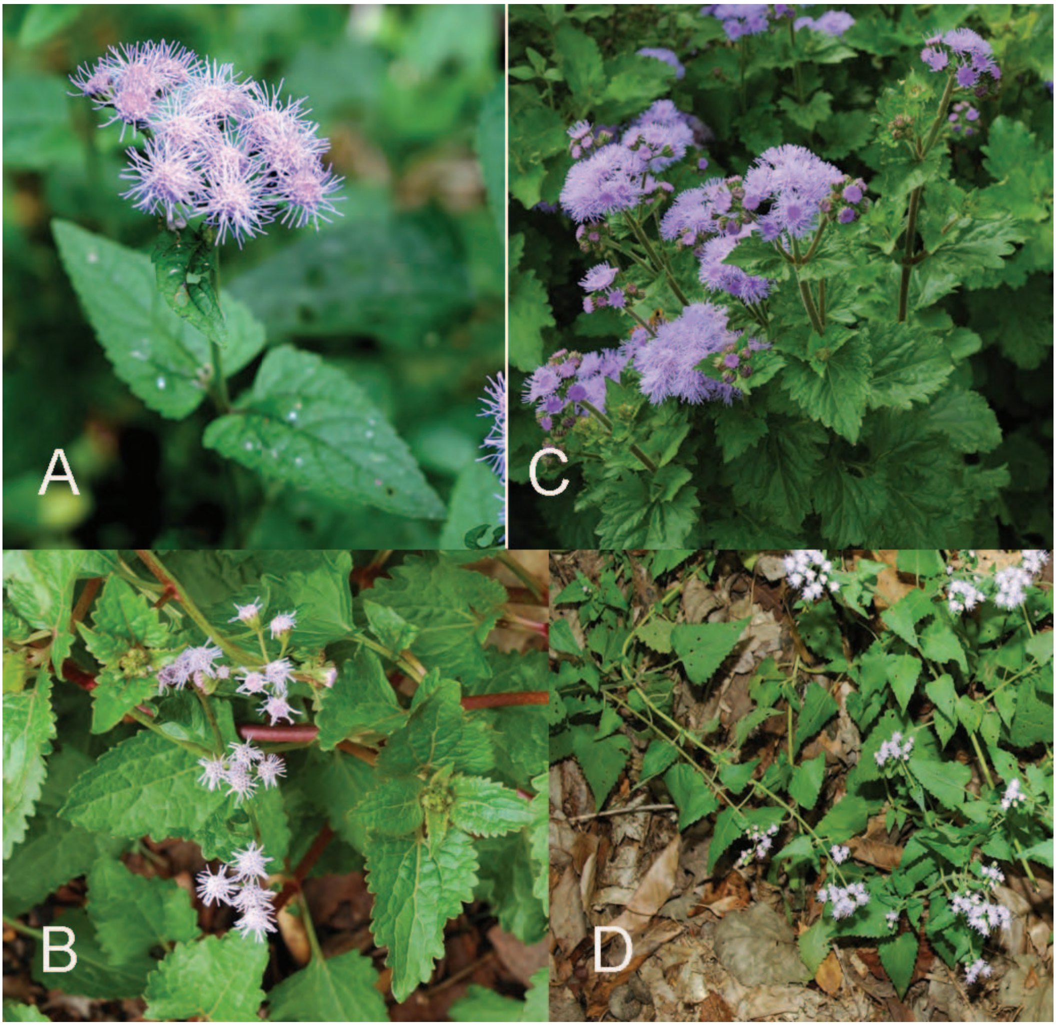

FIG. 1. Images of *Conoclinium* and relatives. (A) *C. coelestinum*. (B) *C. dichotomum*. (C) Artificial hybrid, *Ageratum houstonianum* x *Conoclinium coelestinum*. (D) *Fleischmannia incarnata*.

1980; McVaugh, 1984), by its markedly conical receptacle. Although there are substantial similarities in superficial appearance (Patterson, 1994) as well as in phytochemistry (Wollenweber et al., 1996) between members of the genus and two other genera represented in North America, *Ageratum* L. and *Fleischmannia* Sch.Bip., they are each placed in different subtribes by King and Robinson (1987). Based in part on features of the pappus, *Conoclinium* is placed within Gyptidinae, which has a pappus of bristles and comprises mostly South American genera including the evocatively named *Conocliniopsis* King and H. Rob. *Ageratum* is the type genus for Ageratinae, a decidedly mixed assemblage of genera that are united in having a pappus that is not of bristles, and that includes Mexican, Central American, and South American members and such distinctive genera as *Piqueria* Cav. and *Stevia* Cav. *Fleischmannia* is considered to be sufficiently distinctive by King and Robinson (1987) to be placed in its own, nearly monogeneric subtribe. One of the goals of the present study was to analyze the phylogenetic placement within Eupatorieae of *Conoclinium*, to be able to determine whether its relationships reflect better its habit and chemistry or the micromorphological characters on which the subtribal classification is based.

*Conoclinium* is currently considered to comprise four species, including one described in 1996 (Patterson, 1996). All of the species are diploid, with chromosome numbers of $x = 10$ (Patterson, 1994). Three of the species are restricted to northern Mexico and the southwestern U.S., and the fourth is widespread and common in eastern North America (Patterson, 1994; Patterson and Nesom, 2006). The low species-level diversity is in contrast to other genera of Asteraceae from the eastern U.S., such as *Eupatorium* L. and *Helianthus* L., which are represented there by numerous species. A second goal of the study was to examine the species relationships within *Conoclinium* to seek insight into its apparent lack of species-level diversity.

## Materials and Methods

Taxonomic Sampling. For assessment of the phylogenetic placement of *Conoclinium* a sampling of genera that have been considered to be potential relatives was made (Appendix), which included other members of subtribe Gyptidinae (*Campuloclinium, Conocliniopsis*, *Gyptis*, *Heterocondylus*, *Tamaulipa*); samples with similar overall habit (*Ageratum*, *Fleischmannia* and *Paneroa*); samples from temperate North America (*Carphephorus*, *Eupatorium*, *Eutrochium*) and Mexico (*Chromolaena*, *Critonia*, and *Koanophyllon*) as well as a clear outgroup, *Hofmeisteria*. Most samples were collected as fresh material and either frozen in liquid nitrogen or preserved in silica gel. Study of species relationships within *Conoclinium* involved a broader sampling to include each species, as well as additional sampling within *Ageratum*, *Koanophyllon, Fleischmannia,* and *Paneroa* (Appendix); samples of *Chromolaena* and *Tamaulipa* were used as outgroups. Sampling within *Conoclinium* made use of herbarium material in addition to freshly collected samples.

Molecular Methods. Preparations of total DNA were performed with the DNeasy Plant Minikit (Qiagen, Valencia CA) 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 WI). PCR amplifications were performed in 20 µl reactions using 10-20 ng of genomic DNA, 10× PCR buffer (Promega), 1.8-2.25 mM $MgCl_2$, 0.2 mM each dNTP, 1.25 units of *Taq* polymerase, and 0.2 µM each primer. Amplification and sequencing of the genes *mat*K, *ndh*F, *rbc*L, and the spacer *trn*H-*psb*A generally followed Panero and Crozier (2003). Amplification and sequencing of the ITS and ETS regions was performed as described in Schilling et al. (2007). PCR products were checked on 1% agarose gels before being cleaned with ExoSAP-IT (USB, Cleveland, Ohio, USA). 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, USA) and electrophoresed and detected on an ABI Prism 3100 automated sequencer (University of Tennessee Molecular Biology Resource Facility, Knoxville, Tennessee, USA). 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 using MAFFT v7.308 (Katoh & Standley 2013) implemented in Geneious v. 9.1.7. GenBank accession numbers are provided in the Appendix.

Phylogenetic Analyses. Phylogenetic relationships were analyzed using maximum likelihood and Bayesian approaches. Maximum likelihood was implemented using the RAxML v. 7.2.8 in Geneious. Bayesian analysis was implemented in MRBAYES v. 3.2.6 (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

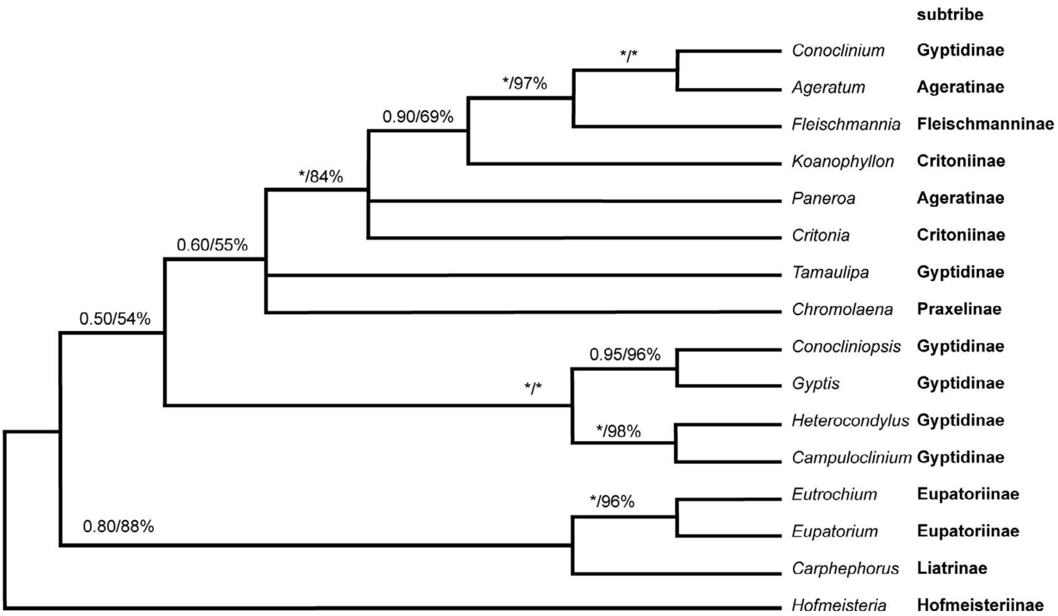

FIG. 2. Tree from maximum likelihood analysis showing the relationships of *Conoclinium* based on the analysis of six markers (plastid *rbc*L, *mat*K, *ndh*F, *trn*H-*psb*A; nuclear ITS, ETS); levels of support, shown as posterior probability/bootstrap %, with *=1.00 or 100%, shown above branches.

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 using Modeltest (Posada and Crandall, 1998).

## RESULTS

PHYLOGENETIC PLACEMENT OF *CONOCLINIUM*. The results of phylogenetic analysis using RAxML and based on DNA sequences from the nuclear ribosomal DNA ITS and ETS regions and the plastid *rbc*L, *mat*K, *ndh*F, and *trn*H-*psb*A regions are shown in Fig. 2. Relative to the outgroup, *Hofmeisteria*, all of the ingroup taxa formed a monophyletic group. Within the ingroup, the North American genera *Carphephorus*, *Eutrochium*, and *Eupatorium* split from the remaining taxa. At the next node, there was a split between the South American samples (*Campuloclinium*, *Conocliniopsis, Gyptis*, and *Heterocondylus*) and another group that contained primarily Mexican genera. Within this mostly Mexican group, there were two successive trichotomies, the first of which split the widespread *Chromolaena* and the monotypic *Tamaulipa* from the rest; the second split the widespread *Critonia* and the monotypic *Paneroa* from a terminal clade. The terminal clade placed the widespread *Koanophyllon* sister to the rest, which had *Fleischmannia* sister to *Ageratum* + *Conoclinium*. Support values (indicated as posterior probability/bootstrap percentage, with *=1.0 or 100%) ranged from moderate to high, and were notably high for the sister group relationship of *Ageratum* and *Conoclinium* (Fig. 2).

RESULTS FROM DNA SPACER REGIONS. Sequences for the *trn*H-*psb*A spacer region were identical in both base pair (bp) composition and length (392 bp) for all 25 samples of *Conoclinium*. There was somewhat greater variability in bp composition within *Fleischmannia*, which also exhibited a consistent length (393 bp) but had 11 variable bp positions. The greatest variability for *trn*H-*psb*A was within *Ageratum*, with individual sequences varying in length from

399-436 bp, from a total of 6 indels, and with 13 variable bp positions. The ITS sequences for *Conoclinium*, which included the entire ITS-1, 5.8SrDNA, and ITS-2 regions, exhibited variability in both length and bp composition. Sequence lengths for the entire region varied from 650-654 bp, with a total of 3 indels, and there were 28 variable bp positions. The ITS sequences from *Fleischmannia* varied in length for the entire region from 642-645 bp, with 7 indels, and there were a total of 83 variable bp positions. The ITS sequences for *Ageratum* varied in length from 651-653 bp, with 6 indels and a total of 66 variable bp positions. The sequences for the ETS region were trimmed to include the initial approximately 400 bp, starting from the 18S rDNA coding region. Within this region, sequence length for *Conoclinium* varied by only two 1-bp indels (although results for DNA sample 2509 suggested that it was polymorphic, with a second sequence that had an 11 bp deletion), and there were 22 variable bp positions. The ETS sequences from *Fleischmannia* were more variable in length for the corresponding region, with 19 indels, and 144 variable bp positions. The ETS sequences for *Ageratum* had 6 indels and there were 61 variable bp positions for the corresponding region.

RELATIONSHIPS OF *AGERATUM*, *FLEISCHMANNIA*, *PANEROA*, AND *CONOCLINIUM*. Alignment of the spacer regions with the near outgroups suggested by the broad multigene analysis (Fig. 2) proved to be straightforward for each of the spacer regions, *trnH-psbA*, ITS, and ETS. Comparative analysis of the individual spacers gave results (not shown) that demonstrated them to be congruent with one another. The combined data set included 1570 aligned positions, of which 346 were potentially parsimony-informative and an additional 183 were variable but parsimony-uninformative. The maximum likelihood and Bayesian approaches gave similar trees with slightly higher support values in the latter, and the results are shown in Fig. 3.

The most significant feature of the spacer gene analysis (Fig. 3) was the placement in a well-supported clade of *Ageratum* and *Conoclinium* (1.0/99%) and each of these two genera was strongly supported as monophyletic: *Ageratum* (1.0/99%) and *Conoclinium* (1.00/100%). Relative to the outgroups (*Chromolaena* and *Tamaulipa*), samples of *Critonia* and *Koanophyllon* formed the initial splits, followed by successive splits of *Fleischmannia* and *Paneroa* leading to the *Ageratum*/*Conoclinium* clade.

RELATIONSHIPS OF *CONOCLINIUM*. Within *Conoclinium* there were several well-supported clades. An initial split (0.8/75%) separated the western samples of *C. mayfieldii* (2429, 2450, 2629) from the rest of the genus (1.0/82%); these appear to represent a species distinct from *C. mayfieldii*, and is here named as *C. gonzaleziae*. A second split separated two terminal clades, the first containing samples of *C. coelestinum*, *C. dichotomum,* and *C. betonicifolium* var. *betonicifolium* (1.00/98%) and the second (1.00/92%) with *C. dissectum*, *C. betonicifolium* var. *integrifolium*, and the eastern samples of *C. mayfieldii* (2424, 2457). The samples identified as *C. dichotomum* (but initially identified as *C. coelestinum*) were separated from those of *C. coelestinum* and *C. betonicifolium* var. *betonicifolium*. Within the terminal clade containing samples of *C. coelestinum* and *C. betonicifolium* var. *betonicifolium* there was no strongly defined separation into the individual species. Instead, there was a polytomy, within which there was a weakly supported (0.64/<50%) clade with samples of *C. coelestinum* from across its range, and two branches with samples of *C. betonicifolium* var. *betonicifolium*. Within *C. betonicifolium* var. *betonicifolium*, two samples (2455, 2456) differed from the third (2425) by a single bp difference in ITS and were otherwise completely identical. Within the other terminal clade, the two samples of *C. mayfieldii* from the eastern part of its range were placed in a basal grade. The samples of *C. betonicifolium* var. *integrifolium*, which were subsequently recognized as *C. oligolepis*, formed a monophyletic group (1.00/96%), whereas those of *C. dissectum* though identical to one another

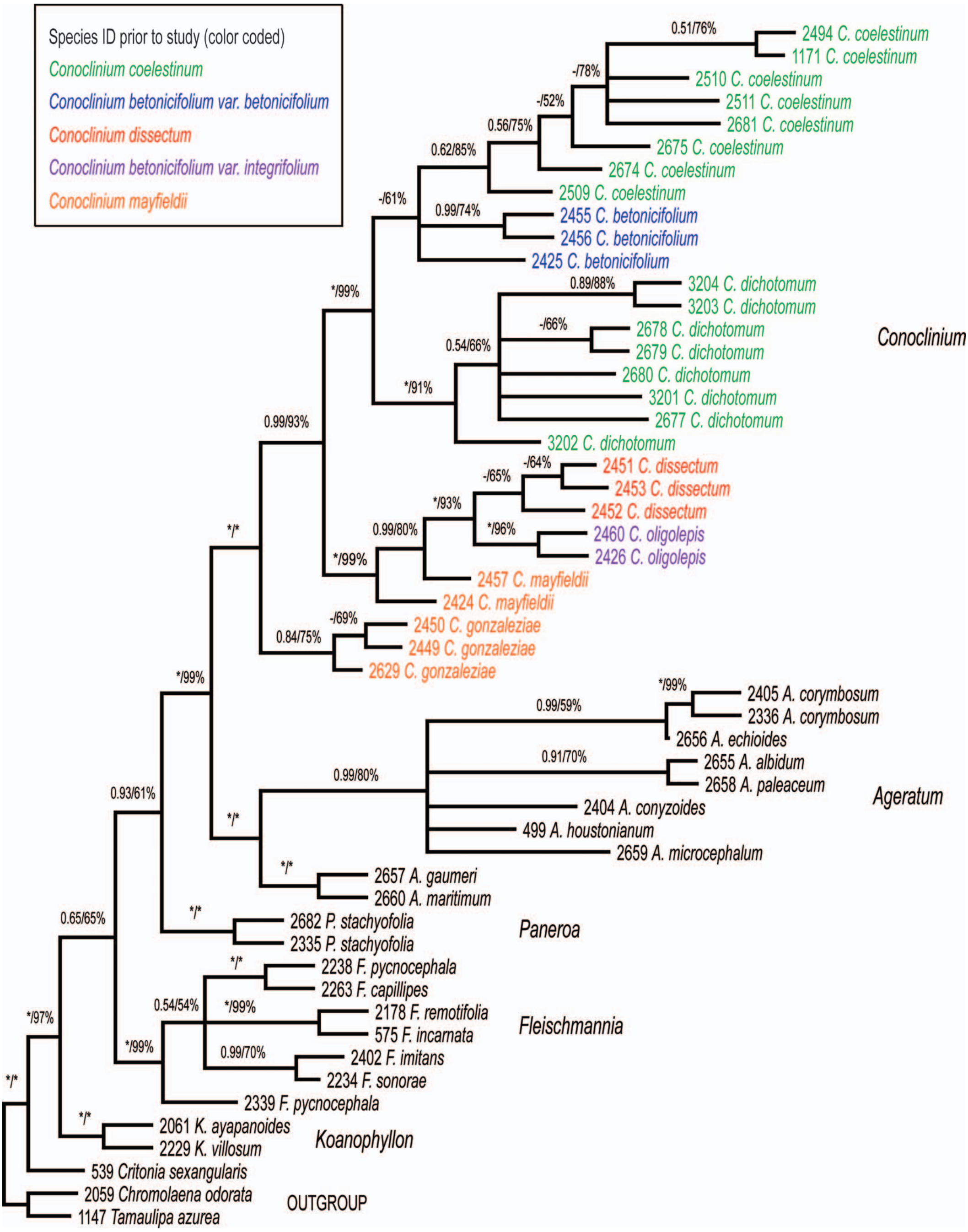

FIG. 3. Tree from maximum likelihood analysis showing the relationships among species of *Conoclinium* based on analysis of three markers (nuclear ITS, ETS, and plastid *trn*H-*psb*A); levels of support, shown as posterior probability/bootstrap %, with *=1.00 or 100%, shown above branches. Samples of *Conoclinium* color-coded by original species identifications of specimens (box).

did not share any derived substitutions relative to *C. betonicifolium* var. *integrifolium*.

Analysis of the ITS and ETS regions from a sample with the morphology of *Conoclinium dissectum* that was in cultivation at the University of Tennessee Horticultural Gardens gave somewhat unexpected results. The sequences of both ITS and ETS regions were polymorphic for both base pair sequence and length, and analysis (not shown) suggested that the polymorphisms could be explained by the presence of characteristic repeats from *C. dissectum* and *C. betonicifolium*. A second sample obtained commercially (sold as *Eupatorium greggii* by Plant Delights Nursery, Raleigh NC) gave identical sequence data. This result would be consistent with the horticultural sample being of hybrid origin.

## DISCUSSION

Phylogenetic analysis of DNA sequence data showed clearly that *Conoclinium* and *Ageratum* are sister taxa, and closely related to *Paneroa* and *Fleischmannia* (Fig. 2, 3). The placement of members considered to be in three different subtribes in a single clade adds to evidence from previous studies (Tippery et al. 2015; Rivera et al. 2016) that subtribal classification of Eupatorieae is in need of revision. *Conoclinium* was clearly supported as monophyletic. The results of the phylogenetic analysis did not completely support the current species level taxonomy of *Conoclinium*, and some adjustments are suggested, notably recognition of *C. dichotomum* and *C. oligolepis* as distinct species, and description of *C. gonzaleziae* as new and distinct from *C. mayfieldii*.

DNA sequence analyses failed to show that *Conoclinium* is closely related to any other member of subtribe in which it is now placed, Gyptidinae, or that the subtribe forms a monophyletic assemblage (Fig. 2). The member of the subtribe that was placed closest to *Conoclinium* was *Tamaulipa*, the only other North American member of the subtribe, but it was placed relatively distant from *Conoclinium* in the phylogenies (Figs. 2, 3). The South American members of tribe Gyptidinae that were sampled, including *Campuloclinium*, *Conocliniopsis*, *Gyptis*, and *Heterocondylus*, were placed in a separate and well supported clade based on the analysis of sequence data (Fig. 2). These results mirror those reported by Rivera et al. (2016). Thus, the morphological characters cited by King and Robinson (1987) for subtribe Gyptidinae, including features of the receptacle, pappus, anther collar thickenings, and corolla lobe cells, do not define a monophyletic assemblage. As a corollary, there was no phylogenetic support for the existence of a biogeographic "Eastern Complex" (King and Robinson, 1987) linking eastern North America and eastern South America. The phylogenetic results also provided further evidence to show that *Conoclinium* and *Fleischmannia* are properly considered distinct from *Eupatorium* (Fig. 2) as has been suggested previously (Schilling et al., 1999; Ito et al., 2000). A provisional adjustment to reflect the phylogenetic results at the subtribal level is to expand Fleischmanniinae to include *Ageratum*, *Conoclinium*, and *Paneroa*.

Although not the focus of this investigation, this study highlights a general lack of basic information, both molecular phylogenetic and taxonomic, for several genera related to *Conoclinium*, notably *Fleischmannia* and *Koanophyllon*. Sequence data are available in GenBank for only seven of the 79 species of *Fleischmannia*, and no comprehensive taxonomic treatment is available for the genus. Similarly, fewer than 10% of the 114 species in *Koanophyllon* are represented by sequence data in GenBank, and again no comprehensive taxonomic treatment is available. Both genera are widespread, exhibit considerable variability in features of the pappus (King and Robinson 1987), and it is possible that neither is monophyletic.

The phylogenetic analysis of the combined data set supported the close relationship of *Conoclinium*, *Ageratum*, *Paneroa*, and *Fleischmannia* that has been suggested by overall habit and phytochemistry. Further evidence supporting the close relationship of

*Ageratum* and *Conoclinium* was the ability to obtain F1 hybrids through crossing (Schilling, unpublished; Fig. 1C). Thus, despite differences in pappus type, *Conoclinium* and *Ageratum* are sister taxa (Fig. 2, 3). Variation in the pappus may have resulted from various processes, but one possibility is that it reflects differences in adaptive features characteristic of the differing environments where the two genera occur. *Conoclinium* occurs where there is a relatively wide expanse of mesic to wet habitats (Wooten and Clewell, 1971). In contrast, many species of *Ageratum* are found in the Mexican highlands where the mesophytic habitats in which they occur are surrounded by highly xerophytic ones, and there may be selective pressure for non-dispersal (Patterson, 1994). Lability in the pappus can be seen in *Fleischmannia*, in which the pappus of capillary bristles can be reduced to as few as five bristles (King and Robinson, 1987).

The clades delimited within *Conoclinium* by molecular phylogenetic analysis did not correspond in all respects with a recent taxonomic treatment (Patterson and Nesom, 2006). Individual species and their phylogenetic placement are discussed in the following paragraphs.

The placement of samples initially identified as *Conoclinium mayfieldii* in two different parts of the tree in the phylogenetic analysis (Fig. 3) was indicative that they represent more than a single species, and this was further supported by morphological and geographical data. Samples from near the type locality in the Sierra Tamaulipas (2424, 2457, see Appendix) are characterized by a pubescent receptacle. In Tamaulipas *C. mayfieldii* occurs in pine-oak forest of the Sierra Tamaulipas and the Sierra Madre Oriental isolated from the reputed populations of *C. mayfieldii* in the pine-oak forest of the Sierra Madre Occidental in Durango, Chihuahua and Sonora, and separated by the Chihuahuan desert. The Sierra Tamaulipas is a mountain range (highest elevation 1554 m) in the Tamaulipan coastal plains surrounded by mesquite woodlands and Tamaulipan scrub, completely isolated from the Sierra Madre Oriental. The lower elevations are dominated by thorn forest and tropical deciduous forest. At mid-level and above are pine-oak forest. *Conoclinium mayfieldii* is found on both limestone and igneous outcrops in the pine-oak forest. The pine-oak forest of the Sierra Tamaulipas is at a much lower elevation than the pine-oak forest of the Sierra Madre Oriental above Cd. Victoria because of its close proximity to the Gulf of Mexico. In the mostly-limestone Sierra Madre Oriental of Tamaulipas, *C. mayfieldii* occurs in the pine-oak forest at a higher elevation. In contrast, samples identified as *C. mayfieldii* from the Sierra Madre Occidental (2449, 2450, 2629, see appendix) have a glabrous receptacle and leaves that are narrower and have somewhat deeper teeth or lobing than the Sierra Tamaulipas material. Unlike the mostly limestone Sierra Tamaulipas and Sierra Madre Oriental, the Sierra Madre Occidental in Chihuahua, Durango, and Sonora is a massive igneous range with abundant surface water. Along the barrancas are found temperate trees of *Acer grandidentatum*, *Ostrya virginiana*, *Prunus serotina*, and *Tilia americana* var. *mexicana*. In this area, the samples that formed a clade sister to the rest of the genus were collected in pine-oak forest at the edge of the barrancas. Based on their phylogenetic placement, as well as the differences in morphology and geographic isolation, the populations formerly included in *C. mayfieldii* from the Sierra Madre Occidental are herein recognized as a new species, *C. gonzaleziae*.

The samples of *Conoclinium betonicifolium* s.l. also did not form a monophyletic group, but were separated between two different clades that corresponded to the taxonomic varieties. Samples of *C. betonicifolium* var. *betonicifolium* were placed with those of *C. coelestinum*, whereas those of *C. betonicifolium* var. *integrifolium* were placed with those of *C. dissectum* (Fig. 3). Both varieties of *C. betonicifolium* have coriaceous leaves, which may merely be a factor of salinity of their habitat. They both have small cypselae and floral parts relative to other species of the genus. The smaller cypselae may be an adaptation to the wetland habitat, and both *C. dissectum* and *C. mayfieldii* with larger cypselae live in drier habitats. Expanded tips of the pappus, which is so distinct in *C. b.* var. *betonicifolium,* is variable or lacking in *C. b.* var. *integrifolium*. The results of the molecular analysis combined with morphology indicate that *C. b.* var. *integrifolium* should be recognized as a distinct species, for which the name *C. oligolepis* Kunze should be used. *Conoclinium oligolepis* is an earlier name than *C. integrifolium* (A. Gray) Small, and issues regarding its typification are discussed below.

*Conoclinium dissectum* was placed as the sister species of *C. oligolepis* (Fig. 3), and these taxa likely reflect recent adaptations to different habitats from a common geographic origin. Both species occupy the basin and range of the Altiplano and Chihuahuan desert and the Tamaulipan thornscrub. Both often grow in patches, but *C. oligolepis* is found in marshes of the basins whereas *C. dissectum* occurs in the desert grasslands.

The samples that would have been identified before the study as *Conoclinium coelestinum* were placed in two different clades (Fig. 3), and slight but consistent difference in morphology between these suggests that they represent two distinct species. Samples from the Florida peninsula were placed in a single, strongly supported clade (1.00; 0.91). This entity was recognized by Chapman as *C. dichotomum*, and we suggest that recognition of this species be reinstated. A primary distinguishing feature is the smaller size of the flowers. The size difference between the samples analyzed in this study and other material of *C. coelestinum* was slight but consistent (corolla length 2.2-2.3 mm in non-Florida populations vs. 1.9-2.0 mm in peninsular Florida ones). There are also differences in flowering time, which begins in late spring in *C. dichotomum* compared to summer-fall in *C. coelestinum*. These differences were retained in plants of the two species grown in a common garden at the University of Tennessee, Knoxville. Material of *Conoclinium* from Cuba, which has been recognized taxonomically as *Eupatorium coelestinum* L. var. *salinum* Griseb., may belong to *C. dichotomum*, and a possible scenario is that they developed in isolation after the species reached Cuba, and subsequently migrated back into Florida.

The placement of samples of *Conoclinium coelestinum* in a single clade with those of *C. betonicifolium* var. *betonicifolium* (Fig. 3) raises questions about the distinctiveness of these two taxa. The samples of *C. coelestinum* were placed in a single though weakly supported (0.62; 85%) clade, but those of *C. betonicifolium* were not (Fig. 3). Morphologically *C. betonicifolium* is distinct with its reclining stems, auriculate leaf bases, and expanded tips of the pappus bristles. Geographically the two taxa are parapatric, with *C. betonicifolium* var. *betonicifolium* occurring along the southern Gulf of Mexico coast from the Yucatan peninsula as far north as southern Texas. The distribution of *C. coelestinum* shows a very slight overlap with *C. betonicifolium* var. *betonicifolium* in southern Texas, and extends north to Ontario and Michigan and east to New York. Furthermore, there is difference in habitat characteristics: although both form patches in marsh communities, *C. betonicifolium* is restricted to saline coastal habitats whereas *C. coelestinum* occurs in freshwater swamps, marshes, and moist terrestrial habitats. Thus there is evidence to suggest the two species be maintained as distinct.

The low amount of sequence-level variability within *Conoclinium* was a notable and somewhat unanticipated result. There are two factors that may explain lack of variability, recent divergence and introgession. The lack of any variation in *Conoclinium* in the *trn*H-*psb*A spacer that is a typically highly variable region in other plants suggests that divergence within *Conoclinium* has been quite recent. The pattern of morphological variability observed by field workers (e.g. Turner, 1997) is consistent with the occurrence of interspecific hybridization, and our results for the garden ornamental of *C. dissectum* indicate that such hybridization could take place. Particularly notable was the lack of variability within the widespread *C. coelestinum* and between it and its apparent sister taxon, *C. betonicifolium*. The most consistent character that separates the two species is the pappus bristles, which are apically expanded in *C. betonicifolium*. It is possible that the change in pappus bristle morphology increased the dispersability of the cypselae and helped *C. coelestinum* to become more widespread in the wet areas of the eastern United States.

A corollary of the lack of sequence level variability is that *Conoclinium coelestinum* appears to be a relatively recent immigrant into eastern North America. This contrasts to other perennial herbaceous Asteraceae, such as *Eupatorium*, *Liatris*, and *Helianthus*, which occupy the same general geographic range, but are represented by numerous species whose ranges and species-level divergence have probably been affected by the advance and ebb of glaciation. A similar lack of variability across a wide geographic distribution was noted for *Fleischmannia incarnata*, in which a survey of four specimens from various parts of its range in eastern North America failed to reveal any variation in the ITS region (Schilling, unpublished data). Differences in the time of initial invasion into the region could explain the different patterns of species level variability observed in these genera.

## TAXONOMY

**Conoclinium gonzaleziae** E. E. Schill. & Panero. MEXICO. DURANGO. Municipio Mezquital: al W de Santa María de Ocotán, 22.91 N, 104.61 W, 2020 m, pine-oak forest, 16 Oct 1984, Martha González and Saturnino Acevedo 1548 (HOLOTYPE: CIIDIR!; ISOTYPE: TEX!).

Similar to *Conoclinium mayfieldii* T.F. Patt., but differing in the glabrous receptacle and in having ovate to lanceolate leaves with deeply crenate or dentate lobes rather than broadly ovate to oblong, shallowly crenate leaves.

Annual or perennial HERBS 30-70 cm tall. STEMS sparsely to densely tomentose to villous with tapered white or translucent trichomes 2.0-3.0 mm long, with a few, scattered, glandular trichomes appearing as resin dots. LEAVES opposite, petiolate, blades lanceolate to ovate, rarely trullate, 2.0-4.0 cm long, 1.0-3.0 cm wide, apices acute, bases obtuse to truncate sometimes cuneate, margins deeply crenate to dentate, rarely shallowly lacerate, lowermost lobe sometimes bilobed, abaxial surface scabrous especially along veins, adaxial surface minutely bullate, sparsely scabrous to moderately velutinous, petioles 0.3-1.0 cm long. INFLORESCENCES of 5-10 capitula arranged in congested simple cymes, pedicels 1.2-2.0 cm long. CAPITULA discoid with 65-90 flowers, 0.5-0.9 cm tall, 0.5-1.0 cm wide. INVOLUCRE campanulate, phyllaries in 3 series, sparsely pubescent on abaxial surface, glabrous on adaxial surface, first and second series 3.5-4.2 mm long, 0.8-0.9 mm wide, narrowly lanceolate, third series 2.7-2.9 mm long, 0.4 mm wide, linear, receptacles conical, alveolate, seldom with one or two scattered enations resembling minute mucros, otherwise glabrous. COROLLAS narrowly campanulate, blue to violet, drying bright magenta pink, 3.0 mm long, tubes 1.6 mm long, lobes 5, 0.5 mm long, resin canals amber-orange flanking vascular strands, abaxial surface with glandular twin trichomes with prominent terminal cells, anthers 5, 1.1-1.2 mm long, appendages broadly obtuse, base of anthers obtuse, anther collar 0.2 mm long, endothecial thickenings 1-3 on radial and tangential walls, style 5.8-6.2 mm long, violet, style branches 3.3-3.5 mm long, papillose and slightly expanded on distal, rounded end, stigmatic area 1.2 mm long, appendage 2.2-2.3 mm long. CYPSELAE 1.6-2.0 mm long, black, ribs 5, with scattered twin trichomes on and in-between ribs, pappus of 22-28 unequal, stramineous bristles, 1.5-3.0 mm long.

Representative specimens: MEXICO. Chihuahua. Municipio Ocampo: Parque Nacional de la Cascada Basaseachic, 108° 12′ 30” W, 28° 10′ N, 17 Sep 1989, *R. Corral Díaz & S. Avalos M. RCD 3419* (TEX); Area of Cascada de Basaseachic at the confluence of Río Basaseachic and Río Durazno, 2 mi S of village of Basaseachic, 107° 55′ W, 28° 03” N, 2000-2050 m, 17-20 Oct 1986, *G. L. Nesom & L. Vorobik 5636* (TEX). Municipio Temósachi: Yepáchic, 108°, 22′ 19.99′ W, 28° 25′ 18.98” N, 19 Sep 1971, C. W. Pennington 170 (TEX). Durango. Municipio Durango: Sierra Madre Occidental, Mesa Los Timones, 1-2 km N of Hwy 40 on rd to Otinapa, ca. 43 km W of Cd Durango, 104° 55′ W 23° 55′ N, 3 Sep 1997, *A. C. Sanders, F. M. Roberts, P. MacKay, T. Thomas, M. Egger, and S. Eliason 21346* (TEX). Arroyo Los Mimbres, on E side of arroyo, between the lowest switchback of Durango Hyw 40, near bridge, 104° 55′ W, 23° 56′ N, 2200 m, 19 Oct 1993, *T. F. Patterson 7467* (TEX). Sonora. Municipio Yécora: La Dura, 10.6 km E of Maycoba on Mex 16, 108°, 34′ 52” W, 28° 24′ 59” N, 1620 m, Sep 15 1999, *T. R. Van Devender & A. L. Reina G. 99-620* (TEX).

*Conoclinium gonzaleziae* is found in rocky slopes in pine-oak forests between 1900 and 2500 m. It is widely distributed in the Sierra Madre Occidental of Chihuahua, Durango, and Sonora.

*Conoclinium gonzaleziae* differs from *C. mayfieldii* in having ovate to lanceolate leaves with deeply crenate or dentate lobes rather than broadly ovate to oblong, shallowly crenate leaves. The conical receptacles in *Conoclinium gonzaleziae* are glabrous whereas *C. mayfieldii* has moderately to densely pubescent alveolate, conical receptacles. *Conoclinium gonzaleziae* in the Sierra Madre Occidental of western Mexico has been long isolated from *C. mayfieldii* in the Sierra Madre Oriental and Sierra Tamaulipas of eastern Mexico.

The species name honors Martha González who has made extensive collections of plants in central and southern Durango that have enhanced our understanding of the distribution and diversity of the flora of the state.

## Designation of Types

Neotypes are required for two species of *Conoclinium*.

**Conoclinium oligolepis** Kunze, Del. Sem. Hort. Lips. (1840) iv. 2. Type: Mexico, “cool region of Mexico”, *Ehrenberg* (B, destroyed). Neotype, here selected: MEXICO. Coahuila: 25 km al N de Zaragoza por la carretera 29 (Zaragoza-Acuña), 13 Sep 1995, *Carranza C-2587* (MEXU; isoneotype: TEX!).

Notes: *Conoclinium oligolepis* is an earlier name than *C. integrifolium* (A. Gray) Small, and a sample was collected by Carl August Ehrenberg in 1837 in a region designated only as a “cool region of Mexico.” Ehrenberg collected in the area of Mineral del Monte in the state of Mexico that is now in the state of Hidalgo. The type was destroyed in the bombing of Berlin. A neotype thus needs to be selected, for which the specimen *Carranza C-2587* (MEXU; isoneotype TEX, DNA 2426 in the analysis) is selected.

**Conoclinium dichotomum** Chapm., Bot. Gaz. 3: 5, 1878. Neotype, here selected: USA. Florida: Peas Creek, July 1878, *A. P. Garber 3* (NY; Isoneotype: NY).

Notes: the original publication did not designate a type, so a neotype must be selected. There are two sheets labeled *A. P. Garber 3* at NY, sheet barcode 8251 is designated as the neotype, and sheet barcode 8252 is designated as an isoneotype. The locality “Peas Creek” has also been referred to as Peace Creek and is now known as Peace River.

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## Appendix. Samples of *Conoclinium* and related genera and outgroups used for DNA analysis. Vouchers at TENN unless otherwise indicated. GenBank accession numbers listed as (ITS, ETS, *trn*H-*psb*A, *rbc*L, *ndh*F, *mat*K) for samples with all six markers, and (ITS, ETS, *trn*H-*psb*A) for other samples; -, sequence not obtained.

### *Ageratum*:

**A. albidum** Hemsl., MEXICO. Oaxaca. *Panero 2493* (TEX), DNA 2655 (MN556263, MN558650, MN558611). **A. conyzoides** L., MEXICO. Oaxaca. *Panero 8827*, DNA 2404 (MN556261, MN558648, MN558609). **A. corymbosum** Zucc. ex Pers., MEXICO. Oaxaca. *Panero 8835*, DNA 2405 (MN556549, MN558649, MN558610); Queretaro. *Hernandez 102249*, DNA 2336 (MN556260, MN558647, MN558608). **A. echioides** Hemsl., MEXICO. Guerrero. *Keil 15315* (TEX), DNA 2656 (MN556264, MN558651, MN558612). **A. gaumeri** B. L. Rob., BELIZE. Belize District. *Worthington 24088* (TEX), DNA 2657 (MN556265, MN558652, MN558613). **A. houstonianum** Mill., Cultivated, *Schilling 04-24*, DNA 1163 (AF177789, MN558687, EU337031, MN558597, EU337042, EU337054). **A. maritimum** Kunth, MEXICO. Quintana Roo. *Webster 17629* (TEX), DNA 2660 (MN556268, MN558655, MN558616). **A. microcephalum** Hemsl., MEXICO. Oaxaca. *Maya 779* (TEX), DNA 2659 (MN556267, MN558654, MN558615). **A. paleaceum** Hemsl., MEXICO. Oaxaca. *Calzada 19576* (TEX), DNA 2658 (MN556266, MN558653, MN558614).

### *Conoclinium*:

**C. betonicifolium** (Mill.) R. M. King & H. Rob., MEXICO. Campeche. *Soule 2081* (TEX), DNA 2425 (MN556285, MN558673, MN558634); U.S.A. Texas. *Calvert 108* (TEX), DNA 2455 (MN556286, MN558674, MN558635); U.S.A. Texas. *Lemke 2973* (TEX), DNA 2456 (MN556287, MN558675, MN558636). **C. coelestinum** DC., U.S.A. Arkansas. *Schilling 04-58*, DNA 1171 (MN556262, MN558688, EU337033, MN558599, EU337044, EU337056); Florida. *Godfrey 84757*, DNA 2509 (MN556280, MN558667, MN558628); *Wunderlin 10041*, DNA 2677 (MN556277, MN558664, MN558625); *Schilling 09-F01*, DNA 2911; Illinois. *Shaw s.n.*, DNA 2511 (MN556282, MN558669, MN558630); *Koelling 193*, DNA 2681 (MN556278, MN558665, MN558626); Louisiana. *Douglas 753*, DNA 2510 (MN556281, MN558668, MN558629); New Jersey. *Martin 8282*, DNA 2674 (MN556275, MN558662, MN558623); Tennessee. *Schilling s.n.*, DNA 2494 (MN556279, MN558666, MN558627); Texas. *Conner 26*, DNA 2675 (MN556276, MN558663, MN558624). **C. dichotomum** Chapm., U.S.A. Florida. *Wunderlin 9853*, DNA 2678 (MN556283, MN558670, MN558631); *McDaniel 9147*, DNA 2679 (MN556284, MN558671, MN558632); *McDaniel 9461*, DNA 2680 (MN556550, MN558672, MN558633); *Schilling CD1*, DNA 3201 (MN556297, -, -); *Schilling CD2*, DNA 3202 (MN556298, -, -); *Schilling CD3*, DNA 3203 (MN556299, -, -); *Schilling CD4*, DNA 3204 (MN556300, -, -). **C. dissectum** A. Gray, MEXICO. Sonora. *Van Devender 2002-610* (TEX), DNA 2451 (MN556288, MN558676, MN558637); Nuevo Leon. *Lavin 4847a* (TEX), DNA 2452 (MN556289, MN558677, MN558638); Durango. *AC San Jens 6736* (TEX), DNA 2453 (MN556290, MN558678, MN558639). **C. gonzaleziae** sp.nov., MEXICO. Chihuahua. *Corral Diaz RCD 3419* (TEX), DNA 2449 (MN556295, MN558683, MN558644); Sonora. *Van Devender 99-620* (TEX), DNA 2450 (MN556296, MN558684, MN558645); Durango. *Patterson 7467* (TEX), DNA 2629 (MN556551, MN558685, MN558646). **C. mayfieldii** T. F. Patt., MEXICO. Tamaulipas. *Patterson 7362* (TEX), DNA 2424 (MN556291, MN558679, MN558640); *Hinton et al. 25030* (TEX), DNA 2457 (MN556292, MN558680, MN558641). **C. oligolepis**

Kunze, MEXICO. Coahuila. *Carranza C-2587* (TEX), DNA 2426 (MN556293, MN558681, MN558642); Nuevo Leon. *Hinton et al 22372* (TEX), DNA 2460 (MN556294, MN558682, MN558643);

### *Fleischmannia*:

***F. capillipes*** (Oerst.) R. M. King & H. Rob., MEXICO. Oaxaca. *Panero 8859*, DNA 2263 (MN556273, MN558660, MN558621). ***F. imitans*** (B. L. Rob.) R. M. King & H. Rob., MEXICO. Oaxaca. *Panero 8869*, DNA 2402 (MN556274, MN558661, MN558622). ***F. incarnata*** (Walter) R. M. King & H. Rob., U.S.A. Tennessee. *Schilling 95-21*, DNA 575 (AF177788, KP454659, EU337032, MN558598, EU337043, EU337055). ***F. pycnocephala*** (Less.) R. M. King & H. Rob., MEXICO. Veracruz. *Dressler 100*, DNA 2338 (MN556271, MN558658, MN558619); Tamaulipas. *Sharp 52104*, DNA 2339 (MN556272, MN558659, MN558620). ***F. remotifolia*** (DC.) R. M. King & H. Rob., BRAZIL. *M.D. de Moraes 758,* DNA 2178 (MN556269, MN558656, MN558617). ***F. sonorae*** (A.Gray) R. M. King & H. Rob., MEXICO. Zacatecas. *Panero 8821*, DNA 2234 (MN556270, MN558657, MN558618).

### *Paneroa:*

***P. stachyofolia*** (B. L. Rob.) E. E. Schill., MEXICO. Oaxaca, *Panero 4453*, DNA 2335 (MN556304, MN558689, EU337034, MN558601, EU337045, EU337057); *Panero 5894*, DNA 2682 (MN605893, MN607174, MN607173)

### Outgroups:

***Campuloclinium hirsutum*** Gardner, BRAZIL. *M.D. de Moraes 720* (TEX) DNA 2103 (KP454329, KP454631, MN558602, MN558590, KP454914, MN558584). ***Carphephorus corymbosus*** (Nutt.) Torr. & A. Gray. U.S.A., *Schilling 2036*, DNA 772 (HQ416307, HQ416400, AY727174, HQ416175, EU337037, EU337049). ***Conocliniopsis prasiifolia*** (DC.) R. M. King & H. Rob., BRAZIL, *M.D. de Moraes 701* (TEX), DNA 2095 (KP454341, KP454645, MN558603, MN558600, KP454925, MN558585). ***Critonia sexangularis*** (Klatt.) R. M. King & H. Rob. MEXICO. Oaxaca. *Panero 2970* (TEX), DNA 539 (KJ637169, KP454649, EU337028, MN558595, EU337039, EU337051). ***Chromolaena odorata*** (L.) R. M. King & H. Rob., *Panero 2004-2*, DNA 2059 (MN556301, KP454641, MN558606, MN558593, KP454921, MN558588). ***Eupatorium hyssopifolium*** L., U.S.A., *Siripun 02-Eup-157*; DNA 870 (DQ236177, HQ416395, AY727172, HQ416170, EU337035, EU337047). ***Eutrochium maculatum*** (L.) E. E. Lamont, U.S.A., Schilling 95-16, DNA 532 (AF177798, HQ416396, EU337026, HQ416171, EU337036, EU337048). ***Gyptis tanacetifolia*** (Gilles ex Hook. & Arn.) D. J. N. Hind. & Flann, ARGENTINA. Buenos Aires. *Panero & Crozier 8378* (TEX), DNA 2065; (KP454365, KP454672, MN558604, MN558591, KP454942, MN558586). ***Heterocondylus pumilus*** (Gardner) R. M. King & H. Rob. BRAZIL. *M.D. de Moraes 715* (TEX), DNA 2101 (KP454370, KP454677, MN558605, MN558592, KP454946, MN558587). ***Hofmeisteria fasciculata*** (Benth.) Walp., MEXICO. Baja California. *Panero 2817* (TEX), DNA 544 (AF374907, MN558690, EU337025, AY215125, AF384731, EU337046). ***Koanophyllon ayapanoides*** (Griseb.) R. M. King & H. Rob. CUBA. *Bennet 7582*, DNA 2061 (KJ637172, KP454686, EU337027). ***Koanophyllon villosum*** (Sw.) R. M. King & H. Rob., CAYMAN ISLANDS. *Hite s.n.*, DNA 2061 (MN556302, KP454689, MN558607, MN558594, KP454955, MN558589). ***Tamaulipa azurea*** (DC.) R. M. King & H. Rob., U.S.A. Texas. *Schilling 1146*, DNA 1146 (MN556303, MN558686, EU337030, MN558596, EU337041, EU337053).