

Independent Origins of Aquatic Eupatorieae (Asteraceae)

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Abstract—Plants that have adapted to grow as submersed aquatics are relatively rare among angiosperms, yet they represent a convergent strategy that has evolved in many groups. Asteraceae tribe Eupatorieae include a number of obligate wetland species, but only three genera (*Gymnocoronis*, *Sclerolepis*, and *Shinnersia*) routinely grow submersed. We conducted a molecular phylogenetic study of Eupatorieae to assess the relationships among aquatic genera and to infer the evolutionary history of the submersed aquatic habit. Two major clades were identified in Eupatorieae, corresponding to base chromosome numbers of $x = 10$ or fewer, and $x = 11$ or greater. We determined that submersed aquatics evolved independently in two subtribes, *Adenostemmatinae* (*Gymnocoronis*; $x = 10$) and *Trichocoroninae* (*Sclerolepis* and *Shinnersia*; $x = 15$), each belonging to a separate major clade, and that the aquatic lineages evolved in allopatry. Sparse taxon sampling precluded a firm assessment of ancestral states, although two widespread genera, *Adenostemma* and *Mikania*, were implicated as likely relatives of the aquatic lineages. Our data also support the continued recognition of *Shinnersia* and *Trichocoronis*, which occasionally have been considered synonymous, as distinct genera.

Keywords—Aquatic angiosperms, bogbutton, bugheal, molecular phylogenetics, Senegal tea.

Aquatic plants generally are uncommon among angiosperms, yet the aquatic habit has evolved independently over 200 times (Cook 1999). Although it is difficult to apply a definition of “aquatic” universally, several genera of Asteraceae Bercht. & J. Presl tribe Eupatorieae Cass. contain species that clearly tolerate inundated soil conditions as well as periods of complete immersion, at least of their vegetative tissues. Worldwide, Cook (1999) recognized 18 Asteraceae genera (e.g. *Bidens* L., *Cotula* L., *Hydropectis* Rydb.), including four Eupatorieae genera (*Gymnocoronis* DC., *Sclerolepis* Cass., *Shinnersia* R. M. King & H. Rob., and *Trichocoronis* A. Gray), as including aquatic species, and he hypothesized that the latter taxa together represented a single origin of the aquatic habit in the family. The evolutionary derivation of aquatic habit in Eupatorieae, however, including the number of independent origins, and the morphology and biogeography of related species, has yet to be examined critically in a phylogenetic context.

In the U. S. A., several Eupatorieae genera (*Eupatorium* L., *Eutrochium* Raf., *Hartwrightia* A. Gray, *Mikania* Willd., *Sclerolepis*, *Shinnersia*) contain species designated as “obligate” wetland plants (i.e. OBL), a category for taxa found almost exclusively in aquatic habitats in at least some part of their ranges (USDA NRCS 2013). Several other genera (*Adenostemma* J. R. Forst. & G. Forst., *Carphephorus* Cass., *Conoclinium* DC., *Liatris* Gaertn. ex Schreb., and *Trichocoronis*) contain “facultative” wetland species (i.e. FACW), which suggests their reduced affinity for aquatic habitats. For the purpose of this study, we exclude *Trichocoronis* from the category of fully aquatic, following USDA NRCS (2013). Likewise, we omit from this category those Eupatorieae (*Carphephorus*, *Eupatorium*, *Eutrochium*, *Hartwrightia*, and *Mikania*) which are designated as OBL but grow only as emergent wetland plants. Comprehensive wetland classification schemes are lacking for species that occur outside of the U. S. A., although several genera (e.g. *Alomia* Kunth, *Bishopiella* R. M. King & H. Rob., *Hofmeisteria* Walp.) notably

include species that inhabit wetlands or survive wet seasons as submersed rhizomes (King and Robinson 1972, 1981; Turner 1997; Esteves 2001).

Two of the fully aquatic Eupatorieae genera consist of one species each (i.e. *Sclerolepis uniflora* and *Shinnersia rivularis*). Taxonomic treatments occasionally consider *Shinnersia* to be congeneric with *Trichocoronis* (e.g. USDA NRCS 2013), whereas *Flora of North America* (Nesom 2006a) supports the taxonomic distinctness established by King and Robinson (1970a, 1987). The two species of *Gymnocoronis* (*G. latifolia* Hook. & Arn. and *G. spilanthoides*) have non-overlapping distributions in Mesoamerica and South America, respectively. Three additional *Gymnocoronis* species formerly considered independent (*G. matudae* R. M. King & H. Rob., *G. nutans* (Greenm.) R. M. King & H. Rob., and *G. sessilis* S. F. Blake; King and Robinson 1987) were merged with *G. latifolia* by Turner (1997), who also noted the morphological similarity between *G. latifolia* and *G. spilanthoides*.

The submersed aquatic habit of *Gymnocoronis*, *Sclerolepis*, and *Shinnersia* represents a marked departure from the growth forms of other Eupatorieae, which are at most only wetland plants that tolerate inundated soil conditions (King and Robinson 1987; Robinson 1913; Cook 1999; Robinson et al. 2009). Taxonomic treatments have placed the aquatic genera consistently within Eupatorieae; however, their relative positions within the tribe have been difficult to ascertain due to abundant morphological homoplasy among genera (King and Robinson 1987; Robinson et al. 2009). Apart from analyses of sparse molecular sequence data for *Sclerolepis* (Ito et al. 2000b; Robinson et al. 2009), the relationships among these unusual aquatic composites have not been investigated phylogenetically.

Subtribal classifications provide some insight into phylogenetic relationships in Eupatorieae. Robinson et al. (2009) recognized 19 subtribes, a slight adjustment from what King and Robinson (1987) established without the aid of molecular data. The aquatic genera on which our study focuses belong

to two subtribes that have been circumscribed consistently (King and Robinson 1987; Robinson et al. 2009), owing to their prominent synapomorphies. *Sclerolepis* and *Shinnersia*, along with *Trichocoronis*, belong to Trichocoroninae R. M. King & H. Rob., a subtribe with floral similarity to Ageratinae Less. but sharing distinct character states such as sessile leaves (King and Robinson 1987; Robinson et al. 2009). Subtribe Adenostemmatinae B. L. Rob., comprising *Gymnocoronis* as well as *Adenostemma* and *Sciadocephala* Mattf., are distinguished by soft receptacles and glandular-viscid appendages on the achene or pappus (King and Robinson 1974, 1987). The fact that these subtribes contain both aquatic (i.e. *Gymnocoronis*, *Sclerolepis*, *Shinnersia*) and semi-aquatic taxa (i.e. *Adenostemma*, *Trichocoronis*) accentuates the need to understand their interrelationships and reconstruct their evolutionary history.

Using the monograph by King and Robinson (1987) as a guide, intergeneric relationships and the validity of Eupatorieae genera and subtribes have been evaluated increasingly by incorporating molecular data (Ito et al. 2000a, 2000b; Schmidt and Schilling 2000; Robinson et al. 2009; Bastos et al. 2011; Schilling 2011; Schilling et al. 2013). The broad sample of existing Eupatorieae sequence data enables fairly robust placement of novel sequences as they are generated. However, molecular data for aquatic Eupatorieae are available only for *Sclerolepis* and are lacking for *Gymnocoronis* and other members of subtribe Adenostemmatinae. The dearth of molecular data for these groups has impeded progress in evaluating their interrelationships, but preliminary studies have indicated a sister relationship between Trichocoroninae and *Ageratina* Spach of subtribe Oxylobinae R. M. King & H. Rob. (Ito et al. 2000a, 2000b; Robinson et al. 2009), rather than with Ageratinae as King and Robinson (1987) had hypothesized previously. Thus it is evident that a more comprehensive study of this group is necessary.

Eupatorieae occur almost entirely in North and South America, with only three genera (*Adenostemma*, *Mikania*, *Stomatanthus* R. M. King & H. Rob.) having broader native ranges (King and Robinson 1987). Of the 170 Eupatorieae genera, 27 are found in North America north of Mexico (Barkley et al. 2006). The three genera with submersed aquatic species have fairly localized native ranges. *Shinnersia* is native to Mexico and Texas (Nesom 2006a), whereas *Sclerolepis* occurs in the eastern United States (Lamont 2006). *Gymnocoronis* is native to Mesoamerica and South America, although weedy, naturalized populations of *G. spilanthoides* exist in Australia, China, Japan, and New Zealand (Webb et al. 1995; Muranaka et al. 2005; Weber et al. 2006; Gao and Liu 2007). *Shinnersia rivularis* likewise has colonized beyond its native range and recently became established in Europe (Eliáš et al. 2009). The narrow native distribution of Trichocoroninae (i.e. temperate Mexico northwards) relative to Adenostemmatinae (primarily tropical), as well as the lack of any range overlap between these subtribes, raises the possibility that the aquatic habit evolved independently in the two groups.

Phylogenetic studies have indicated that Eupatorieae subtribes exhibit some biogeographical structure, with representatives of primarily North American and Mesoamerican groups (e.g. Ageratinae, Alomiinae Less.) resolving as a grade toward more South American lineages (e.g. Gyptidinae R. M. King & H. Rob., Praxelinae R. M. King & H. Rob.)

(Robinson et al. 2009). The phylogeographic structure of Eupatorieae thus could facilitate an understanding of the evolutionary origin of contemporary aquatic genera.

Shinnersia and *Trichocoronis* notably occupy wetland habitats within environments that overall are inhospitable to aquatic plants, namely the arid deserts of southern Texas and northern Mexico. The existence of aquatic plants in challenging environments is far from rare, however, with diverse aquatic floras able to survive freezing temperatures in much of the northern hemisphere, and seasonally dry conditions in northern Australia and elsewhere (Cook 1996). The exceptional nature of arid environments nonetheless provides an impetus for evolutionary novelty, and it would be instructive to elucidate the evolutionary and biogeographical history that culminated in their establishment in these areas.

In this study we investigate the evolutionary origin of the submersed aquatic habit in Eupatorieae and evaluate the relationships among genera by conducting a molecular phylogenetic analysis of *Gymnocoronis*, *Sclerolepis*, *Shinnersia*, and related genera. Our principal objectives are to determine the number of independent origins of the aquatic genera and to evaluate any biogeographical or evolutionary patterns associated with the transition to this unusual habit in the principally terrestrial Asteraceae.

MATERIALS AND METHODS

Nucleotide sequence data were obtained from fresh material or dried herbarium specimens, representing 20 accessions (Appendix 1). Extraction, amplification, and DNA sequencing of the nuclear internal transcribed spacer (ITS) and plastid *matK/trnK* and *rbcL* regions followed previously reported methods (Panero and Crozier 2003; Les et al. 2008; Tippery and Les 2011; Schilling et al. 2013). Sequencing was performed on an ABI PRISM® 3100 genetic analyzer (Applied Biosystems, Foster City, California).

DNA sequence chromatograms were edited, assembled into contigs, and aligned using Geneious ver. 6.0.5 (BioMatters Ltd., Auckland, New Zealand) and Mesquite ver. 2.75 (Maddison and Maddison 2011). Following initial alignment in Geneious using the MUSCLE algorithm (Edgar 2004), nucleotide sequences were aligned manually in Mesquite under the similarity criterion (Simmons 2004). Sequences newly obtained for this study were combined with outgroup sequences retrieved from GenBank (Appendix 1). Sequence data for *rbcL* were deemed to be insufficiently variable and available for too few taxa to be useful phylogenetically, although we did deposit the sequences in GenBank (Appendix 1). The aligned matrix of ITS data consisted of 59 accessions and 776 characters (293 constant, 373 parsimony-informative, 7.2% missing), and the *matK* matrix had 48 accessions and 1,540 characters (1,236 constant, 138 parsimony-informative, 13.3% missing). The combined ITS + *matK* matrix comprised 39 accessions and 2,305 characters (1,552 constant, 464 parsimony-informative, 7.4% missing). Data scored as missing constituted primarily portions of the target genes that were entirely lacking, either in newly generated data or in reference sequences obtained from GenBank; missing data also included character states that were reported as entirely ambiguous (i.e. 'N', symbolizing any of the four DNA nucleotides). Aligned data matrices were uploaded to TreeBASE (study number 15387).

Combinability of ITS and *matK* data was evaluated by conducting constrained analyses on the subset of taxa for which both ITS and *matK* sequences were available. Constrained analyses were run in GARLI ver. 2.0.1019 (Zwickl 2006), after which the site-specific likelihoods were evaluated using the approximately unbiased (AU) test (Shimodaira 2002), conducted using the *scaleboot* package (Shimodaira 2008) in R ver. 3.0.2 (R Core Team 2013). After whole-tree constraint analyses (i.e. where each data set was constrained to the topology produced by the other) indicated that ITS and *matK* topologies were significantly incongruent (see Results), nodes were evaluated individually to ascertain sources of phylogenetic conflict. Subsequent AU tests were conducted only on nodes that received Bayesian posterior probabilities (PP) of 0.80 or greater on either tree. Single-node constraint analyses were conducted on a trimmed data matrix containing only those genera with sequences for both ITS and

matK. After determining that topological incongruence was isolated from the aquatic taxa of interest (see Results), ITS and *matK* data were combined and analyzed as a single matrix. Sequences that were combined for the ITS + *matK* matrix were representative of a single species where possible; otherwise sequences from two species in the same genus were combined and analyzed as one taxon. Topological hypothesis testing also was conducted using the AU test on separate and combined data matrices, with a constraint corresponding to the hypothesis that the submersed aquatic habit had a single evolutionary origin (i.e. *Gymnocoronis* + *Sclerolepis* + *Shinnersia* were constrained to be monophyletic).

Separate and combined molecular data were analyzed using maximum likelihood (ML; Felsenstein, 1973) via GARLI and Bayesian MCMC inference (Yang and Rannala 1997) methods via BEAST ver. 1.8.0 (Drummond and Rambaut 2007). The GTR + I + G model was applied to all analyses, following model selection with jModeltest ver. 2.1.4 (Posada 2008) under the AIC criterion (Akaike 1974). Ten separate likelihood runs were performed using different random starting seeds, and the tree with the maximum likelihood score was compared with the Bayesian consensus tree. Bootstrap analysis was conducted in GARLI using 1,000 replicates. Bayesian analyses each consisted of one MCMC chain and were run for ten million generations, with sampling every 1,000 generations, and the first two million generations excluded as burn-in. Other parameters included a Yule tree prior (Yule 1925; Gernhard 2008). Mixing and effective sample sizes were evaluated using Tracer ver. 1.6 (Rambaut and Drummond 2009), and consensus trees were generated using TreeAnnotator ver. 1.8.0 (Drummond and Rambaut 2007). Divergence times were estimated in BEAST using the combined ITS + *matK* data set, with the age of the root node (i.e. the most recent common ancestor of Perityleae and Eupatorieae) set at 14 million years ago (Ma) (normal distribution, standard deviation = 1), and with other parameters as reported previously (Schilling et al. 2013), based on a rate-calibrated analysis of Asteraceae (Kim et al. 2005). The ingroup was constrained to be monophyletic, in keeping with previous evidence.

RESULTS

[F1], [F2] Independent analyses of ITS (Fig. 1) or *matK* (Fig. 2) data produced largely congruent trees, although several lineages were resolved inconclusively. Both analyses obtained weak support for a clade comprising Hofmeisteriinae R. M. King & H. Rob. and Piqueriinae Benth. & Hook. f., as well as a larger clade that also included Mikaniinae R. M. King & H. Rob., Oxylobinae, and Trichocoroninae. In the *matK* analysis this clade also included Neomirandeiinae R. M. King & H. Rob. For convenience we refer to the clade containing the aforementioned subtribes as ‘major clade 1’ (MC1). Trichocoroninae were strongly supported as monophyletic in both analyses, although their sister relationship with either Mikaniinae (ITS) or Mikaniinae + Oxylobinae (*matK*) was inconsistent between the single-gene analyses.

The second major, well-supported clade (MC2) contained nine subtribes (Figs. 1–2). The well-sampled Eupatoriinae Dumort. and Liatrinae R. M. King & H. Rob. were sister lineages (ITS) or unresolved relative to each other (*matK*). Other subtribes were represented by only one or two species, and their relationships were rather inconsistent, although a number of nodes received strong support. Adenostemmatinae were consistently and strongly supported as monophyletic, with the ITS data supporting Ayapaninae R. M. King & H. Rob. as their sister lineage, whereas *matK* failed to resolve any of four lineages as the sister lineage. Species of Alomiinae were represented only by ITS data, based on which they were resolved as a basal grade toward MC2 (Fig. 1). Additionally, representatives of *Paneroa* E. E. Schill. and *Tamaulipa* R. M. King & H. Rob., for which only *matK* data were available, resolved within MC2 but without clear indication of what lineages comprise their closest relatives.

Besides the aquatic genera, our study included several species for which sequence data had not been analyzed pre-

viously for one or both of ITS and *matK*. *Adenostemma* (novel ITS and *matK* data) resolved consistently and strongly with *Gymnocoronis* in both analyses. Newly added *matK* data for *Austroeupatorium* R. M. King & H. Rob. and *Stomatanthes* resolved these genera as sister lineages, a result congruent with the ITS analysis. Novel ITS data for *Critonia* P. Browne and *Koanophyllon* Arruda resolved these taxa among a diverse assemblage of MC2 subtribes, in a topology similar to that indicated by the *matK* tree. *Chromolaena* DC. (novel *matK* data) also resolved within this assemblage in both ITS and *matK* analyses.

A whole-tree topology test indicated significant incongruence ($p < 0.001$) between ITS and *matK* data, leading us to evaluate congruence at individual nodes. In all, six nodes on the ITS tree and eight nodes on the *matK* tree were resolved differently than on the other tree, at the 0.80 PP cutoff value (Figs. 1–2). Significant incongruence was indicated for two nodes on the ITS tree (*Fleischmannia* + *Koanophyllon* [$p = 0.021$] and *Hartwrightia* + *Trilisa* [$p = 0.022$]), and for one node on the *matK* tree (*Hartwrightia* + *Litrisa* + *Liatris* [$p < 0.001$]). Nodes that were implicated in the evolution of aquatic habit (i.e. those involving Mikaniinae / Oxylobinae / Trichocoroninae and Adenostemmatinae / Ayapaninae), although incongruent between ITS and *matK* trees, were not significantly incongruent after AU analysis.

The combined ITS + *matK* data analysis produced a phylogeny (Fig. 3) that was largely congruent with independent [F3] ITS and *matK* trees. The sister relationship between Trichocoroninae and Mikaniinae received strong support, with an estimated divergence time of 8.9 Ma. *Adenostemma* and *Gymnocoronis* (i.e. Adenostemmatinae) also were monophyletic, and estimated to have diverged about 4.9 Ma. Divergence estimates within Trichocoroninae placed the split of *Sclerolepis* from *Shinnersia* + *Trichocoronis* at 6.2 Ma, and *Shinnersia* from *Trichocoronis* at 3.5 Ma. The topological test of whether data were consistent with a monophyletic origin of aquatic taxa (*Gymnocoronis* + *Sclerolepis* + *Shinnersia*) rejected this hypothesis in all analyses (ITS, *matK*, and combined data; $p < 0.001$).

DISCUSSION

Evolution of the Aquatic Habit—Eupatorieae are exceptionally diverse ecologically, comprising forest, desert, and prairie species in addition to the aquatic and helophytic (wetland) genera that are the focus of our study (Robinson et al. 2009). Our analyses support at least two independent evolutionary origins of the submersed aquatic habit, once in the ancestor of subtribe Trichocoroninae and again in *Gymnocoronis*. These lineages each belong to a different major clade of Eupatorieae, and the intervening lineages between them for the most part lack aquatic species. Of the Eupatorieae genera with obligate wetland representatives, three (*Eupatorium*, *Eutrochium*, and *Hartwrightia*) belong to the clade of Eupatoriinae + Liatrinae and are phylogenetically isolated from both Trichocoroninae and *Gymnocoronis* (Fig. 3). Obligate wetland species also are found in *Mikania*, the genus we determined to be most closely related to Trichocoroninae. Morphologically diverse and geographically widespread, *Mikania* contains predominantly neotropical vines and epiphytes (King and Robinson 1987; Robinson et al. 2009) but also a number of wetland species (USDA NRCS 2013). Although our taxon sampling was limited in

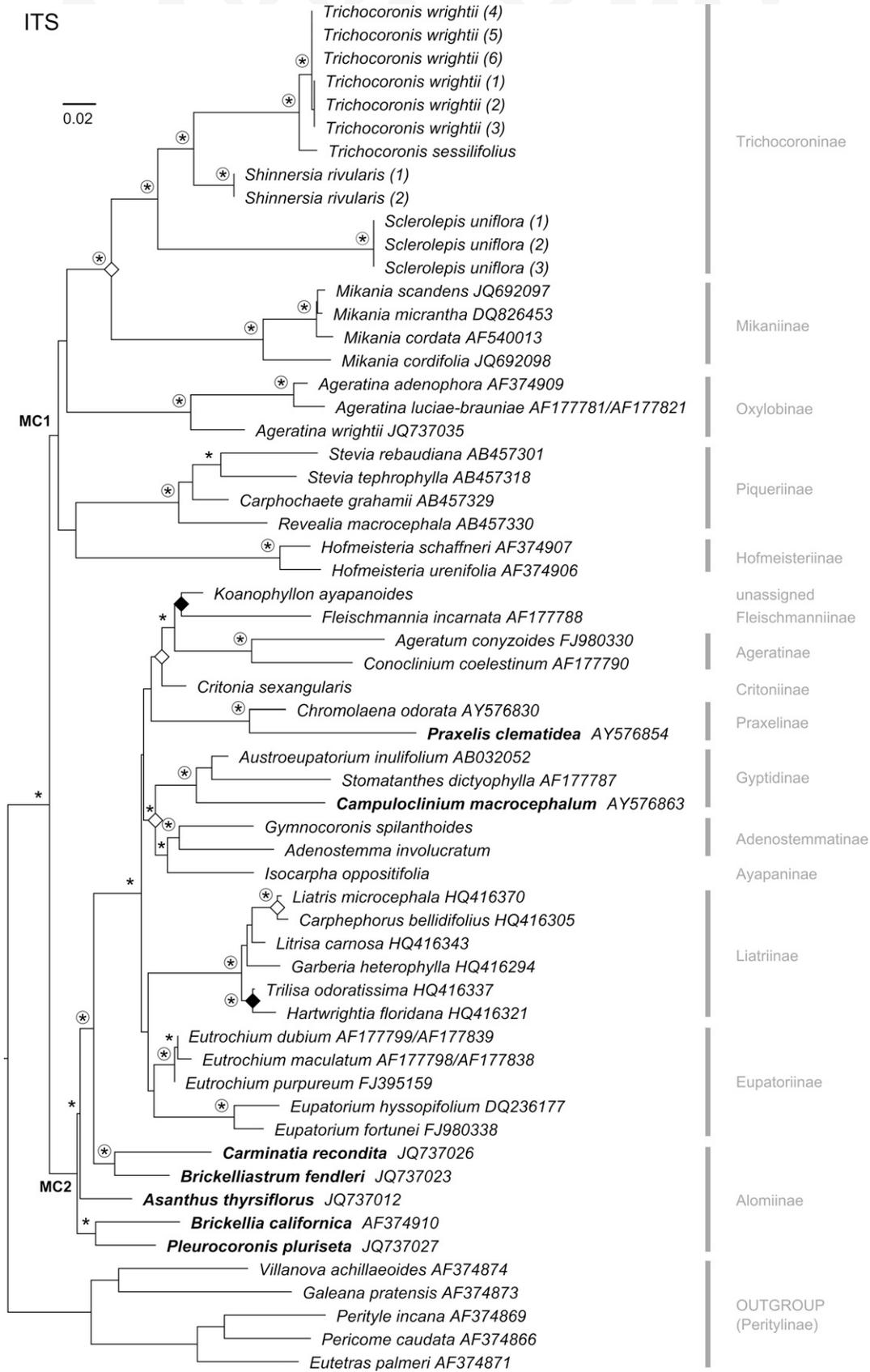


FIG. 1. Phylogeny of Eupatorieae constructed using ITS data. Tree topology reflects the maximum-likelihood phylogram. Asterisks designate nodes with > 0.95 Bayesian PP, and circles indicate ML bootstrap support > 90%. Diamonds indicate nodes that were incongruent between the ITS and *matK* analyses; filled diamonds represent significant incongruence, whereas open diamonds are not significantly incompatible with *matK* data. Species names are followed by GenBank accession numbers for previously published sequences. Genera represented only in the ITS analysis (i.e. lacking *matK* data) are indicated in boldface. 'MC1' and 'MC2' indicate major clades described in the text. Subtribal circumscriptions are indicated at far right.

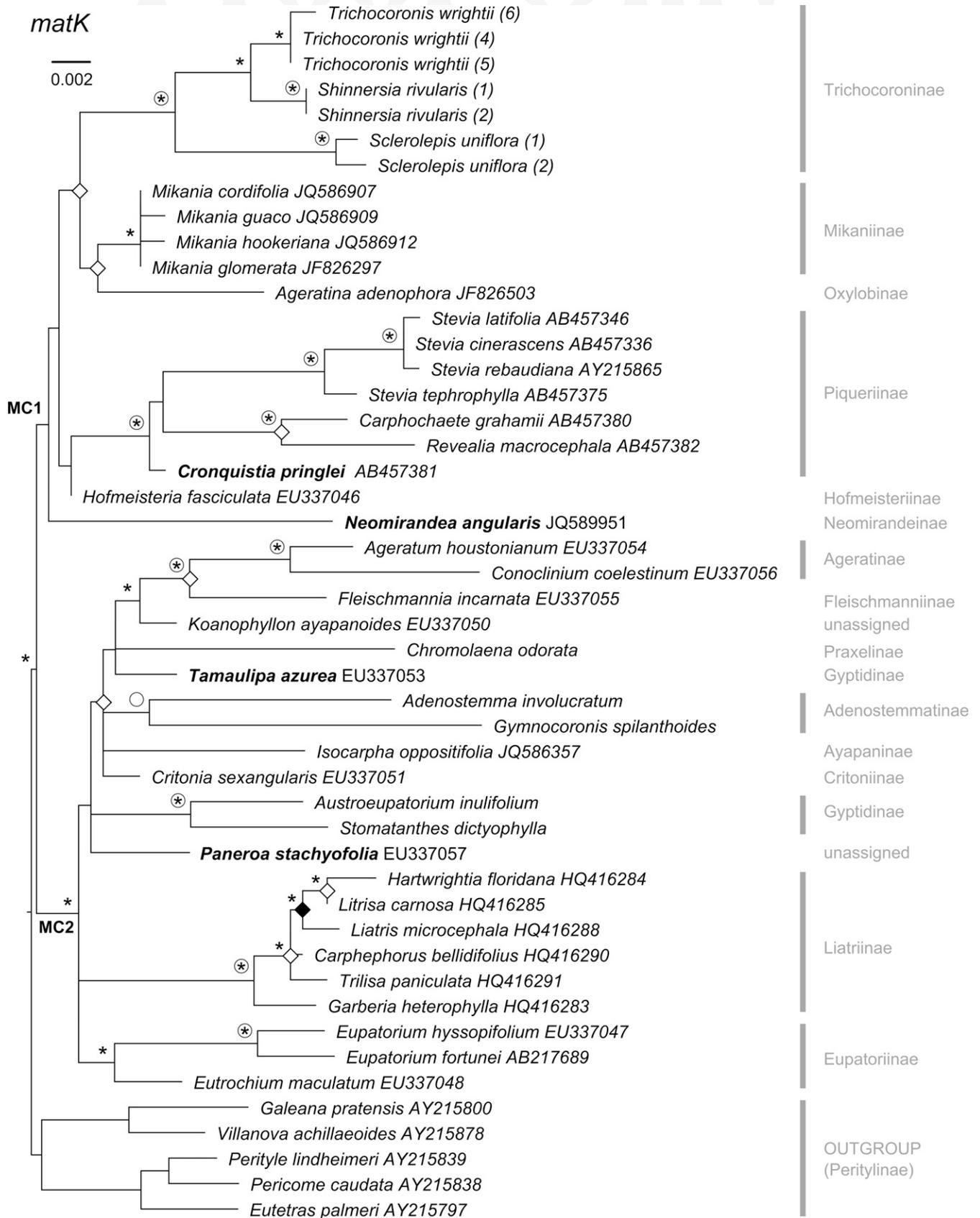


FIG. 2. Phylogeny of Eupatorieae constructed using *matK* data. Tree topology reflects the maximum-likelihood phylogram. Asterisks designate nodes with > 0.95 Bayesian PP, and circles indicate ML bootstrap support > 90%. Diamonds indicate nodes that were incongruent between the ITS and *matK* analyses; filled diamonds represent significant incongruence, whereas open diamonds are not significantly incompatible with ITS data. Species names are followed by GenBank accession numbers for previously published sequences. Genera represented only in the *matK* analysis (i.e. lacking ITS data) are indicated in boldface. 'MC1' and 'MC2' indicate major clades described in the text. Subtribal circumscriptions are indicated at far right.

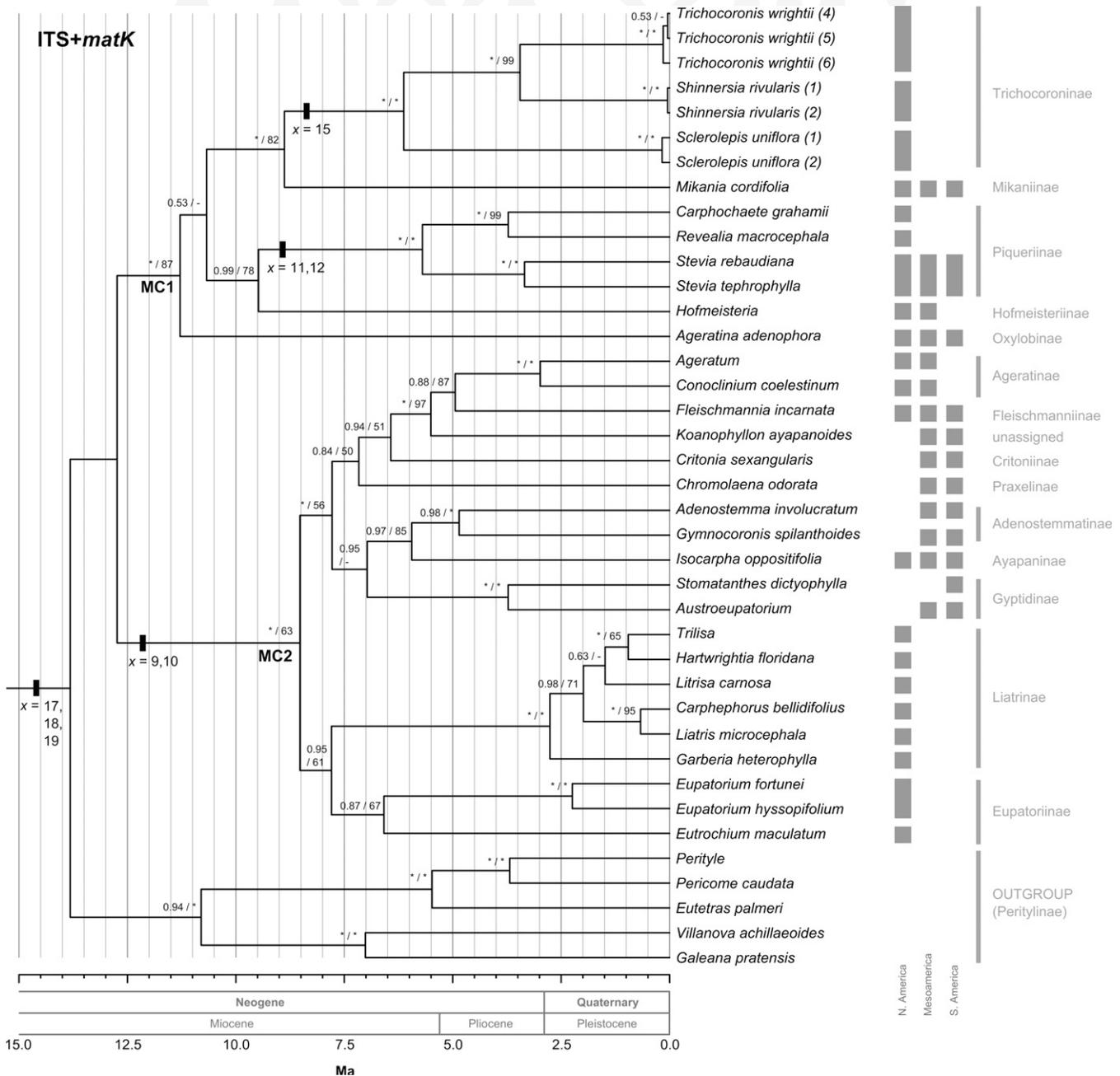


FIG. 3. Phylogeny of Eupatorieae constructed using combined ITS + *matK* data. Tree topology represents the consensus tree obtained from Bayesian analysis. Taxon names are composite representatives of ITS / *matK* source data; species names are given if both sequences were available for the same species, whereas if sequences were combined between two congeneric species only the genus name is given. Nodal support values are given as Bayesian PP / ML bootstrap support. Asterisks indicate support of 1.0 (PP) or 100% (ML), and dashes (-) indicate support < 0.50 or < 50%. 'MC1' and 'MC2' indicate major clades described in the text. Bars indicate putative changes in chromosome base number. Estimated divergence times are shown on the x-axis. Geographic distributions of genera are indicated at right, according to three categories: northern Mexico northward, Mesoamerica (i.e. southern Mexico to Panamá), and South America. Inclusion in the 'Mesoamerica' category was determined following Robinson (2014), and other distributions were obtained from King and Robinson (1987). Subtribal circumscriptions are indicated at far right.

this study, the strong support for *Mikania* as sister to Trichocoroninae suggests that genus as a useful point of comparison for the evolutionary origin of the submersed aquatic habit exhibited by Trichocoroninae. There could well be adaptive advantages shared among the vine/epiphyte and aquatic habits, such as internode flexibility; several plant families notably contain both twining and aquatic representatives (e.g. Araceae Juss., Convolvulaceae Juss., Polygonaceae Juss.; Cook 1999).

Phylogenetic relationships within Trichocoroninae offer two alternative scenarios for the evolution of submersed aquatic habit in *Sclerolepis* and *Shinnersia*. One possibility is that ancestral Trichocoroninae were capable of submersed growth, and the evolution of *Trichocoronis* marked a reversal to more stable wetland habitats where plants could consistently grow emergent. Under the alternative scenario, *Sclerolepis* and *Shinnersia* represent independent derivations of submersed aquatic habit from within a lineage of emergent

wetland plants. At any rate, the submersed habit of *Sclerolepis* and *Shinnersia* is a facultative adaptation in their life history, with sexual reproduction still dependent on periods of low water when plants grow erect and emerged. Thus even a reversal from plants that sometimes grow submersed to those that never grow submersed would not require the novel acquisition of characters related to emergent growth, merely an altered emphasis toward one growth strategy over another. Careful examination of developmental responses to submergence in *Sclerolepis/Shinnersia*, as compared with *Trichocoronis*, could provide a clue as to adaptations that are uniquely present in one group and absent in the other.

The estimated diversification dates for Adenostemmatinae and Trichocoroninae, corresponding to two independent evolutionary origins of aquatic habit, are 4.9 Ma (early Pliocene) and 6.2 Ma (late Miocene), respectively. These dates indicate a relatively recent origin of the aquatic habit, possibly in response to increased availability of suitable environments. Global climate during the late Miocene notably continued a trend of continental aridification (Pound et al. 2011; Eronen et al. 2012), which paradoxically could have driven terrestrial lineages toward evolving an aquatic habit. Several modern-day floras, for example, have diverse aquatic communities along with seasonal water scarcity, such as northern Australia and the Pantanal of South America (Aston 1973; Cook 1996; Pott and Pott 2000; Junk et al. 2006).

Lineages related to each of the aquatic groups in our study, namely *Adenostemma* for *Gymnocoronis* and *Mikania* for Trichocoroninae, are two of the most widespread Eupatorieae genera, with *Mikania* species additionally exhibiting a variety of growth forms. Whereas *Adenostemma* includes only 20 species (Panigrahi 1975; King and Robinson 1987; Orchard 2011), *Mikania* is the largest Eupatorieae genus with over 400 species (King and Robinson 1987). The diversity of ecological forms in *Mikania* increases the difficulty with which ancestral states can be ascribed to the genus, but it also could indicate a potential, in the common ancestor of aquatic and semi-aquatic lineages, for devising novel strategies such as the ability to grow underwater.

Stark ecological shifts during the transition to an aquatic habit are not unheard of; for example, Haloragaceae R. Br. comprise both aquatic (e.g. *Myriophyllum* L.) and woody (*Haloragodendron* Orchard) genera (Moody and Les 2007), and isolated aquatic species also have evolved repeatedly in otherwise terrestrial families (Cook 1999). A more complete understanding of the evolutionary origin of aquatic habit in both *Gymnocoronis* and Trichocoroninae must await more thorough sampling of their related lineages, so that the timing, geography, and morphology of their most recent non-aquatic ancestors might be reconstructed with sufficient confidence.

Biogeography—In addition to having broad phylogenetic distance between them, Trichocoroninae and *Gymnocoronis* are distinctly allopatric. Whereas several MC1 lineages related to Trichocoroninae have relatively restricted ranges also in North America (e.g. *Carphochaete* A. Gray, *Hofmeisteria*), others are widespread throughout the Americas (e.g. *Ageratina*, *Mikania*, and *Stevia* Cav.) (Fig. 3). Ranges of the three Trichocoroninae genera are fairly proximate geographically, with two (*Shinnersia*, *Trichocoronis*) native to Mexico and Texas, and *Sclerolepis* found in the eastern U. S. A. Subtribe Mikaniinae, the putative sister lineage, has a center of diversity in South America (King and Robinson 1987) but also has representative species with ranges that overlap with

Trichocoroninae. The arid environment occupied by *Shinnersia* and *Trichocoronis* contrasts sharply with the distribution of the more distantly related *Sclerolepis*, which grows primarily along the moist Atlantic coastal plain (Kartesz 2011). In our analysis the sister relationship between *Shinnersia* and *Trichocoronis* reflects their sympatry and morphological similarity.

In MC2, the sister genera *Adenostemma* and *Gymnocoronis* are sympatric in Mesoamerica and South America, and several other related lineages have similar distributions (e.g. Ayapaninae, Critoniinae R. M. King & H. Rob., Praxelinae). Indeed, the majority of genera with ranges in Mesoamerica and South America resolved to the clade containing these subtribes, i.e. MC2 excluding Eupatoriinae + Liatrinae. Based on the sympatry of related lineages and the broad distribution of extant species in the genus, we conclude that *Gymnocoronis* likely evolved to be aquatic somewhere within its current range.

Taxonomy—Although *Shinnersia* and *Trichocoronis* often are considered synonymous (e.g. USDA NRCS 2013), our data are consistent with the opinion of several authors (King and Robinson 1970a, 1987; Nesom 2006a; Robinson et al. 2009) who recognize them as independent genera. These genera are separated from each other by substantial phylogenetic distance, and the estimated timing of their divergence (3.5 Ma) is comparable with the divergences of other Eupatorieae genera (e.g. six genera of Liatrinae having diverged in the last 2.6 Ma; Fig. 3; Schilling et al. 2013). Moreover, several morphological characters readily distinguish *Shinnersia* and *Trichocoronis*, including involucre size, corolla shape, and pappus presence/absence (Nesom 2006a). Furthermore, we found no evidence of hybridization among species sampled in our study, although hybridization has been detected elsewhere in Eupatorieae (e.g. Powell 1985; Schilling 2011; Schilling et al. 2013).

As currently circumscribed, *Trichocoronis* contains two species: *T. sessilifolia* and *T. wrightii*, which were monophyletic in the ITS analysis (Fig. 1; *matK* data were unavailable for *T. sessilifolia*). *Trichocoronis wrightii* has a rather sparse distribution, with native populations occupying isolated pockets of suitable habitat in Mexico and Texas, and adventive plants in California (King and Robinson 1970b; Nesom 2006b). Plants from California and Texas were nearly identical in our analyses at both loci surveyed, although a single polymorphism in the ITS sequences separated plants from the two regions. *Sclerolepis* also appears to exhibit little genetic variation across its range, with plants sampled from New Hampshire and the Carolinas differing at only one *matK* site in our study. Unlike the sparse distribution of *Shinnersia* and *Trichocoronis* in arid landscapes, however, *Sclerolepis* occupies a continuous range of wet environments in the eastern United States (Kartesz 2011).

Subtribes for which we evaluated multiple species were monophyletic in our analyses, except for Alomiinae, for which only ITS data were available. Critoniinae, once considered to include both *Critonia* and *Koanophyllon* (King and Robinson 1987), was determined previously to be polyphyletic using plastid data (Schilling 2008), and our newly employed ITS data confirm this result. Additionally, although *Austroeupatorium* and *Stomatanthes* (subtribe Gyptidinae; King and Robinson 1987) were consistently monophyletic in our study, *Tamaulipa* (*matK* data only) was not included in the same subtribal clade. Schilling et al. (2013) previously cast

doubt on the monophyly of Alomiinae and Gyptidinae, and our data further indicate the need for additional phylogenetic study of subtribal relationships. Given the generally low resolution and taxon sampling for these subtribes, we advocate more sampling from these groups and other Eupatorieae before commencing any taxonomic reassignments.

Cytology—Our results indicate a strong phylogenetic signal for base chromosome number in Eupatorieae (cf. Robinson et al. 2009), with nearly all MC2 genera having $x = 10$ (except for *Brickellia* Elliott and *Pleurocoronis* R. M. King & H. Rob., each with $x = 9$) and all MC1 genera having base numbers exceeding $x = 10$ (Watanabe et al. 1995; Schmidt and Schilling 2000; Robinson et al. 2009; Fig. 3). In addition to the molecular phylogenetic evidence presented here, chromosomal data also support the placement of *Gymnocoronis* ($x = 10$) within MC2 and Trichocoroninae ($x = 15$) in MC1. Whereas the $x = 10$ base number is widespread throughout MC2, only the three Trichocoroninae genera have $x = 15$. The specific changes in chromosome base number that accompanied the origin of Trichocoroninae are not immediately apparent, with some related MC1 genera having counts of $x = 17$ or greater (*Ageratina*, *Hofmeisteria*, *Mikania*, *Neomirandea* R. M. King & H. Rob.) and others having $x = 11$ or 12 (*Carphochaete*, *Revealia* R. M. King & H. Rob., and *Stevia*, i.e. Piqueriinae).

The putative ancestral base chromosome number for Eupatorieae is $x = 17$, 18, or 19, with similar numbers characterizing Perityleae and related tribes (Ito et al. 2000b; Schmidt and Schilling 2000; Semple and Watanabe 2009). The phylogeny produced by our study (Fig. 3) is consistent with an ancestral base chromosome number of $x = 17, 18, 19$, followed by independent chromosomal rearrangements to the derived states of $x = 9, 10$ (MC2), $x = 11, 12$ (Piqueriinae), and $x = 15$ (Trichocoroninae). It would be worthwhile to investigate the nature of chromosomal rearrangements in these lineages, particularly to learn if genomic changes might be implicated in the origin and diversification of certain clades.

In conclusion, we were able to support with confidence the monophyly of Adenostemmatinae and Trichocoroninae, and we firmly established that the submersed aquatic habit evolved independently in these two subtribes. By evaluating sequences for other Eupatorieae genera, some of which were generated during this study and others that simply had not been analyzed in combination, we also developed a comprehensive Eupatorieae phylogeny using currently available sequences. Certainly more data are needed to improve the precision with which related lineages and ancestral states can be evaluated, but our study did establish several phylogenetic relationships that were supported strongly with novel molecular data.

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APPENDIX 1. Taxon accessions newly analyzed for this study. Species names, locality information, and voucher numbers are followed by GenBank accession numbers for ITS / matK / rbcL. Sequences not obtained during this study are indicated by dashes (–).

Adenostemma involucratum R. M. King & H. Rob. BRAZIL. Minas Gerais. Moraes 679 (TEX/LL), KJ637168, KJ637185, –. *Austroeupatorium inulifolium* (Kunth) R. M. King & H. Rob. ARGENTINA. Santiago del Estero, Panero 8516 (TEX/LL), –, KJ637186, –. *Chromolaena odorata* (L.) R. M. King & H. Rob. MEXICO. Nuevo Leon, Panero 2059 (TENN), –, KJ637187, –. *Critonia sexangularis* (Klatt) R. M. King & H. Rob. MEXICO. Oaxaca, Panero 2970 (TENN), KJ637169, –, –. *Gymnocoronis spilanthoides* (D. Don) DC. ARGENTINA. Buenos Aires, Panero 8370 (TEX/LL), KJ637170, KJ637188, –. *Isocarpha oppositifolia* (L.) Cass. MEXICO. Tamaulipas, Panero 2004-1 (TENN), KJ637171, –, –. *Koanophyllon ayapanoides* (Griseb.) R. M. King & H. Rob. CUBA. Bennet 7582 (FTG), KJ637172, –, –. *Sclerolepis uniflora* (Walter) Britton, Sterns & Poggenb. U.S.A. New Hampshire: Merrimack Co., (1) Mehrhoff 23699 (CONN), KJ637175, KJ637190, KJ637197; North Carolina: Hoke Co., (2) Schilling 04-15 (TENN), KJ637173, KJ637189, –; South Carolina: Bamberg Co., (3) Schilling 06-06 (TENN), KJ637174, –, –. *Shinnersia rivularis* (A. Gray) King & H. Rob. U.S.A. Texas: San Felipe Co., (1) Schilling & Panero 04-08 (TENN), KJ637176, KJ637191, –; Val Verde Co., (2) Williams 246 (SWT), KJ637177, KJ637192, KJ637198. *Stomatanthes dictyophyllus* (DC.) H. Rob. BRAZIL. Minas Gerais, Moraes 768 (TEX/LL), –, KJ637193, –. *Trichocoronis sessilifolia* (S. Schauer) B. L. Rob. MEXICO. Querétaro, Carranza 3465 (TEX/LL), KJ637178, –, –. *Trichocoronis wrightii* (Torr. & A. Gray) A. Gray U.S.A. California: Riverside Co., (1) Bramlet 2289 (RSA), KJ637184, –, –; (2) 2275 Greene 1408a (RSA), KJ637183, –, –; (3) Greene 1430 (RSA), KJ637182, –, –; Texas: Cameron Co., (4) Carr 18139 (TEX), KJ637180, KJ637196, KJ637200; (5) Schilling & Panero 04-15 (TENN), KJ637181, KJ637194, –; Kleberg Co. (6) Carr 25812 (TEX), KJ637179, KJ637195, KJ637199.