Title
Systematic studies in the marigold (Tageteae) and thoroughwort (Eupatorieae) tribes (Asteraceae)

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Systematic studies in the marigold (Tageteae) and thoroughwort (Eupatorieae) tribes (Asteraceae)

By

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ABSTRACT

The family Asteraceae is currently divided into 50 tribes. Some tribes, such as the marigold and thoroughwort tribes (Tageteae and Eupatorieae, respectively), include species that are native to the New World. Tageteae is more diverse and abundant in the xeric regions of Mexico and adjacent Southwest USA and includes species such as the marigolds (*Tagetes* spp.) and Dahlberg daisies (*Thymophylla tenuiloba*). Several other species have been used as medicines, spices, and garden ornamentals. Most members of Tageteae are characterized by the presence of secretory cavities in the foliage, which stored ethereal oils. Eupatorieae has two main centers of diversity, one in South America and another in Mexico, where it is especially well developed in temperate forests. It includes species that have been used in traditional medicine and as garden ornamentals, as well as *Stevia rebaudiana*, is the source of a sugar substitute. Species of Eupatorieae are characterized by discoid capitula and their often showy style branches.

In the research documented in this dissertation, a variety of systematic studies using molecular data were conducted. In Chapter One, phylogenetic relationships, divergence time, and ancestral ranges of the genera *Adenophyllum* and *Thymophylla* (Tageteae) are estimated using nrDNA (ITS and ETS) and cpDNA (*trn*L-F spacer, *ndh*I gene and *ndh*I-*ndh*G spacer, and *psbA*-*trnH* spacer). The results support the transfer of two species of *Adenophyllum* to *Boeberastrum*, the segregation of two new genera, *Adenophylloides* and *Thymophyllastrum*, and the transfer of *Strotheria gypsophila* to *Thymophylla*.

Chapter Two presents phylogenetic analyses, divergence times, and ancestral character state estimation of the secretory cavities of Tageteae *sensu lato*, using ITS and ETS data. Also, the sclerification of the anther appendages in the tribe is studied. Tageteae *sensu* lato is resolved as paraphyletic and based on the phylogenies estimated, the secretory cavities probably evolved once and were subsequently lost in at least one descendant node. Most species of Tageteae

sensu lato have strongly sclerified anther appendages, but the sclerification is also found in representatives of the tribes Bahieae, Chaenactideae, and Heliantheae. Future phylogenetic and divergence time analyses of Tageteae *sensu lato* should consider using cpDNA data and include more outgroups.

In Chapter Three, a phylogeny of marigolds (*Tagetes*) is estimated using ITS data and sampling almost 50% of the species. The results support the monophyly of *Tagetes* and its sister relationship to *Hydropectis*. It is desirable to conduct additional phylogenetic analyses using more molecular data, especially cpDNA, and including the species of *Tagetes* that were not available in this study.

Finally, in Chapter Four, phylogenetic analyses of the plumeweeds (*Carminatia,* Eupatorieae) based on ITS, ETS, and *psbA-trnH* data, are presented. The results support the monophyly of the genus and the description of a new species, *Carminatia balsana*, which is confined to tropical deciduous forests of the Balsas Basin in Mexico. A taxonomic revision of the genus including taxonomic keys, descriptions, pictures, add distribution maps is rendered.

This dissertation represents an important contribution to the systematics of tribes Tageteae and Eupatorieae of the large family Asteraceae. Each chapter also provides recommendations for future research on these groups.

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Chapter 1

Phylogeny and historic biogeography of the *Adenophyllum*-*Thymophylla* clade (Tageteae, Asteraceae)

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Abstract—*Adenophyllum* and *Thymophylla* are members of the marigold tribe (Tageteae) within Asteraceae. These genera are mostly North American and Mesoamerican (i.e., tropical Mexico and adjacent Central America), with one species of *Adenophyllum* occurring also in Cuba, and one of *Thymophylla* also in Argentina. Species of *Adenophyllum* are often known as dogweeds and some have been used locally as ornamentals and as medicines. Similarly, the Dahlberg Daisy (*T. tenuiloba*) and the pricklyleaves (e.g., *T. pentachaeta*, *T. acerosa*) have been cultivated in gardens for their golden capitula and compact habit, and other Thymophylla species have also been used locally as medicines. Phylogenetic analyses based on limited sampling have shown that each of these genera is monophyletic and distinct from *Dyssodia*, in which they were often included. However, the evolutionary histories of these genera have not been investigated thoroughly. In this study we generated nuclear and plastid DNA sequences of all recognized species and infraspecific taxa of *Adenophyllum*, *Thymophylla*, and their closest relatives (*Boeberastrum*, *Boeberoides*, *Comaclinium*, *Dysodiopsis*, *Dyssodia*, and *Strotheria*) to infer comprehensive phylogenies. In addition, we estimated divergence times and the historical biogeography of the *Adenophyllum*-*Thymophylla* clade. Our results support the transfer of two species of *Adenophyllum* to *Boeberastrum* and the segregation of *A. squamosum* as a new genus, *Adenophylloides*. Similarly, the unique *T. aurantiaca* is segregated as the new genus

Thymophyllastrum, and *Strotheria gypsophila* is transferred to *Thymophylla*. Phylogenetic relationships of the new genera and *Dysodiopsis* within the *Adenophyllum-Thymophylla* clade are still unclear. Additional studies at the interspecific level are also needed, especially to clarify infraspecific boundaries within *Thymophylla*. Based on divergence time analyses, the core *Adenophyllum* and *Thymophylla* clades diverged in the Miocene from ancestors that were probably widespread in the Neotropical and North American deserts regions, respectively, and species diversification took place mostly during the Pleistocene. Adenophyllum and *Thymophylla* are examples of Neotropical and desert adapted lineages, respectively.

***Keywords*—**Compositae, Dahlberg daisy, dogweeds, essential oils, secretory cavities.

INTRODUCTION

Adenophyllum Pers. *nom*. *cons*. and *Thymophylla* Lag. are two members of the tribe Tageteae (also known as the marigold tribe), within the family Asteraceae. These genera comprise about 10 and 18 species, respectively (Panero 2007), and they are predominantly North American, with most species in Mexico. As in most genera of the Tageteae (especially those in the subtribe Tagetinae), *Adenophyllum* and *Thymophylla* have secretory cavities in the leaves and/or bracts, which often appear as translucent dots or glands and contain essential oils, mostly monoterpenes (Strother 2006). When the leaves and/or bracts are crushed or sometimes slightly touched, a pungent or pleasant fragrance is emitted.

Species of *Adenophyllum* are annuals, suffruticose perennials, or shrubs, with mostly opposite leaves that are often pinnatisect and bear secretory cavities near the base and/or near the apex of the lobes. The capitula are almost always radiate and the involucre is composed of bracts that are 1/3 to 2/3 of their length connate, and almost always subtended by an outer series of calycular bracts (Figs. 1–4). Some species occur in xeric scrublands and deserts in

southern California, the Southwest USA, and northern Mexico, and others predominantly inhabit the warm and dry tropical deciduous forests of Mexico, Central America, and Cuba. *Adenophyllum cooperi* (A. Gray) Strother is endemic to the Mojave Desert region in southern California and adjacent Nevada and Arizona.

Thymophylla comprises mostly suffruticose perennials and dwarf shrubs, but also a few annuals. Species in this genus are characterized by their small size (the shoots are shorter than 65 cm high) and small leaves, which are often pinnatisect, and sometimes semi-succulent, prickly, or acerose, and, in a few species, tomentose. In the woolly species, the wool often hides the secretory cavities, which are more visible in the less hairy species. As in *Adenophyllum*, the involucral bracts are connate in Thymophylla and a well-developed calyculus is present in several species. However, the involucre is more strongly connate in *Thymophylla*, with the bracts 2/3 or more of their length fused. The heads in *Thymophylla* are smaller than in *Adenophyllum* and they are almost always radiate and long-pedunculate, with the corollas predominantly yellow, except for a few taxa with white rays (Figs. 4–7).

Species of *Thymophylla* occur in xeric scrublands, deserts, grasslands, and woodlands, often in open and flat areas with limestone or gypsum, from southern California, the Southwest USA, including southeast Kansas, and southwards to south-central Mexico. Moreover, *T. pentachaeta* (DC.) Small var. *belenidium* (DC.) Strother occurs both in North America and in similar arid areas in Argentina (Petenatti and Ariza-Espinar 1997, Strother 2006). There are eight species of *Thymophylla* in the US (Strother 2006). Notably, the region that comprises Texas and adjacent northeastern Mexico (Coahuila, Nuevo Leon, and Tamaulipas) is the richest in Thymophylla, with about 18 taxa (85%) occurring in this area and 9 of them endemics (42%) to this region. In the USA, species of *Adenophyllum*, *Thymophylla* and other related genera (e.g., Dyssodia Cav.) are often known as dogweeds, although the name pricklyleaf has been applied to some

species of *Thymophylla* as well (Spellenberg and Zucker 2019). The essential oils contained in the secretory cavities seem responsible for the claimed medicinal properties of species in both and related genera; at least some species have been used locally in traditional medicine (Felger and Rutman 2016, Villarreal 2003). The herbs are mostly used to make medicinal tea, but in some cases the leaves are also used to make poultices to treat wounds, as in the case of "Arnica" (*Adenophyllum aurantium* (L.) Strother). This species is also locally used as an ornamental (O. Hinojosa-Espinosa pers. obs.). Similarly, some species of *Thymophylla* have been grown and commercialized as garden plants. Perhaps the most important is T. tenuiloba (DC.) Small, from which some cultivars are known as Dahlberg daisy and golden fleece (Strother 2006). However, *T. acerosa* (DC.) Strother and *T. pentachaeta* (DC.) Small are also grown in gardens for their compact and much branched habit, yellow heads, and extended flowering period (Spellenberg and Zucker 2019). Cultivars may also escape cultivation and behave as weeds, as in the case of T. tenuiloba, which is native to Texas and adjacent northeastern Mexico, but it has been introduced in several states of the Southeast USA, Bahamas, Cuba, Africa, and Asia (Strother 2006).

Taxonomic history and generic relationships—*Adenophyllum* is a conserved name that is typified by *Willdenowa glandulosa* Cav. 1791. The name of the original type species for *Adenophyllum* (*A. coccineum* Pers. 1807) was superfluous and illegitimate when published, since *W. glandulosa*, to which *A. coccineum* was referred, had priority (Ghandi 2006). In contrast, *Thymophylla* was validly and effectively published based on *T. setifolia* Lag. 1816. Both genera were originally described as monotypic and few species were added subsequently. However, Hoffman (1884) and Robinson (1913) transferred the species from these and other genera to *Dyssodia*. In contrast, Rydberg (1915) accepted generic status for *Adenophyllum* (but under *Schlechtendalia* Willd. 1803, a name rejected in favor of *Schlechtendalia* Less. 1830) and

Thymophylla. In addition, Rydberg (1915) segregated *A*. *wrightii* A. Gray as the monotypic genus *Trichaetolepis* Rydb., transferred 13 species originally described in *Hymenatherum* Cass. to Thymophylla, and described six new species of *Thymophylla*, recognizing in total 22 species for this genus.

Strother (1969) kept *Adenophyllum* and *Thymophylla* as taxonomic synonyms of *Dyssodia*, which was divided into three subgenera and 13 sections. Seven species were classified in *Dyssodia* subg. *Dyssodia*, 12 species in *Dyssodia* subg. *Clomenocoma* (Cass.) Strother, and 13 species in *Dyssodia* subg. *Hymenatherum* (Cass.) Strother, for a total of 32 species. The species of *Adenophyllum* and *Thymophylla* were assigned to the subgenera *Clomenocoma* and *Hymenatherum*, respectively. After a reevaluation of the morphology, geography, ecology, cytology, and evolutionary relationships within Tageteae by Strother (1977), *Dyssodia* was considered too inclusive and presumably polyphyletic. Therefore Strother (1977) suggested moving *D*. *montana* (Benth.) A. Gray from subg. *Clomenocoma* to subg. *Dyssodia*, and to move sect. *Lebetina* (Cass.) O. Hoffm. from subg. *Clomenocoma* to subg. *Hymenatherum*, or segregating sect. *Lebetina* as a distinct subgenus. Alternatively, Strother (1977) proposed to raise the subgenera to the rank of genus arguing that the subgenera appeared more closely related to other genera within the Tageteae, rather than to each other. For example, *Strotheria* B.L. Turner, *Urbinella* Greenm., and *Hydropectis* Rydb. were considered closely related to subg. *Hymenatherum*, while *Tagetes* L. and *Gymnolaena* (DC.) Rydb., were postulated as closest relatives of subg. *Clomenocoma*.

Robinson (1981) accepted *Adenophyllum* and *Hymenatherum* as distinct genera. *Thymophylla* was treated as a synonym of *Hymenatherum*, although the former has priority over the latter. In contrast, McVaugh (1984) followed a broader circumscription of *Dyssodia*. Later, Strother (1986) proposed a renewed *Dyssodia* narrowing this genus from 32 species to

four. Most species in *Dyssodia* subg. *Clomenocoma* were transferred to a restored *Adenophyllum*. The exceptions were *D*. *montana* and *D*. *grandiflora* (DC.) Strother, which were segregated as the monotypic genera *Comaclinium* Scheidw. & Planch. and *Boeberoides* (DC.) Strother, respectively. Similarly, almost all species of *Dyssodia* subg. *Hymenatherum*, except *Dyssodia tagetoides* Torr. & A. Gray, which was segregated as a monotypic genus (*Dysodiopsis* Rydb.), were transferred to the renewed *Thymophylla*. Moreover, the genus *Boeberastrum* (A. Gray) Rydb. was also restored to include the two species in sect. *Boeberastrum* A. Gray of *Dyssodia* subg. *Dyssodia*.

Turner (1996) did not accept most of the proposed changes by Strother (1986), arguing nomenclatural stability and lack of additional supporting evidence for the recognition of such small generic segregates. However, Baldwin et al. (2002) accepted *Adenophyllum* and *Thymophylla* in their study of the phylogenetic relationships of the helenioid lineages of tribe Heliantheae sensu lato. Finally, the molecular phylogeny of tribe Tageteae by Loockerman et al. (2003) based on ITS and the plastid *ndh*F sequences supported the narrow circumscription of Dyssodia and all segregated/restored genera proposed by Strother (1986). Since the phylogenetic studies of Loockerman et al. (2003) most botanists have been accepted the generic segregates from Dyssodia, including Turner (2009, 2013). The results of Loockerman et al. (2003) supported *Thymophylla* as sister to Strotheria B.L. Turner and *Dysodiopsis* as sister to both, with *Adenophyllum* as successive sister to these three genera. *Dyssodia*, *Gymnolaena*, and the segregates *Boeberoides* and *Comaclinium* appeared as closely related to the clade composed of *Adenophyllum*, *Dysodiopsis*, *Strotheria*, and *Thymophylla*, which we will refer from now on as the *Adenophyllum*-*Thymophylla* clade. Genera such as *Tagetes*, *Hydropectis*, and especially *Urbinella* were distantly related to the *Adenophyllum*-*Thymophylla* clade.

Infrageneric groups, interspecific evolutionary hypotheses, and taxonomic conflicts within Adenophyllum and Thymophylla—Below, to facilitate discussion, state more clearly several hypotheses of phylogenetic relationships, and introduce taxonomic disagreements, the species of these genera have been sorted into informal groupings, which coordinate with the sections of Strother (1969). Moreover, the names under *Adenophyllum* and *Thymophylla* are used, although it should be noted that they correspond to names in *Dyssodia* in Strother (1969) and Turner (1996).

Adenophyllum, group *Lebetina*. Strother (1969) arranged three species in this group, *A*. *porophyllum* (Cav.) Hemsl.), *A*. *wrightii*, and *A*. *anomalum* (Canby & Rose) Strother (Figs. 1–2). They were characterized as annuals with dissected leaves, secretory cavities in the sinuses between the leaf segments, calycular bracts with setaceous appendages, and ray and disk flowers with alike pappus. *Adenophyllum porophyllum* was divided into three varieties, *A*. *porophyllum* var. *porophyllum*, *A*. *porophyllum* var. *cancellatum* (Cass.) Strother, and *A*. *porophyllum* var. *radiatum* (DC.) Strother. The varieties *cancellatum* and *radiatum* were considered more similar in having radiate heads and a pappus composed of an outer series of shorter, erose scales, and an inner series of longer scales, each dissected into bristles. In contrast, var. porophyllum had discoid heads and lacked the outer series of shorter erose scales. Var. *radiatum* was easily distinguished by its small, often inconspicuous rays, and its unique geographic distribution. This is the only taxon of *Adenophyllum* that occurs in Cuba and the Peninsula of Yucatan, where is known from a few collections. It is also known from southern Mexico to Nicaragua in Central America (Strother 1969, 2018) and it represents the southernmost limit of distribution of the genus.

Turner (1996, 2013) did not recognize any varieties and considered var. radiatum as a radiate form of *A*. *porophyllum* that did not merit taxonomic recognition. Moreover, he raised var.

cancellatum to the species level. According to Turner (1996), differences in morphology and geographic distribution between *Adenophyllum porophyllum* and *A*. *cancellatum* (Cass.) Villarreal, and, especially, absence of evidence of introgression in areas of sympatry warranted their recognition as distinct but closely related species.

Turner (2013) added a new species (*A*. *yecoranum* B.L. Turner) to the complex, which had the pappus of var. *porophyllum*, but radiate heads, with larger rays than the var. *radiatum*, but smaller rays than var. *cancellatum*. The species was stated to be confined to the region of Yecora, Sonora, and adjacent Chihuahua, in northwestern Mexico. Moreover, although collections of *A*. *porophyllum* and *A*. *cancellatum* near Yecora were mapped (Turner 2013), the newly described *A*. *yecoranum* appeared to be allopatric to them.

Adenophyllum, group *Trichaetolepis*. This group does not correspond to any section of Strother (1969), but it is convenient as *Trichaetolepis* was based on *A*. *wrightii*, which, according to Strother (1969), is closely related to *A*. *anomalum*, both sharing a chromosome number of *x*=7. Moreover, in these taxa the leaves are pinnatisect with linear segments (Fig 2). However, Strother (1969) also stated that *A*. *wrightii* was morphologically similar to *A*. *porophyllum* var. *cancellatum*.

Two varieties, *A*. *wrightii* var. *wrightii* and *A*. *wrightii* var. *pulcherrimum* (Strother) Strother, were recognized by Strother (1969, 1986). The latter differed from the former by its more conspicuous rays. Moreover, var. *wrightii* was confined to New Mexico, Arizona, and adjacent northern Mexico (Chihuahua), while var. *pulcherrimum* was endemic to central Mexico. Turner (1996) raised var. *pulcherrimum* to species rank emphasizing additional morphological differences between the two taxa and absence of intergrades. Both species were considered closely related and *A. anomalum* was considered superficially similar to *A*. *pulcherrimum* (Strother) Villarreal).

Adenophyllum glandulosum. Strother (1969) placed *A*. *glandulosum* in its own section and stated that it seemed to link the *Lebetina* and *Clomenocoma* groups. This species is characterized by its annual habit, conspicuous calyculate heads with large secretory cavities in the calycular and involucral bracts, and orange to red rays (Fig. 3A). According to Strother (1969), *A*. *glandulosum* differed from all other related species by having a dimorphic pappus, such as in some species of *Tagetes*. In *A*. *glandulosum* the ray flowers have a pappus composed of five erose scales, while the disk pappus is composed of five erose outer scales that alternate with five scabrous aristate smaller scales. For this reason, Turner (1996) considered *A*. *glandulosum* (treated under *Dyssodia*) as intermediate between *Dyssodia* and *Tagetes* and suggested that if *Adenophyllum* was recognized, it would be best treated as monotypic.

Adenophyllum, group *Clomenocoma*. Strother (1969) included in this group predominantly woody species, with calyculate heads, yellow or more frequently orange rays, and a pappus of scales, each divided into bristles. According to Strother (1969) this group seemed monophyletic, although one species was segregated as *Comaclinium montanum* (Strother 1986). Therefore, the current species that would be included in this group are: *A*. *aurantium*, *A*. *appendiculatum* (Lag.) Strother, *A*. *cooperi*, *A*. *porophylloides* (A. Gray) Strother, *A*. *speciosum* (A. Gray) Strother, and *A*. *squamosum* (A. Gray) Strother (Figs 3–4 A-C).

Adenophyllum aurantium and *A*. *appendiculatum* were considered more closely related, as were *A*. *cooperi* and A. *porophylloides*, while *A*. *speciosum* was considered as somewhat in-between this last pair of species. However, Strother (1969) also stated that *A*. *cooperi* and *A*. *porophylloides*, which occur in the xeric regions of southern California, the Southwest USA, and adjacent northeastern Mexico, including Baja California, might have derived from *A*. *speciosum*, which is confined to xeric areas in the southern extreme of the Baja California Peninsula.

As treated by Strother (1969), *A*. *aurantium* was confined to the state of Veracruz near the Gulf of Mexico, while *A*. *appendiculatum* was restricted to Pacific slopes from Colima to central Chiapas. Morphologically, the two species differed by the number and shape of the calycular bracts. *Adenophyllum aurantium* was characterized by having 8–12 lanceolate calycular bracts that graded into the involucral bracts, vs 12–20 subulate calycular bracts that did not grade into the involucral bracts in A. appendiculatum. However, Turner (1996) placed *A*. *appendiculatum* under synonymy of *A*. *aurantium*, arguing that these characteristics did not hold so that even a status of variety was not warranted for these taxa. Strother (2003, 2018) recognized A. appendiculatum as distinct from *A*. *aurantium* and added a few more differences between these taxa: disk florets mostly 40–60, cypselae sparsely pubescent, and pappus scales 9–11 mm long for the former, vs disk florets mostly 20–40, cypselae glabrous, and pappus scales ca. 8 mm long for *A*. *aurantium*.

Strother (1969) regarded *A*. *squamosum* (A. Gray) Strother as a unique species remarkably distinct by the scaly calycular bracts and the large 3–5 pinnatisect leaves. Similarly, Turner (1996) stated that *A*. *squamosum* was clearly distinct and easily recognized from other species. Strother (1969) hypothesized that it might have been related to the lineage that led to A. *cooperi*, *A*. *porophylloides*, and *A*. *speciosum*. Alternatively, Strother (1969) suggested a less probable evolutionary relationship between *A*. *squamosum* and *Boeberoides grandiflora* (DC.) Strother based on similarities in capitulum morphology.

Thymophylla, group *Gnaphalopsis*. Two woolly species with a base chromosome number of x=8 and similar chromatographic profiles were placed in this group (Fig. 4 D-G). *Thymophylla micropoides* (DC.) Strother is confined to northeastern Mexico and adjacent Texas, and it is the type species of the monotypic genus *Gnaphalopsis* DC. *Thymophylla tephroleuca* (S.F. Blake) Strother is endemic to a small area in southern Texas along the Rio Grande, and it is expected at

the Mexican side of the river. It is only known from a few populations composed of several individuals (Turner 1996), and is listed in the Center for Plant Conservation's National Collection of Endangered Plants (Strother 2006).

Although the relationships of *T*. *micropoides* with other species were enigmatic, Strother (1969) postulated that *T*. *micropoides* seemed related to other members of *Dyssodia* subg. *Hymenatherum* based on total morphology and chromosome number. In turn, T. *tephroleuca* was considered a vestige of the line that gave rise to *T*. *micropoides*. Strother (1969) also stated that *T*. *tephroleuca* might have been more closely related to another woolly species, *T*. *setifolia*, although several morphological differences in foliage, indumentum, involucre, and flowers seemed to indicate otherwise.

Thymophylla, group *Aciphyllaea*. Strother (1969) placed *T*. *acerosa* in its own section and stated that it appeared isolated within *Dyssodia* subg. *Hymenatherum* (Fig. 4J-L). According to Strother (1969), this species combined a pappus nearly as in *Dyssodia* subg. *Dyssodia*, the woody habit of sect. *Thymophylla*, and the involucre of sect. *Hymenatherum*. Turner (1972, 1996) related *T*. *gypsophila* (B.L. Turner) Strother to *T*. *acerosa* (Fig. 4M-O). The former is confined to gypsum dunes in Cuatro Cienegas, in northern Mexico, while the latter has a broader distribution from the Southwest USA to central Mexico. According to Turner (1972, 1996) *T*. *gypsophila* differs from the latter by its more robust size, succulent and less crowded leaves, and other minor floral details.

Thymophylla, group *Aurantiacae*. Unclear relationships of *T*. *aurantiaca* (Brandegee) Rydb. (Fig.4 H-I) caused it to be classified in its own section Aurantiacae Strother. However, Strother (1969) postulated that the species appeared to represent an old line derived probably from somewhere close to the origins of sections *Thymophylla* and *Hymenatherum*. This species was considered unique in several morphological features, such as the densely dotted pattern of the

leaves due to the secretory cavities, absence of calycular bracts, and a conic receptacle. Turner (1996) also considered it as a very distinct species. Moreover, this taxon is confined to the Mixteca region in the states of Puebla and Oaxaca, in south-central Mexico, which represents the southernmost distribution of the genus Thymophylla. This area is part of the Tehuacan-Cuicatlan Biosphere Reserve, which is the xeric region with the highest biodiversity in North America and with high levels of endemism (Davila et al. 2002).

Core *Thymophylla*. Strother (1969) included several species in sect. *Thymophylla*: *T*. *setifolia*, *T*. *pentachaeta*, *T*. *tenuiloba*, *T*. *aurea* (A. Gray) Greene, *T*. *tenuifolia* (Cass.) Rydb., and *T*. *concinna* (A. Gray) Strother (Figs. 5-7). Another previously described species, *T*. *greggii*, was treated as a variety of *T*. *setifolia* and it was stated to differ from the latter by the glabrescent peduncles and involucre, and the pappus composed of a small, short crown, vs woolly peduncles and involucre, and pappus of distinct scales in the typical variety (Fig. 5A-H). Strother (1969) stated that the two varieties seemed to intergrade in sympatric regions. However, Turner (1996) treated these two taxa as distinct but sister species and pointed out additional morphological differences, such as smaller heads with involucres 2–4 mm wide, fewer (8–10) and mostly glabrous involucral bracts, fewer rays (8–10), and fewer disk florets (20–50) in *T*. *greggii*, vs larger heads with involucres 5–7 mm wide, more (12–18) and mostly pubescent involucral bracts, more rays (11–15) or none, and more disk florets (50–100) in *T*. *setifolia*. Turner (1996) also stated that these taxa were essentially allopatric and that they did not appear to intergrade in their very small region of sympatry. If treated as two species, *T*. *setifolia* would be endemic to Mexico, occurring in xeric lands from the northeast to the south-central part of the country, while *T*. *greggii* would be confined to a smaller area from Texas to northeastern Mexico.

Strother (1969) recognized a broad *T*. *pentachaeta* (Fig. 5 I-P) composed of four varieties: *T*. *pentachaeta* var. *belenidium*, *T*. *pentachaeta*. var. *hartwegii* (A. Gray) Strother, the typical variety, and *T*. *pentachaeta*. var. *puberula* Rydb. Notably, the varieties were treated as species by Rydberg (1915), who recognized six other species that were treated as synonyms of *T*. *pentachaeta* by Strother (1969). The four varieties were considered clearly circumscribed and easily distinguished, and despite their overlapping geographic distributions, intergrades were rarely seen, as stated by Strother (1969). Moreover, var. *belenidium* was considered as a primitive form from which the other varieties might have derived. Turner (1996) however, stated that the only variety that seemed well marked was var. *hartwegii*. Therefore, only varieties *pentachaeta* and *hartwegii* were recognized by Turner (1996), and varieties *belenidium* and *puberula* were considered synonyms of var. *pentachaeta*.

Based on the chromosome numbers reported by Strother (1969) *T*. *pentachaeta* var. *hartwegii* is a tetraploid with *n*= 26, while the remaining varieties are diploid (*n*=13). However, Strother (1989) suggested that these counts might have been erroneous interpretations of triploids (3*x*= 24) or tetraploids (4*x*= 32). Later, Turner (1996) reported *n*=8, 16, 13, and 26 for var. *pentachaeta* (which included varieties *belenidium* and *puberula*) and *n*= 26 for var. *hartwegii*, and stated that the triploids and tetraploids suggested by Strother (1989) needed verification from proper chromosome counts. Strother (2006) subsequently reported n=16 for varieties *pentachaeta* and *belenidium* only (the latter from a voucher from Argentina), but no longer the previously reported counts of n=13 in Strother (1969). Turner (2009) eventually concluded that all varieties were worthy of specific rank, except for var. *belenidium*, which was placed under the synonymy of *T*. *pentachaeta*.

Among the closest relatives of the *T*. *pentachaeta* complex according to Strother (1969) was *T*. *tenuiloba* (Fig. 6 A-C), which was considered especially close to var. *pentachaeta*. However,

Strother (1969) also postulated that a var. *belenidium*-like ancestor might have given rise to the other varieties of *T*. *pentachaeta* and to *T*. *tenuiloba*. Strother (1969) also cited some herbarium specimens that showed a combination of the habit of var. pentachaeta and the involucre and floral morphology of *T*. *tenuiloba* and that were considered as putative hybrids. Strother (1969) also stated that the *T*. *pentachaeta* complex and *T*. *setifolia* might have been derived from the same lineage. This was based on similarities of the habit (both woody perennials), leaves (both with opposite and pinnatisect leaves with linear segments), and capitula (Strother 1969). The more densely pubescent plants of var. pentachaeta and some specimens of var. puberula, with reduced pappus, were especially considered to approach the radiate forms of T. setifolia or represent hybrids among them (Strother 1969).

Strother (1969) recognized four varieties of *T*. *tenuiloba*, T. *tenuiloba* var. *texana* (Cory) Strother, *T*. *tenuiloba* var. *treculii* (A. Gray) Strother, *T*. *tenuiloba* var. *wrightii* (A. Gray) Strother, and the typical variety. Notably, varieties *treculii* and *wrightii* were treated as species by Rydberg (1915). However, as stated by Strother (1969), varieties *tenuiloba*, *texana*, and *treculii* differed only by minor pappus details and the distribution of secretory cavities in the involucre, while var. *wrightii* differed from the other varieties by its mostly entire leaves and larger rays. Turner (1996) only recognized varieties *tenuiloba* and *wrightii*, and considered varieties *treculii* and *texana* as pappus forms of the more widespread and more variable var. *tenuiloba*. More recently, Nesom (2009) raised var. *wrightii* to species rank and recognized three varieties for *T*. *tenuiloba* (*tenuiloba*, *texana*, and *treculii*). In addition to the distinguishing morphology of *T*. wrightii (A. Gray) Small, Nesom (2009) noted absence of introgression between *T*. *wrightii* and *T*. *tenuiloba* in areas of sympatry. According to Nesom (2009), the varieties *texana* and *treculii* are allopatric and each is sympatric with var. *tenuiloba*. Nesom (2009) also reported intergradation and variation in the pappus scales within populations of these varieties.

Both Strother (1969) and Turner (1996) considered *T*. *mutica* (M.C. Johnst.) Strother (Fig. 6 D-F) to be closely related to *T*. *tenuiloba*. The former differs from the latter by the absence of a well-developed calyculus, its grooved involucre, the scarious involucral bract margins, and more numerous secretory cavities that are more dispersed in the involucre. Strother (1969) postulated that var. *tenuiloba* and *T*. *mutica* might have derived from the same ancestor, which might have resembled *T*. *pentachaeta* var. *belenidium*. Turner (1996) also considered *T*. *mutica* more similar morphologically and closely related to *T*. *tenuiloba*.

The *T*. *tenuiloba* complex also comprises polyploids. Based on the chromosome numbers reported by Strother (2006), varieties *wrightii* and *texana* are diploids (n=8), var. *treculii* is either a tetraploid or pentaploid (*n*=32, 40), and var. *tenuiloba* comprises diploids, triploids, tetraploids, and pentaploids (2*n*=16, 24, 32, 40). However, Strother (1969) originally reported *n*=8, 13, 16, and 26 for var. *tenuiloba*, n=13 and 16 for var. *treculii*, and *n*=8 for varieties *texana* and *wrightii*, and Turner (1996) reported counts of *n*=8, 13, 16, and 26 for var. *tenuiloba* (which included varieties *treculii* and *texana*).

Thymophylla, group *Hymenatherum*. This group includes *T*. *aurea*, *T*. *gentryi* (M.C. Johnst.) Strother, *T*. *tenuifolia* (Cass.) Rydb., and *T*. *concinna*, which are annuals with alternate pinnatisect leaves, without a well-developed calyculus or any calycular bracts. Strother (1969) divided *T*. *aurea* into two varieties, *T*. *aurea* var. *aurea* and *T*. *aurea* var. *polychaeta* (A. Gray) Strother, and stated that they differed mainly by a few characteristics of the pappus (8–10 erose scales in var. *aurea* vs ca. 20 smaller scales, each divided into 3–5 bristles, in var. *polychaeta*). According to Strother (1969), var. *aurea* (Fig. 6 G-I) occurs from eastern Colorado and western Kansas south to western Texas and adjacent northern Mexico (Chihuahua), while var. *polychaeta* has a slightly more southern distribution, occurring from western Texas to Chihuahua, Coahuila, and Durango in north-central Mexico. Notably, the distribution of var. *aurea* represents the

northernmost distributional limit of *Thymophylla*. Strother (1969) also noticed that individuals of both varieties could be found in some locations in Texas and Chihuahua where they might interbreed. Therefore, Strother (1969) stated that recognizing a single polymorphic species might have been justified. In fact, Turner (1996) treated *T*. *aurea* with no varieties, which were considered pappus forms of a widespread single species. Both taxa, however, were treated at the rank of species within *Thymophylla* by Rydberg (1915).

According to Strother (1969), var. aurea might have been derived from var. *polychaeta*, based on the assumption that the pappus of scales divided into bristles was primitive in *Dyssodia* subg. *Hymenatherum*. Moreover, it was postulated that the closest relative of *T*. *aurea* was probably *T*. *gentryi* (Fig. 7 A-C), a localized endemic to north-central Mexico. The latter was stated to differ from *T*. *aurea* by having fewer leaf segments, smaller heads with less flowers, and narrower involucres with less involucral bracts. Turner (1996) also recognized *T*. *gentryi* as distinct from *T*. *aurea*, but suggested that future studies might warrant merging them into a single species because the morphological differences between them were minor and overlapping.

As stated in Strother (1969), *T*. *tenuifolia* (Fig. 7 D-F) was also closely related to *T*. *aurea* and *T*. *gentryi*. *Thymophylla tenuifolia* is the type species of the genus *Hymenatherum* and it is quite similar to both *T*. *aurea* and *T. gentryi*, but it can be distinguished mainly by its outer involucral bracts that are connate almost throughout their length (vs outer involucral bracts connate only at the base in *T*. *aurea* and *T*. *gentryi*). Moreover, *T*. *tenuifolia* is endemic to Mexico, where it occurs from the north to the south-central part of the country. However, the geographic distributions of these taxa overlap in northern Mexico.

Strother (1969) noticed the polymorphic pappus of *T*. *tenuifolia* (which was observed to vary within and among populations), and stated that the geographic distribution of the pappus forms

was almost random and therefore not warranting taxonomic recognition. Thus, five species of *Thymophylla* that were recognized by Rydberg (1915) based on differences of the pappus were treated as synonyms of *T*. *tenuifolia* by Strother (1969) and Turner (1996). Strother (1969) also considered *T*. *tenuifolia* to be closely related to *T*. *concinna* (Fig. 7 G-I). The latter is easily distinguished by its white rays and narrow distribution (Sonora, Mexico, and adjacent Arizona).

The numerous untested hypotheses of interspecific evolutionary relationships encourage further investigation of phylogenetic relationships within the *Adenophyllum-Thymophylla* clade. Moreover, although Loockerman et al. (2003) have shown that genera such as *Tagetes*, *Hydropectis*, *Gymnolaena*, and *Urbinella* are not as closely related to the *Adenophyllum-Thymophylla* clade as previously thought (e.g., Strother 1977), it is still not clear if phylogenetic analyses based on more extensive sampling would support the recognition of the *Adenophyllum-Thymophylla* clade and/or of closely related genera, such as *Boeberoides*, *Comaclinium*, and *Dyssodia sensu stricto*. As Loockerman et al. (2003) focused at the tribal level, generic sampling was limited. For example, only 2–3 species of *Adenophyllum* and *Thymophylla* were included in the analyses. In the case of *Adenophyllum*, they did not sample any species from what we have referred to above as the *Trichaetolepis* group, whose species have the same chromosome number as those of *Boeberastrum*, and that Loockerman et al. (2003) suggested might belong to *Boeberastrum*. Moreover, Loockerman et al. (2003) also suggested that further phylogenetic studies with more samples of *Thymophylla* might show that Strotheria belongs within it.

The *Adenophyllum-Thymophylla* clade comprises approximately 13% of the species of tribe Tageteae and their members are important components of the xeric deserts, scrublands, and dry tropical forests of North America and Central America. Several species have been also used as medicinals and ornamentals. The genera *Adenophyllum* and *Thymophylla* also are the largest

within the tribe after *Pectis* L., *Tagetes*, *Porophyllum* Adans., and *Flaveria* Juss. However, *Adenophyllum* and *Thymophylla* have received less attention by phylogeneticists in comparison to the larger Tageteae genera, for which phylogenetic studies have been conducted recently (Hansen et al. 2016; Hinojosa & Schiavinato 2022, McKown et al. 2005).

The present study focuses on the *Adenophyllum-Thymophylla* clade with the objective of investigating comprehensively the phylogenetic relationships and macroevolution of this branch within the Tageteae tree. Using nuclear and cpDNA sequences of all species and infraspecific taxa of *Adenophyllum* and *Thymophylla* as circumscribed by Strother (1986), we conducted a variety of phylogenetic analyses to: 1) ascertain the monophyly and estimate the divergence times and ancestral biogeography of *Adenophyllum* and *Thymophylla*; 2) infer the closest generic relatives of *Adenophyllum* and *Thymophylla* within the Tageteae; 3) assess the numerous hypotheses of evolutionary relationships among the species within each genus postulated by Strother (1969); and 4) to attempt to resolve the taxonomic disagreement about the circumscription and rank of several infraspecific taxa of *Adenophyllum* and *Thymophylla*.

MATERIALS AND METHODS

Taxonomic sampling and sample collection—To examine all hypotheses of relationships among the species of *Adenophyllum* and *Thymophylla* stated by Strother (1969) and Turner (1996), all species and varieties of these two genera as circumscribed by Strother (1986) were sampled. Moreover, to ascertain whether each of these genera is monophyletic, species of their closest relatives (*Boeberastrum*, *Boeberoides*, *Comaclinium*, *Dysodiopsis*, *Dyssodia*, *Tagetes*, *Gymnolaena*, and *Strotheria*), as evidenced by Loockerman et al. (2003), were included, allowing for the possibility that *Adenophyllum* and/or *Thymophylla* would not be resolved as a clade (Nixon and Carpenter 1993). Finally, two species (*Flaveria anomala* B.L. Rob. and *Varilla*

mexicana A. Gray, from the subtribes Flaveriinae and Varillinae within Tageteae, respectively) that are evidently more distantly related to *Adenophyllum* and *Thymophylla* than to other taxa sampled here (Baldwin 2009, Baldwin et al. 2002, Loockerman et al. 2003) were included as the outgroup. *Varilla mexicana* was used to root the trees.

Once taxon sampling was defined, field work was conducted to collect fresh leaf samples for DNA isolation and to make observation in situ of the morphology and habitat of species of *Adenophyllum*, *Thymophylla*, and their relatives. Several species of these taxa were found during trips to several locations in Mexico and Tucson, Arizona in 2019 and early 2020, but further collecting plans were interrupted by the Covid-19 pandemic. Leaves were immediately preserved in silica gel and vouchers were collected and deposited in the National Herbarium of Mexico (MEXU) and in regional Mexican herbaria (CIIDIR, CH, UAT, and USON) (abbreviations following Thiers 2022). Moreover, leaf samples and voucher duplicates were legally imported and deposited in the herbarium of the University of California, Davis (DAV). Additional samples were obtained from herbarium specimens, with previous authorization of the following institutions: ARIZ, CIIDIR, COCO, HCIB, IBUG, DAV, MEXU, NMS, RENO, and SD. In addition, specimen loans were obtained from MEXU, TEX, and NMC to secure additional samples. At least one sample per taxon was included in the phylogenetic analyses and more samples were included when available for taxa of problematic taxonomic circumscription, with the expectation that samples of the same species should cluster together. A list of taxa, voucher specimens, and GenBank accession numbers is presented in Appendix 1.

***Molecular methods*—**DNA isolations, amplifications, and sequencing were conducted as described in Hinojosa-Espinosa et al. (in press; see also chapter 4). Typically, 1.5–20 mg of dry leaf tissue was used to extract DNA using the DNeasy Plant Kit (Qiagen, Valencia, California) following the manufacturer's protocols with minor modifications. Nuclear rDNA (ITS and ETS)

and plastid DNA markers (*trn*L-F intergenic spacer, *ndh*I and *ndh*I-*ndh*G intergenic spacer, and *psbA*-*trnH* intergenic spacer) were used to estimate phylogenetic relationships. Table 1 summarizes the primers used to amplify and sequence the selected molecular markers. In the case of the ETS, most samples were amplified using the primers of Lopes-Rivera et al. (2016) and the few samples that did not amplify with those primers were successfully amplified using the primers of Markos and Baldwin (2001) and Baldwin and Markos (1998). Similarly, we tried both the primer trnC of Taberlet et al. (1991) and the modified version of Panero and Croizier (2003) to amplify the trnL-F region of the chloroplast genome, achieving successful amplifications with both. PCR products were submitting for sequencing to the UC Davis College of Biological Sciences DNA Sequencing Facility, where an ABI Prism 3730 Capillary Electrophoresis Genetic Analyzer and associated software were used for sequencing and data analyses.

***Sequence editing and alignment*—**Sequencher 5.4.6 (Gene Codes Corporation) was used to assemble contigs and edit the sequences. The first nucleotides of each end of the sequences were usually trimmed until readable bases were obtained. BLAST searches were performed to corroborate taxon and marker identity. As the sequencing of the ndhI and *trn*L-F PCR products often resulted in small, non-overlapping sequences that could not be assembled into contigs, we used MEGA 7.0 (Kumar et al. 2016) to assemble these fragments into complete sequences filled in with missing data. The multiple sequence alignment was conducted on MUSCLE (Edgar 2004) implemented in MEGA 7.0 using default settings and followed by manual adjustments that were minor in most cases.

***Phylogenetic analyses*—**Mesquite 3.6 (Maddison and Maddison 2018) was used to export the alignments in different file formats. Several Bayesian analyses were implemented in MrBayes 3.2. (Huelsenbeck and Ronquist 2001) to compare the phylogenies resulting from

them. First, each of the five data sets was analyzed separately. Next, the ITS and ETS were concatenated into a single data set, and, similarly, the three plastid markers were also concatenated into another data set. Finally, a larger concatenated data set included all five markers. All of the concatenated data sets were partitioned using Mesquite 3.6.

The nucleotide substitution models that best fit the different data sets were evaluated by implementing Reversible Jump-Markov Chain Monte Carlo, in which all the 203 general time reversible substitution models are analyzed during the phylogenetic analyses. The different models are sampled more frequently based on their posterior probabilities and those that fit best the data have the highest probability values (Huelsenbeck et al. 2004). Two simultaneous independent runs of 1–2 million generations were performed using four chains with default heating values. The runs were compared every 1000 generations and sampled every 100–200 generations, discarding the first 25% samples as burn-in. Following Huelsenbeck et al. (2004), the rate variation across sites was modeled as a gamma probability distribution using four categories. Convergence was evaluated by confirming that the average standard deviation of the split frequencies between the two simultaneous runs was lower than 0.01 and that the effective sample size (ESS) was larger than 200. This was assessed by examining the input on the screen in MrBayes and by using Tracer v1.7 (Rambaut et al. 2018). We also confirmed that the Potential Scale Reduction Factor (PSRF) fell between 1–1.1, as this is an indication of good quality samples from the posterior probability distributions (Ronquist et al. 2019). Finally, in the plot of the generations versus the log probabilities of the data we confirmed that the plotted values were randomly distributed, which is another indication of stationarity (Ronquist et al. 2019). FigTree v1.4.4 (Rambaut 2018) was used to edit the majority rule consensus trees that MrBayes uses to summarize the trees sampled from the posterior distribution.

Divergence time estimation—A time calibrated phylogeny was estimated using Bayesian methods implemented in BEAST 2.6.6 (Bouckaert et al. 2019). For these analyses we reduced the data set to have one terminal per species and/or variety (44 terminals in total). We also deleted a few outgroup samples in order to reduce the complexity and computation time of the analyses. Since the sequences of *Boeberastrum littoralis* and *B*. *anthemidifolium* were essentially the same, we excluded the former from the analyses. We also excluded *Gymnolaena serratifolia*, *Tagetes lunulata*, and *Varilla mexicana*. The first two species were not closely related to *Adenophyllum* or *Thymophylla* in our non-calibrated analyses (see Results). The species of *Varilla* was used to root the non-calibrated trees, but for the divergence time analyses we used *Helianthus annuus* L. for rooting purposes and to calibrate the most recent common node of Heliantheae and Tageteae based on Hansen et al. (2016). The sequences of *H*. *annuus* were extracted from GenBank (Appendix 1).

The substitution models were selected based on the results of the RJ-MCMC analyses previously implemented in MrBayes (See Methods 2.4), which showed that the JC model best fit the *trn*L-F and *ndh*I-*ndh*G data sets, the GTR model of Tavare (1986) best fit the ITS data set, and among the substitution models available in BEAST 2.6.6 the HYK model best fit the ETS and *psbA*-*trnH* data sets. Site variation was modeled using a gamma distribution, and substitution rates, transition rates, and nucleotide frequencies were estimated from the analyses. An uncorrelated log normal relaxed clock with estimated clock rate was applied. We used most of the default priors including the Yule model tree prior with birth rate estimated from the analyses. The prior topology was constrained by forcing *Helianthus annuus* as the sister of the rest of the taxa sampled (i.e. Tageteae) and a normal distribution with a mean of 26.6 and standard deviation of 4.5 as set to include the range of the estimated divergence time between the tribes Heliantheae and Tageteae, which Hansen et al. (2016) estimated to have occurred

between 19.7–33.4 million years ago (Ma) with a mean age of 26.55 Ma. Two independent MCMC chains of 100 million generations, logging and sampling trees and parameters every 10,000 generations, were performed using the CIPRES portal (Miller et al. 2010). Parameters and posterior densities from the two independent runs were compared and analyzed in Tracer 1.7. After verifying that both runs converged on the posterior probabilities and reached stationarity, the sampled trees were combined into a single tree file using the BEAST package LogCombiner. Another BEAST package, TreeAnnotator, was employed to construct a Maximum Clade Credibility (MCC) tree, by discarding 25% of the initial trees as burn-in, and annotating the mean heights of each node. FigTree was used to visualize and edit the time-calibrated MCC tree.

Historical biogeography of the Adenophyllum-Thymophylla clade—Biogeographic analyses were conducted in RASP 4.0 (Yu et al. 2020). We used the MCC calibrated tree and 10,000 trees derived from the divergence time analyses conducted in BEAST 2.6.6 as input data. We focused the analyses on the *Adenophyllum-Thymophylla* clade and excluded most of the outgroup taxa using the pruning option of RASP; only *Boeberastrum*, which resolved as sister to the *Trichaetolepis* group of *Adenophyllum* on the one hand, and *Boeberoides* and *Comaclinium*, which resolved as more closely related to the *Adenophyllum-Thymophylla* clade than *Boeberoides*, were not removed. A total of 41 terminals were included in the analyses. Only the native distribution of the taxa was considered, discarding adventive occurrences of the cultivated species (mainly *T. tenuiloba*). The terminals were coded as present/absent in seven biogeographic units based on the ecological regions of North America level I from EPA (https://www.epa.gov/eco-research/ecoregions-north-america), with minor modifications. These included the use of the Pacific Domain biogeographic unit for Central America following Castellano-Morales et al. (2018), who based their assessment on Morrone (2005, 2014) and Gamez et al. (2017); the use of the Neotropical region following Escalante et al. (2022), and the

use of another xeric region that we defined as Argentinian Deserts. The seven ranges are the following: A) North American Deserts; B) Temperate Sierras; C) Southern Semi-arid Highlands; D) Neotropical; E) Great Plains; F) Pacific Domain; and G) Argentinian Deserts.

We used the statistical dispersal-extinction cladogenesis (S-DEC) biogeographic model (Ree and Smith 2008, Beaulieu et al. 2013), which, as implemented in RASP 4.0, uses multiple phylogenetic trees (instead of a single summary tree) to account for phylogenetic uncertainty. We used the default setting of 100 random trees from the 10,000 sampled trees resulting from the combined runs from the divergence time analyses implemented in BEAST 2.6.6. Since the taxa with the widest geographic ranges span five biogeographic units, the maximum number of unit areas was set to six. We did not set any range or dispersal restrictions.

RESULTS

The unaligned ITS sequences ranged from 633 base pairs (bp) in *T*. *setifolia* var. *greggii* to 649 bp in *Dyssodia papposa* (Vent.) Hitchc. In *Adenophyllum*, they ranged from 638 bp in *A*. *yecoranum* and varieties *cancellatum* and *radiatum* of *A*. *porophyllum* to 646 bp in the two varieties of *A*. *wrightii*. In *Thymophylla* the longest ITS sequences (641 bp) were found in *T*. acerosa and *T*. *aurantiaca*. The aligned ITS data matrix had 167 terminals and 694 characters, of which 397 (57.2%) were variable. The nucleotide substitution model M198 (following Huelsenbeck et al. (2004) terminology) best fit the ITS data set. This model is very similar to the widely used GTR model of Tavaré (1986), but it differs from it in which the substitution rates of the transversions AC and GT are equal. The majority rule consensus tree from phylogenetic analysis of the ITS data set is shown in Figures S3-S4.

The unaligned ETS sequences ranged from 427 bp in *Comaclinium montanum* to 448 bp in *Varilla mexicana*. In *Adenophyllum*, they ranged from 437 bp in *A*. *glandulosum* to 444 bp in *A*.

anomalum, while in *Thymophylla*, the shortest sequences (433 bp) were found in *T*. *aurantiaca* and the longest sequences (441 bp) were in *T*. *micropoides* and *T*. *setifolia* var. *setifolia*. The ETS data matrix had 167 taxa and 456 characters, of which 277 (60.7%) were variable. The M85 substitution model best fit the data. This model is similar to the 2-parameter model of Kimura (1980), but it differs from it in which the substitution rates of the transversions AC, CG, and GT are equal. The majority rule consensus tree from phylogenetic analyses of the ETS data set is shown in Figures S4-S5.

The unaligned sequences of the plastid *trn*L intron and *trn*L-F intergenic spacer ranged from 749 bp in *Tagetes lunulata* to 859 bp in *Boeberoides grandiflora*. In *Adenophyllum*, the sequences ranged from 828 bp in *A*. *speciosum* to 856 bp in *A*. *squamosum*. Notably, *A*. *appendiculatum* and *A*. *aurantium* shared a small (six bp) insertion and small (eight bp) deletion not found in any other species in the genus. The sequences in *Thymophylla* ranged from 805 bp in *T*. *aurantiaca* to 822 bp in *T*. *mutica* and the varieties of *T*. *tenuiloba*. The aligned data set had 121 taxa and 930 characters, of which only 99 (10.6%) were variable. The 2-parameter substitution model M4 best fit the data set. This model is similar to a JC model, except that the transition AT has a different substitution rate from the the other transitions and transversions. Figures S5-S6 show the majority rule consensus tree from the phylogenetic analysis of the *trn*LF data set.

The unaligned sequences of the plastid *ndh*I gene and *ndh*I-*ndh*G intergenic spacer ranged from 910 bp in *Dyssodia pinnata* and *D*. *tagetiflora* to 964 bp in *Thymophylla acerosa*, *T*. *tenuifolia*, and the two varieties of *T*. *aurea*. In *Adenophyllum*, the sequences ranged from 915 bp in the two varieties of *A*. *wrightii* to 944 bp in *A*. *appendiculatum* and *A*. *aurantium*, which shared a 25 bp insertion. In *Thymophylla*, the shortest sequences (932 bp), were found in *T*. *aurantiaca*, which did not share two small insertions (five bp and 20 bp) found in all other

species of *Thymophylla* and in *Strotheria*. Also, *T*. *acerosa*, the two varieties of *T*. *aurea*, *T*. *gentryi*, and samples 2 and 4 of *T*. *tenuifolia* shared an insertion of 15 bp. The aligned data matrix had 103 taxa and 997 characters, of which only 95 (9.5%) were variable. As in the previous data set the substitution model M4 best fit the data. Figures S9-S10 show the majority rule consensus tree from the phylogenetic analysis of this data set.

The sequences of the plastid *psbA*-*trnH* intergenic spacer exhibited the widest size range among the markers used due to several indels. The sequences ranged from 298 bp in *Comaclinium montanum*, which had a large deletion of 265 bp, to 522 bp in sample 4 of *A*. *cooperi* and sample 2 of *A*. *porophylloides*, which shared an insertion of 24 bp. The samples of these two species of *Adenophyllum* shared another insertion of 28 bp. Similarly, a small insertion of 13 bp was shared by *A*. *glandulosum*, *A*. *aurantium*, and one sample of *A*. *appendiculatum*. Also, the two varieties of *A*. *wrightii* shared an insertion of 14 bp. In *Thymophylla*, the sequences varied from 398 bp in *T*. *tephroleuca* to 461 bp in *T*. *aurantiaca*. In addition, except for *T*. *aurantiaca*, all other species of *Thymophylla* and *Strotheria* shared a deletion of 45 bp, and *T*. *acerosa*, the two varieties of *T*. *aurea*, *T*. *gentry*, *T*. *gypsophila*, and the samples 2 and 4 of *T*. *tenuifolia* shared an insertion of 9 bp. The data matrix of the *psbA*-*trnH* intergenic spacer had 142 taxa and 723 characters, of which only 188 (26%) were variable. The nucleotide substitution model that best fit the data was the 3-parameter model M52. This model is similar to the 3-parameter model of Kimura (1981), but it differs from it in which the substitution rates of the transversions AG, CT, and GT are equal. The majority rule consensus tree from phylogenetic analysis of this data set is shown in Figures S11-S12.

The concatenated nrDNA (ITS and ETS) data set had 167 terminals and 1,150 characters, of which 674 (59.27%) were variable. The majority rule consensus tree from phylogenetic analysis of this data set is shown in Figures 8–9. The concatenated cpDNA (*trn*L-F, *ndh*I-*ndh*G, and *psbA*-

trnH intergenic spacers) data set had less terminals (154, but included at least one sample per taxon) and more characters (2,650) than the nrDNA data set. however, the cpDNA data set had less variation, as only 382 (14.4%) characters were variable. The majority rule consensus tree from phylogenetic analyses of the concatenated plastid data set is shown in Figures 10–11. Finally, the concatenated nrDNA plus cpDNA data set had 167 terminals and 3,800 characters, of which 1,056 (27.78%) were variable. Figures 12–13 show the majority rule consensus tree from phylogenetic analysis of the concatenated nrDNA and cpDNA data set.

Due to higher character variation in the nrDNA data sets than in the cpDNA data sets, the former were more useful for resolving species relationships than the latter. The majority rule consensus trees from the ITS and ETS analyses were considerably congruent and the majority rule consensus tree from the concatenated analyses of these data sets (Figures 8–9) was mostly resolved, with the majority of nodes strongly supported. Variation in the cpDNA sequence alignments was minimal except for more insertions and/or deletions than in the nrDNA data sets. These resulted in majority rule consensus trees with numerous polytomies (Figures S7-S12); however, some clades were also resolved and in some instances these clades were congruent with those resolved from the nrDNA phylogenies. Moreover, the majority rule consensus tree of the concatenated cpDNA data sets yielded a more resolved topology (Figures 10–11). Similarly, the analyses based on the concatenation of the nrDNA and cpDNA data sets yielded a highly resolved majority rule consensus tree, with most of the clades strongly supported (Figures 12-13). We will focus mostly on the consensus trees from the concatenated analyses and refer to the consensus from individual partitions when necessary.

Phylogenetic relationships of Adenophyllum—The majority rule consensus trees of the concatenated nrDNA, concatenated cpDNA, and concatenated nrDNA plus cpDNA resolved *Adenophyllum* as polyphyletic. Most taxa of this genus were resolved in a large core

Adenophyllum clade including the type species (*A*. *glandulosum*), but *A*. *anomalum* and the two varieties of *A*. *wrightii* (i.e, the *Trichaetolepis* group) were resolved in a different clade that included the two species of *Boeberastrum*. Notably, the nrDNA and cpDNA sequences of Boeberastrum anthemidifolium and *B*. *littoralis* were essentially the same. Moreover, *A*. *squamosum* from the *Clomenocoma* group was resolved as a distinct lineage from the core *Adenophyllum* clade. The nrDNA sequences of *A*. *squamosum* differed by several sites from those of the species of the *Clomenocoma* group, and the cpDNA sequences were also different and had a couple of insertions not seen in any other species of the *Clomenocoma* group. Within the core *Adenophyllum* clade the *A*. *porophyllum* complex was resolved as a clade (here called the *Lebetina* clade), and usually with *A*. *porophyllum* var. *porophyllum* as sister to the remaining taxa. The sequences of the nuclear and plastid markers of the typical variety were always different from the other taxa in this complex, although by a few sites. Notably, var. *radiatum*, which was considered a form of var. *porophyllum* by Turner (1996) was resolved as a subclade nested within the clade composed of var. *cancellatum* and *A*. *yecoranum* in the majority rule consensus tree for concatenated nrDNA and nrDNA plus cpDNA (Figures 8 and 12). The *Clomenocoma* group of *Adenophyllum*, excluding *A*. *squamosum*, was resolved as a clade in all consensus trees from the analyses of the three concatenated data sets (Figures 8, 10, and 12). We will refer to this monophyletic group as the *Clomenocoma* clade. This clade comprised two main subclades, one composed of *A*. *cooperi*, *A*. *porophylloides*, and *A*. *speciosum* (i.e., the xeric *Clomenocoma* group), and the other composed of *A*. *aurantium*, often nested within *A*. *appendiculatum*. The sequences of *A*. *cooperi* and *A*. *porophylloides* were essentially the same. Notably, the nrDNA and cpDNA sequences of sample 4 of *A*. *cooperi* and sample 2 of *A*. *porophylloides* were even more similar, and they shared a small insertion in the *psbA-trnH* sequences not seen in other samples. The cpDNA sequences of the plastid markers of *A*.

appendiculatum and *Adenophyllum aurantium* were essentially the same, but they differed by a very few sites in the ITS and ETS sequences.

Phylogenetic relationships of Thymophylla—The majority rule consensus trees from concatenated ITS and ETS, as well as the one from concatenated nrDNA plus cpDNA resolved a large *Thymophylla* clade with *Strotheria* nested within it, and with T. aurantiaca as sister to all other *Thymophylla* species. This core *Thymophylla* clade was strongly supported in the consensus tree in the nrDNA (Figure 9), but weakly supported in the consensus tree from the concatenated nrDNA plus cpDNA (Figure 13). However, the clade composed of all *Thymophylla* species, excluding *T*. *aurantiaca*, in the consensus tree from concatenated nrDNA plus cpDNA was strongly supported. Similarly, in the consensus tree from concatenated cpDNA, *Strotheria* was nested within the TA clade, and, notably, *T*. *aurantiaca* was sister to the core *Adenophyllum* clade. In general, the phylogenies estimated using cpDNA resolved a non-monophyletic *Thymophylla sensu* Strother (1986), with *T*. *aurantiaca* never clustering with the other Thymophylla species. Both the nrDNA and cpDNA sequences of this species were always notably different from those of the other species of *Thymophylla*. The majority rule consensus trees from ITS (Figure S4), concatenated nrDNA (Figure 9), and concatenated nrDNA plus cpDNA (Figure 13) supported A. squamosum as the sister of the core *Thymophylla* clade, but in the majority rule consensus tree from concatenated cpDNA, *Tagetes lunulata* was resolved as sister to *Thymophylla*, although without a significant posterior probability value (Figure 11). The consensus tree from the ETS analyses (Figure S6) supported a clade composed by A. squamosum and *Dysodiopsis tagetoides* as the sister of the core *Thymophylla* clade.
The *Gnaphalopsis* group of *Thymophylla* was not resolved as monophyletic in any of the analyses performed, as *T*. *micropoides* and *T*. *tephroleuca* were always resolved in distinct

clades. We referred to these monophyletic groups as the *Gnaphalopsis* and *Tephroleuca* clades (Figures 9 and 13).

The *Aciphyllaea* group (*T*. *acerosa* and *T*. *gypsophila*) was resolved as monophyletic with strong support in the majority rule consensus trees from concatenated ITS and ETS (Figure 9), and concatenated nrDNA plus cpDNA (Figure 13) analyses. Notably, *T*. *acerosa* was nested within *T*. *gypsophila* in these consensus trees. Although there were several differences in the nrDNA sequences of these two species, the sequences of sample 3 of *T*. *gypsophila* were usually more similar to those of *T*. *acerosa* than those of *T*. *gypsophila*. However, these similarities were in found in low-read-quality regions of the sequences of sample 3 of *T*. *gypsophila*. Moreover, the consensus tree from the ITS analyses resolved these two species as sister clades (Figure S4). On the other hand, in the consensus tree of the concatenated cpDNA data sets, the *Aciphyllaea* group was resolved in a strongly supported clade that also included species of the *Hymenatherum* group (Figure 11). This clade was also resolved in the majority rule consensus tree from trnL-F and ndhI-ndhG intergenic spacers (Figures S8-S10). However, the relationships among the terminals constituting this clade were mostly ambiguous.

The two varieties of *T*. *setifolia* were resolved in distinct clades in the majority rule consensus trees from concatenated nrDNA (Figure 9), and concatenated nrDNA plus cpDNA (Figure 13). Similarly, in the consensus tree from concatenated cpDNA (Figure 11), the samples of *T*. *setifolia* var. *greggii* resolved in a clade that included *T*. *micropoides* and *T*. *pentachaeta* var. *puberula*. This clade was also resolved in the consensus trees from *ndh*I-*ndh*G (Figure S10) and *psbA*-*trnH* (Figure S12), although in the former the clade included one sample of *T*. *setifolia* as well. Within the *T*. *setifolia* var. *setifolia* clade, the radiate and discoid forms of this variety composed two sister subclades in the consensus tree from the concatenated ITS and ETS analyses, but only the subclade comprising the discoid forms was strongly supported (Figure

11). It is also notable that in the majority rule consensus tree from ETS analysis the radiate forms of *T*. *setifolia* were resolved in the subclade composed of the discoid forms (Figure S6). The *T*. *pentachaeta* complex was resolved as a monophyletic group with strong support in the majority rule consensus tree from concatenated nrDNA (Figure 9) and concatenated nrDNA plus cpDNA (Figure 11). Notably, a subclade was composed of varieties *hartwegii* and *belenidium*, but there was not any resolution within this subclade. In general, the DNA sequences of these two varieties were more similar and in the case of the ETS sequences they were identical. In contrast, the sequences of the three samples of var. *pentachaeta* were different at least a few sites, but ITS and ETS sequences were more similar to those of var. puberula. Surprisingly, the samples of this variety resolved in a clade that also included *Thymophylla micropoides* and *T*. *setifolia* var. *greggii* in the majority rule consensus trees from concatenated cpDNA (Figure 11) and ndhI-ndhG (Figure S10) and *psbA*-*trnH* (Figure S12) analyses.

The four varieties of *T*. *tenuiloba* plus *T*. *mutica* (i.e., the *T*. *tenuiloba* complex), were resolved as a monophyletic group with strong support in all majority rule consensus trees (Figures 9, 11, and 13, Figures S4, S6, S8, S10, and S12). However, a clade composed of the varieties of *T*. *tenuiloba* only, was weakly supported and resolved in the majority rule consensus trees of the ITS (Figure S4), concatenated nrDNA (Figure 9), and concatenated nrDNA plus cpDNA analyses (Figure 13). The samples of the four varieties of *T*. *tenuiloba* failed to form distinct subclades in all analyses. However, the samples of var. *wrightii* were resolved as a subclade in some majority rule consensus trees (Figures 9 and 13, and Figures S4, and S6), but this subclade also included one sample of the var. *tenuiloba*.

A clade composed of all taxa of the *Hymenatherum* group of *Thymophylla*, except for *T*. *concinna*, was resolved with strong support in the majority rule consensus trees from ITS (Figure S4), ETS (Figure S6), concatenated nrDNA (Figure 9), and concatenated nrDNA plus cpDNA

(Figure 13). The ITS and ETS sequences of *Thymophylla concinna* were notably different from those of the other taxa of the *Hymenatherum* clade, and this species was resolved as the successive sister to all other species of *Thymophylla* after *T. aurantiaca* in the consensus trees from ETS (Figure S6), concatenated nrDNA (Figure 9), and concatenated nrDNA plus cpDNA (Figure 13). The *psbA-trnH* sequences of *Thymophylla* concinna were also different from all other taxa of the *Hymenatherum* clade. Indeed T. concinna was resolved in a clade that also included *Adenophyllum anomalum* and *Comaclinium montanum* in the majority rule consensus tree from *psbA-trnH* analyses (Figure S11). The *ndh*I-*ndh*G sequences of *T. concinna* were also different from those of the other species of the *Hymenatherum* group, although at a few sites. In contrast, the *trn*L-F sequences of *T. concinna* were more similar to those of *T. tenuifolia*. Within the *Hymenatherum* group, the *T. aurea* complex did not resolve as monophyletic. For example, some samples of *T. aurea* var. *polychaeta* were nested within *T. tenuifolia* (Figures 9 and 13, and Figures S4 and S6). Notably, both the nrDNA and the cpDNA sequences of those samples of *T. aurea* var. *polychaeta* were more similar to the sequences of *T. tenuifolia* than to the sequences of other samples of var. *polychaeta*. Similarly, the samples of *T. gentryi* were resolved as monophyletic only in the majority rule consensus tree of the *psbA-trnH* analyses (Figure S12). On the other hand, the majority rule consensus tree from concatenated cpDNA resolved a strongly supported clade composed of the samples of the *T. aurea* complex, *T. tenuifolia*, and the *Aciphyllaea* group (Figure 11). However, the relationships of the terminals within this clade were mostly equivocal.

Dyssodia clade and other relatives—The phylogenies estimated from ITS, ETS, concatenated nrDNA, and concatenated nrDNA plus cpDNA strongly support a clade composed of the species of *Dyssodia sensu stricto* (i.e., sensu Strother 1986). *Dyssodia* decipiens was resolved as sister to the other three species, and the samples of the two varieties of *Dyssodia pinnata* resolved in a

polytomy. The sequences of these two varieties were essentially the same. Notably, *D*. *papposa* was polyphyletic, with samples 1–3 and 4–5 resolved as distinct subclades. When we collected the specimens of samples 4–5 of D. papposa (Appendix 1), we noticed that the involucre was greener than usual, among other minor morphological features. The majority rule consensus tree from concatenated cpDNA also resolved the Dyssodia clade, but it was weakly supported and with less resolution among the terminals composing the clade (Figure 10).

The majority rule consensus tree from concatenated nrDNA (Figure 8), concatenated nrDNA and cpDNA (Figure 12), ITS (Figure S3), and ETS (Figure S4) supported a sister relationship between *Boeberoides* and *Gymnolaena*. These analyses also supported a sister relationship between the Dyssodia clade and Boeberoides-Gymnolaena. Moreover, the cpDNA sequences of *Dyssodia*, *Boeberoides*, and *Gymnolaena* were very similar and the phylogenetic analyses of the plastid markers supported a close relationship among these taxa. The majority rule consensus tree from the concatenated cpDNA resolved *Gymnolaena* as sister to *Dyssodia* and *Boeberoides* as sister to both. However, the sister relationship between *Gymnolaena* and *Dyssodia* was weakly supported (Figure 10).

Comaclinium montanum was resolved as sister to the clade composed of *Dyssodia*, *Boeberoides*, and *Gymnolaena* in the majority rule consensus tree from the concatenated nrDNA (Figure 8), concatenated nrDNA plus cpDNA (Figure 12), and ITS (Figure S3). However, the majority rule consensus tree from concatenated cpDNA resolved *C. montanum* as more distantly related to *Dyssodia*, *Boeberoides*, and *Gymnolaena*, albeit weakly (Figure 10).

Phylogenetic relationships of *Dysodiopsis tagetoides* were equivocal. The majority rule consensus tree from ITS (Figure S3) and concatenated nrDNA (Figure 8) resolved it as sister to the core *Adenophyllum* clade, although only the latter was strongly supported. However, the majority rule consensus tree from concatenated nrDNA plus cpDNA (Figure 12) resolved a

strongly supported clade comprising a trichotomy of 1) Dysodiopsis, 2) the core *Adenophyllum* clade, and 3) *Thymophylla* plus *A*. *squamosum*. In contrast, the majority rule consensus tree from concatenated cpDNA (Figure 10) resolved *Dysodiopsis* as a distinct lineage with unclear relationships.

Divergence time estimation—The topology of the MCC calibrated tree (Figure 14) was almost identical to the topology of the majority rule consensus tree from the non-calibrated analyses of the concatenated nrDNA plus cpDNA data sets conducted in MrBayes 3.2 (Figure 14). Among the differences are that the MCC tree was fully resolved with more strongly supported nodes and, notably, *Thymophylla aurantiaca* was resolved as sister to the core *Adenophyllum* clade. Based on the MCC calibrated tree, core *Adenophyllum* and *T*. *aurantiaca* diverged at a mean age of 11.16 Ma [6.24−16.39 Ma, 95% credible interval] and the most recent common ancestor (MRCA) of core *Adenophyllum* originated at a mean age of 6.74 [3.63−10.36 Ma, 95% credible interval]. The MRCA of the *Clomenocoma* group of *Adenophyllum* is inferred at a mean age of 4.38 Ma [2.14−7 Ma, 95% credible interval] and the MRCA of the *Lebetina* group at a mean age of 1.12 Ma [0.43−1.99 Ma, 95% credible interval].

The MCRA of *Boeberastrum sensu lato* (i.e., including the *Trichaetolepis* group of *Adenophyllum*) is estimated at a mean age of 9.32 Ma [4.54−14.37 Ma, 95% credible interval], and the MRCA of this node is estimated at a mean age of 19.07 Ma. [14.35−33 Ma, 95% credible interval]. *Adenophyllum anomalum* diverged from *B. anthemidifolium* at 7 Ma [3.41−11.43 Ma, 95% credible interval].

The MCRA of *A*. *squamosum* is inferred at a mean age of 12.37 Ma [7−17.85 Ma, 95% credible interval]. The node from which *Dysodiopsis* and core *Thymophylla* descended is weakly supported; it is estimated, however, at a mean age of 11.51 Ma [6.62−16.86 Ma, 95% credible interval].

The MRCA of core *Thymophylla* is estimated at a mean age of 6.1 Ma [3.29−9.14 Ma, 95% credible interval] and the MRCA of the *Aciphyllaea* group at a mean age of 1.9 Ma [0.73−3.54 Ma, 95% credible interval]. *Strotheria gypsophila* diverged at a mean age of 4.57 Ma [3.15−8.51 Ma, 95% credible interval]. The MRCA of the *Hymenatherum* group is estimated at a mean age of 3.41 Ma [1.81−5.19 Ma, 95% credible interval]. The split between *T*. *mutica* and *T*. *tenuiloba* is estimated at 1.14 Ma [0.41−1.86 Ma, 95% credible interval]. The lineages leading to *T*. *tephroleuca* and *T*. *setifolia* var. *greggii* diverged at a mean age of 3.42 Ma [1.87−5.12 Ma, 95% credible interval] and 3.18 Ma [1.78−4.83 Ma, 95% credible interval], respectively. The MRCA of the pentachaeta complex is estimated at a mean age of 2 Ma [1.78−4.83 Ma, 95% credible interval]. The lineage leading to *T*. *setifolia* var. *setifolia* diverged at a mean age of 2.47 Ma [1.31−3.74 Ma, 95% credible interval]. Although the node from which *T*. *micropoides* descended is weakly supported, it is inferred at a mean age of 2.24 Ma [1.22−3.46 Ma, 95% credible interval]. The split of *T*. *mutica* and the *T*. *tenuiloba* complex is estimated at a mean age of 1.24 Ma [0.63−2 Ma, 95% credible interval].

Among the outgroups, the MCRA of *Dyssodia* and *Boeberoides* is estimated at a mean age of 6.82 Ma [3.−11.25 Ma, 95% credible interval] and the node from which these genera and *Comaclinium* derived is inferred at a mean age of 11.75 Ma [6.37−17.71 Ma, 95% credible interval]. Finally, the MCRA of *Flaveria* (represented by *F*. *anomala*) is estimated at a mean age of 24 Ma [15.61−37.26 Ma, 95% credible interval].

Historical biogeography—The ancestral range estimation under the S-DEC model is presented in Figure 15, which shows up to three of the most probable ancestral ranges at each node to facilitate visualization. A total of 48 dispersal events and 5 vicariance events are inferred; however, the vicariance events occur at nodes in which the probability of the ancestral ranges is lower than 0.9. No extinction events were estimated. Most of the speciation events

(i.e., 22) are inferred in the North American deserts, followed by the Neotropical region (11), Great Plains (5), Southern Semi-arid Highlands (4), and Temperate Sierras (2). No speciation events are estimated for the Pacific Domain and the Argentinian deserts.

The Neotropical region is inferred as the most probable ancestral range for the *Adenophyllum*-*Thymophylla* clade, core *Adenophyllum*, and its *Lebetina* subclade. The combination of the Neotropical region and North American deserts (AD range) is estimated as the most likely ancestral range for the ancestor of the *Clomenocoma* subclade within *Adenophyllum*.

Although there are several probable ranges at the node of the MRCA of the *Boeberastrum* clade, the state with higher probability is a combined range (ABCD). Also, it is inferred that the MRCA of *B*. *anthemidifolium* and *A*. *anomalum* occurred in the North American deserts, while the MRCA of *A*. *wrightii* was more likely widespread in the Temperate Sierras, Southern Semi-Arid Highlands, and the Neotropical region (BCD state). The most probable range for the MRCA of *A*. *squamosum* is the Neotropical region, although the ancestral range reconstruction at this node shows several other areas with lower probabilities.

The most probable ancestral range for core *Thymophylla* and several nodes within this clade, including the MRCA of the *Aciphyllaea* group, the Hymenatherum group (excluding *T*. *concinna*), and the *T*. *pentachaeta* and *T*. *tenuiloba* complexes, is the North American deserts, in which most of the speciation events are inferred. This area is also the most likely ancestral range for isolated lineages within *Thymophylla*, such as those of *T*. *concinna* and *Strotheria gypsophila*. However, the combined range composed of the North American deserts and the Great Plains (AE) was the most likely ancestral area for some nodes within the core *Thymophylla* clade.

DISCUSSION

***Systematics of Adenophyllum*—**Our results support transfer of the species of the Trichaetolepis group of *Adenophyllum* to *Boeberastrum* as suggested by Loockerman et al. (2003). Although these species of *Adenophyllum* differ in several morphological features from those of *Boeberastrum*, especially in the morphology of the involucre, they all share the chromosome number of *x*=7. In addition, the species of the Trichaetolepis group and *Boeberastrum* spp. are predominantly annuals bearing pinnatisect leaves with very narrow segments (Figure 2A,F and I). The exceptions are *A*. *anomalum*, which according to our observations in the field and in the herbarium is an early blooming perennial, and *B*. *littoralis*, which has broader leaf segments.

The species of the *Boeberastrum* clade, including the *Trichaetolepis* group of *Adenophyllum*, have a similar geographic distribution. *Boeberastrum anthemidifolium* and *B*. *littoralis* are endemic to the Baja California peninsula; *A*. *anomalum* occurs in adjacent northwestern Mexico, in the deserts and tropical deciduous forests from Sonora to Nayarit; *A*. *wrightii* var. *wrightii* is restricted to the semi-arid highlands and temperate forests of SW USA (New Mexico, Arizona) and adjacent northern Mexico (Chihuahua), while var. *pulcherrimum* is distributed in western and central Mexico.

Boeberastrum anthemidifolium and *B*. *littoralis* differ mainly in leaf morphology (leaves dissected with linear segments in *B*. *anthemidifolium* vs entire and spatulate to shallowly lobed in *B*. *littoralis*). Also, *B*. *littoralis* is confined to coastal dunes in the southernmost extreme of the Baja California peninsula, while *B*. *anthemidifolium* is widespread in the peninsula. However, both the nrDNA and cpDNA sequences analyzed of these taxa were essentially the same and, therefore, they are probably best treated as varieties of the same species.

Since our results indicate a sister relationship between the two varieties of *A*. *wrightii*, it seems equally justifiable to keep treating them as infraspecific taxa (e.g., Strother 1969, 1987, 2006) or as sister species (e.g. Turner 1996, Villarreal 2003, Carnahan 2019). However, unlike the case of *B*. *anthemidifolium* and *B*. *littoralis* discussed above, we did find a few but constant differences in the nrDNA (both ITS and ETS), and the plastid *ndh*I-*ndh*G and *psbA*-*trnH* DNA sequences between these two varieties. They also differ in morphology as mentioned in the introduction, and they are clearly allopatric. These facts may warrant the recognition of two closely related species as proposed by Turner (1996), at least until additional studies focused at the infraspecific level support a different treatment.

We also propose to segregate *A*. *squamosum* to a new genus. Both analyses based on nrDNA and cpDNA sequences clearly suggest that this species belongs to a different lineage from the core *Adenophyllum* clade (Figures 8, 10, and 12). Although the heads of *A*. *squamosum* resemble in size and color those of the *Clomenocoma* group of *Adenophyllum*, there are several morphological differences that distinguish *Adenophyllum squamosum* (Strother 1969).

Our results support a narrowed circumscription of *Adenophyllum* to comprise the species in the core *Adenophyllum* clade only (Figures 8, 10, and 12). The *Lebetina* and *Clomenocoma* groups were resolved as subclades and therefore they could be recognized as two monophyletic sections. Within the *Lebetina* clade, our results support the view of Strother (1969), who considered *A*. *porophyllum* var. *radiatum* more closely related to var. *cancellatum*, instead of being a radiate form of var. *porophyllum* (e.g., Turner 1996, 2013). Variety cancellatum differs in morphology from var. radiatum by its conspicuous rays vs the highly reduced rays of var. *radiatum*. Moreover, they are clearly allopatric. Var. *cancellatum* is a taxon of the Central Plateau of Mexico that is also found in the semi-arid highlands and tropical dry forests of western and northeastern Mexico, while var. *radiatum* is confined to southern Mexico

(Veracruz, Oaxaca, and Chiapas), including the Yucatan peninsula, and to adjacent Cuba and Central America (Guatemala to Nicaragua). There were also a few site differences in both the nrDNA and cpDNA sequences analyzed of the samples of these two varieties. This evidence warrants the recognition of var. *radiatum* as distinct from var. *porophyllum* and we propose to treat it as a distinct species.

We also propose to follow Turner (1996) in treating ho treated *Adenophyllum porophyllum* var. *porophyllum* and *A. porophyllum* var. *cancellatum* as two distinct species. In addition to the morphological differences stated in the introduction and in Turner (1996), we found a few differences in the nrDNA and cpDNA sequences of the samples of these two taxa. Moreover, we did not observe any morphological intergradation in areas of sympatry also noticed by Turner (1996). Indeed, we collected specimens of the two taxa in the same location (*Hinojosa-Espinosa 716*, *717* (DAV, MEXU)), growing next to each other, and we used DNA samples from these specimens in the analyzed data sets (Appendix 1). In the estimated phylogenies from concatenated nrDNA (Figure 8), concatenated nrDNA plus cpDNA (Figure 12), ITS (Figure S1), and ETS (Figure S3) the samples of *A*. *porophyllum* var. *porophyllum* were always resolved as a distinct group from those of var. *cancellatum*, which were often intermixed with samples of var. *radiatum* and *A*. *yecoranum*.

Since *A*. *yecoranum* was usually nested within *A*. *cancellatum* (Figures 8 and 12), it would seem best to treat the former as a synonym of the latter. There were, however, a few differences in the nrDNA and cpDNA sequences analyzed of these two taxa. Moreover, in *A*. *yecoranum*, the exterior series of pappus scales is dissected into bristles, but not in *A*. *cancellatum* (Turner 2013). In addition, the region of Yecora and adjacent Chihuahua in northwestern Mexico is an area that harbors several localized endemics (Turner 2013). For

these reasons we suggest to tentatively treat *Adenophyllum yecoranum* as a variety of *A*. *cancellatum* until additional studies support a different disposition for these taxa.

Within the *Clomenocoma* clade, our results support the view of Turner (1996) in treating *A*. *appendiculatum* as a synonym of *A*. *aurantium*. Indeed, Strother (1999) indicated that these two species were probably conspecific. However, we noticed in the field and in the herbarium that the specimens of *A*. *aurantium* from Veracruz near the Gulf of Mexico appear to have a more robust, shrubby habit and smaller heads (Figure 3B-F) than those from the Pacific slopes that fall into the concept of A. appendiculatum sensu Strother (1969, 1999, 2018). There were also a few differences in nrDNA sequences between *A*. *aurantium* and *A. appendiculatum*, with the latter nested within the former (except in the phylogenies lacking resolution). The restricted distribution of *A*. *aurantium*, and the allopatric distribution and differences in morphology between *A*. *aurantium* and *A*. *appendiculatum* seem to warrant the recognition of two varieties.

Strother (1969) emphasized that *A*. *porophylloides* and *A*. *cooperi* were very closely related and our results support that view at the level that it seems best to treat them as varieties of the same species. These taxa are quite similar in overall morphology and both the nrDNA and cpDNA sequences analyzed were essentially the same. They are also similar in habitat (North American deserts) and their geographic ranges overlap in the SW USA. Notably, the two samples (Adenophyllum_porophylloides2 and Adenophyllum_cooperi4) that were usually clustered together (Figures 10 and 12) come from a sympatric region in Arizona. This suggest that introgression between these two taxa occurs in the area of sympatry. The analyzed nrDNA and cpDNA sequences of *A*. *speciosum* were also quite similar to those of *A*. *porophylloides* and *A*. *cooperi*, but not identical. In morphology, *A*. *speciosum* differs in some features, including habit and leaf segment morphology. *Adenophyllum speciosum* also differs in geographic distribution, being allopatric with respect to *A*. *porophylloides* and *A*. *cooperi*.

Systematics of Thymophylla—Our findings support the transfer of *Strotheria gypsophila* to *Thymophylla* as first suggested by Loockerman et al. (2003). Strotheria is especially similar to the perennial shrubby species of *Thymophylla* in having a small and woody habit, strongly connate involucre, and a chromosome number of *x*=8 (Turner 1996); however, it is unique in having reduced succulent leaves and small capitula with four disk flowers only. It is also a localized endemic, confined to a small gypsum area in Nuevo Leon (northeastern Mexico). Although it is clearly nested within *Thymophylla*, our results show that this species is somewhat isolated; it is not sister to a particular species or subgroup of *Thymophylla*, but is sister to a large subclade that contains most species of *Thymophylla*.

In contrast to the inclusion of Strotheria within *Thymophylla*, we suggest to segregate *T. aurantiaca* to a new monotypic genus. The analyzed nrDNA and, especially, the cpDNA sequences of *T. aurantiaca* were always different from the rest of the species of the genus. The phylogenies based on the concatenated cpDNA sequences and the divergence time analyses (the latter based on less copious taxon sampling) support a closer phylogenetic relationship between *T. aurantiaca* and the core *Adenophyllum* clade (Figures 10, 14, S7 and S9). Although the phylogenies estimated using ITS and ETS resolved T. aurantiaca as sister to the remaining taxa of *Thymophylla*, *T. aurantiaca* has a longer branch, which suggests a considerable amount of evolutionary change, and the clade without *T. aurantiaca* is strongly supported (Figure 9, S4, and S6). Moreover, based on morphology Strother (1969) indicated that the relationships of this species within *Dyssodia* subg. *Hymenatherum* were unclear. In addition, *T. aurantiaca* is confined to the Tehuacan-Cuicatlan Valley in south-central Mexico, a region known for its high level of plant diversity and endemism (Davila et al. 2002). For all these reasons we propose to exclude this species from *Thymophylla*.

It is not surprising that the *Gnaphalopsis* group of *Thymophylla* was not resolved as monophyletic. Strother (1969) tentatively placed the woolly species, *T*. *tephroleuca* and *T*. *micropoides* in this group, emphasizing their unclear relationships. However, the phylogenies from combined nrDNA plus cpDNA (Figure 12) support a close relationship between *T*. *micropoides* and the *T*. *tenuiloba* complex. Although these taxa differ strikingly in leaf morphology and indumentum they have a similar biogeographic distribution; they are confined to the Great Plains of Texas and northeastern Mexico and the adjacent Chihuahuan Desert. *Thymophylla tephroleuca* also occurs in the Texan Great Plains. In general, our results support an isolated position of this species within *Thymophylla*, where it is clearly nested.

Within the *Aciphyllaea* group, the nested position of *T*. *acerosa* within *T*. *gypsophila* in the most resolved phylogenies (Figures 9, 13 and S6) suggests that the two taxa are perhaps best treated as varieties of the same species. They are very similar in morphology and we noticed their similar if not identical fragrance in the field. However, there are some morphological differences between them. *Thymophylla gypsophila* is a more robust shrub with larger capitula than those of *T*. *acerosa* (Figure 4 J-O), and it is confined to a small area of gypsum dunes in Cuatro Cienegas, northern Mexico. There were also differences in the ITS and ETS sequences in the samples of these two species, except for sample 3 of *T*. *gypsophila*, which, unexpectedly, had ETS sequences that were more similar to those of *T*. *acerosa* than those of *T. gypsophila*, and, for this reason, this sample grouped with the samples of *T*. *acerosa* (Figures 9, 13, S4). In this case, the similarity appears to reflect the low quality of the sequence of sample 3 rather than a true phylogenetic affinity. Usually, this sample had ambiguities at the nucleotide sites where *T*. *acerosa* and the other samples of *T*. *gypsophila* differed. For this reason and because *T*. *acerosa* and *T*. *gypsophila* are otherwise resolved as two sister taxa, we propose to tentatively keep recognizing them as two distinct but sister species. The ITS phylogeny also

resolved the samples of *Thymophylla acerosa* and *T*. *gypsophila*, including the sample 3, as two distinct closely related clusters (Figure S2).

Our findings clearly support the recognition of *T*. *setifolia* var. *setifolia* and *T*. *setifolia* var. *greggii* as distinct species (Figures 9, 11, and 13). However, it was unexpected to find they are not sister species. Although Rydberg (1915) and more recently Turner (1996) recognized them as separate species, at least Turner (1996) considered them very closely related. The two taxa are very similar in overall morphology; however, based on our results their phenetic resemblance might be due to convergent evolution.

Thymophylla setifolia has both discoid and radiate capitula unlike *T*. *greggii*, which has radiate capitula only (Figure 5 A-H). The radiate forms of *T*. *setifolia* are confined to northern Mexico, while the discoid forms are more widespread, ranging from northern to south-central Mexico. In some trees, the discoid forms were nested within the radiate forms (Figure 13, S2), or as two closely related groups (Figure 9); however, in the ETS phylogeny (Figure S4), the radiate forms resolved as isolated lineages while the discoid forms resolved within the T. pentachaeta complex, supporting the view of Strother (1969), who stated that *T*. *setifolia* was closely related to *T*. *pentachaeta*. This inconsistency might be an indication of incomplete lineage sorting (ILS), or an indication of hybridization between the radiate forms of *T*. *setifolia* and other species of *Thymophylla*, as the populations with radiate capitula are sympatric with several species of *Thymophylla* in northern Mexico. This might explain why the more isolated populations of *T*. *setifolia* from south-central Mexico always have discoid capitula, but further studies at the population level are required to fully document this hypothesis.

Within the *T*. *pentachaeta* complex, the phylogenies from concatenated cpDNA sequences (Figure 11) and plastid *ndh*G-*ndh*I (Figure S8) and *psbA*-*trnH* (Figure S10) data sets support the recognition of *T*. *pentachaeta* var. *puberula* as a distinct species, as treated by Turner (2009). In

contrast, the evidence from concatenated nrDNA sequences (Figure 9), concatenated cpDNA plus nrDNA sequences (Figure 13), and ITS (Figure S4) suggest a sister relationship between *T*. *puberula* (= T. *pentachaeta* var. *puberula*) and *T*. *pentachaeta*, although this relationships is weakly supported. Regarding their morphology, these two taxa are extremely similar and it is in general difficult to sort specimens out without a solid understanding of the group. Moreover, the discrepancy between cpDNA and nrDNA might be due to ILS and/or hybridization and requires further study, but in the meantime we propose to follow Turner (2009) and treat these taxa as two distinct, putatively closely related species.

Our results do not support Turner's (1996, 2009) treatment of *T*. *pentachaeta* var. *belenidium* as a synonym of var. *pentachaeta*. In contrast, our findings suggest that the varieties *belenidium* and *hartwegii* are best treated as the same taxon. The analyzed nrDNA and cpDNA sequences of these two varieties were essentially the same. They are also quite similar in overall morphology, but not identical. Variety *hartwegii* has a more compact, cushion-like habit, and narrower heads with fewer florets than those of var. *belenidium*, which is more robust and has broader capitula with more florets (Strother 1969, 2006). Their morphological differences are subtle and perhaps more evident in the field (Figure 5 L-P). Moreover, var. *belenidium* is more widespread in northern Mexico and adjacent USA, and it is also disjunct in Argentina, where it is thought to be native (Diego Gutierrez, com. pers.). Based on these morphological and geographical differences between the varieties *hartwegii* and *belenidium*, we propose to recognize one species with two varieties, until further studies support a different taxonomic treatment.

Although samples of var. *belenidium* from several regions in North America (Arizona, Nevada, and Texas in the USA, and Baja California and Coahuila of northern Mexico) were analyzed in this study, it is desirable to study samples from South America to determine

whether there are differences at the molecular level or not with the North American populations. At least the few available herbarium specimens from Argentina that we could examine seemed to be extremely similar if not identical in morphology to the specimens from North America.

Within the *Thymophylla tenuiloba* complex, our results do not support the recognition of *T*. *tenuiloba* var. *wrightii* at the specific level as recently treated by Nesom (2009) nor any of the other three varieties. As Strother (1969) stated, the varieties *texana*, *treculii* and *tenuiloba* differ in pappus characteristics. Only var. wrightii has additional distinguishing features, mainly simple leaves vs. pinnatisect leaves with linear segments of the other three varieties, and it is also more restricted geographically (coastal plains in southeastern Texas) than the other three (Texas and adjacent Mexico). Also, the nrDNA and cpDNA sequences analyzed here do not provide resolution at the infraspecific level. Therefore, we provisionally suggest following Turner (1996) in recognizing only two varieties (var. *tenuiloba* and var. *wrightii*) of *T*. *tenuiloba*.

Thymophylla mutica, which was resolved as sister to the *T*. *tenuiloba* complex with strong support (Figures 9, 13), may also warrant treatment as a variety of *T*. *tenuiloba*. However, in addition to the morphological differences between *T*. *mutica* and *T*. *tenuiloba*, such as absence of a calyculus in the former, which is well-developed in the latter (Figure 6A-F), we also found that the ITS and ETS sequences of *T*. *mutica* were different a few sites from the sequences of *T*. *tenuiloba*. Moreover, *T*. *mutica* is allopatric with respect of *T*. *tenuiloba* and its varieties. For these reasons, we suggest to keep recognizing *T*. *mutica* as a distinct species pending the results of another investigation more focused on relationships at the interspecific level.

The *Hymenatherum* group of *Thymophylla* is not resolved as monophyletic unless *T*. *concinna* is not considered a member of the group. Both the nrDNA and cpDNA sequences of this species were considerably different from those of the other taxa of the *Hymenatherum*

group. *Thymophylla concinna* differs from other species of the *Hymenatherum* group by having white rays. It is also confined to the Sonoran Desert in northwestern Mexico and adjacent Arizona. If *T*. *aurantiaca* is excluded from the genus as we discussed above, the phylogenies from concatenated nrDNA (Figure 9), concatenated cpDNA (Figure 11), and concatenated nrDNA plus cpDNA (Figure 13) would strongly support *T*. *concinna* as sister to the rest of the *Thymophylla* species. The ITS phylogeny, however (Figure S2), suggests a closer relationship between *T*. *concinna* and the *Aciphyllaea* group of *Thymophylla*, although the relationship is weakly supported. Nevertheless, *T*. *concinna* and the species of the *Aciphyllaea* group share the strongly connate involucre without a well-developed calyculus.

With respect to the *T*. *aurea* complex, based on the results of the concatenated nrDNA (Figure 9) and combined nrDNA plus cpDNA analyses (Figure 13), *T*. *gentryi* is nested within *T*. *aurea*, which suggests treating the former as a synonym of the latter. Although Turner (1996) tentatively recognized *T*. *gentryi* as distinct from *T*. *aurea*, Turner (1996) suggested that they might be conspecific. Based on our results, we also propose to follow Turner (1996) in treating T. aurea without varieties. If *T*. *gentryi* and *T*. *aurea* are the same species as supported by our analyses, then *T*. *aurea* has a wider distribution, reaching central Durango in northern Mexico, so populations of this species with a pappus of erose scales (i.e., *T*. *aurea* var. *aurea*) are no longer confined to the Southwest USA and adjacent northernmost Mexico as previously thought (e.g., Strother 1969, 2006).

Our results also indicate that *T*. *tenuifolia* is extremely closely related to *T*. *aurea*, and perhaps they might be treated as a single species. Although these two taxa are also very similar in morphology, there are morphological and geographic differences that warrant the recognition of two distinct taxa. The main morphological difference between these two species is the strongly connate, uniseriate involucre of *T*. *tenuifolia* (Figure 7D), which is more similar to that

of *Thymophylla concinna* (Figure 7G) than to the involucre of *T*. *aurea* (Figure 6I), which is composed of two series of phyllaries that are connate up to 2/3 of their length (Turner 1996, Strother 1969). On another matter, the cluster composed of samples of *T*. *tenuifolia* and *T*. *aurea* var. *polychaeta* that were resolved in some phylogenies (Figures 9, 13, S2 and S4) might be due to ILS and/or hybridization. It is likely that *T*. *tenuifolia* and *T*. *aurea* hybridize in sympatric areas (i.e., the Mexican portion of the Chihuahuan Desert); however, additional studies are required to test this hypothesis.

Relationships among outgroups—Here, we briefly highlight some of the phylogenetic findings for the outgroups. Most of our analyses indicate that *Boeberoides grandiflora* is sister to *Gymnolaena* and might warrant transfer to that genus. Indeed, Rydberg (1915) transferred *Dyssodia seleri* B.L. Rob. & Greenm., a synonym of *B*. *grandiflora*, to *Gymnolaena*. The monotypic *Boeberoides* is distinguished in part by its calyculus, which is composed of several series of bracts. The heads of *Boeberoides* are also larger than those of any species of *Gymnolaena*. However, besides these features, *Boeberoides* is very similar in morphology to *Gymnolaena*. Both genera have opposite, simple leaves with pinnate venation and numerous, pellucid, dot-like, secretory cavities. They are also similar in geographic distribution, occurring in the tropical dry forests of Mexico, although *Boeberoides* is confined to the western and central portion of the Balsas Basin in the states of Guerrero, Mexico State, and Morelos, while *Gymnolaena* species are restricted to more southerly dry forests of Oaxaca and adjacent Chiapas.

Our results strongly support monophyly of *Dyssodia sensu stricto*. Based on morphology, it is surprising that *D*. *tagetiflora* and *D*. *pinnata* were not resolved as sister species. They are very similar in overall morphology and sometimes are confused in herbarium collections. Notably, it is possible that samples 4 and 5 of *D*. *papposa*, which come from central Durango in northern

Mexico, represent a different taxon. Both the nrDNA and cpDNA sequences of these samples were always different from the other samples (1–3) of *D*. *papposa*. However, besides a greener involucre, we have not found any morphological differences from typical *D*. *papposa* so more detailed studies are still needed to conclude whether these samples represent an undescribed species or not.

It is clear that *Dysodiopsis* belongs to the *Adenophyllum*-*Thymophylla* clade, but is still not understood whether *Dysodiopsis* is more closely related to *Adenophyllum sensu stricto*, *Thymophylla*, or *A*. *squamosum*. Our results, however, support further the recognition of *Dysodiopsis* as a monotypic genus distinct from *Dyssodia*. Although in overall morphology *Dysodiopsis* appears most similar to *Adenophyllum*, it is unlike any species of *Adenophyllum* in having simple leaves and more strongly connate involucres. It is also different from *Adenophyllum* in geographic distribution since it is confined to the Great Plains of the eastern United States.

Similarly, our study supports the recognition of *Comaclinium montanum* as distinct from *Dyssodia*. However, as some phylogenies show that *Comaclinium*, *Boeberoides*, *Gymnolaena*, and *Dyssodia* s.s. are a strongly supported clade, *Dyssodia* s.s. might be re-expanded to include these three genera. The reason why Strother (1977, 1986) segregated genera from *Dyssodia sensu lato* was that some previously infrageneric groups appear to be more closely related to other taxa of the Tageteae than to each other. The analyses of Loockerman et al. (2003), based on limited taxon sampling, supported the dismemberment of *Dyssodia*. However, if analyses with denser taxon sampling, such as those conducted here, show that at least some of the segregates are closely related to *Dyssodia*, it might be worth considering treating them in *Dyssodia* again. However, to fully evaluate phylogenetic relationships of *Dyssodia*, species of *Schizotrichia* Benth. should be sampled in future phylogenetic analyses. This Peruvian endemic

genus has been considered potentially related to *Dyssodia* and *Comaclinium* (Strother 1977) and the phylogenetic analyses of Loockerman et al. (2003) support a close relationship of this genus with *Comaclinium*, *Dyssodia*, *Gymnolaena*, and *Boeberoides*.

***Divergence times and historical biogeography*—**Our divergence time estimates suggest a Neogene origin for the taxa studied, specifically a Miocene origin with most species diversification in the Pliocene and Pleistocene. These patterns are congruent with previously estimated divergence times and species diversification for *Pectis* and *Porophyllum* (Hansen et al. 2016) and for other North American genera of the Asteraceae. Some examples are *Florestina* Cass. and relatives in Bahieae (Soto-Trejo et al., 2017), *Asanthus* R.M. King & H. Rob. and *Brickellia* Elliott in Eupatorieae (Schilling et al. 2013, 2015), and *Perityle* Benth. in Perityleae (Lichter-Marck 2021). The divergence of Flaveriinae from Tagetinae evidently took place in the Oligocene and the lineage bifurcation that gave rise to the Boeberastrum clade on the one hand, and to the rest of the Tagetinae on the other, evidently occurred in the early Miocene. The splitting of *Comaclinium* from its MRCA, shared with *Dyssodia* and *Boeberoides*, evidently took place in the mid Miocene.

The *Adenophyllum-Thymophylla* clade evidently originated in the Miocene probably from a Neotropical ancestor. Similarly, the core *Adenophyllum* clade appears to have originated in the late Miocene, with most of the species diversification occurring in the Pleistocene (Figure 14). Based on our results, the ancestor of the core *Adenophyllum* clade was probably a Neotropical lineage that dispersed towards the North American Deserts by the end of the Miocene into the Pleistocene. The *Clomenocoma* group of *Adenophyllum* evidently originated in the Pleistocene and according to our biogeographic analyses, a vicariance event separated the Neotropical species (*A*. *aurantium* and *A*. *appendiculatum*) from those of xeric areas (*A*. *speciosum* and allies). This scenario is consistent with the formation of the Trans-Mexican Volcanic Belt, which

is inferred to date from the late Miocene to Pleistocene (Ferrari et al. 2012), and limits the northernmost distribution of the species of the Neotropical region and North American deserts. The split between *A*. *glandulosum* and the *Lebetina* clade also occurred in the late Miocene in the Neotropical region, from which the ancestor of the *Lebetina* clade probably dispersed towards other biogeographic areas. It is likely that this ancestral lineage was widespread in the Neotropical region prior to isolation and differentiation of descendent lineages, as in the case of the Neotropical species of *Florestina* (Soto et al. 2017). The Neotropical species of *Adenophyllum* occur predominantly in tropical deciduous forests (Rzedowski and Calderon de Rzedowski 2013), and it has been postulated that during the Pleistocene such forest became patchy in distribution and may have functioned as climate refugia (Galvin et al. 2014), with separation of forest populations promoting speciation. This may explain why some species have a narrow distribution in the Neotropical region, such as *A. aurantium* (sensu Strother (1969)), which is confined to the tropical deciduous forests of Veracruz in the Gulf of Mexico, and *A*. *glandulosum*, which is restricted to the same type of vegetation in the Balsas Basin.

The *Boeberastrum* clade also originated in the Miocene. Our results suggest that the ancestor was widespread in North America, although this must be taken with caution since the ancestral range at this node is highly uncertain (Figure 15). Our results indicate that the split between typical *Boeberastrum* and *A*. *anomalum* occurred in the late Miocene, while the *Trichaetolepis* group of *Adenophyllum* (i.e., A. wrightii) evidently originated later in the Pleistocene from an ancestor that was likely widespread in current Northwestern Mexico and adjacent Southwestern USA. It is possible that the split of typical *Boeberastrum* (e.g., *B*. *anthemidifolium* and *B*. *littoralis*) and A. *anomalum* was due to the separation of the Peninsula of Baja California, as the former is restricted to this peninsula. However, our analyses did not

estimate such a vicariance event. Perhaps the removal of *B*. *littoralis* from the analyses contributed to the ambiguity at the node of the *Boeberastrum* clade.

According to our results, the core *Thymophylla* clade also originated near the end of the Miocene and continued its diversification into the Pleistocene. The MRCA of the core *Thymophylla* species was likely a desert-adapted lineage. Our results suggest that this ancestral lineage was widespread in the North American Deserts, where most of the speciation events took place. Then the ancestral lineage dispersed towards the Great Plains and Temperate Sierras, where additional but fewer speciation events occurred. The switch to the Great Plains habitat may have promoted speciation of the species that are currently predominant in this region, such as *T*. *tenuiloba* and *T*. *tephroleuca*. Our results suggest that the ancestrally desert-adapted lineage of *Thymophylla* was favored by the spreading of the North American deserts and origin of the scrublands, which are estimated to have arisen at Middle Miocene to early Pliocene (Graham 2011). The xeric conditions of the North American deserts and their large range of climatic, topographic, and micro-habit conditions have been considered fundamental factors in promoting plant adaptation to aridity and speciation, in part through large population fragmentation (Stebbins 1952).

The core *Thymophylla* clade is a remarkable example of a North American desert lineage that has undergone rapid speciation (Figures 14–15) and morphological adaptation to xeric conditions. *Thymophylla* is one of largest genera of the tribe Tageteae, only surpassed in species number by *Pectis*, *Porophyllum*, *Tagetes*, and *Flaveria*. Essentially, all species of *Thymophylla* occur in the arid scrublands and deserts of Northern Mexico and adjacent USA. Among the xeric-associated features exhibited by *Thymophylla* species are the small size (all spp. are 5-60 cm high), perennial, underground, woody stems (e.g. *T*. *setifolia*), reduced leaves, often stiff and fibrous (e.g. *T*. *pentachaeta*), or semi-succulent (e.g. *T*. *concinna*, *T*. *gypsophila*), dense woolly

indumentum (e.g., *Thymophylla micropoides*, *T*. *tephroleuca*), and ephemeral life cycle (e.g. *T*. *concinna*, *T*. *aurea*, *T*. *tenuifolia*) (Figures 4–7).

Finally, since our ancestral range estimation suggests that the ancestor of *T. pentachaeta* was also probably widespread in the North American desert region, a long-distance dispersal event must have occurred from there to the Argentinian deserts, where *T*. *pentachaeta* var. *belenidium* separately occurs. Based on our results, this dispersal event probably occurred during the early Pleistocene. However, all samples of *T*. *pentachaeta* var. *belenidium* used in this investigation came from North American populations and it is desirable that future studies include samples from the South American populations for more robust and accurate phylogenetic and macroevolutionary estimations.

TAXONOMIC CHANGES

The following taxonomic changes and new names are supported or proposed based on the results of the phylogenetic analyses conducted in this study (see Discussion for details).

1) *Adenophylloides* Hinojosa, *gen*. *nov*. Type species: *Dyssodia squamosa* A. Gray, Proc. Amer. Acad. Arts. 19: 38. 1884. ≡ *Adenophylloides squamosum* (A. Gray) Hinojosa.

Perennial scandent herbs, 1–5 m high. Stems green, 4–6 sided, glabrous or puberulent to sparsely pilose, fistulose, branched above, becoming terete and woody at base. Leaves opposite or the uppermost alternate, petiolate, the petioles dilated and connate at base forming a irregularly lobed disc around the node, the disc lobes linear-lanceolate to bristle-like, the largest bearing a gland-like secretory cavity, blades ternate-trifoliolate, the segments ovate to lanceolate, petiolulate, base oblique to rounded, margin serrate, with constrictions due to marginal secretory cavities, apex acute, pinnately veined, glabrous or sparsely puberulent. Capitulescence monochasial; peduncles 10 cm long or often longer and thus the capitula

appearing solitary, bracteate, dilated and fistulose below capitula; bracts spirally arranged, broadly ovate, with a short petiolar base, crowded below the involucre and simulating a calyculus but grading into the involucral bracts, pale green, often purple at the apex, scaly, each with a dark-purple secretory cavity at the abaxial surface, the margins and base often pilose, the petiolar base becoming longer. Capitula radiate, showy; involucre campanulate to cup-shaped; involucral bracts oblong-lanceolate to lanceolate-ovate, connate, distally free, with a distinct oblong base and dilated into an ovate or lanceolate scarious apex, bearing a large gland-like secretory cavity, with additional smaller secretory cavities at the margins towards the base, pale green to purple at apex, puberulent to pilose; receptacle convex, conspicuously hirsute. Ray florets 13, pistillate, fertile; ray laminas orange to orange-red, obovate to elliptic, tube greenish-white; style yolk yellow, divided into two recurved branches, each with an apical acute appendage. Disk florets 80–200, perfect, fertile; corollas narrowly funnelform, with five subulate lobes, distally red-orange to yolk yellow, proximally greenish-white; anthers orange, with acute apical appendages; style branches bearing sweeping hairs on the abaxial surface, and two papillate stigmatic lines at the margins of the adaxial surface, the apex with caudate, recurved appendages. Cypselae narrowly clavate, striate, black at maturity, appressed silky, with a distinct carpopodium at the base; pappus of short, narrow scales in two series, each scale dissected into 5–8, unequal bristles.

A monotypic genus endemic to the Pacific west coast of Mexico. Its relationships are still unclear, but based on the molecular phylogenetic analyses conducted here, it appears more closely related to either *Thymophylla* or *Dysodiopsis* than to *Adenophyllum*. However, in overall morphology, *Adenophylloides* is most similar to *A. aurantium*, which is a more shrubby species, with pinnatisect leaves with 5 or more lobes, and a more clearly defined calyculus subtending

the involucre. The ternate-trifoliolate leaves of *Adenophylloides* are unique within the *Adenophyllum-Thymophylla* clade.

2) *Adenophylloides squamosum* (A. Gray) Hinojosa, *comb*. *nov*. (Figure 4 A-C). Basionym: *Dyssodia squamosa* A. Gray, Proc. Amer. Acad. Arts. 19: 38. 1884. Lectotype: *Gregg 1061* (GH picture!) ≡ *Clomenocoma squamosa* (A. Gray) Rydb., N. Amer. Fl. 34: 165. 1915. = *Adenophyllum squamosum* (A. Gray) Strother, Sida 11: 378. 1986.

3) *Adenophyllum cancellatum* var. *yecoranum* (B.L. Turner) Hinojosa, *stat*. *nov*. Basionym: *Adenophyllum yecoranum* B.L. Turner, Phytologia 95: 18. 2013. Holotype: *Reina 98-1732* (TEX picture!). Isotype (MEXU picture!).

4) *Adenophyllum porophylloides* var. *cooperi* (A. Gray) Hinojosa, *stat*. *nov*. Basionym: *Dyssodia cooperi* A. Gray, Proc. Amer. Acad. Arts. 9: 201. 1874. Holotype: *Cooper* s.n. (GH picture!).

5) *Adenophyllum radiatum* (DC.) Hinojosa, *comb*. et *stat*. *nov*. Basionym: Dyssodia porophyllum var. *radiata* DC., Prodr. 5: 639. 1836. Holotype: Sessé & Mociño, Fl. Mex. Icon. t. 636 (G). Ilustr. (not seen, but as noted by Strother (1969), the protologue leaves no doubt about the proper use for this name).

6) *Boeberastrum anomalum* (Canby & Rose) Hinojosa, *comb*. *nov*. Basionym: *Hymenatherum anomalum* Canby & Rose. Contr. U.S. Nat. Herb. 1: 105. 1891. Holotype: *Palmer 346* (US picture!).

7) *Boeberastrum anthemidifolium* var. *littoralis* (Brandegee) Hinojosa, *comb et stat*. *nov*. Basionym: *Dyssodia littoralis* Brandegee, Zoë 5: 163. 1903. Lectotype: *Brandegee* s.n.(UC!).

8) *Boeberastrum wrightii* (A. Gray) Hinojosa, *comb*. *nov*. Basionym: *Adenophyllum wrightii* A. Gray. Pl. Wright. 2: 92. 1853. Holotype: *Wright 1240* (GH picture!).

9) *Boeberastrum pulcherrimum* (Strother) Hinojosa, *comb*. et *stat*. *nov*. Basionym: *Dyssodia neomexicana* var. *pulcherrima* Strother. Univ. Calif. Publ. Bot. 48: 43. 1969. Holotype: *Powell 588* (TEX picture!).

10) *Thymophylla strotheria* (B.L. Turner) Hinojosa, *comb*. *nov*. Basionym: *Strotheria gypsophila* B.L. Turner, Amer. J. Bot. 59: 180. 1972. Holotype: *Turner 6214* (TEX picture!). Isotype: DAV! A new specific epithet was needed since gypsophila was already occupied by *T*. *gypsophila* B.L. Turner. The selected specific epithet honors Dr. John L. Strother, a remarkable synantherologist and Tageteae scholar, for whom *Strotheria* was named.

11) *Thymophyllastrum* Hinojosa, *gen*. *nov*. Type species: *Hymenatherum aurantiacum* Brandegee, Zoe 5: 258. 1908. ≡ *Thymophyllastrum aurantiacum* (Brandegee) Hinojosa.

Perennial herbs, 5–30 cm high. Stems woody at base, with ascending, spreading branches. Leaves opposite, the uppermost alternate, sessile or with a petiolar base, pinnatisect, with 3–7 lobes at each side, the uppermost leaves reduced and often simple, lobes linear, glabrous, terete and semi-succulent when fresh, with numerous dot-like or ovate, gland-like, translucent secretory cavities. Capitulescence monochasial, the capitula at the tips of leafy branches and appearing solitary; peduncles conspicuous, 2–16 cm long, bracteate. Capitula radiate, involucre turbinate to campanulate, biseriate; involucral bracts subequal, connate, lanceolate to elliptic-lanceolate, green to dark purple, provided with gland-like secretory cavities on the abaxial surface, margins and apex scarious; receptacle conic, naked. Ray florets 8, pistillate and fertile; ray lamina yellow, sometimes white with a yellow base, oval-orbicular to oblong-oblanceolate, truncate-erose to emarginate at the apex; style yellow, terete, divided into two branches. Disk florets 28–60; corollas yellow, funnelform, with five apical lobes; anthers brownish, with apical, lanceolate, yellow appendages; style branches recurved, with short, conic apical appendages.

Cypselae narrowly clavate, black at maturity, sparsely puberulent with straight, ascending hairs; pappus of narrow scales, each dissected into 3–5 bristles.

A monotypic genus endemic to the Tehuacan-Cuicatlan Biosphere Reserve in south-central Mexico. Its phylogenetic relationships are still unclear within the *Adenophyllum-Thymophylla* clade, although the molecular phylogenetic analyses conducted here suggest that the genus is phylogenetically closest to *Adenophyllum*. In morphology, it seems more similar to the perennial and annual species of *Thymophylla* that have pinnatisect leaves and linear lobes. These species, however, have sparser secretory cavities in the leaves, sometimes a well-developed calyculus, and, especially, a flat receptacle. Based on morphology only, the affinities of Thymophyllastrum with other species of *Thymophylla* were also unclear (Strother 1969).

12) *Thymophyllastrum aurantiacum* (Brandegee) Hinojosa comb. nov. (Figure 4 H-I). Basionym: *Hymenatherum aurantiacum* Brandegee, Zoe 5: 258. 1908. Holotype: *Purpus 2532* (UC!) ≡ *Dyssodia aurantiaca* (Brandegee) B.L. Rob. Proc. Amer. Acad. Arts. 49: 507. 1913. ≡ *Thymophylla aurantiaca* (Brandegee) Rydb. N. Amer. Fl. 34: 175. 1915.

CONCLUSIONS

The comprehensive phylogenetic analyses conducted here support narrowing the circumscription of *Adenophyllum* to comprise only the species within the core *Adenophyllum* clade. The species of the *Trichaetolepis* group are more closely related to *Boeberastrum* and therefore they are transferred to this genus. *Adenophyllum squamosum* is not a member of the core *Adenophyllum* clade and is segregated as the new monotypic genus *Adenophylloides*. *Adenophyllum porophyllum* var. *radiatum* is raised to the specific level, as *A*. *radiatum*, while *A*. cooperi and A. yecoranum are treated at the varietal level under *A*. *porophylloides* and *A*. *cancellatum*, respectively. As a result, *Adenophyllum* now comprises seven species and six

varieties (Appendix 2), while *Boeberastrum* is expanded to comprise four species and two varieties, and the genus is no longer endemic to Baja California.

Thymophylla aurantiaca is segregated as the monotypic genus *Thymophyllastrum*, and *Strotheria gypsophila*, which is clearly nested within *Thymophylla*, is transferred to this genus. Our phylogenetic analyses support the recognition of the two varieties of *T. setifolia* as distinct species, which are not closely related. Based our results we also propose to treat the varieties puberula and belenidium of *T. pentachaeta* as distinct species. *Thymophylla pentachaeta* var. *hartwegii* is recognized as part of *T. belenidium*, keeping its rank of variety. Similarly, *T. gentryi* is considered a synonym of *T. aurea*, which is proposed to be treated without varieties. We propose to keep treating *T. mutica* as a distinct species and to recognize only the varieties *tenuiloba* and *wrightii* of *T. tenuiloba* until additional studies are conducted probably most productively focused at the interspecific level, that might support a different taxonomic treatment. With all of these changes, *Thymophylla* is composed of 15 species and four varieties (Appendix 2).

Additional phylogenetic analyses are also needed to clarify the sister groups of *Dysodiopsis* and the new proposed genera, *Adenophylloides* and *Thymophyllastrum*. It is clear that these taxa belong to the *Adenophyllum-Thymophylla* clade, but their relationships within the clade are equivocal. The use of additional molecular markers such as those derived from phylogenomics may help elucidate the relationships of these genera.

Based on divergence time analyses conducted here most clades were estimated to have originated during the Miocene, with species diversification occurring relatively recently, mainly during the Pleistocene. The ancestral ecological region for *Adenophyllum* and *Thymophylla* is probably the Neotropical region and the North American deserts, respectively. Dispersal events have contributed predominantly to the current distribution of *Adenophyllum* and *Thymophylla*

species. Finally, this investigation represents an important contribution to the systematic understanding of the tribe Tageteae, by focusing on one of the main understudied branches of the tribe to perform comprehensive phylogenetic analyses that allow us to better understand the evolution and phylogeny of the Adenophyllum and Thymophylla clades.

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Appendix 1. Taxa, terminals, and voucher information for plant material used in this study (ITS, ETS, *trn*L-F, *ndh*I-*ndh*G spacer and ndhI gene, *psbA*-*trnH*; — not available; *used in divergence time analyses; **used in historical biogeography analyses). See Taxonomic Changes for updated taxonomy based on results of this study.

Adenophyllum anomalum (Canby & Rose) Strother, Adenophyllum_anomalum1, *Benitez 2577*** (MEXU), Adenophyllum_anomalum2, *Gonzalez 6563* (CIIDIR), Adenophyllum_anomalum3, *Hinojosa-Espinosa 727* (DAV, MEXU, USON), Adenophyllum_anomalum4, *Reina 186* (DAV), Adenophyllum_anomalum5, *Webster 24380* (DAV). ***Adenophyllum appendiculatum*** (Lag.) Strother, Adenophyllum_appendiculatum1, *Hinojosa-Espinosa 729*** (DAV, MEXU, ECOSUR), Adenophyllum_appendiculatum2, *Santiago 121* (MEXU), Adenophyllum_appendiculatum3, *Soto 8161* (MEXU), Adenophyllum_appendiculatum4, *Soto 15609* (MEXU), Adenophyllum_appendiculatum5, *Soto 18671* (MEXU), Adenophyllum_appendiculatum6, *Velasco 702* (MEXU), Adenophyllum_appendiculatum7, *Velasco 3132* (MEXU). ***Adenophyllum aurantium*** (L.) Strother, Adenophyllum_aurantium1, *Hinojosa-Espinosa 761*** (DAV, MEXU), Adenophyllum_aurantium2, *Ventura 18313* (MEXU). ***Adenophyllum cooperi*** (A. Gray) Strother, Adenophyllum_cooperi1, *Andre 22192*** (MEXU), Adenophyllum_cooperi2, *Andre 36973* (RENO), Adenophyllum_cooperi3, *Everett 23209* (DAV), Adenophyllum_cooperi4, *Lehto 22974* (SD). ***Adenophyllum glandulosum*** (Cav.) Strother, Adenophyllum_glandulosum1, *Espin 666*** (MEXU), Adenophyllum_glandulosum2, *Hinojosa-Espinosa 630* (MEXU), Adenophyllum_glandulosum3, *Martinez 24035* (MEXU), Adenophyllum_glandulosum4, *Mota 99* (MEXU), Adenophyllum_glandulosum5, *Reyes 814* (MEXU). ***Adenophyllum porophylloides*** (A. Gray) Strother, Adenophyllum_porophylloides1, *Everett 23032* (DAV),

Adenophyllum_porophylloides2, *Gust 2421*** (RENO), Adenophyllum_porophylloides3, *Leon 3267* (MEXU), Adenophyllum_porophylloides4, *McNair s.n.* (DAV), Adenophyllum_porophylloides5, *Medel 1028* (HCIB). ***Adenophyllum porophyllum* var. cancellatum** (Cass.) Strother, Adenophyllum_p_cancellatum1, *Breckon 1212* (DAV), Adenophyllum_p_cancellatum2 *Hernandez 11356* (MEXU), Adenophyllum_p_cancellatum3, *Hinojosa-Espinosa 706*** (CIIDIR, DAV, MEXU), Adenophyllum_p_cancellatum4, *Hinojosa-Espinosa 717* (DAV, MEXU), Adenophyllum_p_cancellatum5, *Hinojosa-Espinosa 719* (DAV, MEXU), Adenophyllum_p_cancellatum6, *Hinojosa-Espinosa 742* (DAV, MEXU, UAT). Adenophyllum_p_cancellatum7, *Kishler 813* (MEXU), Adenophyllum_p_cancellatum8, *Retana 52* (CIIDIR), Adenophyllum_p_cancellatum9, *Villaseñor 2218* (MEXU). *Adenophyllum porophyllum* (Cav.) Hemsl. **var**. ***porophyllum***, Adenophyllum_p_porophyllum1, *Cornejo 5014*** (MEXU), Adenophyllum_p_porophyllum2, *Hinojosa-Espinosa 668* (DAV, MEXU), Adenophyllum_p_porophyllum3, *Hinojosa-Espinosa 716* (DAV, MEXU), Adenophyllum_p_porophyllum4, *McNair 2302* (ARIZ), Adenophyllum_p_porophyllum5, *Onofre 3900* (MEXU), Adenophyllum_p_porophyllum6, *Villasenor 1883* (MEXU), Adenophyllum_p_porophyllum7. *Adenophyllum porophyllum* **var**. ***radiatum*** (DC.) Strother, Adenophyllum_p_radiatum1, *Linares 3880* (MEXU), Adenophyllum_p_radiatum2, *Lopez 1449* (MEXU), Adenophyllum_p_radiatum3, *Martinez 1432*** (MEXU), Adenophyllum_p_radiatum4, *Ventura 9309* (MEXU). ***Adenophyllum speciosum*** (A. Gray) Strother, Adenophyllum_speciosum1, *Calzada 25258*** (MEXU), Adenophyllum_speciosum2, *Campos 4499* (CIIDIR), Adenophyllum_speciosum3, *Hinojosa-Espinosa 772* (DAV, MEXU). ***Adenophyllum squamosum*** (A. Gray) Strother, Adenophyllum_squamosum1, *Carrillo 2906*** (IBUG), Adenophyllum_squamosum2, *McVaugh 26241* (MEXU), Adenophyllum_squamosum3, *Sanders 10677* (MEXU). ***Adenophyllum wrightii* var. *pulcherrimum*** (Strother) Strother,

Adenophyllum_w_pulcherrimum1, *Hinojosa-Espinosa 722*** (DAV, MEXU), Adenophyllum_w_pulcherrimum2, *Soule 2471* (ARIZ), Adenophyllum_w_pulcherrimum3, *Ramírez 106* (IBUG). *Adenophyllum wrightii* A. Gray **var. *wrightii***, Adenophyllum_w_wrightii1, *Spellenberg 13847*** (NMC), Adenophyllum_w_wrightii2 *Spellenberg 13848* (NMC), Adenophyllum_w_wrightii3, *Stavast s.n.* (NMC), Adenophyllum_w_wrightii4, *Tonne 99-183* (NMC). ***Adenophyllum yecoranum*** B.L. Turner, Adenophyllum_yecoranum1, *Reina 96-558*** (ARIZ), Adenophyllum_yecoranum2, *Tenorio 4565* (MEXU). ***Boeberastrum*** *anthemidifolium* (Benth.) Rydb., Boeberastrum_anthemidifolium1, *Domínguez 4830*** (HCIB), Boeberastrum_anthemidifolium2, *Nixon 928* (MEXU). *Boeberastrum littoralis* (Brandegee) Rydb., Boeberastrum_littoralis, *León 12270* (HCIB). ***Boeberoides*** *grandiflora* (DC.) Strother, Boeberoides_grandiflora1, *Benz 719*** (MEXU), Boeberoides_grandiflora2, *Hinojosa-Espinosa 72* (MEXU). ***Comaclinium*** *montanum* (Benth.) Strother, Comaclinium_montanum1, *Hinojosa-Espinosa 757*** (CH, MEXU), Comaclinium_montanum2, *Sinaca 2264* (MEXU). ***Dysodiopsis*** tagetoides (Torr. & A. Gray) Rydb., Dysodiopsis_tagetoides1, *Gostel 447*** (BRIT), Dysodiopsis_tagetoides2, *Gostel 493* (BRIT), Dysodiopsis_tagetoides3, *Webster 33291* (DAV). ***Dyssodia*** *decipiens* (Bartl.) M.C. Johnst., Dyssodia_decipiens1, *Hinojosa-Espinosa 728* (CH, DAV, MEXU), Dyssodia_decipiens2, *Lopez 1575* (MEXU), Dyssodia_decipiens3, *Suárez 270* (MEXU). Dyssodia papposa (Vent.) Hitchc., Dyssodia_papposa1, *Céspedes 487* (MEXU), Dyssodia_papposa2, *Garcia 7949** (MEXU), Dyssodia_papposa3, *Hinojosa-Espinosa 684* (DAV, MEXU), Dyssodia_papposa4, *Hinojosa-Espinosa 700* (CIIDIR), Dyssodia_papposa5, *Hinojosa-Espinosa 702* (CIIDIR). *Dyssodia pinnata* (Cav.) B.L. Rob. var. *pinnata*, Dyssodia_pinnatapin1, *Castaneda 853* (MEXU), Dyssodia_pinnatapin2, *Villaseñor 2169* (MEXU). *Dyssodia pinnata* var. *glabrescens* Strother, Dyssodia_pinnatagla1, *Hinojosa-Espinosa 749* (DAV, MEXU), Dyssodia_pinnatagla2, *Yahara 1438* (MEXU). *Dyssodia tagetiflora* Lag., Dyssodia_tagetiflora1,

Hinojosa-Espinosa 287 (MEXU), Dyssodia_tagetiflora2, *Hinojosa-Espinosa 674* (DAV, MEXU), Dyssodia_tagetiflora3, *Hinojosa-Espinosa 678* (DAV, MEXU), Dyssodia_tagetiflora4, Rubio 677 (MEXU). ***Flaveria*** *anomala* B.L. Rob., Flaveria_anomala, *Hinojosa-Espinosa 753** (DAV, MEXU). ***Gymnolaena*** *serratifolia* (DC.) Rydb., Gymnolaena_serratifolia, *Messer 172* (MEXU). ***Helianthus*** *annuus* L., [KX671853*; HQ688886*; AY216183,* AY216058*; AF383671,* AF383796*; AY215554*]. ***Strotheria*** *gypsophila* B.L. Turner, Strotheria_gypsophila1, *Hinojosa-Espinosa 752*** (DAV, MEXU), Strotheria_gypsophila2, *Moore 1286* (MEXU). ***Tagetes*** *lunulata* Ortega, Tagetes_lunulata, *Hinojosa-Espinosa 677* (DAV). ***Thymophylla*** *acerosa* (DC.) Strother, Thymophylla_acerosa1, *Hinojosa-Espinosa 672*** (DAV), Thymophylla_acerosa2, *Hinojosa-Espinosa 704* (CIIDIR, DAV, MEXU), Thymophylla_acerosa3, Levin 816 (DAV), Thymophylla_acerosa4, *Tiehm 14700* (RENO), Thymophylla_acerosa5, *Villaseñor 2198* (MEXU), Thymophylla_acerosa6 *Webster 34388* (DAV), Thymophylla_acerosa7, *Yahara 1724* (MEXU). ***Thymophylla aurantiaca*** (Brandegee) Rydb., Thymophylla_aurantiaca1, *Hinojosa-Espinosa 690*** (DAV, MEXU), Thymophylla_aurantiaca2, *Panero 2603* (MEXU), Thymophylla_aurantiaca3, *Tenorio 17663* (MEXU), Thymophylla_aurantiaca4, *Tenorio 21185* (MEXU). ***Thymophylla aurea*** (A. Gray) Greene, **var**. ***aurea***, Thymophylla_a_aurea1, *Pealand 4679* (COCO), Thymophylla_a_aurea2, *Spellenberg 14979*** (NMC), Thymophylla_a_aurea3, *Weber 17527* (TEX). *Thymophylla aurea* **var**. ***polychaeta*** (A. Gray) Strother, Thymophylla_a_polychaeta1, *Chiang 8816* (MEXU), Thymophylla_a_polychaeta2, *Spellenberg 14185*** (NMC), Thymophylla_a_polychaeta3, *Villarreal 8228* (MEXU), Thymophylla_a_polychaeta4, *Villarreal 17735* (MEXU). ***Thymophylla concinna*** (A. Gray) Strother, Thymophylla_concinna1, *Reina 96-06*** (MEXU), Thymophylla_concinna2, *Webster 23801* (DAV). ***Thymophylla gentryi*** (M.C. Johnst.) Strother, Thymophylla_gentryi1, *Cronquist 10773* (MEXU), Thymophylla_gentryi2, *Hinojosa-Espinosa 705*** (CIIDIR, DAV, MEXU), Thymophylla_gentryi3, *Strother 577* (MEXU).

Thymophylla gypsophila (B.L. Turner) Strother, Thymophylla_gypsophila1, *Chiang 9512*** (MEXU), Thymophylla_gypsophila2, *Henrickson 2260* (MEXU), Thymophylla_gypsophila3, *Hinojosa-Espinosa 711* (CIIDIR, DAV, MEXU). ***Thymophylla micropoides*** (DC.) Strother, Thymophylla_micropoides1, *Helkamp 65-4*** (DAV), Thymophylla_micropoides2, *Hinton 24265* (MEXU), Thymophylla_micropoides3, *Yahara 1462* (MEXU). ***Thymophylla mutica*** (M.C. Johnst.) Strother, Thymophylla_mutica1, *Hinojosa-Espinosa 739*** (DAV, MEXU, UAT), Thymophylla mutica2, *Strother 540* (MEXU). ***Thymophylla pentachaeta*** **var**. ***belenidium*** (DC.) Strother, Thymophylla_p_belenidium1, *Atwood 25001* (DAV), Thymophylla_p_belenidium2, *Breedlove 60791* (MEXU), Thymophylla_p_belenidium3, *Hinojosa-Espinosa 671*** (DAV), Thymophylla_p_belenidium4, *Hinojosa-Espinosa 713* (CIIDIR, DAV, MEXU), Thymophylla_p_belenidium5, *Pitzer 3926* (DAV). *Thymophylla pentachaeta* **var**. ***hartwegii*** (A. Gray) Strother, Thymophylla_p_hartwegii1, *Balleza 5842* (MEXU), Thymophylla_p_hartwegii2, *Hinojosa-Espinosa 703*** (CIIDIR, DAV, MEXU), Thymophylla_hartwegii3, *Salas 650* (MEXU). *Thymophylla pentachaeta* (DC.) Small ***var***. ***pentachaeta***, Thymophylla_p_pentachaeta1, *Hinojosa-Espinosa 670* (DAV), Thymophylla_p_pentachaeta2, *Hinojosa-Espinosa 737*** (DAV, MEXU, UAT), Thymophylla_p_pentachaeta3, *Webster 33601* (DAV). *Thymophylla pentachaeta* **var**. ***puberula*** (Rydb.) Strother, Thymophylla_p_puberula1, *Garcia 474*** (MEXU), Thymophylla_p_puberula2, *Hinojosa-Espinosa 754* (DAV, MEXU), Thymophylla_p_puberula3, *Hinton 18455* (LL/TEX), Thymophylla_p_puberula4, *Hinton 27077* (LL/TEX), Thymophylla_p_puberula5, *Luckow 2683* (MEXU), Thymophylla_p_puberula6, *Yahara 1466* (MEXU). ***Thymophylla setifolia*** **var**. ***greggii*** (A. Gray) Strother, Thymophylla_s_greggii1, *Carranza 713* (MEXU), Thymophylla_s_greggii2, *Webster 34416*** (DAV), Thymophylla_s_greggii3, *Yahara 1745* (MEXU). *Thymophylla setifolia* Lag. **var**. ***setifolia***, Thymophylla_s_setifolia_d1, *Castaneda 809*** (MEXU), Thymophylla_s_setifolia_d2, *Garcia*

7738 (MEXU), Thymophylla_s_setifolia_r1, *Hinojosa-Espinosa 740*** (DAV, MEXU), Thymophylla_s_setifolia_d3, *Hinojosa-Espinosa 747* (DAV, MEXU), Thymophylla_s_setifolia_r2, *Villaseñor 498* (MEXU), Thymophylla_s_setifolia_d4, *Villaseñor 1289* (MEXU). ***Thymophylla tenuifolia*** (Cass.) Rydb., Thymophylla_tenuifolia1, *Balleza 8988*** (MEXU), Thymophylla_tenuifolia2, *Balleza 11221* (MEXU), Thymophylla_tenuifolia3, *Hinojosa-Espinosa 701* (CIIDIR, DAV, MEXU), Thymophylla_tenuifolia4, *Hinojosa-Espinosa 751* (CIIDIR, DAV, MEXU), Thymophylla_tenuifolia5, *Yahara 1535* (MEXU). ***Thymophylla tenuiloba*** (DC.) Small **var**. ***tenuiloba***, Thymophylla_t_tenuiloba1, *Martinez 177*** (MEXU), Thymophylla_t_tenuiloba2, *Martinez 940* (MEXU), Thymophylla_t_tenuiloba3, *Ramos 47* (DAV). *Thymophylla tenuiloba* **var**. ***texana*** (Cory) Strother, Thymophylla_t_texana1, Henderson 63-914** (TEX), Thymophylla_t_texana2, Strother 164 (TEX). *Thymophylla tenuiloba* **var**. ***treculii*** (A. Gray) Strother, Thymophylla_t_treculii1, *Graham 1959* (TEX), Thymophylla_t_treculii2, *Webster 11171*** (DAV). Thymophylla tenuiloba **var**. ***wrightii*** (A. Gray) Strother, Thymophylla_t_wrightii1, *Carr 22854* (MEXU), Thymophylla_t_wrightii2, *Carr 11793*** (TEX). ***Thymophylla tephroleuca*** (S.F. Blake) Strother, Thymophylla_tephroleuca1, *Atha 337* (TEX), Thymophylla tephroleuca2, *Miller* s.n. (MEXU), Thymophylla tephroleuca3, *Turner 80-65M* (TEX). ***Varilla*** *mexicana* A. Gray, var. *mexicana*, Varilla_mexicanamex3, *Turner 15049* (DAV).

Appendix 2. List of accepted taxa in the *Adenophyllum-Thymophylla* and *Boeberastrum* clades based on results of this study. *Not effectively published yet.

***Adenophyllum-Thymophylla* clade:**

Adenophylloides Hinojosa*

1. *Adenophylloides squamosum* (A. Gray) Hinojosa*

Adenophyllum Pers. nom. cons.

1. *Adenophyllum aurantium* (L.) Strother

    A. aurantium var. *aurantium*

    A. aurantium var. *appendiculatum* (Lag.) Hinojosa*

2. *Adenophyllum cancellatum* (Cass.) Villarreal

    A. cancellatum var. *cancellatum*

    A. cancellatum var. *yecoranum* (B.L. Turner) Hinojosa*

3. *Adenophyllum glandulosum* (Cav.) Strother

4. *Adenophyllum porophylloides* (A. Gray) Strother

    A. porophylloides var. *porophylloides*

    A. porophylloides var. *cooperi* (A. Gray) Hinojosa*

5. *Adenophyllum porophyllum* (Cav.) Hemsl.

6. *Adenophyllum radiatum* (DC.) Hinojosa*

7. *Adenophyllum speciosum* (A. Gray) Strother

Thymophyllastrum Hinojosa*

1. *Thymophyllastrum aurantiacum* (Brandegee) Hinojosa*

Appendix 2. Continuation.

Thymophylla Lag.

1. *Thymophylla acerosa* (DC.) Strother
2. *Thymophylla aurea* (A. Gray) Greene (including *T*. *aurea* var. *polychaeta* and *T*. *gentryi*)
3. *Thymophylla belenidium* (DC.) Cabrera

    T. *belenidium* var. *belenidium*

    T. *belenidium* var. *hartwegii* (A. Gray) Hinojosa*
4. *Thymophylla concinna* (A. Gray) Strother
5. *Thymophylla greggii* A. Gray
6. *Thymophylla gypsophila* (B.L. Turner) Strother
7. *Thymophylla micropoides* (DC.) Strother
8. *Thymophylla mutica* (M.C. Johnst.) Strother
9. *Thymophylla pentachaeta* (DC.) Small
10. *Thymophylla puberula* Rydb.
11. *Thymophylla setifolia* Lag.
12. *Thymophylla strotheria* (B.L. Turner) Hinojosa*
13. *Thymophylla tenuifolia* (Cass.) Rydb.
14. *Thymophylla tenuiloba* (DC.) Small

    T. *tenuiloba* var. *tenuiloba* (including varieties *texana* and *treculii*)

    T. *tenuiloba* var. *wrightii* (A. Gray) Strother
15. *Thymophylla tephroleuca* (S.F. Blake) Strother

Appendix 2. Continuation.

***Boeberastrum* clade:**

Boeberastrum (A. Gray) Rydb.

1. *Boeberastrum anomalum* (Canby & Rose) Hinojosa*

2. *Boeberastrum anthemidifolium* (Benth.) Rydb.

    B. *anthemidifolium* var. *anthemidifolium*

    B. *anthemidifolium* var. *littoralis* (Brandegee) Hinojosa*

3. *Boeberastrum pulcherrimum* (Strother) Hinojosa*

4. *Boeberastrum wrightii* (A. Gray) Hinojosa*

Table 1. Sets of primers used to amplify and sequence the nuclear and plastid DNA markers employed in this study. For simplicity, shorter names (*trn*L-F, *ndh*I-*ndh*G, and *psbA*-*trnH*), are used when referring to the plastid markers.

Molecular marker	Primers	Sequence 5'–3'	Reference
ITS	Forward (ITS5) Reverse (ITS4)	GGAAGTAAAAGTCGTAACAAGG TCCTCCGCTTATTGATATGC	White et al. (1990)
ETS	Forward (Ast-1 modified) Reverse (18S Alt) Forward (Ast-1) Reverse (18S-ETS)	CGTAAAGGTGTGTGAGTGGTTT TGAGCCATTCGCAGTTTCACAGTC CGTAAAGGTGCATGAGTGGTG ACTTACACATGCATGGCTTAATCT	Rivera et al. (2016) Markos and Baldwin (2001) Baldwin and Markos (1998)
*trn*L intron and *trn*L-*trn*F intergenic spacer	Forward (trnC) Forward (trnC-Aster) Reverse (trnF)	ATTTGAACTGGTGACACGAG CGAAATTGGTAGACGCTACG ATTTGAACTGGTGACACGAG	Taberlet et al. (1991) Panero and Crozier (2003)
*ndh*I and *ndh*I-*ndh*G intergenic spacer	Forward (ndhGF) Reverse (ndhAexon2R)	CCGACCCTAGAAAGACTAAAAG CGTCCCAACTTCTTTCACTG	Panero and Crozier (2003)
psbA-*trnH* intergenic spacer	Forward (psbAF) Reverse (trnHR)	GTTATGCATGAACGTAATGCTC CGCGCATGGTGGATTCACAAATC	Sang et al. (1997)

FIGURES

Fig. 1. *Adenophyllum*, *Lebetina* group. A-C, A. *porophyllum* var. *porophyllum* (*A*. *porophyllum sensu* Turner 1996). Note herbaceous habit and discoid capitula. D-G: *A. porophyllum* var. *cancellatum* (*A*. *cancellatum sensu* Turner 1996). Note conspicuous rays, pellucid secretory cavities in the leaves, and pappus of outer scales and inner scales extending into bristles. H: *A*. *porophyllum* var. *radiatum* (*A*. *porophyllum* sensu Turner 1996). Note smaller rays. A-G by OHE, H by O.M. Montiel 2005 (http://www.tropicos.org/Image/74329).

Fig 2. *Adenophyllum*, *Trichaetolepis* group. A-C: *A*. *wrightii* var. *wrightii*. Note smaller rays. D-F: *A*. *wrightii* var. *pulcherrimum* (*A*. *pulcherrimum* sensu Turner 1996). Note conspicuous rays, purplish disk corolla with purplish lobes, and involucre subtended by few calycular bracts. G-I: A. anomalum. Note secretory cavities in the involucre, essentially without calycular bracts, and pinnatisect leaves with linear segments. A-C, by Russ Kleimann 2008 (https://www.wnmu.edu/academic/nspages/gilaflora/adenophyllum_wrightii.html), D-I by OHE.

Fig 3. *Adenophyllum*, type species and *Clomenocoma* group. A: *A*. *glandulosum*, note eight rays. B-D: *A*. *appendiculatum* (*A*. *aurantium sensu* Turner 1996), from tropical deciduous forest in Chiapas. E-H: *A*. *aurantium*, from tropical deciduous forest in Veracruz, note woody habit and purplish secretory cavities. I-K. *A*. *speciosum*, note secretory cavities and calyculus. L-M. *A*. *cooperi*, note calyculus and coarsely toothed simple leaves. N-O: *A*. *porophylloides*, note inconspicuous rays and pinnatisect leaves. A-K by OHE, L, M, and N-O by © 2005 James M. Andre, © 2017 John Doyen, and © 2014 Keir Morse, respectively (https://calphotos.berkeley.edu/).

Fig. 4. *Adenophyllum*, *Clomenocoma* group and *Thymophylla* (*Gnaphalopsis* (D-G), *Aurantiacae* (H-I), and *Aciphyllaea* (J-O) groups). A-C: *A*. *squamosum*. Note trifoliolate leaf, and capitulum subtended by numerous bracts with dark purplish secretory cavities. D-E: *T*. *micropoides*, note woolly indumentum. F-G: *T*. *tephroleuca*, note yellowish secretory cavities in bracts and leaves. H-I: *T*. *aurantiaca*, a rare white-rayed form (the yellow-rayed forms are more common). J-L: *T*. *acerosa*, note shrubby habit and needle-shape leaves. M-O: *T*. *gypsophila*, in gypsum soil. A-C and H-M by OHE, D-E by ©2017 Wynn Anderson (https://calphotos.berkeley.edu), F-G by © Joey Santore (https://www.inaturalist.org/taxa/169750-Thymophylla-tephroleuca).

Fig. 5. Core *Thymophylla* and the *T*. *pentachaeta* complex. A-F: *T*. *setifolia* var. *setifolia*, radiate and discoid forms. G-H: *T*. *setifolia* var. *greggii* (T. greggii sensu Turner 1996), note less hairy peduncles and involucre. I-K: *T*. *pentachaeta* var. *pentachaeta* (sensu Strother 1969), note calyculus. L-N: *T*. *pentachaeta* var. *belenidium* (sensu Strother 1969), note narrower capitula. O-P: *T*. *pentachaeta* var. *hartwegii* (sensu Strother 1969), note smaller and thiner capitula. A-F and I-P by OHE, G-H by Patrick Alexander 2010 (https://swbiodiversity.org/seinet/taxa/index.php?taxon=163599).

Fig. 6. *Thymophylla tenuiloba* complex (A-F) and *Hymenatherum* group. A-C: *Thymophylla tenuiloba* var. *tenuiloba*, note calyculus. D-F: T. mutica, note secretory cavities in the involucre and absence of calyculus. G-I: *Thymophylla aurea* var. *aurea* (sensu Strother 1969), note absence of calyculus. A-C and G-H by © 2015, 2014 Richard Spellenberg (https://calphotos.berkeley.edu), D-F by OHE.

Fig. 7. *Thymophylla*, *Hymenatherum* group. A-C. *T*. *gentryi*, near type locality. D-F. *T*. *tenuifolia*, note strongly connate involucre with gland-like secretory cavities. G-I: *T*. *concinna*, the only species of *Thymophylla* with only white rays. A-F by OHE, G-I by Sue Carnahan 2019 (https://swbiodiversity.org/seinet/taxa/index.php?taxon=374).

Fig. 8. Majority rule consensus tree from the Bayesian analyses of the concatenated nrDNA (ITS and ETS), focusing on *Adenophyllum*. Deep blue nodes with pp ≥ 0.95.

Fig. 9. Majority rule consensus tree from the Bayesian analyses of the concatenated nrDNA (ITS and ETS), focusing on the core *Thymophylla* clade. Deep blue nodes with pp ≥ 0.95.

Fig. 10. Majority rule consensus tree from the Bayesian analyses of the concatenated cpDNA data set (*trn*L-F, *ndh*I-*ndh*G, and *psbA*-*trnH* intergenic spacers), focusing on *Adenophyllum*. Deep blue nodes with pp ≥ 0.95. Some pp values annotated next to the nodes for clarification.

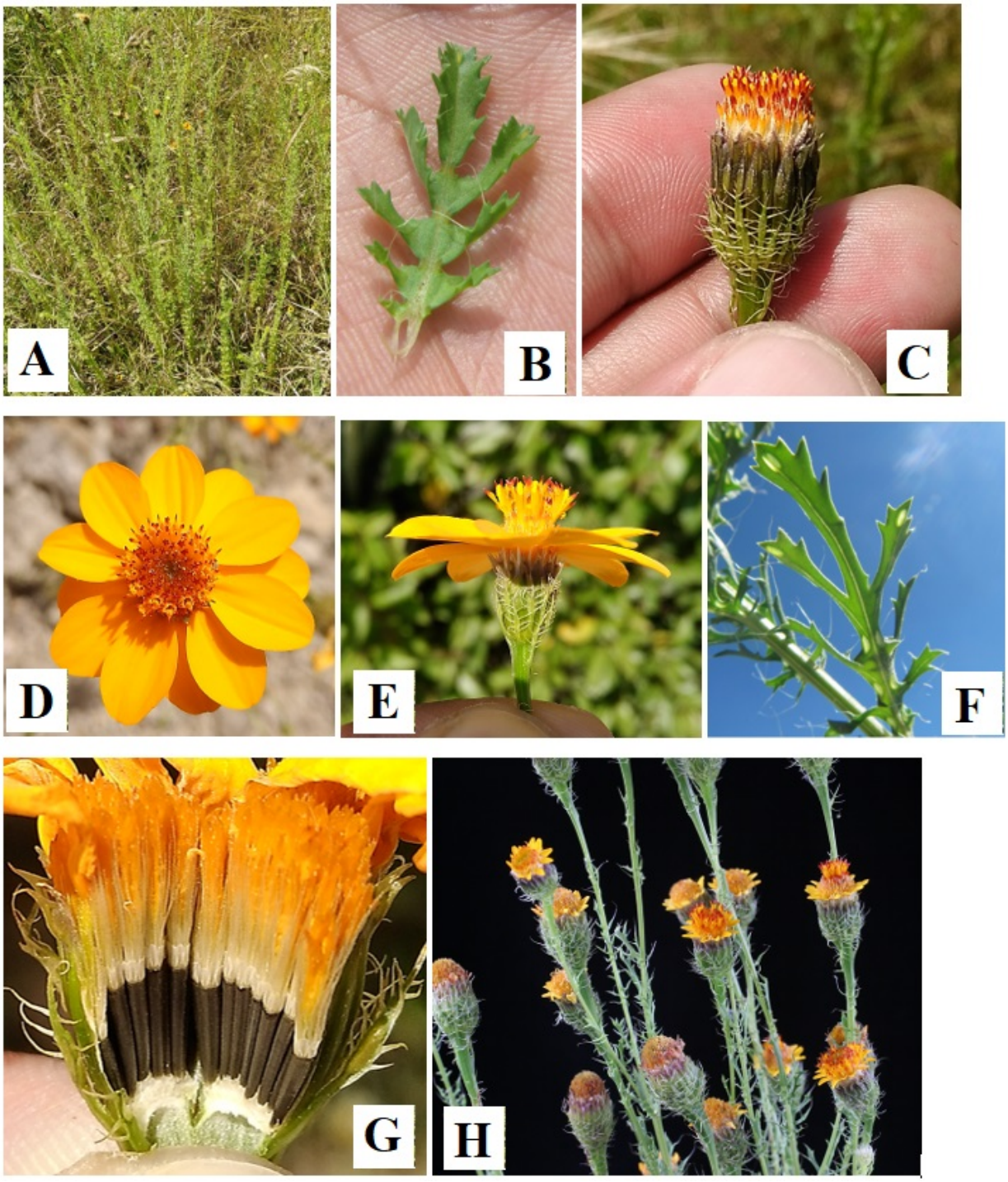
Fig. 11. Majority rule consensus tree from the Bayesian analyses of the concatenated cpDNA (*trn*L-F, *ndh*I-*ndh*G, and *psbA*-*trnH* intergenic spacers), focusing on the core *Thymophylla* clade. Deep blue nodes with pp ≥ 0.95. A pp value annotated next to the *T*. *gentryi* node for clarification.

Fig. 12. Majority rule consensus tree from the Bayesian analyses of the concatenated nrDNA (ITS, ETS) and cpDNA (*trn*L-F, *ndh*I-*ndh*G, and *psbA*-*trnH* intergenic spacers), focusing on *Adenophyllum*. Deep blue nodes with PP ≥ 0.95.

Fig. 13. Majority rule consensus tree from the Bayesian analyses of the concatenated nrDNA (ITS, ETS) and cpDNA (*ndh*I-*ndh*G, *psbA*-*trnH*, and *trn*L-F intergenic spacers), focusing on the core *Thymophylla* clade. Note *Strotheria* nested within *Thymophylla*. Deep blue nodes with PP ≥ 0.95. Some PP values annotated next to the nodes for clarification.

Fig. 14. Time-calibrated MCC tree from two independent runs, each 100 million MCMC generations from the Bayesian analyses of nrDNA (ETS and ITS) and cpDNA (*trn*L-F, *ndh*I-*ndh*G, *psbA*-*trnH*) sequences conducted in BEAST 2.6.6. All nodes with PP 0.95–1.0, except when indicated. Bars at nodes show the 95% credible intervals. Time-scale units in millions of years. Note *Strotheria* nested within the core *Thymophylla* clade, *T*. *aurantiaca* as sister to the core *Adenophyllum* clade and *A*. *squamosum* as sister to *Dysodiopsis* and core *Thymophylla*.

Fig. 15. Ancestral range estimation for the *Adenophyllum*-*Thymophylla* clade under the S-DEC model and depicted in the calibrated tree from the divergence time analyses (all nodes with 0.95 PP or higher unless indicated). Pie charts at each node show the relative probability of ancestral states. For clarification, only up to three areas with probability above 0.5 are shown. Legend at the right indicate combined ranges. White wedges represent uncertainty (several tiny ie slices).



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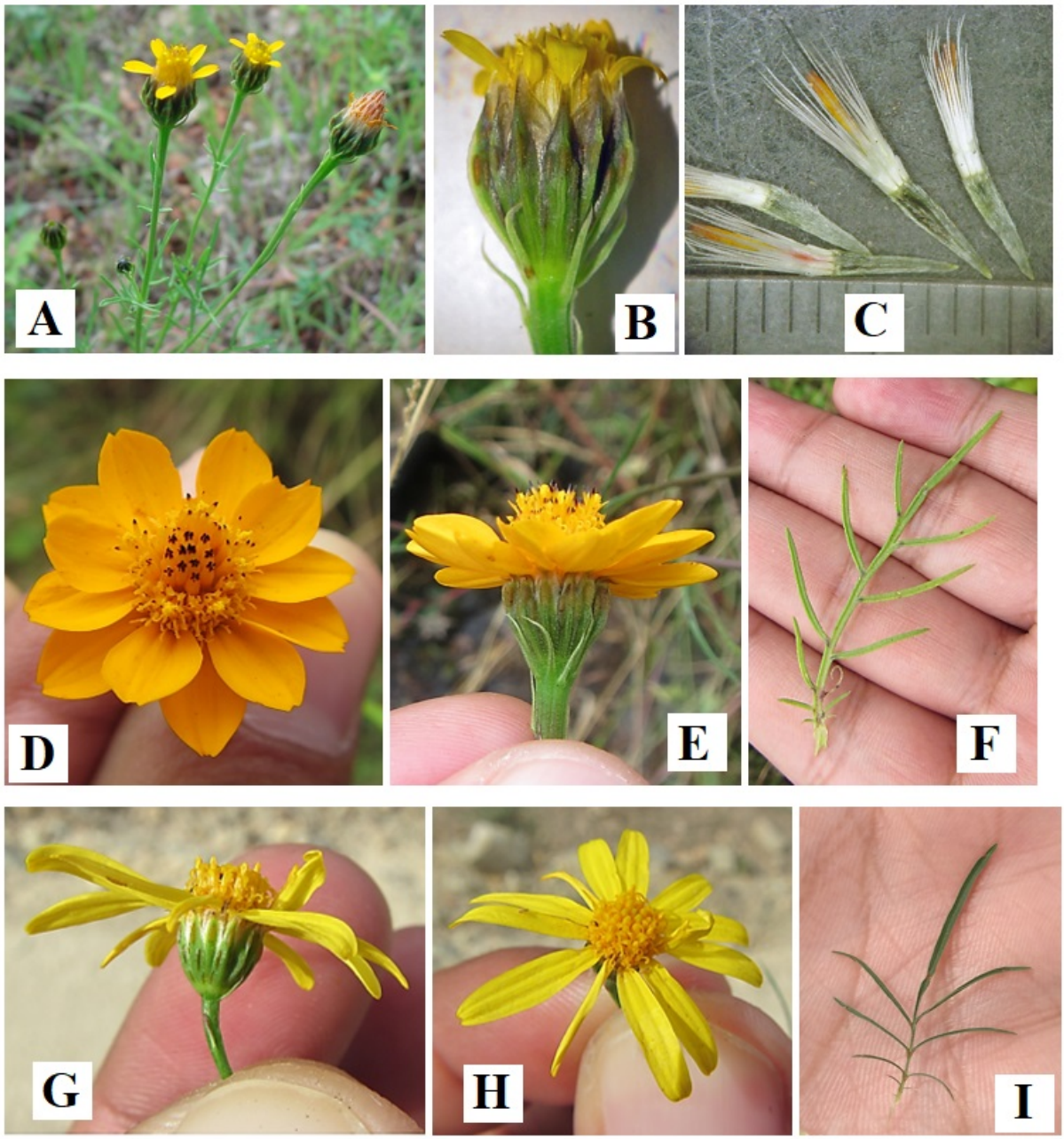

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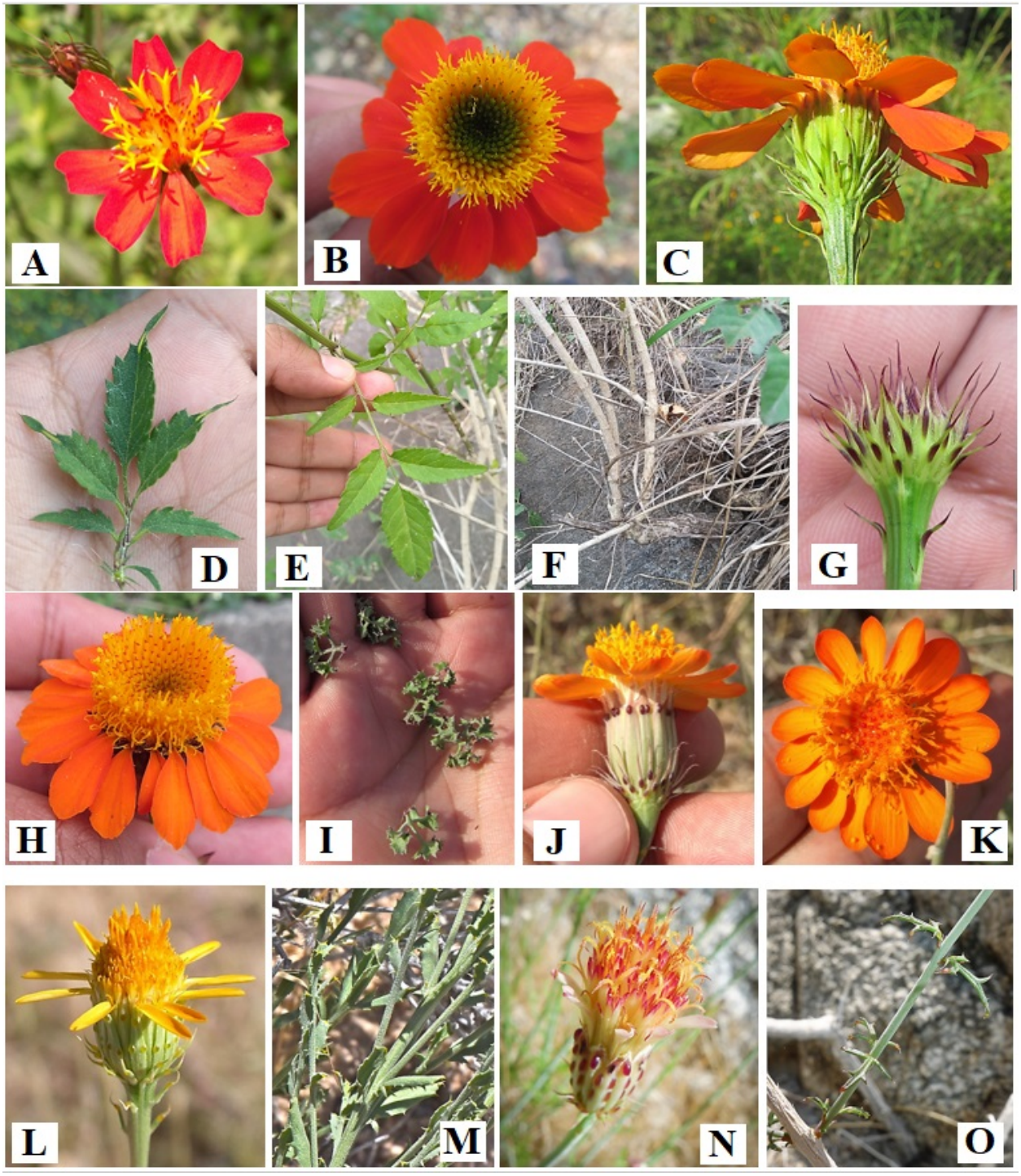

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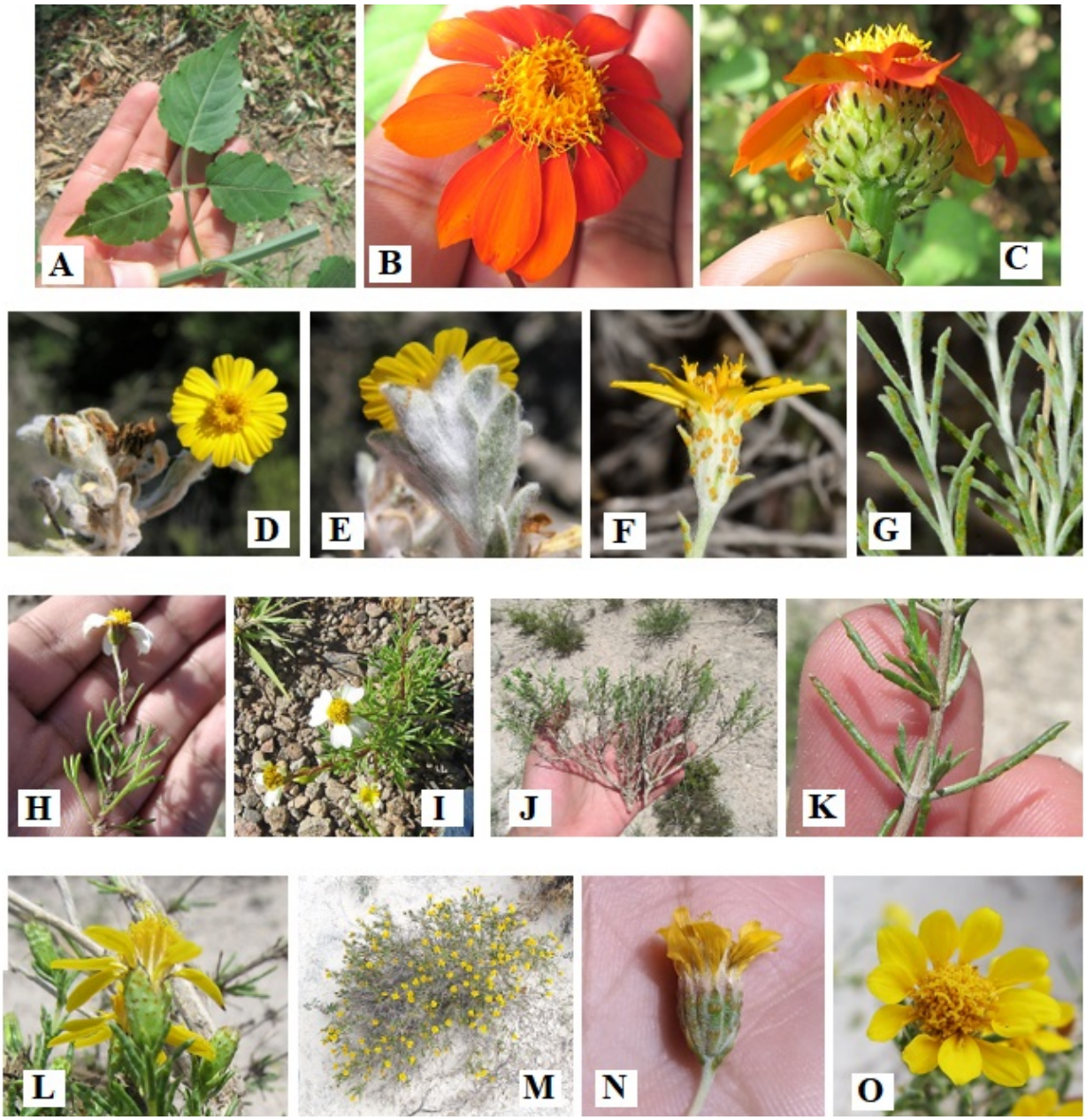

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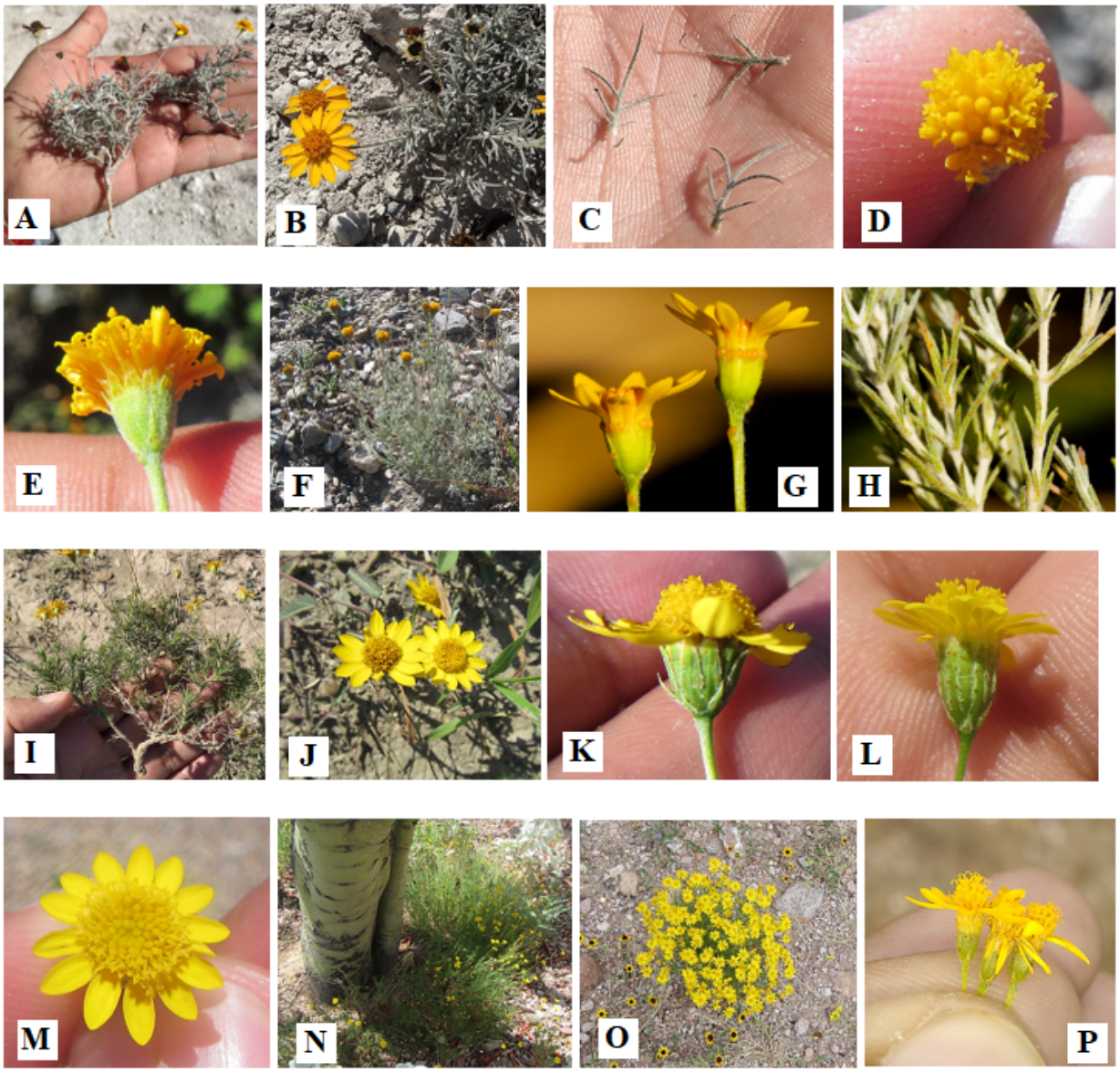

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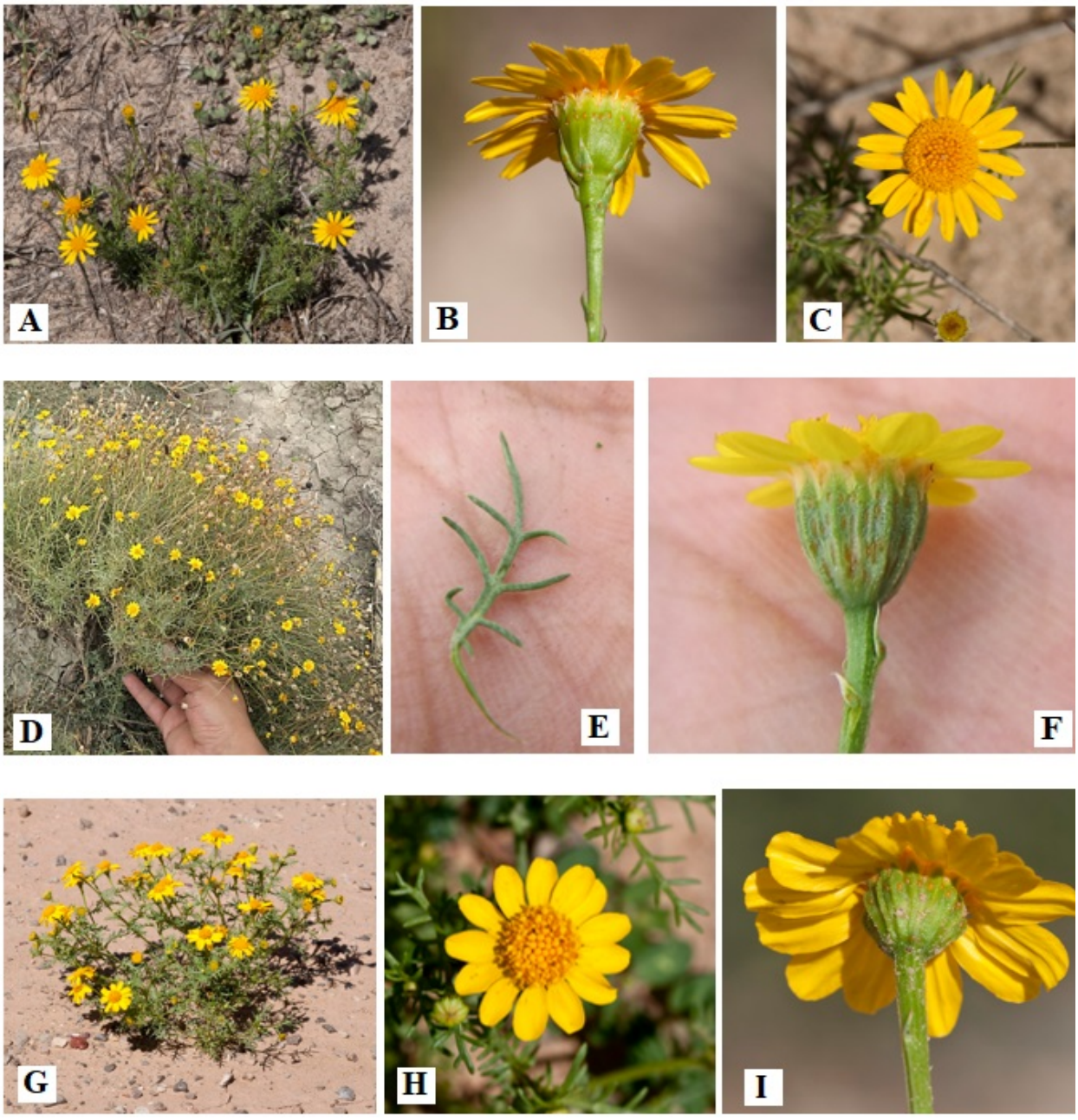

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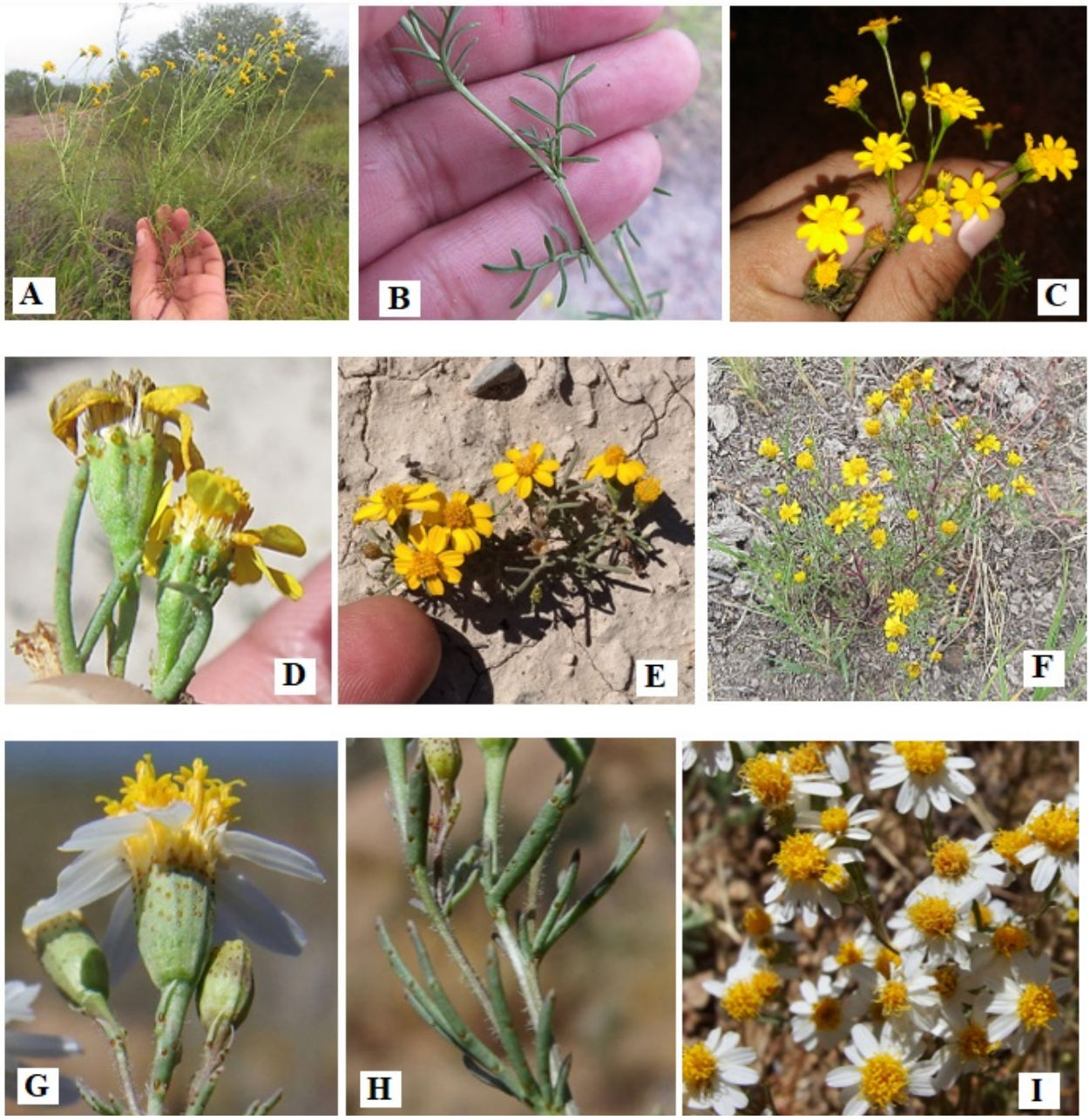

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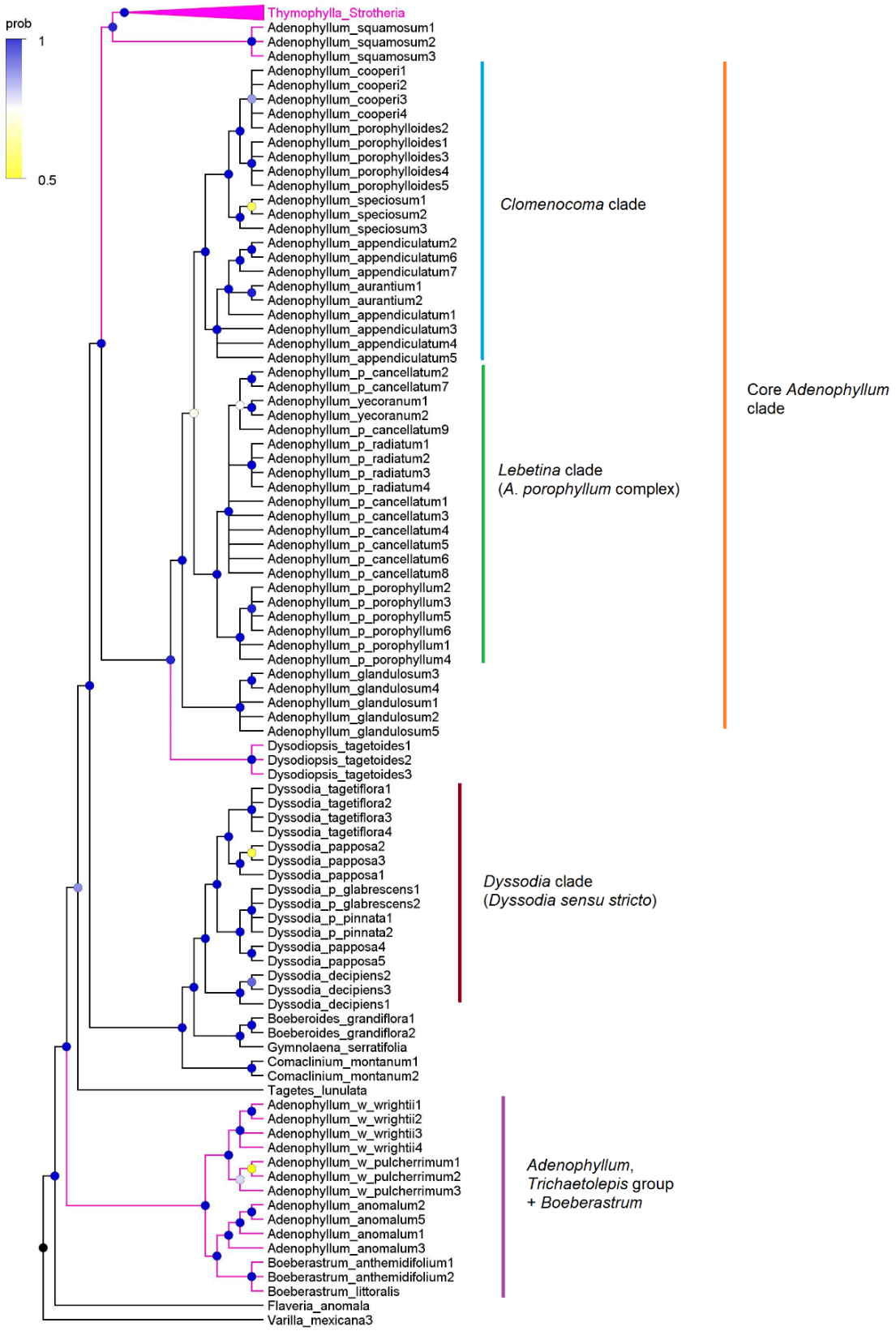
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Adenophyllum_squamosum2
Adenophyllum_squamosum3
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Adenophyllum_cooperi2
Adenophyllum_cooperi3
Adenophyllum_cooperi4
Adenophyllum_porophylloides2
Adenophyllum_porophylloides1
Adenophyllum_porophylloides3
Adenophyllum_porophylloides4
Adenophyllum_porophylloides5
Adenophyllum_speciosum1
Adenophyllum_speciosum2
Adenophyllum_speciosum3
Adenophyllum_appendiculatum2
Adenophyllum_appendiculatum6
Adenophyllum_appendiculatum7
Adenophyllum_aurantium1
Adenophyllum_aurantium2
Adenophyllum_appendiculatum1
Adenophyllum_appendiculatum3
Adenophyllum_appendiculatum4
Adenophyllum_appendiculatum5
Adenophyllum_p_cancellatum2
Adenophyllum_p_cancellatum7
Adenophyllum_yecoranum1
Adenophyllum_yecoranum2
Adenophyllum_p_cancellatum9
Adenophyllum_p_radiatum1
Adenophyllum_p_radiatum2
Adenophyllum_p_radiatum3
Adenophyllum_p_radiatum4
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Adenophyllum_p_cancellatum3
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Adenophyllum_p_porophyllum4
Adenophyllum_glandulosum3
Adenophyllum_glandulosum4
Adenophyllum_glandulosum1
Adenophyllum_glandulosum2
Adenophyllum_glandulosum5
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Dysodiopsis_tagetoides2
Dysodiopsis_tagetoides3
Dyssodia_tagetiflora1
Dyssodia_tagetiflora2
Dyssodia_tagetiflora3
Dyssodia_tagetiflora4
Dyssodia_papposa2
Dyssodia_papposa3
Dyssodia_papposa1
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Dyssodia_p_glabrescens2
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Dyssodia_p_pinnata2
Dyssodia_papposa4
Dyssodia_papposa5
Dyssodia_decipiens2
Dyssodia_decipiens3
Dyssodia_decipiens1
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Boeberoides_grandiflora2
Gymnolaena_serratifolia
Comaclinium_montanum1
Comaclinium_montanum2
Tagetes_lunulata
Adenophyllum_w_wrightii1
Adenophyllum_w_wrightii2
Adenophyllum_w_wrightii3
Adenophyllum_w_wrightii4
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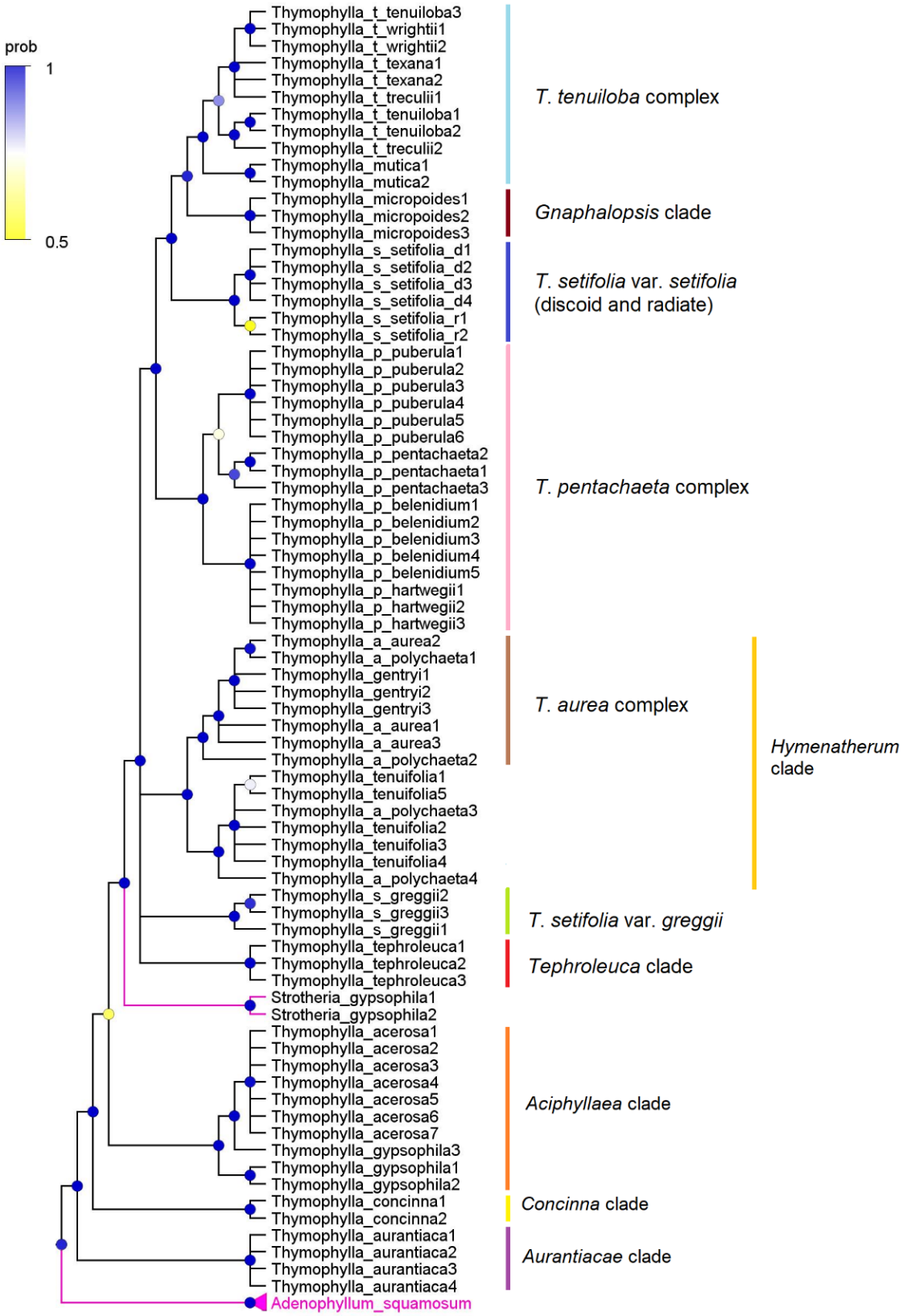
Adenophyllum_w_pulcherrimum2
Adenophyllum_w_pulcherrimum3
Adenophyllum_anomalum2
Adenophyllum_anomalum5
Adenophyllum_anomalum1
Adenophyllum_anomalum3
Boeberastrum_anthemidifolium1
Boeberastrum_anthemidifolium2
Boeberastrum_littoralis
Flaveria_anomala
Varilla_mexicana3
Clomenocoma clade
Lebetina clade
(A. porophyllum complex)
Dyssodia clade
(Dyssodia sensu stricto)
Adenophyllum,
Trichaetolepis group
+ Boeberastrum
Core Adenophyllum
clade

Fig. 8.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0.5
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Thymophylla_t_wrightii1
Thymophylla_t_wrightii2
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Thymophylla_t_texana2
Thymophylla_t_treculii1
Thymophylla_t_tenuiloba1
Thymophylla_t_tenuiloba2
Thymophylla_t_treculii2
Thymophylla_mutica1
Thymophylla_mutica2
Thymophylla_micropoides1
Thymophylla_micropoides2
Thymophylla_micropoides3
Thymophylla_s_setifolia_d1
Thymophylla_s_setifolia_d2
Thymophylla_s_setifolia_d3
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Thymophylla_p_puberula1
Thymophylla_p_puberula2
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Thymophylla_p_hartwegii3
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Thymophylla_gentryi1
Thymophylla_gentryi2
Thymophylla_gentryi3
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Thymophylla_tenuifolia4
Thymophylla_a_polychaeta4
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Thymophylla_s_greggii3
Thymophylla_s_greggii1
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Thymophylla_tephroleuca2
Thymophylla_tephroleuca3
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Strotheria_gypsophila2
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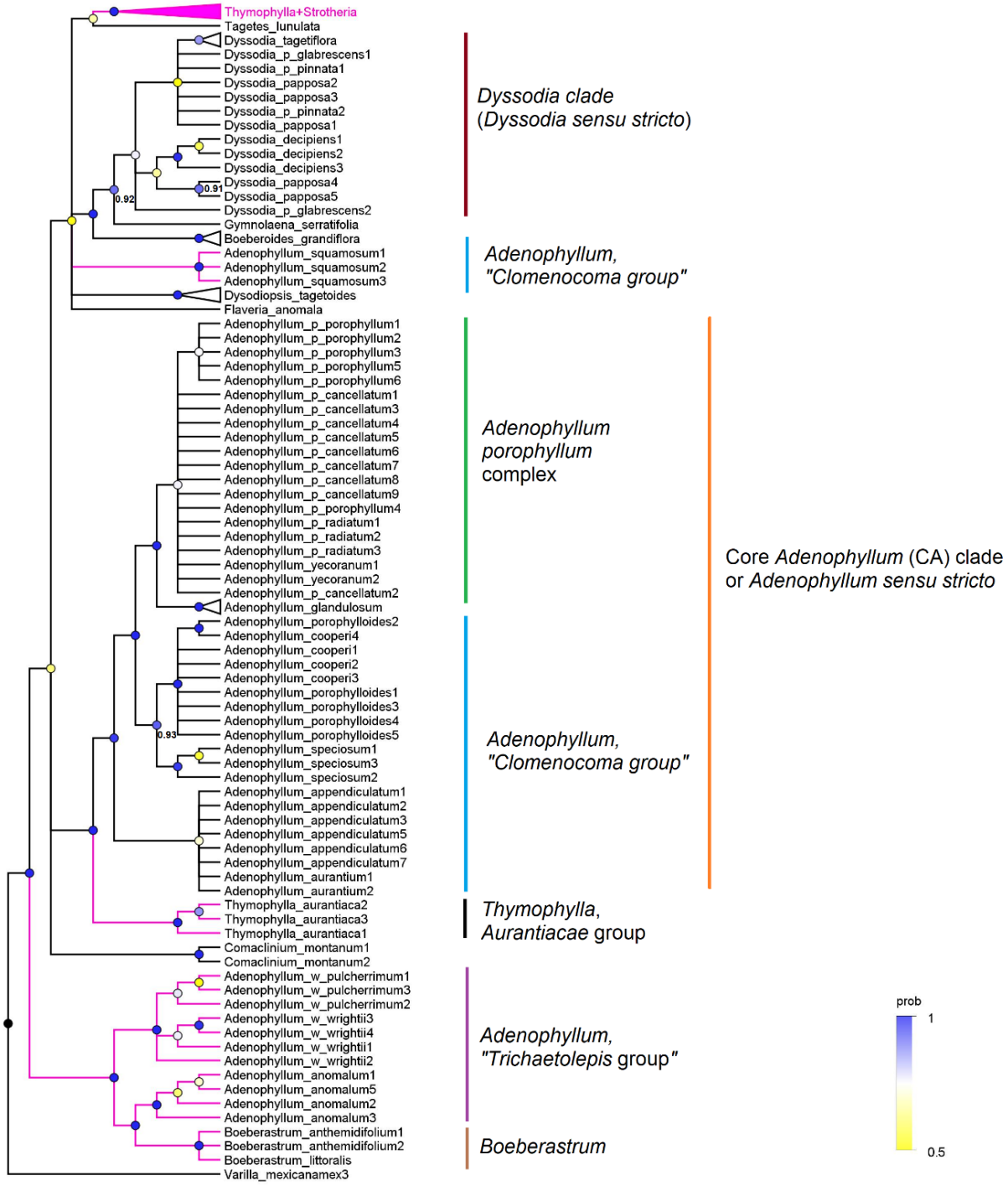
Thymophylla_acerosa7
Thymophylla_gypsophila3
Thymophylla_gypsophila1
Thymophylla_gypsophila2
Thymophylla_concinna1
Thymophylla_concinna2
Thymophylla_aurantiaca1
Thymophylla_aurantiaca2
Thymophylla_aurantiaca3
Thymophylla_aurantiaca4
Adenophyllum_squamosum
T. tenuiloba complex
Gnaphalopsis clade
T. setifolia var. setifolia
(discoid and radiate)
T. pentachaeta complex
T. aurea complex
Hymenatherum
clade
T. setifolia var. greggii
Tephroleuca clade
Aciphyllaea clade
Concinna clade
Aurantiacae clade

Fig. 9.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Gymnolaena_serratifolia
Boeberoides_grandiflora
Adenophyllum_squamosum1
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Adenophyllum_squamosum3
Dysodiopsis_tagetoides
Flaveria_anomala
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Comaclinium_montanum2
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Adenophyllum_w_pulcherrimum2
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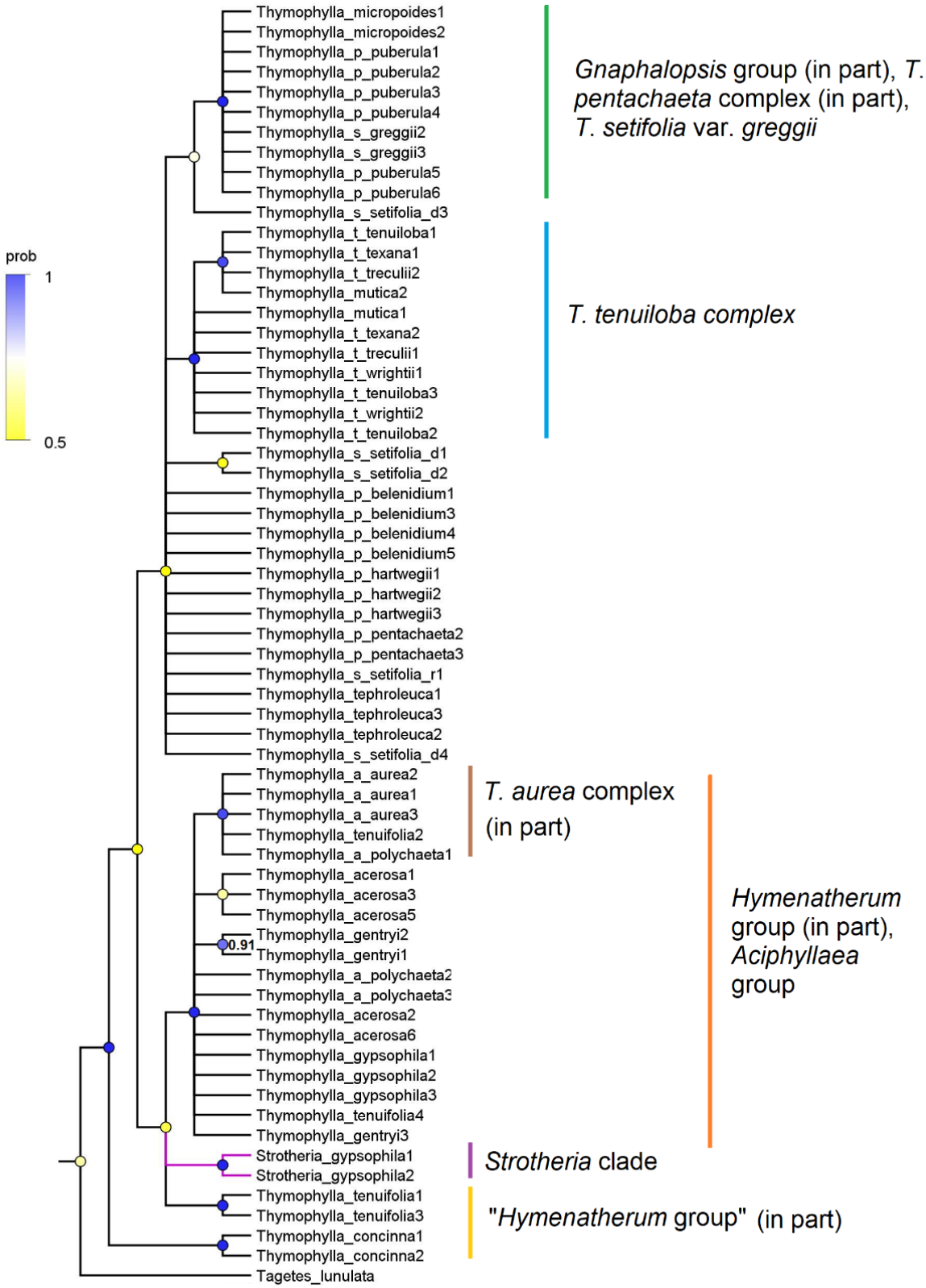
Adenophyllum_anomalum2
Adenophyllum_anomalum3
Boeberastrum_anthemidifolium1
Boeberastrum_anthemidifolium2
Boeberastrum_littoralis
Varilla_mexicanamex3
0.92
0.91
0.93
Dyssodia clade
(Dyssodia sensu stricto)
Adenophyllum,
"Clomenocoma group"
Adenophyllum
porophyllum
complex
Adenophyllum,
"Clomenocoma group"
Thymophylla,
Aurantiacae group
Adenophyllum,
"Trichaetolepis group"
Boeberastrum
Core Adenophyllum (CA) clade
or Adenophyllum sensu stricto
prob
1
0.5

Fig. 10.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0.5
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Thymophylla_micropoides2
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Thymophylla_p_puberula4
Thymophylla_s_greggii2
Thymophylla_s_greggii3
Thymophylla_p_puberula5
Thymophylla_p_puberula6
Thymophylla_s_setifolia_d3
Thymophylla_t_tenuiloba1
Thymophylla_t_texana1
Thymophylla_t_treculii2
Thymophylla_mutica2
Thymophylla_mutica1
Thymophylla_t_texana2
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Thymophylla_p_pentachaeta3
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Thymophylla_tephroleuca1
Thymophylla_tephroleuca3
Thymophylla_tephroleuca2
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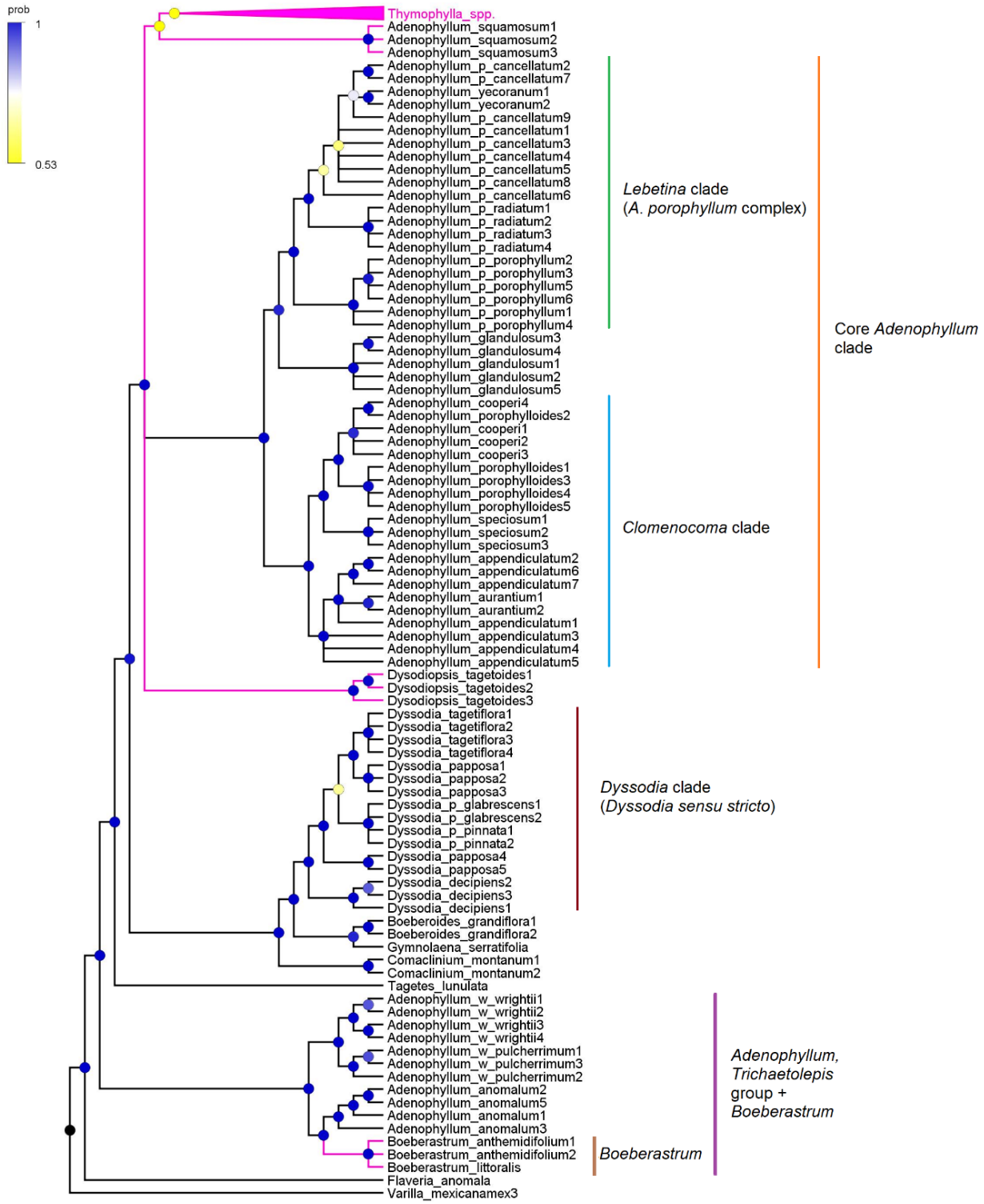
Thymophylla_tenuifolia4
Thymophylla_gentryi3
Strotheria_gypsophila1
Strotheria_gypsophila2
Thymophylla_tenuifolia1
Thymophylla_tenuifolia3
Thymophylla_concinna1
Thymophylla_concinna2
Tagetes_lunulata
Gnaphalopsis group (in part), T. pentachaeta complex (in part), T. setifolia var. greggii
T. tenuiloba complex
T. aurea complex (in part)
Hymenatherum group (in part), Aciphyllaea group
Strotheria clade
"Hymenatherum group" (in part)

Fig. 11.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Adenophyllum_squamosum3
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Adenophyllum_p_cancellatum7
Adenophyllum_yecoranum1
Adenophyllum_yecoranum2
Adenophyllum_p_cancellatum9
Adenophyllum_p_cancellatum1
Adenophyllum_p_cancellatum3
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Adenophyllum_p_cancellatum5
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Adenophyllum_p_radiatum4
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Adenophyllum_p_porophyllum5
Adenophyllum_p_porophyllum6
Adenophyllum_p_porophyllum1
Adenophyllum_p_porophyllum4
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Adenophyllum_glandulosum4
Adenophyllum_glandulosum1
Adenophyllum_glandulosum2
Adenophyllum_glandulosum5
Adenophyllum_cooperi4
Adenophyllum_porophylloides2
Adenophyllum_cooperi1
Adenophyllum_cooperi2
Adenophyllum_cooperi3
Adenophyllum_porophylloides1
Adenophyllum_porophylloides3
Adenophyllum_porophylloides4
Adenophyllum_porophylloides5
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Adenophyllum_aurantium2
Adenophyllum_appendiculatum1
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Adenophyllum_appendiculatum5
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Dysodiopsis_tagetoides2
Dysodiopsis_tagetoides3
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Dyssodia_tagetiflora2
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Dyssodia_papposa2
Dyssodia_papposa3
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Dyssodia_p_pinnata2
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Dyssodia_papposa5
Dyssodia_decipiens2
Dyssodia_decipiens3
Dyssodia_decipiens1
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Boeberoides_grandiflora2
Gymnolaena_serratifolia
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Comaclinium_montanum2
Tagetes_lunulata
Adenophyllum_w_wrightii1
Adenophyllum_w_wrightii2
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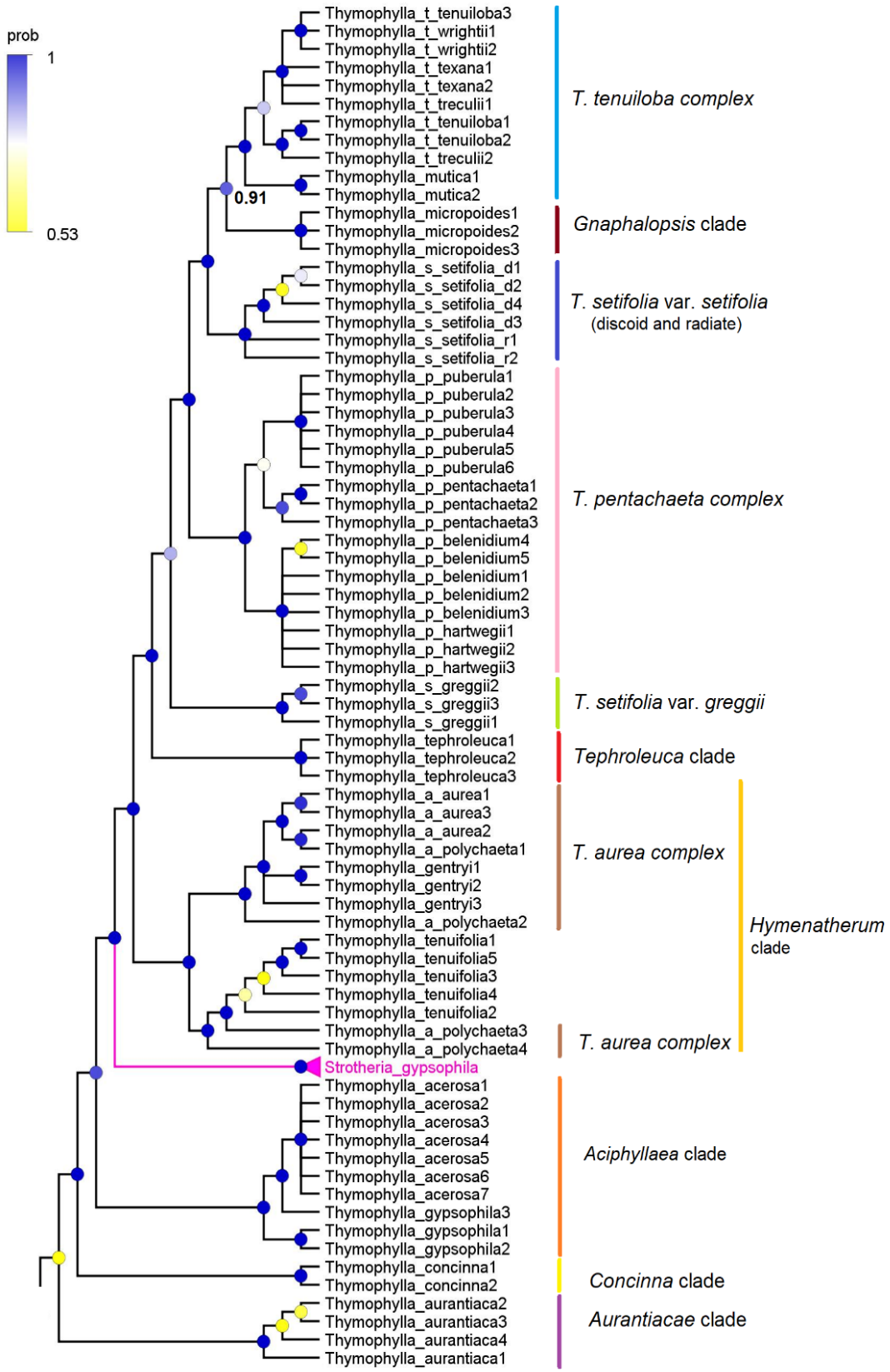
Adenophyllum_w_pulcherrimum2
Adenophyllum_anomalum2
Adenophyllum_anomalum5
Adenophyllum_anomalum1
Adenophyllum_anomalum3
Boeberastrum_anthemidifolium1
Boeberastrum_anthemidifolium2
Boeberastrum_littoralis
Flaveria_anomala
Varilla_mexicanamex3
Lebetina clade
(A. porophyllum complex)
Clomenocoma clade
Dyssodia clade
(Dyssodia sensu stricto)
Boeberastrum
Adenophyllum,
Trichaetolepis
group +
Boeberastrum
Core Adenophyllum
clade

Fig. 12.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1
0.53
0.91
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Thymophylla_t_wrightii1
Thymophylla_t_wrightii2
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Thymophylla_t_texana2
Thymophylla_t_treculii1
Thymophylla_t_tenuiloba1
Thymophylla_t_tenuiloba2
Thymophylla_t_treculii2
Thymophylla_mutica1
Thymophylla_mutica2
Thymophylla_micropoides1
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Thymophylla_micropoides3
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Thymophylla_s_setifolia_d2
Thymophylla_s_setifolia_d4
Thymophylla_s_setifolia_d3
Thymophylla_s_setifolia_r1
Thymophylla_s_setifolia_r2
Thymophylla_p_puberula1
Thymophylla_p_puberula2
Thymophylla_p_puberula3
Thymophylla_p_puberula4
Thymophylla_p_puberula5
Thymophylla_p_puberula6
Thymophylla_p_pentachaeta1
Thymophylla_p_pentachaeta2
Thymophylla_p_pentachaeta3
Thymophylla_p_belenidium4
Thymophylla_p_belenidium5
Thymophylla_p_belenidium1
Thymophylla_p_belenidium2
Thymophylla_p_belenidium3
Thymophylla_p_hartwegii1
Thymophylla_p_hartwegii2
Thymophylla_p_hartwegii3
Thymophylla_s_greggii2
Thymophylla_s_greggii3
Thymophylla_s_greggii1
Thymophylla_tephroleuca1
Thymophylla_tephroleuca2
Thymophylla_tephroleuca3
Thymophylla_a_aurea1
Thymophylla_a_aurea3
Thymophylla_a_aurea2
Thymophylla_a_polychaeta1
Thymophylla_gentryi1
Thymophylla_gentryi2
Thymophylla_gentryi3
Thymophylla_a_polychaeta2
Thymophylla_tenuifolia1
Thymophylla_tenuifolia5
Thymophylla_tenuifolia3
Thymophylla_tenuifolia4
Thymophylla_tenuifolia2
Thymophylla_a_polychaeta3
Thymophylla_a_polychaeta4
Strotheria_gypsophila
Thymophylla_acerosa1
Thymophylla_acerosa2
Thymophylla_acerosa3
Thymophylla_acerosa4
Thymophylla_acerosa5
Thymophylla_acerosa6
Thymophylla_acerosa7
Thymophylla_gypsophila3
Thymophylla_gypsophila1
Thymophylla_gypsophila2
Thymophylla_concinna1
Thymophylla_concinna2
Thymophylla_aurantiaca2
Thymophylla_aurantiaca3
Thymophylla_aurantiaca4
Thymophylla_aurantiaca1
T. tenuiloba complex
Gnaphalopsis clade
T. setifolia var. setifolia
(discoid and radiate)
T. pentachaeta complex
T. setifolia var. greggii
Tephroleuca clade
T. aurea complex
Hymenatherum
clade
T. aurea complex
Aciphyllaea clade
Concinna clade
Aurantiacae clade

Fig. 13.

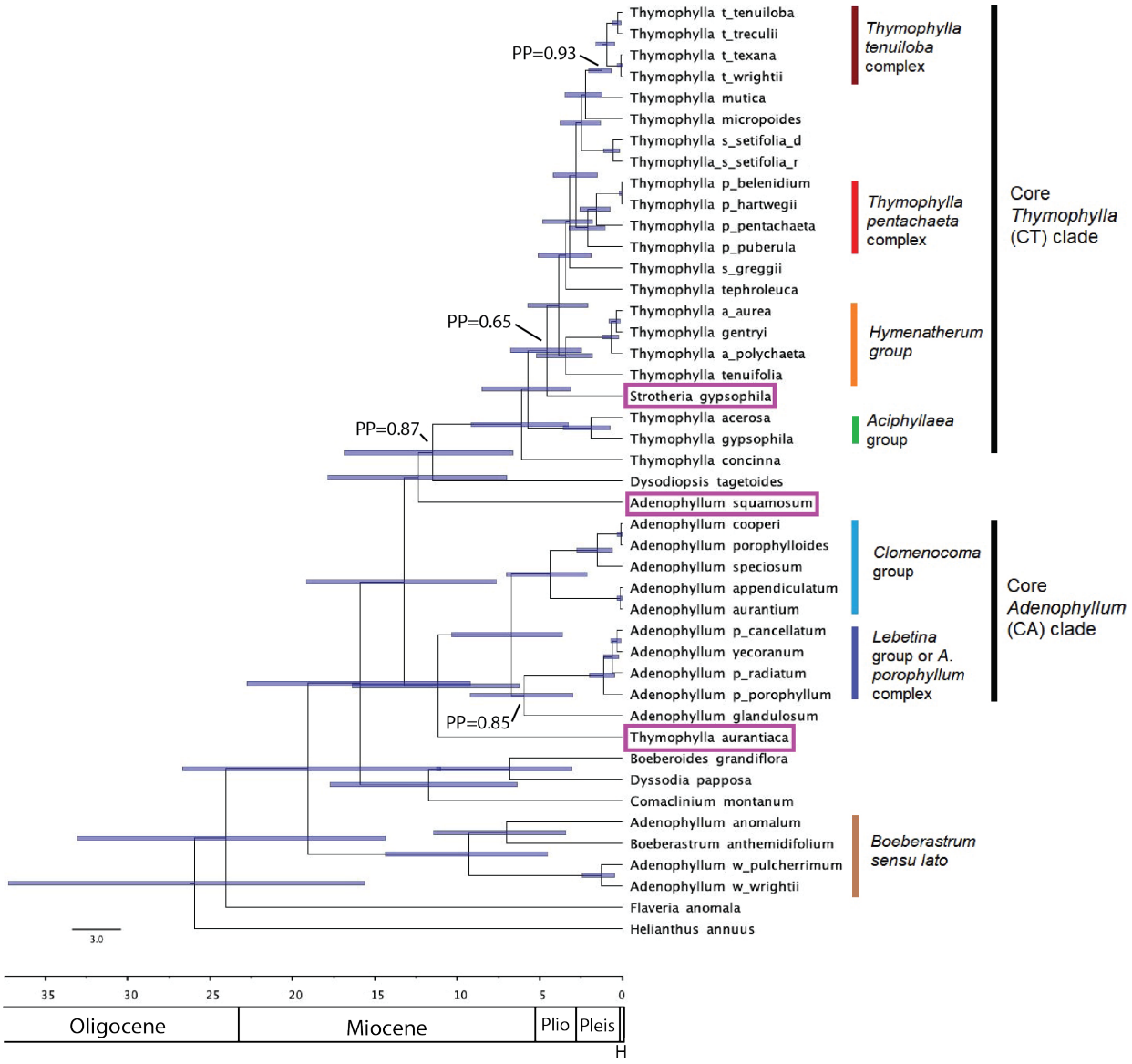

Thymophylla t_tenuiloba
Thymophylla t_treculii
Thymophylla t_texana
Thymophylla t_wrightii
Thymophylla mutica
Thymophylla micropoides
Thymophylla s_setifolia_d
Thymophylla_s_setifolia_r
Thymophylla p_belenidium
Thymophylla p_hartwegii
Thymophylla p_pentachaeta
Thymophylla p_puberula
Thymophylla s_greggii
Thymophylla tephroleuca
Thymophylla a_aurea
Thymophylla gentryi
Thymophylla a_polychaeta
Thymophylla tenuifolia
Strotheria gypsophila
Thymophylla acerosa
Thymophylla gypsophila
Thymophylla concinna
Dysodiopsis tagetoides
Adenophyllum squamosum
Adenophyllum cooperi
Adenophyllum porophylloides
Adenophyllum speciosum
Adenophyllum appendiculatum
Adenophyllum aurantium
Adenophyllum p_cancellatum
Adenophyllum yecoranum
Adenophyllum p_radiatum
Adenophyllum p_porophyllum
Adenophyllum glandulosum
Thymophylla aurantiaca
Boeberoides grandiflora
Dyssodia papposa
Comaclinium montanum
Adenophyllum anomalum
Boeberastrum anthemidifolium
Adenophyllum w_pulcherrimum
Adenophyllum w_wrightii
Flaveria anomala
Helianthus annuus
PP=0.93
PP=0.65
PP=0.87
PP=0.85
3.0
Thymophylla tenuiloba complex
Thymophylla pentachaeta complex
Hymenatherum group
Aciphyllaea group
Core Thymophylla (CT) clade
Clomenocoma group
Lebetina group or A. porophyllum complex
Core Adenophyllum (CA) clade
Boeberastrum sensu lato
35
30
25
20
15
10
5
0
Oligocene
Miocene
Plio
Pleis
H

Fig. 14.


(AD) Adenophyllum anomalum
(A) Boeberastrum anthemidifolium
(BCD) Adenophyllum w pulcherrimum
(BC) Adenophyllum w wrightii
(D) Adenophyllum appendiculatum
(D) Adenophyllum aurantium
(A) Adenophyllum cooperi
(A) Adenophyllum porophylloides
(A) Adenophyllum speciosum
(D) Adenophyllum glandulosum
(ACDE) Adenophyllum p cancellatum
(D) Adenophyllum yecoranum
(D) Adenophyllum p radiatum
(D) Adenophyllum p porophyllum
(D) Thymophylla aurantiaca
(BD) Adenophyllum squamosum
(E) Dysodiopsis tagetoides
(A) Strotheria gypsophila
(AE) Thymophylla a aurea
(A) Thymophylla gentryi
(AE) Thymophylla a polychaeta
(ABC) Thymophylla tenuifolia
(AE) Thymophylla micropoides
(D) Thymophylla mutica
(AE) Thymophylla t tenuiloba
(AE) Thymophylla t treculii
(AE) Thymophylla t texana
(E) Thymophylla t wrightii
(ABC) Thymophylla s setifolia d1
(ABC) Thymophylla s setifolia r1
(ACEG) Thymophylla p belenidium
(AC) Thymophylla p hartwegii
(ACE) Thymophylla p pentachaeta
(ABCE) Thymophylla p puberula
(AE) Thymophylla s greggii
(E) Thymophylla tephroleuca
(ACE) Thymophylla acerosa
(A) Thymophylla gypsophila
(A) Thymophylla concinna
(D) Bocberoides grandiflora
(DF) Comaclinium montanum
Boeberastrum clade
Core Adenophyllum clade
Core Thymophylla clade
PP=0.87
PP=0.93
PP=0.65
19 18 15 12 9 6 3 0
Mya
*
AB
ABC
ABCD
ABCE
ABD
AC
ACD
ACDE
ACE
ACEG
ACG
AD
ADE
AE
BC
BCD
BD
DE
DF
A North American Deserts
B Temperate Sierras
C Southern Semi-arid Highlands
D Neotropical
E Great Plains
F Pacific Domain
G Argentinian Deserts

Fig. 15.

SUPPLEMENTARY FIGURES

Fig. S1. Majority rule consensus tree from the Bayesian analyses of ITS sequences focusing on *Adenophyllum*. Dark blue nodes with posterior probabilities ≥ 0.95.

Fig. S2. Majority rule consensus tree from the Bayesian analyses of ITS sequences focusing on the core *Thymophylla* clade. Note *Strotheria* nested within *Thymophylla*. Dark blue nodes with posterior probabilities ≥ 0.95.

Fig. S3. Majority rule consensus tree from the Bayesian analyses of ETS sequences focusing on *Adenophyllum*. Dark blue nodes with posterior probabilities ≥ 0.95.

Fig. S4. Majority rule consensus tree from the Bayesian analyses of ETS sequences focusing on the core *Thymophylla* clade. Note *Strotheria* nested within *Thymophylla*. Dark blue nodes with posterior probabilities ≥ 0.95.

Fig. S5. Majority rule consensus tree from the Bayesian analyses of plastid *trn*L-F intergenic spacer focusing on the Boeberastrum and core *Adenophyllum* clades. Also note *Thymophylla aurantiaca* nested in *Adenophyllum*. Dark blue nodes with posterior probabilities ≥ 0.95.

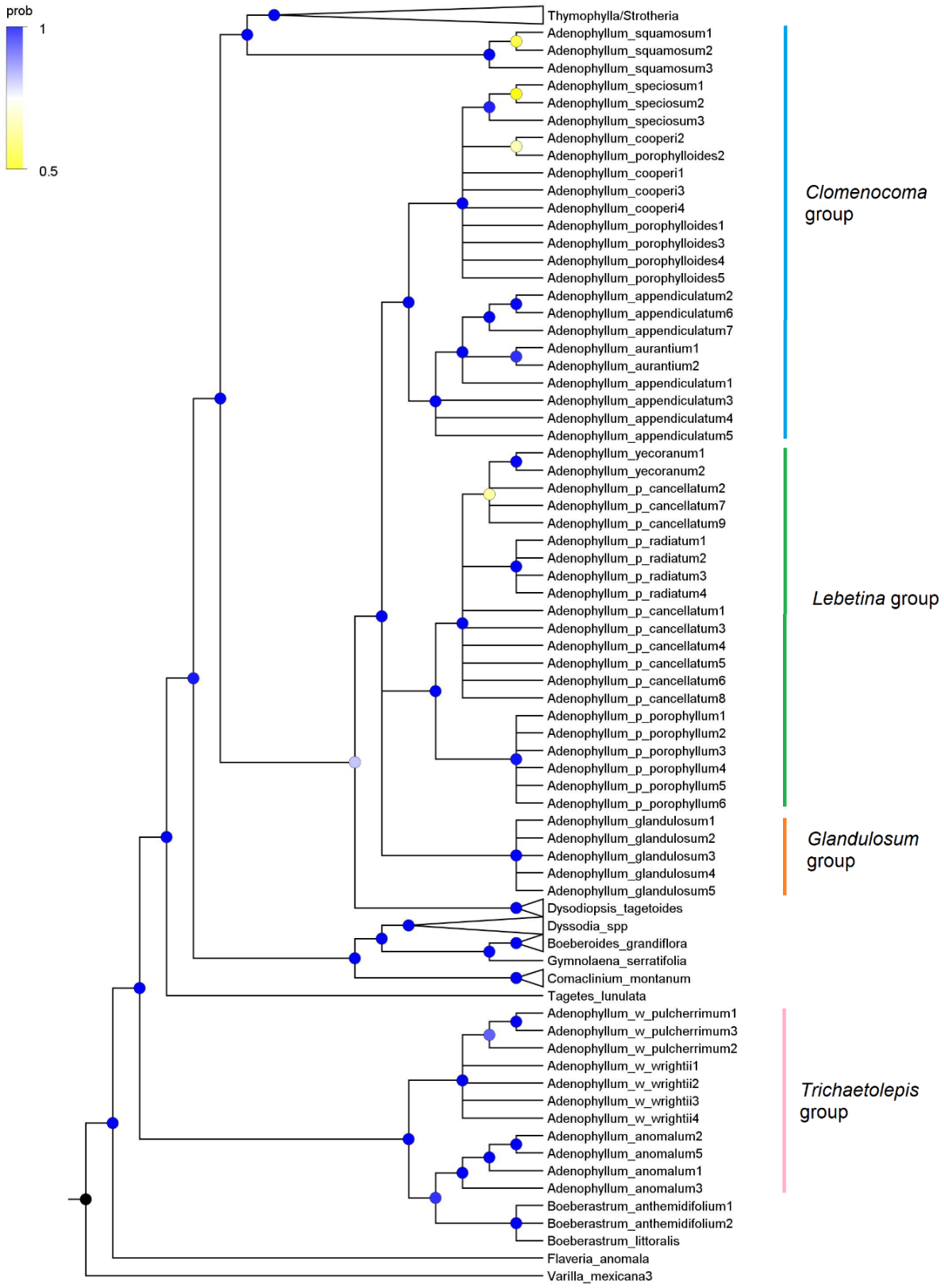
Fig. S6. Majority rule consensus tree from the Bayesian analyses of plastid *trn*L-F intergenic spacer focusing on the core *Thymophylla* clade. Note *Strotheria gypsophila* nested within *Thymophylla*. Dark blue nodes with posterior probabilities ≥ 0.95.

Fig. S7. Majority rule consensus tree from the Bayesian analyses of plastid *ndh*I-*ndh*G intergenic spacer and *ndh*I gene focusing on *Adenophyllum*. Note *Thymophylla aurantiaca* nested within the core *Adenophyllum* clade. Dark blue nodes with posterior probabilities ≥ 0.95.

Fig. S8. Majority rule consensus tree from the Bayesian analyses of plastid *ndh*I-*ndh*G intergenic spacer and *ndh*I gene focusing on the core *Thymophylla* clade. Note *Strotheria* nested within *Thymophylla*. Dark blue nodes with posterior probabilities ≥ 0.95.

Fig. S9. Majority rule consensus tree from the Bayesian analyses of plastid *psbA*-*trnH* intergenic spacer focusing on the core *Adenophyllum* clade. Also note the clades of *Thymophylla aurantiaca*, *T*. *concinna*, *A. anomalum* and *Comaclinium*. Dark blue nodes with posterior probabilities ≥ 0.95.

Fig. S10. Majority rule consensus tree from the Bayesian analyses of plastid *psbA*-*trnH* intergenic spacer focusing on the core *Thymophylla* clade. Note *Strotheria gypsophila* nested within *Thymophylla*. Dark blue nodes with posterior probabilities ≥ 0.95.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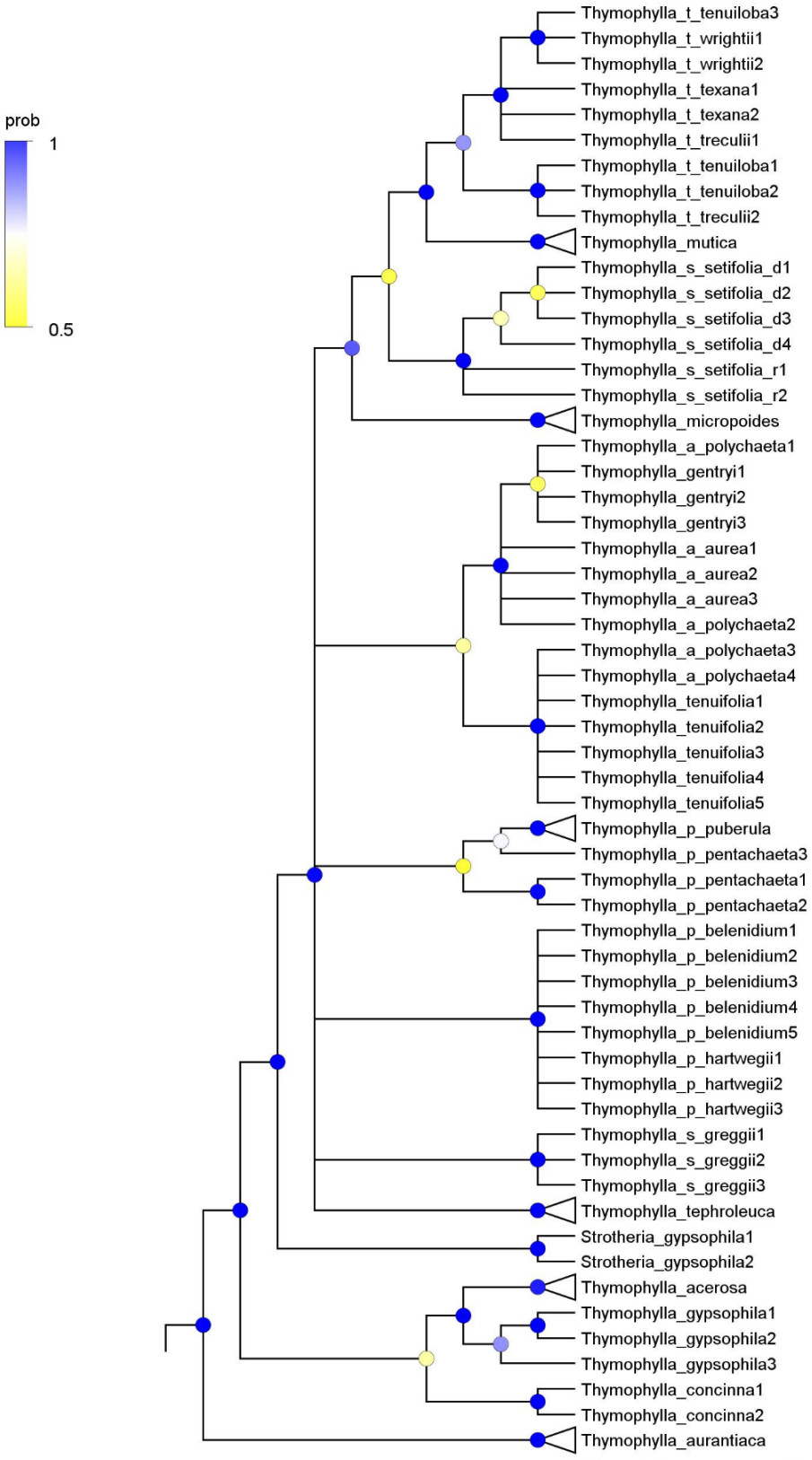
0.5
Thymophylla/Strotheria
Adenophyllum_squamosum1
Adenophyllum_squamosum2
Adenophyllum_squamosum3
Adenophyllum_speciosum1
Adenophyllum_speciosum2
Adenophyllum_speciosum3
Adenophyllum_cooperi2
Adenophyllum_porophylloides2
Adenophyllum_cooperi1
Adenophyllum_cooperi3
Adenophyllum_cooperi4
Adenophyllum_porophylloides1
Adenophyllum_porophylloides3
Adenophyllum_porophylloides4
Adenophyllum_porophylloides5
Adenophyllum_appendiculatum2
Adenophyllum_appendiculatum6
Adenophyllum_appendiculatum7
Adenophyllum_aurantium1
Adenophyllum_aurantium2
Adenophyllum_appendiculatum1
Adenophyllum_appendiculatum3
Adenophyllum_appendiculatum4
Adenophyllum_appendiculatum5
Adenophyllum_yecoranum1
Adenophyllum_yecoranum2
Adenophyllum_p_cancellatum2
Adenophyllum_p_cancellatum7
Adenophyllum_p_cancellatum9
Adenophyllum_p_radiatum1
Adenophyllum_p_radiatum2
Adenophyllum_p_radiatum3
Adenophyllum_p_radiatum4
Adenophyllum_p_cancellatum1
Adenophyllum_p_cancellatum3
Adenophyllum_p_cancellatum4
Adenophyllum_p_cancellatum5
Adenophyllum_p_cancellatum6
Adenophyllum_p_cancellatum8
Adenophyllum_p_porophyllum1
Adenophyllum_p_porophyllum2
Adenophyllum_p_porophyllum3
Adenophyllum_p_porophyllum4
Adenophyllum_p_porophyllum5
Adenophyllum_p_porophyllum6
Adenophyllum_glandulosum1
Adenophyllum_glandulosum2
Adenophyllum_glandulosum3
Adenophyllum_glandulosum4
Adenophyllum_glandulosum5
Dysodiopsis_tagetoides
Dyssodia_spp
Boeberoides_grandiflora
Gymnolaena_serratifolia
Comaclinium_montanum
Tagetes_lunulata
Adenophyllum_w_pulcherrimum1
Adenophyllum_w_pulcherrimum3
Adenophyllum_w_pulcherrimum2
Adenophyllum_w_wrightii1
Adenophyllum_w_wrightii2
Adenophyllum_w_wrightii3
Adenophyllum_w_wrightii4
Adenophyllum_anomalum2
Adenophyllum_anomalum5
Adenophyllum_anomalum1
Adenophyllum_anomalum3
Boeberastrum_anthemidifolium1
Boeberastrum_anthemidifolium2
Boeberastrum_littoralis
Flaveria_anomala
Varilla_mexicana3
Clomenocoma group
Lebetina group
Glandulosum group
Trichaetolepis group

Fig. S1.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0.5
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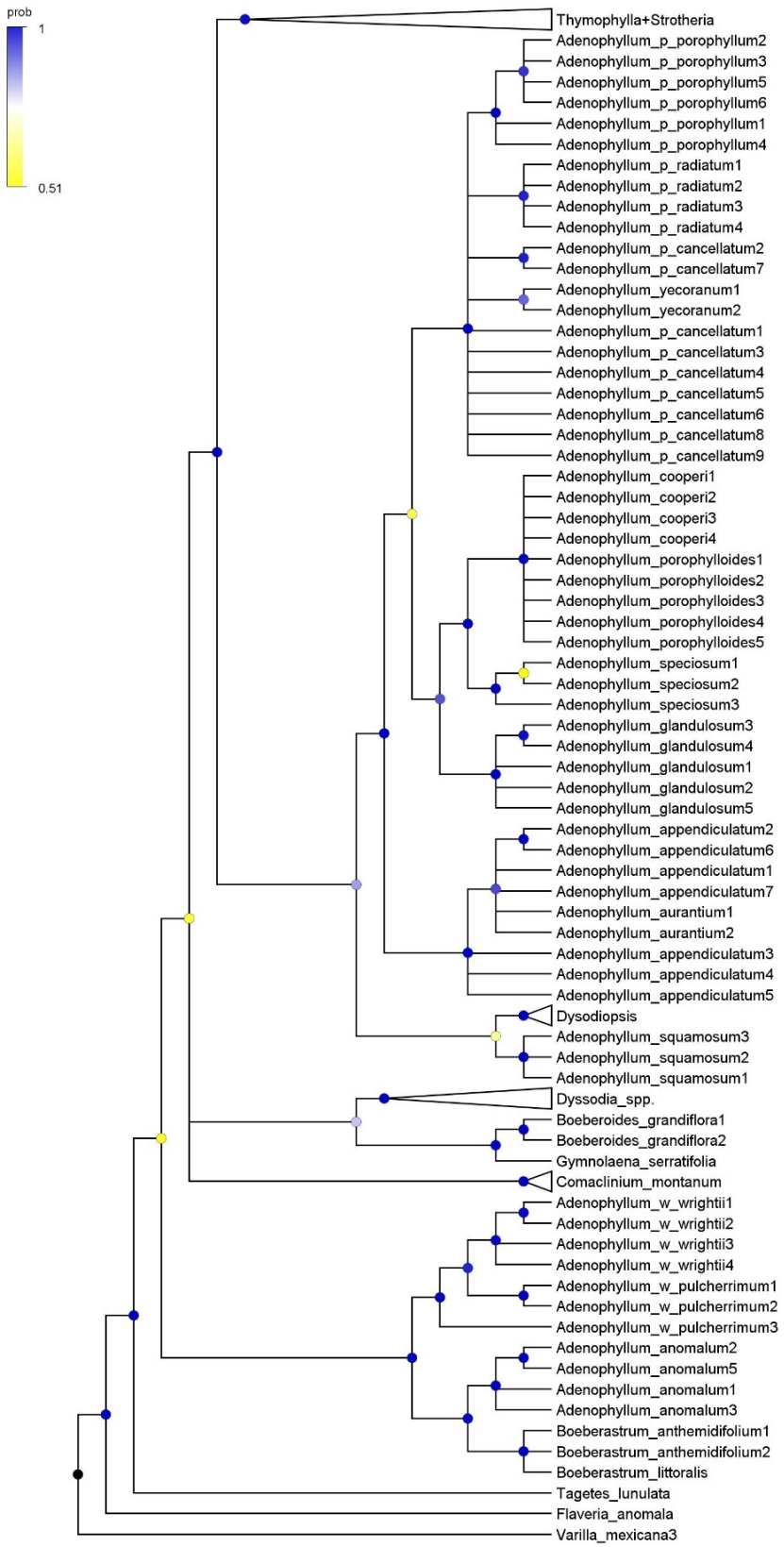
Thymophylla_t_wrightii1
Thymophylla_t_wrightii2
Thymophylla_t_texana1
Thymophylla_t_texana2
Thymophylla_t_treculii1
Thymophylla_t_tenuiloba1
Thymophylla_t_tenuiloba2
Thymophylla_t_treculii2
Thymophylla_mutica
Thymophylla_s_setifolia_d1
Thymophylla_s_setifolia_d2
Thymophylla_s_setifolia_d3
Thymophylla_s_setifolia_d4
Thymophylla_s_setifolia_r1
Thymophylla_s_setifolia_r2
Thymophylla_micropoides
Thymophylla_a_polychaeta1
Thymophylla_gentryi1
Thymophylla_gentryi2
Thymophylla_gentryi3
Thymophylla_a_aurea1
Thymophylla_a_aurea2
Thymophylla_a_aurea3
Thymophylla_a_polychaeta2
Thymophylla_a_polychaeta3
Thymophylla_a_polychaeta4
Thymophylla_tenuifolia1
Thymophylla_tenuifolia2
Thymophylla_tenuifolia3
Thymophylla_tenuifolia4
Thymophylla_tenuifolia5
Thymophylla_p_puberula
Thymophylla_p_pentachaeta3
Thymophylla_p_pentachaeta1
Thymophylla_p_pentachaeta2
Thymophylla_p_belenidium1
Thymophylla_p_belenidium2
Thymophylla_p_belenidium3
Thymophylla_p_belenidium4
Thymophylla_p_belenidium5
Thymophylla_p_hartwegii1
Thymophylla_p_hartwegii2
Thymophylla_p_hartwegii3
Thymophylla_s_greggii1
Thymophylla_s_greggii2
Thymophylla_s_greggii3
Thymophylla_tephroleuca
Strotheria_gypsophila1
Strotheria_gypsophila2
Thymophylla_acerosa
Thymophylla_gypsophila1
Thymophylla_gypsophila2
Thymophylla_gypsophila3
Thymophylla_concinna1
Thymophylla_concinna2
Thymophylla_aurantiaca

Fig. S2.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0.51
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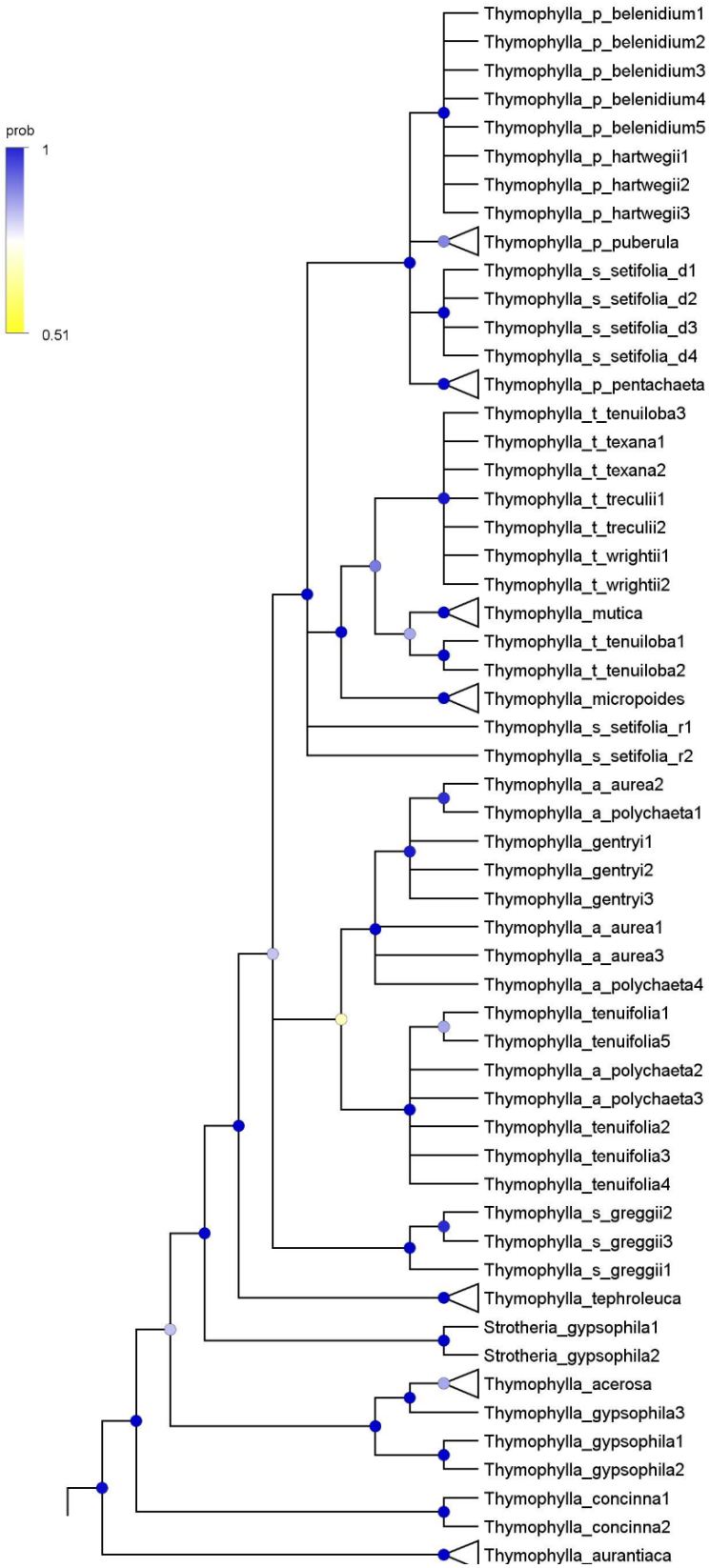
Adenophyllum_p_porophyllum3
Adenophyllum_p_porophyllum5
Adenophyllum_p_porophyllum6
Adenophyllum_p_porophyllum1
Adenophyllum_p_porophyllum4
Adenophyllum_p_radiatum1
Adenophyllum_p_radiatum2
Adenophyllum_p_radiatum3
Adenophyllum_p_radiatum4
Adenophyllum_p_cancellatum2
Adenophyllum_p_cancellatum7
Adenophyllum_yecoranum1
Adenophyllum_yecoranum2
Adenophyllum_p_cancellatum1
Adenophyllum_p_cancellatum3
Adenophyllum_p_cancellatum4
Adenophyllum_p_cancellatum5
Adenophyllum_p_cancellatum6
Adenophyllum_p_cancellatum8
Adenophyllum_p_cancellatum9
Adenophyllum_cooperi1
Adenophyllum_cooperi2
Adenophyllum_cooperi3
Adenophyllum_cooperi4
Adenophyllum_porophylloides1
Adenophyllum_porophylloides2
Adenophyllum_porophylloides3
Adenophyllum_porophylloides4
Adenophyllum_porophylloides5
Adenophyllum_speciosum1
Adenophyllum_speciosum2
Adenophyllum_speciosum3
Adenophyllum_glandulosum3
Adenophyllum_glandulosum4
Adenophyllum_glandulosum1
Adenophyllum_glandulosum2
Adenophyllum_glandulosum5
Adenophyllum_appendiculatum2
Adenophyllum_appendiculatum6
Adenophyllum_appendiculatum1
Adenophyllum_appendiculatum7
Adenophyllum_aurantium1
Adenophyllum_aurantium2
Adenophyllum_appendiculatum3
Adenophyllum_appendiculatum4
Adenophyllum_appendiculatum5
Dysodiopsis
Adenophyllum_squamosum3
Adenophyllum_squamosum2
Adenophyllum_squamosum1
Dyssodia_spp.
Boeberoides_grandiflora1
Boeberoides_grandiflora2
Gymnolaena_serratifolia
Comaclinium_montanum
Adenophyllum_w_wrightii1
Adenophyllum_w_wrightii2
Adenophyllum_w_wrightii3
Adenophyllum_w_wrightii4
Adenophyllum_w_pulcherrimum1
Adenophyllum_w_pulcherrimum2
Adenophyllum_w_pulcherrimum3
Adenophyllum_anomalum2
Adenophyllum_anomalum5
Adenophyllum_anomalum1
Adenophyllum_anomalum3
Boeberastrum_anthemidifolium1
Boeberastrum_anthemidifolium2
Boeberastrum_littoralis
Tagetes_lunulata
Flaveria_anomala
Varilla_mexicana3

Fig. S3.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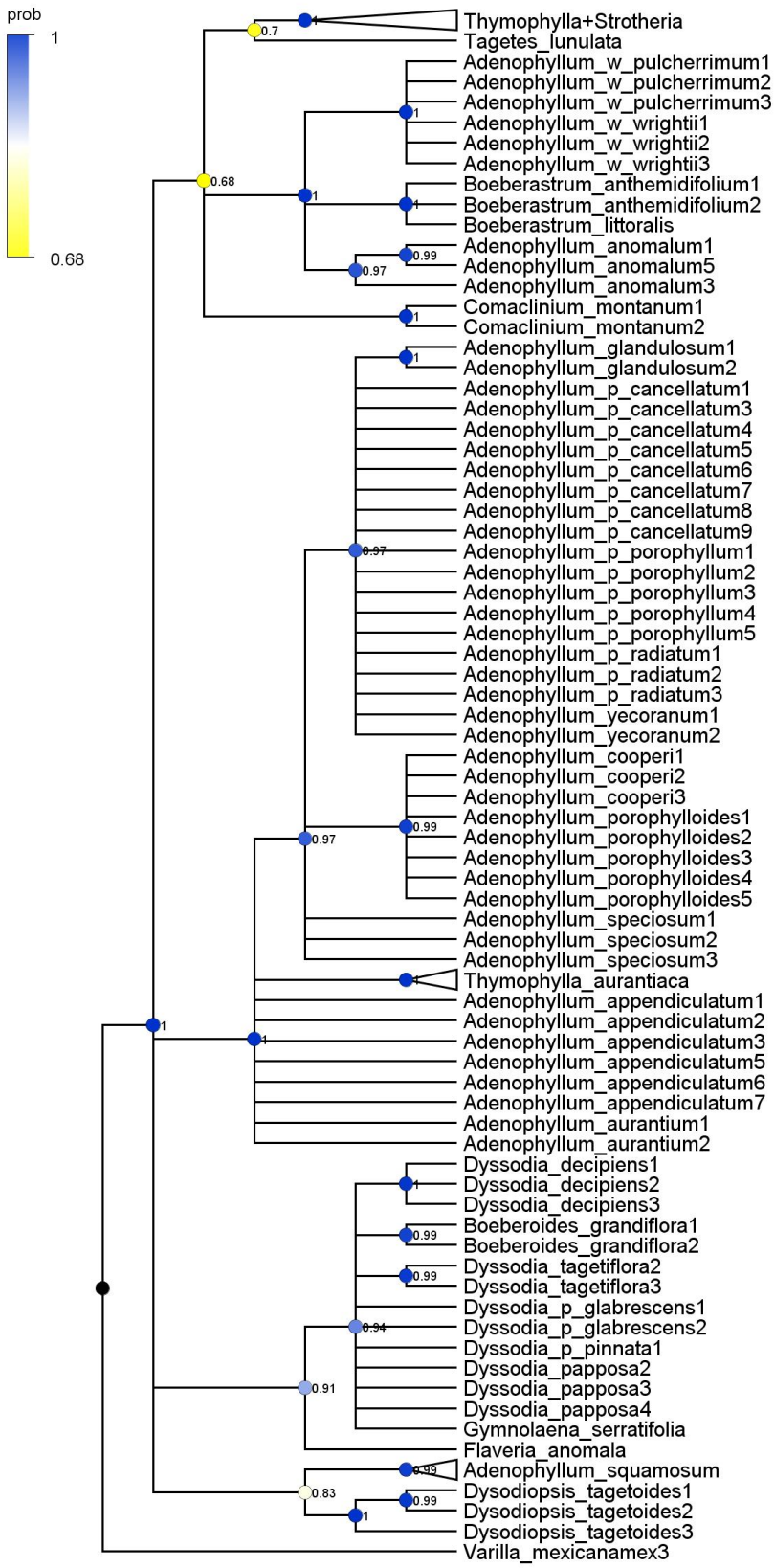
Thymophylla_p_belenidium2
Thymophylla_p_belenidium3
Thymophylla_p_belenidium4
Thymophylla_p_belenidium5
Thymophylla_p_hartwegii1
Thymophylla_p_hartwegii2
Thymophylla_p_hartwegii3
Thymophylla_p_puberula
Thymophylla_s_setifolia_d1
Thymophylla_s_setifolia_d2
Thymophylla_s_setifolia_d3
Thymophylla_s_setifolia_d4
Thymophylla_p_pentachaeta
Thymophylla_t_tenuiloba3
Thymophylla_t_texana1
Thymophylla_t_texana2
Thymophylla_t_treculii1
Thymophylla_t_treculii2
Thymophylla_t_wrightii1
Thymophylla_t_wrightii2
Thymophylla_mutica
Thymophylla_t_tenuiloba1
Thymophylla_t_tenuiloba2
Thymophylla_micropoides
Thymophylla_s_setifolia_r1
Thymophylla_s_setifolia_r2
Thymophylla_a_aurea2
Thymophylla_a_polychaeta1
Thymophylla_gentryi1
Thymophylla_gentryi2
Thymophylla_gentryi3
Thymophylla_a_aurea1
Thymophylla_a_aurea3
Thymophylla_a_polychaeta4
Thymophylla_tenuifolia1
Thymophylla_tenuifolia5
Thymophylla_a_polychaeta2
Thymophylla_a_polychaeta3
Thymophylla_tenuifolia2
Thymophylla_tenuifolia3
Thymophylla_tenuifolia4
Thymophylla_s_greggii2
Thymophylla_s_greggii3
Thymophylla_s_greggii1
Thymophylla_tephroleuca
Strotheria_gypsophila1
Strotheria_gypsophila2
Thymophylla_acerosa
Thymophylla_gypsophila3
Thymophylla_gypsophila1
Thymophylla_gypsophila2
Thymophylla_concinna1
Thymophylla_concinna2
Thymophylla_aurantiaca

Fig. S4.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Adenophyllum_w_pulcherrimum3
Adenophyllum_w_wrightii1
Adenophyllum_w_wrightii2
Adenophyllum_w_wrightii3
Boeberastrum_anthemidifolium1
Boeberastrum_anthemidifolium2
Boeberastrum_littoralis
Adenophyllum_anomalum1
Adenophyllum_anomalum5
Adenophyllum_anomalum3
Comaclinium_montanum1
Comaclinium_montanum2
Adenophyllum_glandulosum1
Adenophyllum_glandulosum2
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Adenophyllum_p_cancellatum3
Adenophyllum_p_cancellatum4
Adenophyllum_p_cancellatum5
Adenophyllum_p_cancellatum6
Adenophyllum_p_cancellatum7
Adenophyllum_p_cancellatum8
Adenophyllum_p_cancellatum9
Adenophyllum_p_porophyllum1
Adenophyllum_p_porophyllum2
Adenophyllum_p_porophyllum3
Adenophyllum_p_porophyllum4
Adenophyllum_p_porophyllum5
Adenophyllum_p_radiatum1
Adenophyllum_p_radiatum2
Adenophyllum_p_radiatum3
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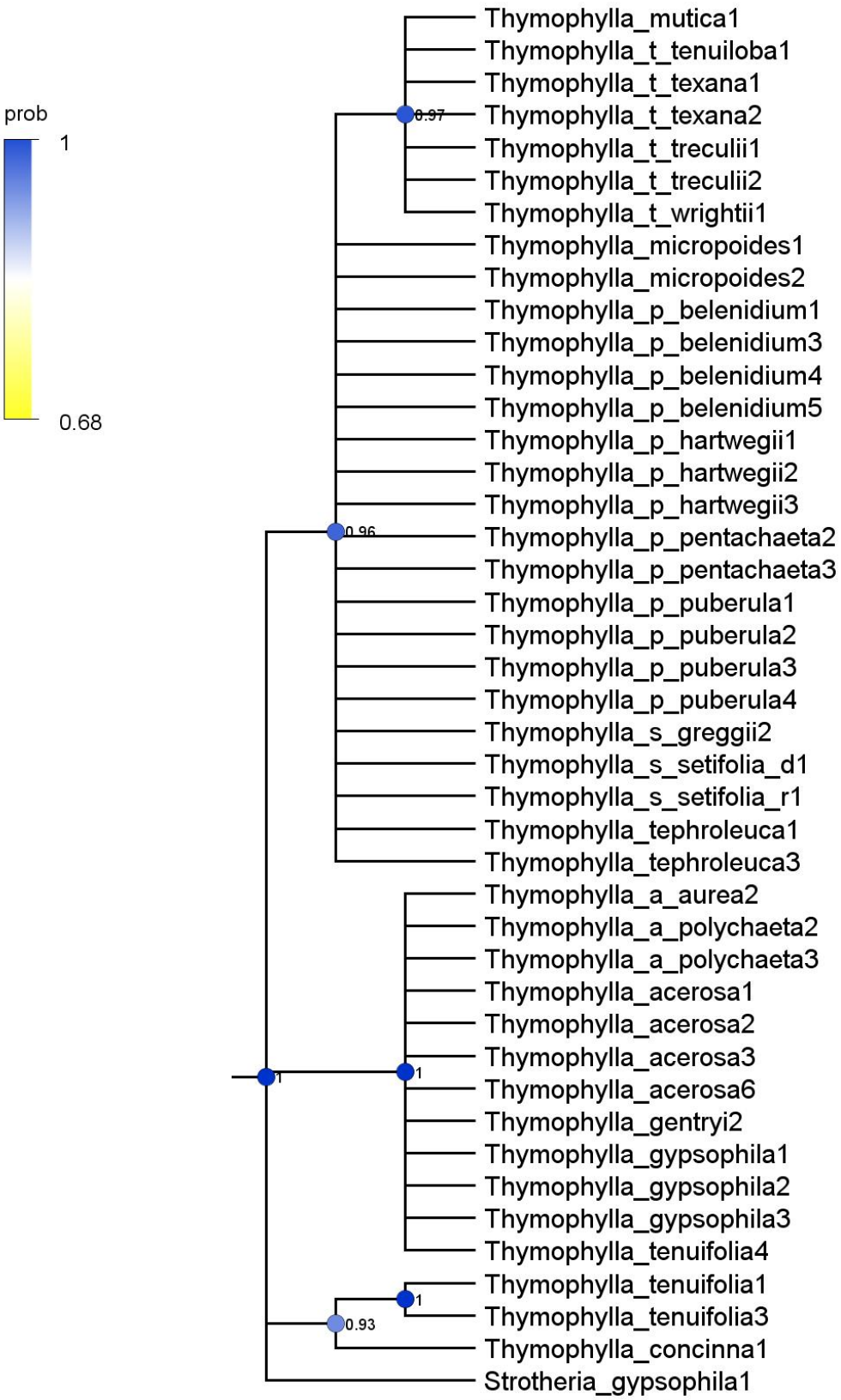
Adenophyllum_yecoranum2
Adenophyllum_cooperi1
Adenophyllum_cooperi2
Adenophyllum_cooperi3
Adenophyllum_porophylloides1
Adenophyllum_porophylloides2
Adenophyllum_porophylloides3
Adenophyllum_porophylloides4
Adenophyllum_porophylloides5
Adenophyllum_speciosum1
Adenophyllum_speciosum2
Adenophyllum_speciosum3
Thymophylla_aurantiaca
Adenophyllum_appendiculatum1
Adenophyllum_appendiculatum2
Adenophyllum_appendiculatum3
Adenophyllum_appendiculatum5
Adenophyllum_appendiculatum6
Adenophyllum_appendiculatum7
Adenophyllum_aurantium1
Adenophyllum_aurantium2
Dyssodia_decipiens1
Dyssodia_decipiens2
Dyssodia_decipiens3
Boeberoides_grandiflora1
Boeberoides_grandiflora2
Dyssodia_tagetiflora2
Dyssodia_tagetiflora3
Dyssodia_p_glabrescens1
Dyssodia_p_glabrescens2
Dyssodia_p_pinnata1
Dyssodia_papposa2
Dyssodia_papposa3
Dyssodia_papposa4
Gymnolaena_serratifolia
Flaveria_anomala
Adenophyllum_squamosum
Dysodiopsis_tagetoides1
Dysodiopsis_tagetoides2
Dysodiopsis_tagetoides3
Varilla_mexicanamex3

Fig. S5.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0.97
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Thymophylla_t_treculii1
Thymophylla_t_treculii2
Thymophylla_t_wrightii1
Thymophylla_micropoides1
Thymophylla_micropoides2
Thymophylla_p_belenidium1
Thymophylla_p_belenidium3
Thymophylla_p_belenidium4
Thymophylla_p_belenidium5
Thymophylla_p_hartwegii1
Thymophylla_p_hartwegii2
Thymophylla_p_hartwegii3
0.96
Thymophylla_p_pentachaeta2
Thymophylla_p_pentachaeta3
Thymophylla_p_puberula1
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Thymophylla_s_greggii2
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Thymophylla_s_setifolia_r1
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Thymophylla_a_polychaeta2
Thymophylla_a_polychaeta3
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Thymophylla_acerosa3
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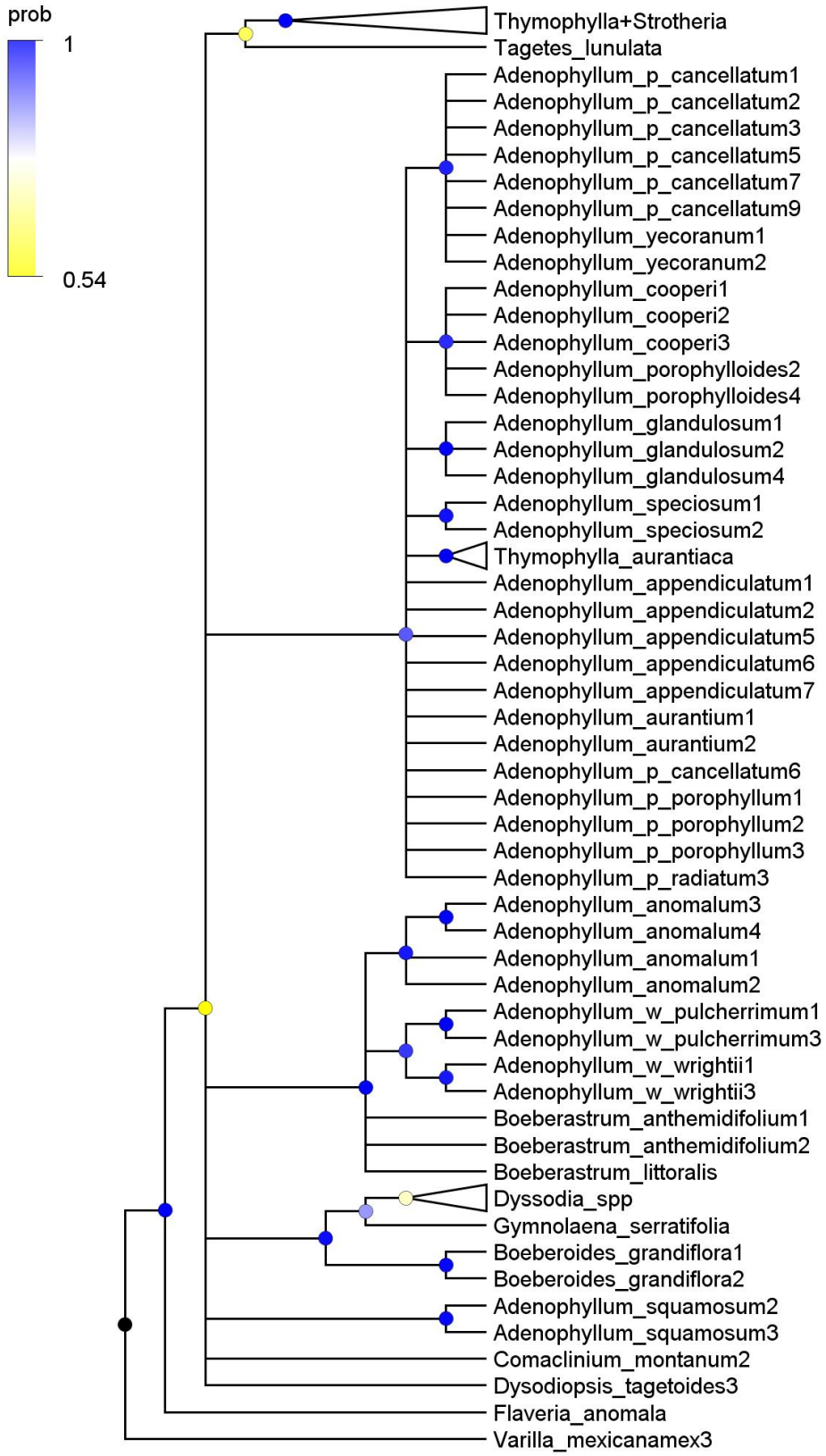
Fig. S6.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Adenophyllum_anomalum2
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Adenophyllum_w_pulcherrimum3
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Adenophyllum_w_wrightii3
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Dyssodia_spp
Gymnolaena_serratifolia
Boeberoides_grandiflora1
Boeberoides_grandiflora2
Adenophyllum_squamosum2
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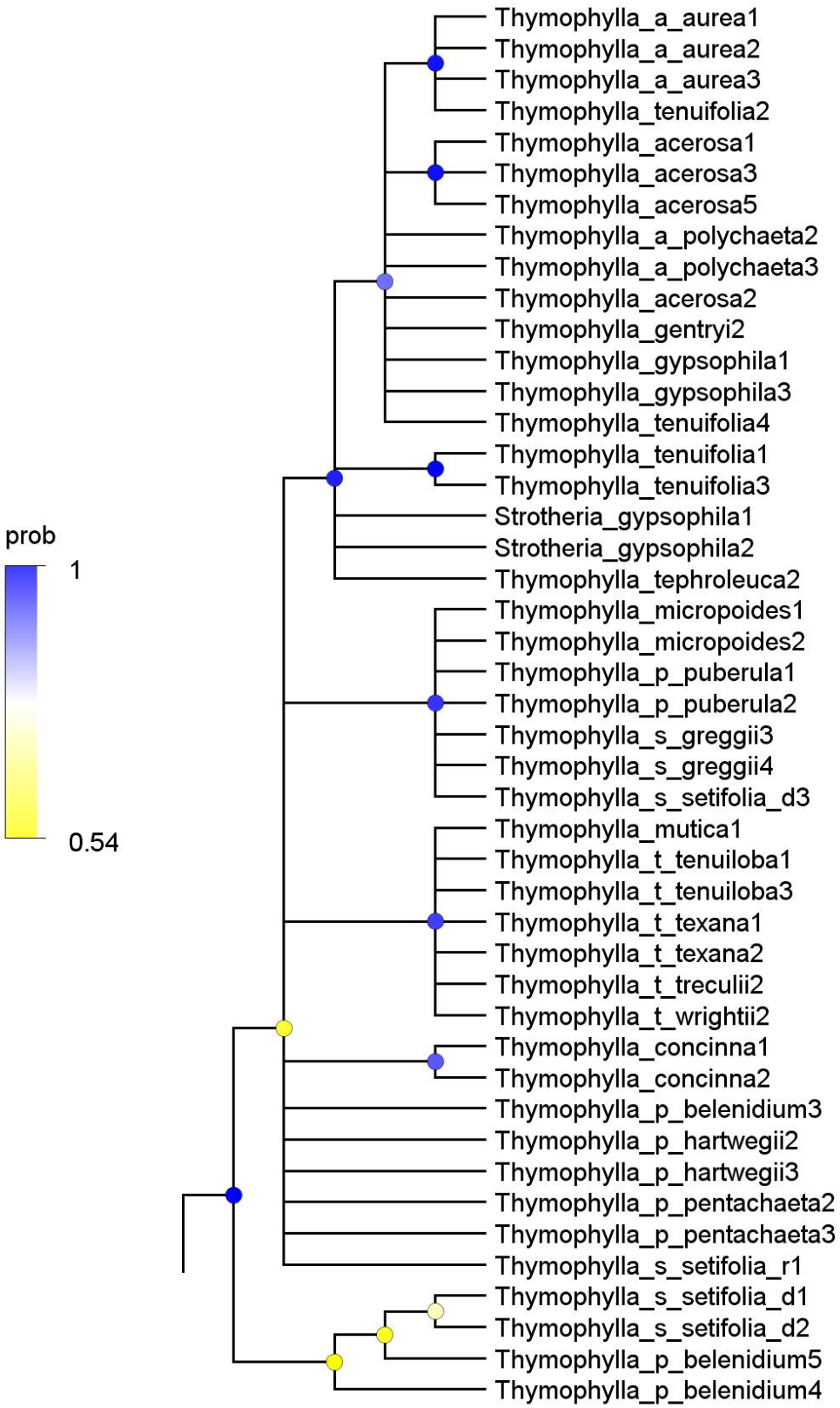
Fig. S7.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Thymophylla_micropoides2
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Thymophylla_s_greggii3
Thymophylla_s_greggii4
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Thymophylla_mutica1
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Thymophylla_t_texana1
Thymophylla_t_texana2
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Thymophylla_t_wrightii2
Thymophylla_concinna1
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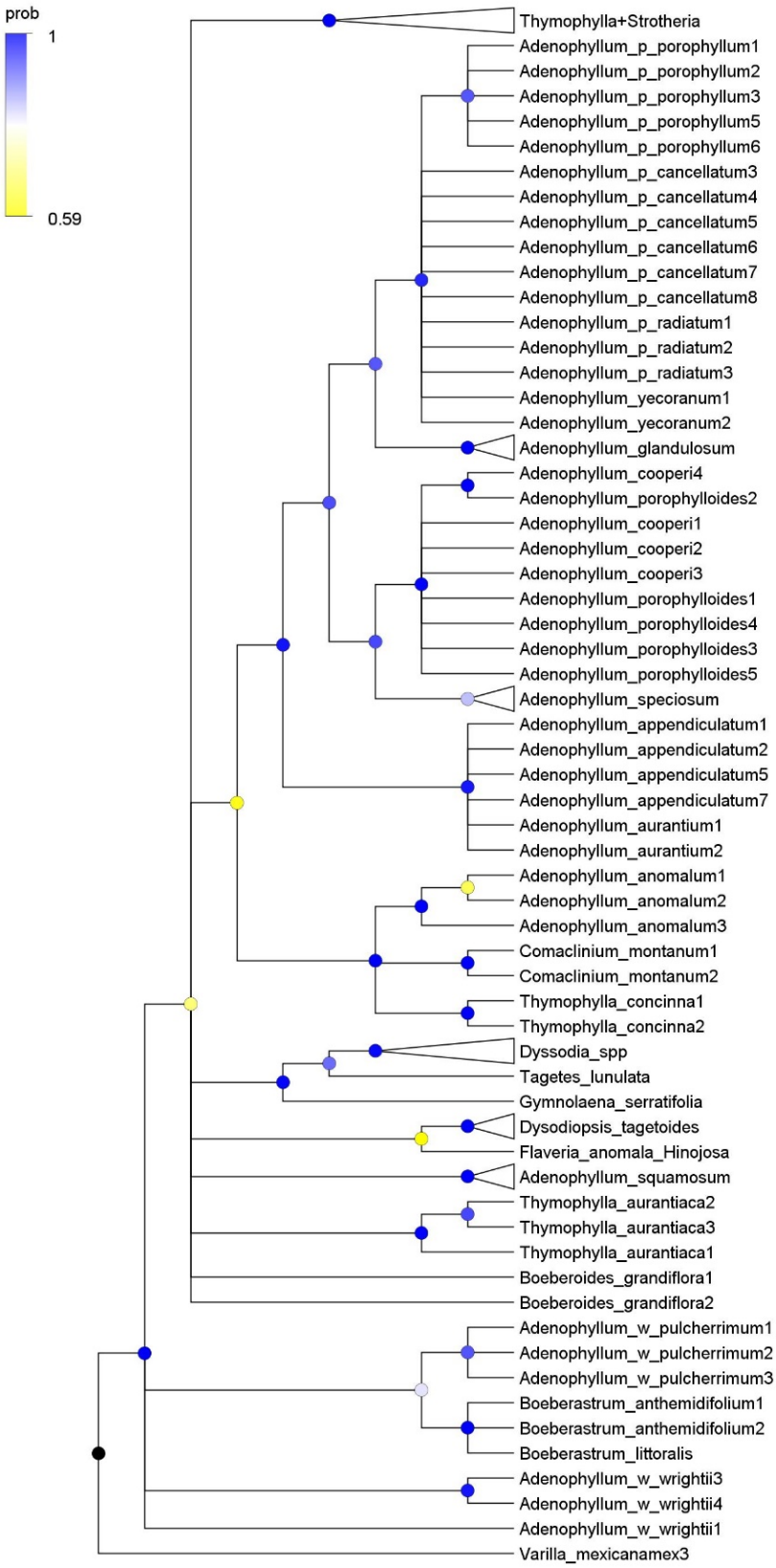
Fig. S8.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Adenophyllum_aurantium1
Adenophyllum_aurantium2
Adenophyllum_anomalum1
Adenophyllum_anomalum2
Adenophyllum_anomalum3
Comaclinium_montanum1
Comaclinium_montanum2
Thymophylla_concinna1
Thymophylla_concinna2
Dyssodia_spp
Tagetes_lunulata
Gymnolaena_serratifolia
Dysodiopsis_tagetoides
Flaveria_anomala_Hinojosa
Adenophyllum_squamosum
Thymophylla_aurantiaca2
Thymophylla_aurantiaca3
Thymophylla_aurantiaca1
Boeberoides_grandiflora1
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Adenophyllum_w_pulcherrimum1
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Adenophyllum_w_pulcherrimum3
Boeberastrum_anthemidifolium1
Boeberastrum_anthemidifolium2
Boeberastrum_littoralis
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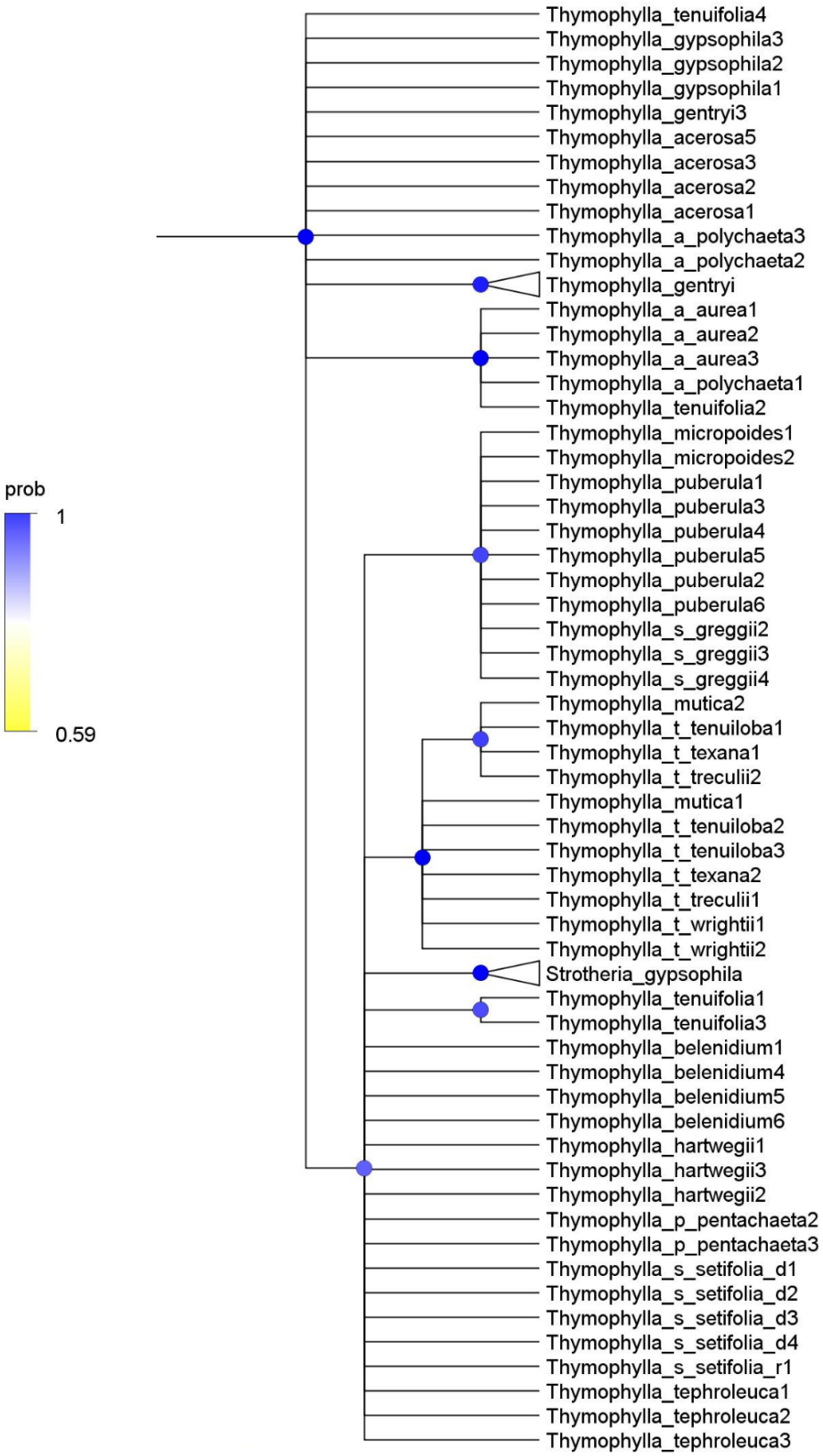
Varilla_mexicanamex3

Fig. S9.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Thymophylla_s_setifolia_r1
Thymophylla_tephroleuca1
Thymophylla_tephroleuca2
Thymophylla_tephroleuca3

Fig. S10.

Chapter 2

A systematic study of the marigold tribe, Tageteae *sensu lato* (Asteraceae)

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***Abstract*—**The tribe Tageteae includes popular cultivated species such as the marigold, Tagetes erecta L. The tribe is notable for the presence of secretory cavities filled with ethereal oils, which are responsible for the bitterness, spiciness, odors, and fragrances of many of its species. In its traditional sense, the tribe included only genera with secretory cavities, but previous phylogenetic analyses based on ITS sequences and limited taxon sampling showed that some genera lacking these structures were nested within the tribe and a broader classification including these other genera (Tageteae sensu lato) was proposed. In this study, we conducted phylogenetic analyses using ITS and ETS sequences and sampled all genera and ca. 75% of the species of Tageteae s.l. In addition, we estimated a calibrated phylogeny and use it to investigate the evolutionary history of the secretory cavities. We also studied the nature and presence of sclerified anther apical appendages in Tageteae s.l. Our results show that the tribe as currently circumscribed is paraphyletic. The genera with secretory cavities constitute a clade that also includes taxa lacking the cavities. It is more probable that the secretory cavities evolved once and eventually were lost at least once and that the sclerified anther appendages are symplesiomorphic. The use of additional sources of evidence such as plastid sequences are desirable to estimate a more robust and well-supported phylogeny that allow us to investigate more thoroughly the evolution of these and other characters in the tribe.

***Keywords*—**Anther apical appendages, ethereal oils, Flaveriinae, Heliantheae alliance, secretory cavities, Tagetinae.

INTRODUCTION

The tribe Tageteae is one of the 11 tribes within the Heliantheae alliance clade of the family Asteraceae (Susanna et al., 2020). Currently the tribe is composed of 32 genera and approximately 270 species (Panero 2007, Susanna et al. 2020). All species of Tageteae are native to the New World, although a few have been introduced in the Old World, mainly through horticulture. The center of diversity and endemism of the tribe is Mexico, where approximately 84% of the genera and 63% of the species of the tribe occur, and around 31% of the genera and ca. 41% of the species are endemic (Villasenor 2018). However, a more natural center of diversity is the region from the Southwest USA and the adjacent eastern Great Plains southwards to the xeric and semi-arid highlands and tropical deciduous forest of Mexico. Almost all genera are represented in this region, except for a pair of poorly known monotypic Cuban genera (*Harnackia* Urb. and *Leiscaillea* Griseb.) and *Schizotrichia* Benth., a small genus endemic to Peru (Strother 1977).

Most species of Tageteae are annuals, although there are also perennial herbs to shrubs. Most taxa are terrestrial, but there are also a few subaquatic species that occur mostly in shallow pools or on the banks of water bodies. In addition, some species from xeric region and salty soils exhibit succulence. The leaves are predominantly opposite and frequently once- or more- pinnately lobed. The capitula are almost always radiate, and the rays are mostly yellow to orange or red, rarely white to purple. The fruits (cypselae) are usually blackish at maturity due to phytomelanin and are almost always crowned by a pappus of scales, bristles, or scales dissected into bristles.

One of the most distinctive characteristics of many members of the tribe Tageteae is the presence of secretory cavities in the foliage, which are filled with ethereal oils, mostly monoterpenes (Strother 2006). These cavities often appear as pellucid dots, translucent lines, or gland-like structures (Figure 1). The ethereal oils are related to the medicinal, perfuming, ritual, insecticidal, and culinary uses of many species. Perhaps the most well-known are the species of Tagetes, especially the marigold, T. erecta L. (Figure 1), which was cultivated in Mesoamerica before European contact (Hinojosa and Schiavinato 2022, see Chapter 3). Another important medicinal species in Mexico is *Chrysactinia mexicana* A. Gray, which is known as *Hierba de San Nicolas* and is used to make medicinal infusions (Figure 1). In addition, the leaves of several species of Porophyllum, especially *Porophyllum macrocephalum* DC. (Figure 1), are eaten as greens in Mexico, where they are known as *papalo* or *papaloquelite*. The hispanicized word *papaloquelite* (from the Nahuatl or Aztec language) means butterfly greens, and it alludes to the look of the opposite leaves of some species. The leaves have a particular bitter taste due to the ethereal oils.

In other taxa, the ethereal oils give off a strong pungency and the plants are usually avoided by grazing animals. For instance, the smell of the fetid marigolds (*Dyssodia* spp., from the Greek *dyssos*- bad, and Latin *odor*- smell) is often considered unpleasant or even nauseating; however, they may be used as medicinals as well (Spellenberg et al. 2019). In addition to the uses related to ethereal oils, several species are used as ornamentals due to the beauty of the capitula. Some species are widely cultivated, such as *Tagetes erecta* L., but others are used more locally, such as *Boeberoides grandiflora* (DC.) Strother (Figure 1).

Traditionally, the tribe Tageteae only included taxa that had secretory cavities in their foliage. Strother (1977) stated that the naturalness of the Tageteae appeared never to have been doubted. Indeed, based on morphology, it would be understandable to assume that all

species within the Heliantheae alliance that have secretory cavities constitute a monophyletic group and these cavities are a synapomorphy. Despite this, the cladistic analyses based on morphology of Karis (1993) failed to resolve a monophyletic Tageteae, but only three terminals representing three genera of the traditional Tageteae (i.e., species bearing secretory cavities) were sampled.

The molecular phylogenetic analyses of Baldwin et al. (2002) showed that some genera previously classified in the tribe Helenieae (Arnicastrum Greenm., Clappia A. Gray, *Jamesianthus* S.F. Blake & Sherff, *Pseudoclappia* Rydb., and *Oxypappus* Benth.) were nested within the traditional Tageteae. Baldwin et al. (2002) presented a broader classification for the tribe, which was divided into four subtribes: Flaveriinae, Jaumeinae, Pectidinae, and Varillinae. We will refer to this classification as Tageteae *sensu lato* (s.l.). However, Baldwin et al.'s (2002) taxon sampling within the traditional Tageteae was limited to six species. The subtribe Pectidinae included the genera with secretory cavities, and with reservations, the five genera lacking secretory cavities mentioned above.

Based on nrDNA (ITS) and cpDNA (ndhF) sequences, Loockerman et al. (2003) resolved the Tageteae as a monophyletic group. Unfortunately, their analyses did not include any of the five genera without secretory cavities classified in Pectidinae by Baldwin et al. (2002), nor from the subtribes Jaumeinae or Varillinae. Nevertheless, their analyses sampled at least one species of all genera with secretory cavities. They found support for 1) the dismemberment of *Dyssodia sensu lato*; 2) the inclusion of *Adenopappus* Benth. and *Vilobia* Strother in *Tagetes*; and 3) a sister relationship between *Pectis* and *Porophyllum* and the segregation of *Bajacalia* from the latter. They also hypothesized a Mexican origin for the tribe and an ancestral chromosome number of x=12.

Panero (2007) presented a classification of Tageteae s.l. similar to that of Baldwin et al. (2002), except for two aspects: 1) *Clappia* and *Coulterella* Vasey & Rose were placed in their own subtribe; and 2) *Arnicastrum*, *Jamesianthus*, and *Pseudoclappia* were not assigned to any subtribe. Panero (2007) stated that most members of the Tageteae s.l. share striate cypselae with well-developed carpopodia, glabrous and strongly sclerified anther appendages, and a tendency to have a pappus of bristles or scales dissected into bristles. Baldwin (2009) rendered a systematic overview of the Heliantheae alliance clade. In this contribution, the current understanding of the phylogenetic relationships within this clade, to which Tageteae belongs, is discussed and summarized. More recently, Susanna et al. (2020) presented an updated classification of Asteraceae, in which seven subtribes within Tageteae s.l. were listed. This classification is mostly congruent with that of Panero (2007), except that the subtribes Pectidinae and Tagetinae were listed. Tagetinae Less. (1831) is often treated as a synonym of Pectidinae Less. (1830) (e.g., Robinson 1981, Strother 2006, Panero 2007). However, if these subtribes are considered synonyms, the name Tagetinae Dumort. (1829) has nomenclatural priority according to the *Indices Nominum Supragenericorum Plantarum Vascularium* (http://www.plantsystematics.org/reveal/pbio/fam/famAQ-AZ.html), and therefore this is the name that will be used in this manuscript.

Phylogenetic studies using molecular data and focused at the generic level have contributed to the understanding of the systematics of the Tageteae *sensu lato*. Mckown et al. (2005) inferred a phylogeny of *Flaveria*. They found two strongly supported subclades within *Flaveria* and estimated that the C4 photosynthesis pathway evolved twice. Hansen et al. (2016) investigated the phylogenetic relationships of the C_4 genus *Pectis* and its sister Porophyllum. They estimated a mean age of 11.27 Ma. for the divergence of *Pectis* and *Porophyllum* and a mean age of 24.34 Ma. for the origin of the tribe Tageteae. More recently, Hinojosa and

Schiavinato (2022) estimated phylogenetic relationships of the marigold genus *Tagetes* based on ITS sequences and Bayesian approaches. They resolved a monophyletic *Tagetes* and corroborated the inclusion of *Adenopappus* and *Vilobia* within the genus and the sister relationship between *Tagetes* and *Hydropectis* Rydb.

Recent studies focused above the tribal level provide additional insights to our understanding of the evolution of the marigold tribe. Mandel et al. (2019) resolved Tageteae sister to Millerieae using hundreds of low-copy nuclear loci and maximum likelihood approaches (ML). They estimated the split between Tageteae and Millerieae at a mean age of 20 Ma. However, only two species of Tageteae and one of Millerieae were sampled. In contrast, based on 11 chloroplast loci and ML approaches, Rivera et al. (2021) resolved a polyphyletic Tageteae, since *Dyssodia papposa* (Vent.) Hitchc. was nested within Bahieae. The other seven sampled species of Tageteae were resolved as a clade, which was sister to a paraphyletic Bahieae (with nested members of the tribe Chaenactideae and D. papposa). Rivera et al. (2021) estimated an origin for Tageteae (excluding *D*. *papposa*) at a mean age of 26.69 Ma.

In this study we investigate the phylogenetic relationships of the Tageteae sensu lato using nrDNA and the densest taxon sampling of the tribe to date. We address the hypothesis that the traditional Tageteae or *sensu stricto* (i.e., composed of genera with secretory cavities only) is monophyletic and the presence of secretory cavities is a synapomorphy for the clade. In addition, we estimate divergence times and reconstruct the evolutionary history of the secretory cavities. Also, we investigate if the presence of sclerified anther appendages is shared by all members of the Tageteae *sensu lato*.

Materials and methods

Sampling strategy—We sampled broadly within Tageteae including representatives of all subtribes, and as many genera and species as possible. However, we did not sample below the species level (e.g. at the rank of variety). Taxonomic concepts in *Adenophyllum* Pers. and *Thymophylla* Lag. followed the proposal of *Hinojosa-Espinosa* et al. (in prep., see Chapter 1). We tentatively recognized the new generic segregates *Adenophylloides* Hinojosa and *Thymophyllastrum* Hinojosa, the new specific segregate, *Adenophyllum radiatum* (DC.) Hinojosa, and *Thymophylla strotheriae* (B.L. Turner) Hinojosa, which is based on *Strotheria gypsophila* B.L. Turner. However, it is important to note that these names have not been effectively published yet. In addition, we sample four species from closely related tribes as outgroups: *Bahia ambrosioides* Lag. (Bahieae), *Chaenactis douglasii* (Hook.) Hook. & Arn. (Chaenactidinae), *Galinsoga quadriradiata* Ruiz. & Pav. (Millerieae), and *Helianthus annuus* L. (Heliantheae *sensu stricto*).

Molecular markers and methods—ITS was selected, taking advantage of the numerous sequences for species of Tageteae that are available at GenBank. We also selected ETS, which has been shown to be the source of additional phylogenetic characters that provide phylogenetic resolution comparable to that of ITS, both yielding considerably congruent topologies within the Tageteae (Mckown et al. 2005, Ma, in prep., Chapter 1) and other tribes (e.g., Baldwin and Markos, 1998). A total of 113 ITS and 20 ETS sequences were extracted from GenBank (Appendix 1). We also generated new ITS and ETS sequences for 87 and 88 species of Tageteae s.l. (Appendix 1). Leaves were removed from herbarium specimens from several institutions (ARIZ, CIIDIR, COCO, HCIB, IBUG, DAV, MEXU, NMS, RENO, SD, TEX, and UC). A few leaf samples and voucher specimens were also collected during field work in Mexico conducted

during 2019 and early 2020 and imported to the University of California, Davis herbarium (DAV), with appropriate valid permits.

DNA extraction, PCR, and sequencing—Molecular methods followed Hinojosa-Espinosa et al. 2022, in press., Chapter 4). In summary, DNA was extracted using the DNeasy Plant Kit (Qiagen, Valencia, California) using 1.5–20 mg of dry leaf tissue, which was finely ground by hand. Amplification and sequencing of the ITS region were conducted using the ITS5 (GGAAGTAAAAGTCGTAACAAGG) and ITS4 (TCCTCCGCTTATTGATATGC) primers of White et al. (1990). For the ETS region we used the modified Ast-1 (CGTAAAGGTGTGTGAGTGGTTT) and 18S-Alt (TGAGCCATTCGCAGTTTCACAGTC) primers of Lopes-Rivera et al. (2016), and in the few cases when these primers failed, we used the primer Ast-1 (CGTAAAGGTGCATGAGTGGTG) of Markos and Baldwin (2001) in conjunction with the primer 18-S (ACTTACACATGCATGGCTTAATCT) of Baldwin and Markos (1998). PCR reactions were conducted using the Taq PCR Core Kit (Qiagen, Valencia, California). The amplification protocol for all markers followed that of Rivera et al. (2016) with minor modifications. PCR samples were separated by gel electrophoresis and extracted and purified from gel slices using QIAquick Gel Extraction Kits (Qiagen, Valencia, California). PCR products were submitting for sequencing to the UC Davis College of Biological Sciences DNA Sequencing Facility.

Sequence editing and alignment—Sequencher 5.6 (Gene Codes Corporation, Ann Arbor, MI USA) was used to edit the sequences and to conduct BLAST searches to corroborate taxon and marker identity. MUSCLE v5 (Edgar 2021) was used to align the sequences using the CIPRES portal (Miller et al. 2010) followed by manual adjustments. Since the ITS sequences of *Harnackia* and *Lescaillea* extracted from GenBank were too short (288 and 359 bp, respectively), these were aligned separately, then inserted into the alignment produced by Muscle 5.0 and manually aligned. Gaps were mostly 1-4 bp in length and were treated as

missing data. Mesquite 3.70 (Maddison and Maddison 2021) was also used to export the alignments in different formats for phylogenetic analyses.

Data sets and phylogenetic analyses—The ITS data set contains all genera of Tageteae s.l. and 200 species, which represent 75% of species the tribe. The aligned ITS data set is composed of 204 terminals and 862 sites, of which 528 (61.25%) are variable. Notably, *Arnicastrum glandulosum* Greenm. and *A*. *guerrerense* Villasenor share a large insertion of 159 bp, which is larger in *A*. *glandulosum* by 121 additional bp. Also, *Jamesianthus* shared 47 bp of this insertion with *Arnicastrum* spp. In contrast, the ITS sequences of *Harnackia* and *Lescaillea* have 387 bp missing in the ITS1 region and 163 and 117 bp missing in the ITS2 region, respectively. The ETS data set contains 28 genera and 108 species of Tageteae s.l., which represent ca. 90% and 40% of the genera and species of the tribe, respectively. The aligned ETS data set includes 112 terminals and 403 characters, of which 338 (83%) were variable. The nrDNA concatenated data set includes 204 terminals and 1,265 characters, of which 866 (68.45%) were variable.

Bayesian analyses were conducted in MrBayes 3.2.7 (Ronquist et al. 2012) using the CIPRES portal. Three phylogenetic analyses were performed on the following data sets: 1) ITS; 2) ETS; and 3) nrDNA (i.e., a concatenated and partitioned ITS and ETS data set). No a priori model tests were performed to select a single nucleotide substitution model, but rather all general time reversible substitution models (i.e., 203 in total, including common models such as JC, HYK, TYK, and the widely used GTR of Tavare 1986) were evaluated during the Bayesian phylogenetic analyses by implementing Reversible Jump-Markov Chain Monte Carlo (RJ-MCMC) in MrBayes 3.2.7. This is a Bayesian approach to model selection that takes into account model selection uncertainty, as all 203 substitution models are analyzed during the MCMC and the models that best fit the data are sampled more frequently during the MCMC simulations and have higher posterior probabilities (Huelsenbeck et al. 2004). Following the nomenclature of Huelsenbeck et

al. (2004), the submodels M136 and M85 best fit the ITS and ETS data. The M136 submodel is similar to the GTR model of Tavare (1986), except the former assumes equal rates for the AC, AT, and GT substitution types, and the M85 submodel is similar to the model of Kimura (1981), except the former assumes that the rates AC and CG are equal (Huelsenbeck et al. 2004).

Two simultaneous and independent runs of 20 million generations were performed, sampling parameters every 2,000 generations and estimating nucleotide frequencies and substitution rates from the analyses. By default MrBayes summarizes and combines the sampled trees from the two runs in a majority-rule consensus (MRC) tree. Before using these consensus trees, Tracer 1.7. (Rambaut et al. 2018) was used to analyze the output files for MCMC convergence diagnostics. After corroborating that the two independent simultaneous runs had converged on the posterior distributions of the sampled parameters and reached stationarity, we proceeded to edit the MRC trees. FigTree (http://tree.bio.ed.ac.uk/software/figtree/) was employed to visualize and edit the phylogenetic trees. Further editing was performed in Adobe Illustrator (Adobe Inc., San Jose, California).

Divergence time analyses—Bayesian divergence time analyses were conducted in BEAST 2.6.6 (Bouckaert et al. 2019) using the CIPRES gateway. We used the same concatenated nrDNA data set to estimate divergence times. However, we reduced the number of terminals to include only the type species of each genus of the Tageteae s.l., except for the largest genera (*Pectis*, *Porophyllum*, *Tagetes*, *Flaveria*, *Thymophylla* and *Adenophyllum*), for which 3–5 species representing major lineages were included. Also, since the ETS sequence for the type of *Tagetes* (*T. erecta* L.) was not available for this study, we sampled the closely related *T. patula* L. instead. Indeed, Turner (1996) and Strother (1999) did not recognize *T. patula* as distinct from *T. erecta*. In addition, we follow a similar approach to that of Hansen et al. (2016), and included five more species representing major clades in Asteraceae, based on the well-supported phylogeny of

Mandel et al. (2019). The divergence time data set included 64 species, 55 of them representing all genera and main lineages of the Tageteae s.l.

Since the substitution models M136 and M85 are not available in BEAST 2.6.6, we used the GTR model with four gamma categories, and frequencies, substitution rates, and transition rates estimated during the analyses. The clock model was a relaxed uncorrelated lognormal clock with rates estimated during the analyses. We used most of the default priors, including the Yule tree prior with speciation rate estimated during the analyses. However, we changed the uniform distribution prior for the mean hyperparameter of the lognormal distribution to a normal distribution. In addition, we forced *Mutisia clematis* L. as the sister to the remaining taxa sampled, and calibrated this node based on the Mutisioideae capitula fossil date of 47.5 Ma (Barreda et al. 2012). Following Hansen et al. (2016), a lognormal distribution with a mean of 2.0, standard deviation of 0.5, and offset of 44.5 Ma were set to provide a minimum age of 47.5 Ma for the most recent common ancestor of the taxa sampled. Two independent MCMC chains of 80 million generations, sampling trees and parameters every 8,000 generations, were conducted using the CIPRES portal (Miller et al. 2010). We used Tracer 1.7. to verify that the two independent runs converged on the posterior probabilities and reached stationarity before combining the sampled trees into a single tree file. This was executed using the BEAST2.6.6 package LogCombiner, by discarding 25% of the initial sampled trees as burn-in. TreeAnnotator (another BEAST package) was used to construct a Maximum Clade Credibility (MCC) tree from the combined trees annotating the mean heights of each node. FigTree was used to visualize and edit the time-calibrated MCC tree and Adobe Illustrator was employed for additional editing.

Ancestral character state reconstruction—Analyses of the ancestral state reconstruction of the secretory cavities were conducted in RASP 4.0 (Yu et al. 2020) using the package Bayes Trait

(Meade and Pagel, 2018). A random subsample of 1000 trees from the combined trees and the MCC calibrated tree from the divergence analyses were used as input. Species outside the Heliantheae alliance clade were removed from the analyses. Secretory cavities were coded as present (A), absent (B), or uncertain (AB), as in the case of *Clappia* in which it is unclear whether the dot-like secretory structures in the leaves are homologous to those found in Tageteae s.s. Two independent MCMC chains of 1,010,000 generations sampling every 100 generations and discarding the first 10,000 trees were applied.

Morphological studies of the anther appendages—We studied the sclerification of the appendages of the anthers for 27 species of Tageteae representing almost all genera and all subtribes recognized for the Tageteae s.l. by Panero (2007). Samples for only four genera (*Arnicastrum*, *Harnackia*, *Lescaillea*, and *Schizotrichia*) of the tribe *sensu* Panero (2007) were not available for this study. We studied if this feature could be a synapomorphic character for the Tageteae s.l. as implied by Panero (2007). We tested for the presence of lignin in the anther appendages by treating the anthers with saturated HCl-Phloroglucinol, which stains lignified tissue pink-red. We expect that such lignification is confined to the Tageteae s.l. if the trait is a synapomorphy for the tribe. Therefore, we also studied the anther appendages of five species from members of other tribes within the Heliantheae alliance: *Bahia absinthifolia* Benth. (Bahieae), *Chaenactis douglasii* (Hook.) Hook. & Arn. (Chaenactideae), *Lasianthaea* sp. (Heliantheae), and *Carminatia recondita* McVaugh and *Stevia lucida* Lag. (both in Eupatorieae).

One to three disk florets were extracted from herbarium specimens deposited at the University of California, Davis herbarium (DAV), National Herbarium of Mexico (MEXU), and University of Texas at Austin (TEX). Next, 200 ml of HCl-Phloroglucinol solution was prepared using 35% HCl, which was diluted down to 20% with DI water. Then phloroglucinol was added until saturation. Subsequently, the florets were rehydrated by boiling them in 20 ml of water for

45 seconds. Then the corollas were dissected to remove the anthers, which were transferred to clean microscope slides. The excess water was removed with clean paper towels and a drop of HCl-Phloroglucinol phloroglucinol solution was added. After 5-10 minutes a clean coverslip was placed over the anthers. We used an Olympus compound microscope (Olympus Corp., Tokyo) for anther appendage examination and a mounted ProgRess digital camera (Jenoptik AG, Germany) for image capture.

RESULTS

Phylogeny of the Tageteae s.l.—The majority-rule consensus trees from the Bayesian analyses of the ITS (Figure 2) and ETS (Figure 3) are considerably congruent, although they differ in node support and in the phylogenetic position of certain lineages as described below. In general, most relationships at the species level, and in some instances at the rank of genus, were resolved but the relationships above the genus level were mostly uncertain. The majority-rule consensus tree from the concatenated ITS and ETS data sets (Figure 4) yielded higher support, but most of the deeper nodes are still weakly supported.

The monophyly of the tribe Tageteae s.l. is not supported since one or more members of the tribes Bahieae, Chaenactideae, and Millerieae are nested within the clade that included Tageteae s.l. The majority-rule consensus tree from the concatenated ITS and ETS data set resolved a strongly supported clade composed of the subtribes Flaveriinae, Coulterellinae, and Varillinae, and the representatives of the tribes Bahieae, Chaenactideae, and Millerieae. This clade is also resolved in the ITS and the ETS majority-rule consensus trees, but it is weakly supported in the latter and it does not include the members of the Chaenactideae and Millerieae.

The subtribe Flaveriinae was resolved as paraphyletic in the ETS phylogeny since Coulterellinae (represented by *Coulterella capitata* Vasey & L. Rose) was nested within *Sartwellia* A. Gray. However, Coulterellinae was sister to Flaveriinae in the majority-rule consensus tree from the ITS plus ETS phylogenetic analyses, and sister to Varillinae (represented by *Varilla* spp.) in the ITS majority-rule consensus tree. Within Flaveriinae, *Haploësthes* A. Gray was not resolved as monophyletic. In the majority-rule consensus tree of the ITS phylogenetic analyses, *Flaveria macdougallii* M.E. Theroux, Pinkava & D.J. Keil was nested within *Haploësthes*. The clade comprising these taxa (i.e., *Haploësthes* plus *F. macdougalli*) was weakly supported. In the majority-rule consensus tree of the ETS phylogenetic analyses, *Coulterella capitata*, *Sartwellia*, and *Flaveria macdougallii* were nested within *Haploësthes*. The majority-rule consensus tree of the combined ITS plus ETS phylogenetic analyses resolved *Sartwellia* and *F. macdougallii* nested within *Haploësthes*. Jaumeinae (represented by *Jaumea carnosa*) was resolved as an isolated lineage within the clade constituted of Coulterellinae, Flaveriinae, Varillinae, and the tribes Chaenactideae and Bahieae, and Millerieae in the nrDNA majority-rule consensus tree, and most consistently resolved with Coulterellinae, Flaveriinae, and Varillinae across the ITS, ETS, and combined nrDNA analyses.

The subtribe Tagetinae sensu Panero (2007) is not inferred as monophyletic, since genera lacking secretory cavities (*Arnicastrum*, *Clappia*, and *Jamesianthus* in the ITS and nrDNA consensus trees, and *Pseudoclappia* and *Oxypappus* in the ETS consensus tree) are nested within its clade. Two major strongly supported subclades were resolved in the ITS and concatenated nrDNA consensus trees. One of them is composed by *Pectis* and its sister genus *Porophyllum*, and the other includes all remaining Tagetinae plus the genera lacking secretory cavities.

Most genera of Tagetinae are resolved as monophyletic and the phylogenetic relationships inferred for *Adenophyllum*, *Boeberastrum* (A. Gray) Rydb., *Boeberoides* (DC.) Strother, *Dyssodia*, *Flaveria*, *Pectis*, *Porophyllum*, *Tagetes*, and *Thymophylla* are consistent with previous molecular phylogenetic studies (Hansen et al. 2016, Hinojosa and Schiavinato 2022, Hinojosa-Espinosa et al. in prep., McKown et al. 2005, Loockerman et al. 2003). The sister relationships among 1) *Arnicastrum* and *Jamesianthus* (Baldwin et al. 2002); 2) *Pectis* and *Porophyllum*; and 3) *Tagetes* and *Hydropectis* are also corroborated. The inclusion of *Strotheria gypsophila* within *Thymophylla* and the transfer of *Adenophyllum anomalum* Canby & Rose and *Adenophyllum wrightii* A. Gray to *Boeberastrum* (Hinojosa-Espinosa et al. in prep., Chapter 1) are also corroborated. *Comaclinium* (Benth.) Strother and *Schizotrichia* are inferred as sister genera in the ITS phylogeny with low support, but with strong support in the consensus from the concatenated nrDNA analyses. They are also inferred as members of a clade composed of *Dyssodia*, *Boeberoides*, and *Gymnolaena* (DC.) Rydb. The monotypic genera *Leucactinia* Rydb. and *Urbinella* Greenm. are inferred as sister taxa, with Bajacalia Loockerman, R.K. Jansen & B.L. Turner as sister to both. The proposed segregate Adenophylloides (Hinojosa-Espinosa et al. in prep., Chapter 1) is corroborated as a distinct lineage from *Adenophyllum*, and *Thymophyllastrum*, another proposed segregate from *Thymophylla* (Hinojosa-Espinosa et al. in prep.), is resolved as sister to *Thymophylla*, although its lineage has a longer branch.

Divergence times estimation—The topology and supporting values of the MCC calibrated tree (Figure 5) is highly congruent with those of the majority-rule consensus trees from the non-calibrated analyses of the concatenated nrDNA, except for a few exceptions discussed below. The most recent common ancestor (MRCA) of the non-monophyletic Tageteae s.l. is inferred at a mean age of 31.55 Ma; however, within this clade the representatives of the tribes Bahieae, Chaenactideae, and Millerieae are also included.

The subtribes Coulterellinae, Flaveriinae, Jaumeinae, and Varillinae shared a MRCA with the representatives of the Bahieae and Chaenactideae, and this ancestor is estimated at a mean age of 25.68 Ma. Varillinae and Jaumeinae are resolved as sister subtribes, but this relationship is weakly supported. Flaveriinae and Coulterellinae shared a MRCA estimated at a mean age of 15.22 Ma. The MRCA of Flaveriinae originated at a mean age of 8.36 Ma. The MRCA of the Tagetineae clade (including *Arnicastrum*, *Clappia*, and *Jamesianthus*) is strongly supported and estimated at a mean age of 24.78 Ma. *Oxypappus*, is the sister to Tagetinae, but their MRCA (estimated at a mean age of 26.1 Ma) is not strongly supported. However, the MRCA of *Pseudoclappia*, *Oxypappus*, and Tagetinae is strongly supported and inferred at a mean age of 27.48 Ma.

Within the Tagetinae clade, the MRCA of *Pectis* and *Porophyllum* is estimated at a mean age of 19.49 Ma. *Arnicastrum* and its sister *Jamesianthus*, whose MRCA is estimated at a mean age of 3.72 Ma. are resolved as sister to the *Pectis*/*Porophyllum* subclade, although this relationship is not well supported. *Adenophyllum wrightii*, which Hinojosa-Espinosa et al. (in prep., Chapter 1) proposed to transfer to *Boeberastrum*, shared a MRCA with *B*. *anthemidifolium* inferred at a mean age of 9.34 Ma. The MRCA of the *Bajacalia*, *Leucactinia*, and *Urbinella* subclade (BLU) is estimated at a mean age of 12.97 Ma. *Clappia* shared a MRCA with the BLU clade, but this relationship is not well supported. *Hydropectis* is resolved as sister to Tagetes, but the relationship is weakly supported. In addition, the Cuban *Harnackia* and *Lescaillea*, whose MRCA is estimated at a mean age of 6 Ma, are resolved as sister to the Hydropectis/Tagetes subclade, although this relationship is not well supported either. The MRCA of the Dyssodia clade (including *Comaclinium*, *Schizotrichia*, *Gymnolaena* and *Boeberastrum*) had a mean age of 11.31 Ma. and the MRCA of Tagetes is estimated at a mean age of 11.54 Ma. The closely related

Adenophyllum-*Thymophylla* clade (including *Dysodiopsis* (A. Gray) Rydb. and the proposed segregates *Adenophylloides* and *Thymophyllastrum*) had a mean age of 14.44 Ma.

Ancestral state reconstruction of the secretory cavities—The MCC tree showing the reconstruction of the ancestral states for presence or absence of secretory cavities is shown in Figures 6–7. The most probable state at all nodes within the Tagetinae clade is presence of secretory cavities, except for the MRCA of *Arnicastrum* and *Jamesianthus*, in which the trait is inferred as absent. Presence of secretory cavities is also inferred as the most probable state for the MRCA of Tagetinae and *Oxypappus*, and for the MRCA of *Pseudoclappia* and *Oxypappus*/ Tagetinae. However, at these two nodes the probability for absence of secretory is 30.86 and 0.38, respectively. Similarly, there is a probability of 0.35 that the absence of secretory cavities is the ancestral state for the MRCA of *Pectis* and *Porophyllum*.

Anther appendages sclerification—The apical appendages of the sampled species were mostly concave and usually lanceolate to broadly ovate in shape, occasionally oblong elliptic, and rarely reduced and emarginate. Since the morphology of the appendages was quite similar in most species studied (Appendix 2), only the lignification for selected taxa is shown in Figure 7. Most, but not all species studied of Tageteae s.l. tested positive for lignin as the cells strongly stained pink-red. In some cases the staining and cell wall thickening was stronger in the apical and marginal cells. Strongly lignified apical appendages were also found in representatives from the tribes Bahieae, Chaenactideae, and Heliantheae. In these species the appendages were also concave. Moreover, the abaxial surface of the appendages of *Bahia absinthifolia*, *Chaenactis douglasii*, and *Coulterella capitata* were characterized by the presence of glandular trichomes. Apart from *C. capitata*, glandular trichomes were not found in any other studied species of Tageteae s.l.

In contrast, the anther appendages of the Tageteae s.l. representatives, *Adenophyllum glandulosum* (Cav.) Strother, *C. capitata*, *Hydropectis aquatica* (S. Watson) Rydb., *Oxypappus scaber* Benth., *Pectis multiflosculosa* (DC.) Sch. Bip. and *Urbinella palmeri* Greenm. stained only slightly pink-red or did not stain at all (*C. capitata*). Similarly, the appendages of the Eupatorieae species stained only slightly pink-red. In all these cases, the cell walls were more homogeneous and thin in all cells constituting the appendages. Moreover, the appendages in Eupatorieae were flat, not concave. Notably, the apical appendage of *Pectis multiflosculosa* was emarginate. Since the appendages of A. glandulosum did not react to HCl-phloroglucinol, we also tested *A. cancellatum* (Cass.) Villarreal and in this case the appendages strongly stained pink-red (Figure 7). In these two species the anther appendages also differed in shape and cell wall thickness.

Discussion

Phylogenetic relationships of Tageteae s.l.—In this study we present the most densely sampled phylogeny for the Tageteae to date. The results support the hypothesis that Tageteae s.l. is paraphyletic. According to our results, the subtribes Coulterellinae, Jaumeinae, Flaveriinae, and Varillinae of the tribe Tageteae s.l. are more closely related to the tribes Bahieae, Chaenactideae, and Millerieae than to the subtribe Tagetinae. The species in all these subtribes (and tribes) lack the characteristic secretory cavities of Tageteae s.s., in the narrowest sense. Therefore, these subtribes might warrant removal from the Tageteae. In addition, our results support the view that the Tageteae is not closely related to any other single tribe, such as Millerieae (e.g. Mandel et al. 2019), but to a clade composed of multiple tribes, as previously resolved by Rivera et al. (2021). On the other hand, the nesting of the representatives of the tribes Bahieae, Chaenactideae, and Millerieae within the Tageteae s.l., may also be the result of scarce outgroup sampling (one species per tribe). We noticed great intergeneric variation in the

ITS and ETS sequences of the taxa sampled, and lacking additional outgroup representatives from these tribes may have had an impact on the accuracy of the sequence alignment. Therefore, future phylogenetic analyses including more members of tribes Bahieae, Chaenactideae, and Millerieae may resolve a monophyletic Tageteae s.l. These analyses should also include representatives of tribe Neurolaeneae, since Panero and Crozier (2016) found this tribe closely related to Tageteae, Bahieae, and Chaenactideae.

The subtribe Tagetinae is also paraphyletic if it is circumscribed narrowly to include only genera with secretory cavities (e.g., Panero 2007), but it is evidently monophyletic if the subtribe also includes *Clappia*, *Arnicastrum*, and *Jamesianthus* (Baldwin et al. 2002). Based on our results, Tagetinae is also monophyletic if the subtribe is expanded to include the genera *Oxypappus* and *Pseudoclappia* in addition to *Arnicastrum*, *Clappia*, and *Jamesianthus* as proposed by Baldwin et al. (2002). *Pseudoclappia* and *Oxypappus* might be seen as the closest relatives of the traditional Tageteae that lack secretory cavities. Additional studies may show that this is also true for *Arnicastrum* and *Jamesianthus*, since the ETS consensus tree showed that *Arnicastrum* and *Jamesianthus* are sister to Tagetinae. Only ITS showed that these eglandular genera are nested within Tagetinae, but combining ITS and ETS results in an unclear phylogenetic position for the *Arnicastrum-Jamesianthus* subclade (i.e., an unsupported sister relationship to *Pectis-Porophyllum*). *Clappia* on the other hand, is clearly nested within Tagetinae in all analyses and therefore it must be considered a true member of this clade unless further evidence contradicts this hypothesis.

The close relationship between *Clappia* and *Arnicastrum-Jamesianthus* found by Baldwin et al. (2002) and noted by them as only weakly supported in their parsimony trees is not supported by any of our analyses. However, our ITS and ETS analyses corroborated the sister relationship between *Arnicastrum* and *Jamesianthus* (Baldwin et al. 2002). Panero (2007) did

not assign these genera to a subtribe, and based on our results *Arnicastrum* and *Jamesianthus* might be justifiably classified in their own subtribe. However, due to their unclear phylogenetic position within the Tagetinae clade, we suggest to treat them in Tagetinae. It is notable that the inserted 120 bp segment in the ITS region in *A*. *guerrerense*, which was considered as probably the largest ITS insertion in the angiosperms (Baldwin et al. 2002) is even larger in the type species, *A*. *glandulosum* (280 bp).

Although the limits to resolution within the subtribe Tagetinae blur the understanding of phylogenetic relationships among its members, some hypotheses of relationship were strongly supported and our study is also the source of new testable hypothesis. For instance, our results suggest that the Tagetinae clade is composed of two main subclades, one of them composed of *Pectis* and its sister genus *Porophyllum* and the other constituted by the remaining members of the Tagetinae (including the genera lacking secretory cavities). *Pectis* had been seen as an isolated lineage within the Tageteae (e.g., Strother 1977). This hypothesis was not supported by the analyses of Hansen et al. (2016), and our results support the view that both *Pectis* and *Porophyllum* represent a distinct lineage within the tribe.

Our study also corroborates the close relationship among *Bajacalia*, *Leucactinia*, and *Urbinella* found by Loockerman et al. (2003). They suggested that *Leucactinia* and *Urbinella* are congeneric and since they are resolved as sister taxa in our analyses as well, these monotypic genera arguably might be treated in the same genus if not for their morphological differences. However, it is desirable to corroborate these results with other sources of evidence, such as plastid DNA sequences and/or low-copy nuclear genes. *Leucactinia* and *Urbinella* are endemic to north-central Mexico, while *Bajacalia* is confined to the Peninsula of Baja California.

Boeberoides may also be transferred to *Gymnolaena* as proposed by Hinojosa-Espinosa et al. (in prep., Chapter 1) based on the results of phylogenetic analyses using nrDNA and cpDNA

sequences and morphological similarities between the two genera. Indeed, *Dyssodia sensu stricto* might be re-expanded in part to include *Comaclinium*, *Schizotrichia*, *Boeberoides*, and *Gymnolaena* since this clade seems well-supported based on nrDNA and cpDNA sequences (Hinojosa-Espinosa et al. (in prep., Chapter 1). However, we could not generate ETS sequences of *Schizotrichia*, as samples of this Peruvian genus were unavailable. Therefore, additional samples and data are needed to fully investigate the potential expansion of *Dyssodia sensu stricto* to include the genera *Boeberoides*, *Gymnolaena*, *Comaclinium*, and *Schizotrichia*.

ETS sequences were not available for the Cuban genera *Harnackia* and *Lescaillea* and their ITS sequences have almost 50% missing data. Thus, their apparent close relationship to the *Tagetes*/*Hydropectis* subclade requires further investigation. Likewise, the sister relationship between *Coulterella* and Flaveriinae must be taken with caution since the ETS sequence of *Coulterella*, which came from an herbarium specimen that yielded poor quality DNA, was noisy and had several ambiguities. Moreover, the ITS consensus tree suggests a sister relationship between *Coulterella* and Varillinae. On the other hand, *Coulterella* is similar to *Flaveria* in its succulent habit, reduced heads, and in some chemistry compounds such as thiophenes (Robinson 1981). Therefore, the sister relationship among *Coulterella* and Flaveriinae may not be misleading. The phylogenetic relationships of *Coulterella* remain unclear and warrant further investigation.

The *Adenophyllum*-*Thymophylla* clade, which also includes *Strotheria* and *Dysodiopsis*, is corroborated and strongly supported as monophyletic by our results. Similarly, the transfer of Strotheria and the species of the Trichaetolepis group of *Adenophyllum* to *Thymophylla* and *Boeberastrum*, respectively, are also corroborated and supported by our results. However, the relationships of *Dysodiopsis* and the new genera *Adenophylloides* and *Thymophyllastrum*, proposed by Hinojosa-Espinosa et al. (in prep., Chapter 1), are still unclear within this clade.

Additional examples of genera whose relationships within Tagetinae are not resolved are *Chrysactinia* A. Gray and *Nicolletia* A. Gray. Each of these genera were resolved as monophyletic isolated lineages within the Tagetinae.

Within the subtribe Flaveriinae our results suggest that *Haploësthes* and *Sartwellia* may warrant treatment as congeneric. Moreover, the fact that *Flaveria mcdougallii* is nested within the *Haploësthes-Sartwellia* clade makes *Flaveria* paraphyletic. In general, these three genera are quite similar morphologically, differing mainly by features of the pappus. Perhaps *Flaveria* may warrant re-circumscription to include *Sartwellia* and *Haploësthes*. Alternatively, since *Haploësthes* has nomenclatural priority, both *Sartwellia* and *F. mcdougallii* could be transferred to *Haploësthes*. According to our results, *Flaveria* is monophyletic if *F. mcdougallii* is excluded from the genus.

Since we used nrDNA data only, the hypotheses discussed above require testing from other sources of evidence, such as plastid DNA sequence data and/or low copy targeted nuclear genes (e.g., Mandel et al. 2019), before any taxonomic changes are made. The use of additional characters may also help to clarify the ambiguous or weakly supported relationships within the Tageteae based on ITS and ETS. The use of ITS and ETS provided a reasonable level of resolution at the species and sometimes at the genus level within Tageteae and also resolved phylogenetic relationships among the major lineages of Asteraceae in the divergence time analyses (Figure 5), but our results suggest that these markers lacked adequate variation to resolve relationships above the rank of the genus in the marigold tribe.

Divergence times estimation—The estimated mean age for the MRCA of Tageteae s.l. (31.55 Ma.) is older than the estimated age by Mandel et al. (2019) and Rivera et al. (2021) (20 Ma and 26.69 Ma, respectively). However, the MRCA of Tageteae s.l. in our analyses also includes representatives of the tribes Bahieae, Chaenactideae, and Millerieae, but the estimated mean

age (27.48) for the MRCA of *Pseudoclappia*, *Oxypappus*, and Tagetinae is more congruent with the mean age inferred by Rivera et al. (2021). We also estimated an older mean age for the origin of the *Pectis-Porophyllum* MRCA (19.49 Ma.) than Hansen et al. (2016), who inferred a mean age of 15.92 Ma, despite using the same fossil age for calibration and a similar approach to estimate divergence times (i.e., adding representatives of major clades of Asteraceae) as Hansen et al. (2016). The estimated ages for the species diversification in *Dyssodia* and the *Adenophyllum-Thymophylla* clade were also slightly older than those estimated by Hinojosa-Espinosa et al., (in prep., Chapter1). This might be due to differences in taxon sampling, as Hansen et al. (2016) focused on *Pectis* and *Porophyllum* and Hinojosa-Espinosa et al. on *Adenophyllum* and *Thymophylla* rather than focusing on a few species from all genera of the tribe.

The slightly older ages might be also due to the data used to infer the calibrated phylogenies. Hansen et al. (2016) used several plastid markers rather than ITS and ETS as used in this study. Both ITS and ETS are more rapidly evolving than coding regions of plastid genes, such as ndhF (Baldwin, com. pers.), which may lead older estimations. In addition, alignment of ITS and ETS sequences throughout the Asteraceae is more challenging than alignment of slower evolving plastid genes, which are also more conserved and have a higher percentage of unsaturated sites. Therefore, future divergence time analyses of Tageteae s.l. should consider using plastid genes (e.g., ndhF, matK) rather than nrDNA sequences. The resulted divergence time estimations from such analyses should be compared with our results based on nrDNA sequences, which are described below.

Our estimations suggest that the MRCA of *Pseudoclappia*, *Oxypappus*, and the Tagetinae clade (Figure 5) originated in the Miocene and that resolved lineage diversification continued into the Pliocene. Since the Tagetinae are more diverse and abundant in the xeric regions of

Mexico and adjacent USA (Strother 1977), it is possible that the origin and expansion of the North American deserts during the Miocene (Graham 2011) favored speciation and lineage diversification of the Tagetinae clade. Moreover, the postulated formation of climate change refugia in the tropical deciduous forests of Mexico (Galvin et al. 2014) may have also favored species diversification in those lineages of the Tagetinae clade that predominantly occur in this type of vegetation, such as *Adenophyllum* (Hinojosa-Espinosa et al. in prep., Chapter 1). Additional examples of genera that are restricted to the tropical deciduous forests of Mexico are *Boeberoides* and its sister genus *Gymnolaena*.

Ancestral character reconstruction of the secretory cavities—The most probable scenario for the evolution of the secretory cavities that have been traditionally considered diagnostic of the tribe Tageteae is that they originated only once in the MRCA of subtribe Tagetinae in the Miocene, ca. 25 Ma. The descendants from this node inherited the secretory cavities, including the MRCA of *Pectis* and *Porophyllum*, until they were subsequently lost in the lineage leading to the MRCA of *Arnicastrum* and *Jamesianthus* on the one hand, and likely to the lineage leading to *Clappia* on the other. However, additional morphological studies are needed to clarify if the dot-like secretory structures in *Clappia* are homologous to the secretory cavities of the traditional Tageteae. If they are homologous, then the secretory structures in *Clappia* should be lined with an epithelium of secretory parenchyma cells as this is the anatomical constitution of secretory cavities of typical Tageteae genera (Martinez-Quesada et al. 2022). If these parenchyma cells are absent, that would suggest that the secretory structures in *Clappia* are homologous to the resinous canals found in other members of the Heliantheae alliance, as Robinson (1981) stated.

To our current understanding, secretory cavities containing ethereal oils in the family Asteraceae are confined to the Tagetinae clade. Based on our results, these structures would

have evolved only once in the family. However, a different pattern of evolution may be postulated if further phylogenetic studies resolve phylogenetic relationships among the genera that are ambiguous or weakly supported in this study. Further analyses would also be useful to test additional evolutionary hypothesis of character evolution within Tageteae. For instance, Strother (1977) provided a list of ancestral and derived character states within the tribe. This list includes traits such as ray color, in which the yellow and red colors were considered ancestral states and the white and purple colors the derived states. A more resolved and strongly supported phylogeny of the tribe would also help to test hypothesis of historical biogeography.

Anther appendage sclerification—The results from the morphological study of the anther apical appendages suggest that this characteristic is not a synapomorphy of Tageteae s.l., but rather a symplesiomorphy, since it is also shared at least with members of the Bahieae, Chaenactideae, and Heliantheae *sensu stricto*. In addition, not all members of Tageteae s.l. shared the strong lignification of the anther appendages. However, there is a possibility that the absence of lignification in the anther appendages of *Coulterella capitata* is due to the quality of the sample, since the florets used were extracted from an old herbarium specimen. Using fresher samples to test for lignin presence as generally done in this study may result in a positive test. It is also desirable to sample additional species for a broader understanding of the distribution of this feature in the Tageteae and related tribes.

The function of anther appendage lignification is another topic that requires further consideration. According to Jeffrey (2009) the sclerified apical appendages may have a defensive function against pest pressure. Jeffrey also stated that the non-asteroid Asteraceae in which ornithophily is common, have anther appendages that are also sclerified, and therefore the lignification may be a response to large pollinators. However, this does not seem to be the case for apical appendages in the Tageteae s.l., in which the flowers are not pollinated by large

pollinators such as birds. Jeffrey (2009) also suggested that the sclerified apical appendages of the anthers in the Heliantheae alliance may protect the young style branches and provide support for their subsequent elongation (Jeffrey 2009). Since the apical appendages are concave and fully cover the opening of the anther tube before style elongation, the hypothesis that anther sclerification protects the young style branches seems more plausible for the Tageteae.

CONCLUSIONS

The phylogenetic analyses conducted in this study do not support the monophyly of the tribe Tageteae s.l., since the subtribes Coulterellinae, Jaumeinae, Flaveriinae, and Varillinae, are supported as more closely related to other closely related tribes in the Heliantheae alliance. Similarly, the subtribe Tagetinae *sensu* Panero (2007) is also evidently paraphyletic. However, the subtribe is monophyletic if the genera lacking secretory cavities that are nested within it (*Clappia*, *Arnicastrum*, and *Jamesianthus*) or closely related to it (*Oxypappus*, *Pseudoclappia*) are included in Tagetinae, as treated by Baldwin et al. (2002).

Our results also suggest that *Flaveria* and *Haploësthes* are paraphyletic. One way to deal with this is transferring *F. mcdougallii* and *Sartwellia* to *Haploësthes*. Similarly, the monotypic genera *Leucactinia* and *Boeberoides* may warrant transfer to their respective sister genera, that is *Urbinella*, and *Gymnolaena*.

Although the phylogeny presented in this study is based on the densest sampling of the Tageteae to date, by including ca. 75% of the estimated species and 100% of the genera at least in the ITS analyses, it remains necessary to sample more species of the Peruvian genus *Schizotrichia*, and to obtain ETS sequences for this genus and for the Cuban genera *Harnackia* and *Lescaillea*. In addition, other sources of phylogenetic evidence are desirable, such as plastid DNA sequences and/or additional low-copy nuclear gene sequences. Adding more characters

may help to elucidate the phylogenetic relationships that are still ambiguous or unsupported within the subtribe Tagetinae.

The divergence time analyses based on nrDNA sequences yielded older ages estimations in comparison with previous studies. This may be due to the rapidly evolving nature of the ITS and ETS sequences and future divergence time analyses should consider using plastid gene sequences, which evolve slower and the sequences can be aligned throughout the Asteraceae with more accuracy. The results of such analyses should be compared with ours based on nrDNA, which suggest that the clade of the traditional Tageteae (i.e., composed of the subtribe Tagetinae and five genera lacking secretory cavities), originated in the Miocene at a mean age of 24.78 Ma and continued its lineage diversification into the Pleistocene. The secretory cavities would have evolved once in the MRCA of this clade and subsequently were lost in at least the MRCA of *Jamesianthus* and *Arnicastrum*.

The strong sclerification seen in the apical appendages of the anthers in the Tageteae s.l. and related tribes is due to lignin. In addition to lignification, the marginal and terminal cells of the appendages are also thickened. Since members of other tribes, including Bahieae, Chaenactideae, and Heliantheae, have lignified anther apical appendages, this characteristic is probably a symplesiomorphy rather than a synapomorphy for the Tageteae s.l.

Finally, this investigation is an important contribution to the systematics of the tribe Tageteae. However, further phylogenetic studies are still required for a better understanding of the phylogeny and macroevolution of the marigold tribe.

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Appendix 1. Taxa, vouchers and GenBank accessions used in the phylogenetic analyses [ITS; ETS; —not available]. *Name not effectively published yet.

Adenophylloides** *squamosum* (A. Gray) Hinojosa, *Sanders 10677* (MEXU). ***Adenophyllum *anomalum* (Canby & Rose) Strother, *Gonzalez 6563* (CIIDIR). *Adenophyllum aurantium* (L.) Strother, *Ventura 18313* (MEXU). *Adenophyllum cancellatum* (Cass.) Villarreal, *Hinojosa-Espinosa 742* (DAV, MEXU, UAT). *Adenophyllum glandulosum* (Cav.) Strother, *Hinojosa-Espinosa* 630 (MEXU). *Adenophyllum porophylloides* (A. Gray) Strother, *Leon 3267* (MEXU). *Adenophyllum porophyllum* (Cav.) Hemsl., *Hinojosa-Espinosa 686* (DAV, MEXU). *Adenophyllum pulcherrimum* (Strother) Villarreal, *Hinojosa-Espinosa 722* (DAV, MEXU). **Adenophyllum radiatum* (DC.) Hinojosa, *Martinez 143321* (MEXU). *Adenophyllum speciosum* (A. Gray) Strother, *Campos 4499* (MEXU). *Adenophyllum wrightii* (A. Gray) Strother, *Stavast* s.n. (NMC). ***Arnicastrum*** *glandulosum* Greenm., *Bye 8729* (CIIDIR). *Arnicastrum guerrerense* J.L. Villasenor [AF374924;—]. ***Bahia*** *ambrosioides* Lag. [KX260975; KX261014]. ***Bajacalia*** *crassifolia* (S. Watson) Loockerman, B.L. Turner & R.K. Jansen, *Rebman 34803* (SD). *Bajacalia tridentata* (Benth.) Loockerman, B.L. Turner & R.K. Jansen, *Webster 19585* (DAV). ***Boeberastrum*** *anthemidifolium* (Benth.) Rydb., *Dominguez 4830* (HCIB). *Boeberastrum littoralis* (Brandegee) Rydb., *Leon 12270* (HCIB). ***Boeberoides*** *grandiflora* (DC.) Strother, *Hinojosa-Espinosa 72* (MEXU). ***Chaenactis*** *douglasii* (Hook.) Hook. & Arn. [GU818511; GU818134]. ***Chrysactinia*** *acerosa* S.F. Blake, *Turner 6321* (TEX). *Chrysactinia luzmariae* Rzed. & Calderón, *Zamudio 10786* (TEX). *Chrysactinia mexicana* A. Gray, *Hinojosa-Espinosa 748* (DAV, MEXU). *Chrysactinia pinnata* S. Watson, *Webster 20498* (DAV). *Chrysactinia truncata* S. Watson, *Yahara 1458* (TEX). ***Clappia*** *suaedifolia* A. Gray, *Hinojosa-Espinosa 766* (DAV, MEXU, UAT). ***Comaclinium*** *montanum* (Benth.) Strother, *Sinaca 2264* (MEXU). ***Coulterella*** *capitata* Vasey & L. Rose, *Panero 2841* (UC) [AF374927], *Johnson 48* (DAV). ***Dysodiopsis*** *tagetoides* (Torr. & A. Gray) Rydb., *Gostel 447* (BRIT). ***Dyssodia*** *decipiens* (Bartl.) M.C. Johnst.,

Lopez 1575 (MEXU). *Dyssodia papposa* (Vent.) Hitchc., *Hinojosa-Espinosa 684* (DAV, MEXU). *Dyssodia pinnata* (Cav.) B.L. Rob., *Hinojosa-Espinosa 749* (DAV, MEXU). *Dyssodia tagetiflora* Lag., *Hinojosa-Espinosa 678* (DAV, MEXU). ***Flaveria*** *angustifolia* A. Gray [DQ122482; DQ122427]. *Flaveria anomala* B.L. Rob., *Hinojosa-Espinosa 753* (DAV, MEXU). *Flaveria australasica* Hook. [DQ122530; DQ122402]. *Flaveria bidentis* (L.) Kuntze [DQ122543; DQ122410]. *Flaveria brownii* A.M. Powell [DQ122499; DQ122419]. *Flaveria campestris* J.R. Johnst. [DQ122533; DQ122413]. *Flaveria chloraefolia* A. Gray [DQ122493; DQ122425]. *Flaveria cronquistii* A.M. Powell [DQ122475; DQ122431]. *Flaveria floridana* J.R. Johnst. [DQ122484; DQ122421]. *Flaveria kochiana* B.L. Turner [DQ122538; DQ122404]. *Flaveria linearis* Lag., Kennecky 6294 (DAV). *Flaveria mcdougallii* M.E. Theroux, Pinkava & D.J. Keil [DQ122546; DQ122451]. *Flaveria oppositifolia* (DC.) Rydb. [DQ122495; DQ122416]. *Flaveria palmeri* J.R. Johnst. [DQ122535; DQ122411]. *Flaveria pringlei* Gand. [DQ122462; DQ122438]. *Flaveria pubescens* Rydb. [DQ122497; DQ122415]. *Flaveria ramosissima* Klatt [DQ122523; DQ122414]. *Flaveria robusta* Rose [DQ122476; DQ122430]. *Flaveria sonorensis* A.M. Powell [DQ122520; DQ122429]. *Flaveria trinervia* (Spreng.) C. Mohr, Webster 10220 (DAV). *Flaveria vaginata* B.L. Rob. & Greenm. [DQ122537; DQ122409]. ***Galinsoga*** *quadriradiata* Ruiz & Pav. [GU818550; GU818169]. ***Gochnatia*** *vernonioides* Kunth [MN457808; MN457770]. ***Gymnolaena*** *chiapasana* Strother, Cronquist 11219 (MEXU). *Gymnolaena oaxacana* (Greenm.) Rydb., *Torres 430* (MEXU). *Gymnolaena serratifolia* (DC.) Rydb., Messer 172 (MEXU). ***Haploësthes*** *fruticosa* B.L. Turner, *Moore 1934* (TEX). *Haploësthes greggii* A. Gray, *Moore 1476* (TEX). *Haploësthes hintoniana* B.L. Turner, *Moore 2001* (TEX). *Haploësthes robusta* I.M. Johnst., *Henrickson 17440* (TEX); *Powell 2619* (TEX). ***Harnackia*** *bisecta* Urb. [AF413615; —]. ***Helianthus*** *annuus* L., *Schilling 660* [KX671853; HQ688886]. ***Hydropectis*** *aquatica* (S. Watson) Rydb.), *Soule 2796* (DAV). *Hydropectis stevensii* McVaugh, *Perez-Calix 4607* (TEX). ***Jamesianthus*** *alabamensis* S.F. Blake &

Sherff, Kral *44062* (TEX). ***Jaumea*** *carnosa* (Less.) A. Gray, *Park 196* (DAV); *Hinojosa-Espinosa 773* (DAV). ***Lescaillea*** *equisetiformis* Griseb. [AF413616; —]. ***Leucactinia*** *bracteata* (S. Watson) Rydb., Poole 2497 (TEX). ***Nicolletia*** *edwardsii* A. Gray, *Hinojosa-Espinosa 709* (CIIDIR, DAV, MEXU). *Nicolletia occidentalis* A. Gray, *Andre 24940* (SD). *Nicolletia trifida* Rydb., *Webster 33811* (DAV). ***Oxypappus*** *scaber* Benth., *Sundberg 2944* (TEX). ***Pectis*** *angustifolia* Torr. [KJ524916.1; —]. *Pectis arida* D.J. Keil [KJ525031.1; —]. *Pectis barberi* Greenm. [KJ525069.1; —]. *Pectis berlandieri* DC. [KJ524921.1; —]. *Pectis bonplandiana* Kunth [KJ524922.1; —]. *Pectis brevipedunculata* Sch. Bip. [KJ525030.1; —]. *Pectis canescens* Kunth [KJ525029.1; —]. *Pectis capillipes* DC. [KJ524925.1; —]. *Pectis carthusianorum* Less. [KJ524926.1; —]. *Pectis ciliaris* L. [KJ524983.1; —]. *Pectis coulteri* Harv. & A. Gray [KJ524930.1; —]. *Pectis cubensis* (A. Rich.) Griseb. [KJ524932.1; —]. *Pectis cylindrica* (Fernald) Rydb. [KJ524934.1; —]. *Pectis decemcarinata* McVaugh [KJ525050.1; —]. *Pectis depressa* Fernald [KJ525063.1; —]. *Pectis diffusa* Hook. & Arn. [KJ525048.1; —]. *Pectis elongata* Kunth var. *floribunda* (A. Rich.) D.J. Keil [KJ524938.1; —]. *Pectis ericifolia* D.J. Keil [KJ524941.1; —]. *Pectis exilis* D.J. Keil [KJ525049.1; —]. *Pectis exserta* McVaugh [KJ525055.1; —]. *Pectis filipes* Harv. & A. Gray [KJ524943.1; —]. *Pectis glaucescens* (Cass.) D.J. Keil [KJ524948.1; —]. *Pectis graveolens* Klatt [KJ525028.1; —]. *Pectis haenkeana* (DC.) Sch. Bip. [KJ525056.1; —]. *Pectis holochaeta* (S.F. Blake) D.J. Keil [KJ525057.1; —]. *Pectis humifusa* Sw. [KJ524952.1; —]. *Pectis imberbis* A. Gray [KJ524955.1; —]. *Pectis incisifolia* I.M. Johnst. [KJ524956.1; —]. *Pectis latisquama* A. Gray [KJ524957.1; —]. *Pectis leavenworthii* Standl. [KJ525047.1; —]. *Pectis leonis* Rydb. [KJ524959.1; —]. *Pectis liebmannii* Sch. Bip. ex Hemsl. [KJ525059.1; —]. *Pectis linearifolia* Urb. [KJ524961; —]. *Pectis linearis* La Llave [KJ524962.1; —]. *Pectis linifolia* L. [KJ525052.1; —]. *Pectis longipes* A. Gray [KJ524965.1; —]. *Pectis luckoviae* D.J. Keil [KJ525053.1; —]. *Pectis multiceps* Urb. [KJ524967.1; —]. *Pectis multiflosculosa* (DC.) Sch. Bip. [KJ524969.1; —]. *Pectis multiseta* Benth. [KJ525061.1; —]. *Pectis odorata* Griseb.

[KJ525026.1; —]. *Pectis oligocephala* Sch. Bip. [KJ524974.1; —]. *Pectis papposa* Harv. & A. Gray var. *papposa* [KJ524977.1; —]. *Pectis papposa* Harv. & A. Gray var. *grandis* D.J. Keil, *Hinojosa-Espinosa 710* (ETS only) (CIIDIR, DAV, MEXU). *Pectis portoricensis* Urb. [KJ524978.1; —]. *Pectis pringlei* Fernald [KJ524979.1; —]. *Pectis propetes* Greenm. [KJ524980.1; —]. *Pectis prostrata* Cav. [KJ524984.1; —]. *Pectis purpurea* Brandegee var. *sonorae* D.J. Keil [KJ524985.1; —]. *Pectis pusilla* Urb. [KJ524986.1; —]. *Pectis repens* Brandegee [KJ525039.1; —]. *Pectis saturejoides* (Mill.) Sch. Bip. [KJ525037.1; —]. *Pectis sessiliflora* (Less.) Sch. Bip. [KJ524990.1; —]. *Pectis sinaloensis* Fernald [KJ524992.1; —]. *Pectis* stella Malme [KJ525025.1; —]. *Pectis* sp., *Castro 4667* (ETS only) (CIIDIR). *Pectis stenophylla* A. Gray [KJ524994.1; —]. *Pectis tenuicaulis* Urb. [KJ524996.1; —]. *Pectis tenuifolia* (DC.) Sch. Bip. [KJ524997.1; —]. *Pectis uniaristata* DC. [KJ524999.1; —]. *Pectis vandevenderi* B.L. Turner [KJ525070.1; —]. *Pectis vollmeri* Wiggins [KJ525001.1; —]. ***Porophyllum*** *amplexicaule* Engelm. ex A. Gray [KJ525002.1; —]. *Porophyllum angustissimum* Gardner [KJ525003.1; —]. *Porophyllum calcicola* B.L. Rob. & Greenm. [KJ525004.1; —]. *Porophyllum coloratum* (Kunth) DC. var. *obtusifolium* (DC.) McVaugh [KJ525006.1; —]. *Porophyllum filiforme* Rydb. [KJ525008.1; —]. *Porophyllum gracile* Benth., Rebman 29921 (SD). *Porophyllum lanceolatum* DC. [KJ525011.1; —]. *Porophyllum leiocarpum* (Urb.) Rydb. [KJ525012.1; —]. *Porophyllum lindenii* Sch. Bip. [KJ525015.1; —]. *Porophyllum linifolium* (Ard.) DC. [KJ525043.1; —]. *Porophyllum macrocephalum* DC., Clark s.n. (DAV). *Porophyllum maritimum* Brandegee [KJ525062.1; —]. *Porophyllum pausodynum* B.L. Rob. & Greenm. [KJ525018.1; —]. *Porophyllum punctatum* (Mill.) S.F. Blake [KJ525020.1; —]. *Porophyllum ruderale* (Jacq.) Cass., *Webster 32113* (DAV). *Porophyllum scoparium* A. Gray [KJ525022.1; —]. *Porophyllum tagetoides* (Kunth) DC., *Hinojosa-Espinosa 685* (DAV, MEXU). *Porophyllum viridiflorum* (Kunth) DC. [KJ525023.1; —]. *Porophyllum zimapanum* B.L. Turner [KJ525024.1; —]. ***Pseudoclappia*** *arenaria* Rydb., *Carr 20044* (TEX). *Pseudoclappia watsonii* A.M.

Powell & B.L. Turner, *Powell 6517* (TEX). ***Sartwellia*** *flaveriae* A. Gray, Moore 3692 (TEX). *Sartwellia gypsophila* A.M. Powell & B.L. Turner, *Moore 2427* (TEX). *Sartwellia mexicana* A. Gray, *Henrickson 22701* (TEX). *Sartwellia puberula* Rydb., *Stuessy 937* (DAV). ***Schizotrichia*** *jelskii* (Hieron.) Strother ex Loockerman, B.L. Turner & R.K. Jansen [AF413606; —]. ***Tagetes*** *campanulata* Griseb., *Soule 3553* (TEX) [AF413574; —]. *Tagetes erecta* L., Hansen 126 (TEX) [KJ525046.1; —]. *Tagetes filifolia* Lag. [DQ862118.1; —]. *Tagetes foetidissima* DC. [DQ862119.1; —]. *Tagetes lacera* Brandegee, *Medel 2014-03* (HCIB). *Tagetes laxa* Cabrera [KC800431.1; —]. *Tagetes lemmonii* A. Gray, *Reina 1120* (HCIB). *Tagetes lucida* Cav., *Hinojosa-Espinosa 676* (DAV, MEXU). *Tagetes lunulata* Ortega, *Hinojosa-Espinosa 723* (DAV, MEXU). *Tagetes micrantha* Cav., *Hinojosa-Espinosa 724* (DAV, MEXU). *Tagetes multiflora* Kunth [KC800434.1; —]. *Tagetes minuta* L. [AF413576; —]. *Tagetes moorei* H. Rob. [KC800433.1; —]. *Tagetes nelsonii* Greenm., *Hinojosa-Espinosa 731* (CH, DAV, MEXU). *Tagetes palmeri* A. Gray, *Soule 3362* (TEX) [AF413577; —]. *Tagetes parryi* A. Gray [KC800427.1; —]. *Tagetes patula* L. [DQ862121.1; AF319761]. *Tagetes persicifolia* (Benth.) B.L. Turner, *Sundberg 2954* (TEX), [AF413580; —]. *Tagetes praetermissa* (Strother) H. Rob., *Balls 6183* (UC) [AF413581; —]. *Tagetes pringlei* S. Watson, *Soule 2798* (TEX) [AF413578; —]. *Tagetes subulata* Cerv., *Rebman 30705* (SD). *Tagetes tenuifolia* Cav. (*sensu* Turner 1996, *T. lunulata sensu* Rzedowski 2005), *Hinojosa-Espinosa 677* (DAV).

****Thymophyllastrum*** *aurantiacum* (Brandegee) Hinojosa, *Hinojosa-Espinosa 691* (DAV, MEXU). ***Thymophylla*** *acerosa* (DC.) Strother, *Hinojosa-Espinosa 672* (DAV). *Thymophylla aurea* (A. Gray) Greene, *Pealand 4679* (COCO). *Thymophylla belenidium* (DC.) Cabrera, *Atwood 25001* (DAV). *Thymophylla concinna* (A. Gray) Strother, *Webster 23801* (DAV). *Thymophylla gentryi* (M.C. Johnst.) Strother, *Hinojosa-Espinosa 705* (CIIDIR, DAV, MEXU). *Thymophylla greggii* A. Gray, *Yahara 1745* (MEXU). *Thymophylla gypsophila* (B.L. Turner) Strother, *Henrickson 2260* (MEXU). *Thymophylla micropoides* (DC.) Strother, *Hinton 24265* (MEXU). *Thymophylla mutica* (M.C.

Johnst.) Strother, *Hinojosa-Espinosa 739* (DAV, MEXU, UAT). *Thymophylla pentachaeta* (DC.) Small, *Hinojosa-Espinosa 737* (DAV, MEXU, UAT). *Thymophylla puberula* Rydb., *Garcia 474* (MEXU). *Thymophylla setifolia* Lag., *Garcia 7738* (MEXU). **Thymophylla strotheria* (B.L. Turner) Hinojosa, *Moore* 1286 (MEXU). *Thymophylla tenuifolia* (Cass.) Rydb., *Hinojosa-Espinosa 751* (DAV, MEXU). *Thymophylla tenuiloba* (DC.) Small var. *tenuiloba*, *Martinez 177* (MEXU, UAT). *Thymophylla tephroleuca* (S.F. Blake) Strother, Turner *80-65M* (DAV). ***Urbinella*** *palmeri* Greenm., *Castro 4623* (CIIDIR). ***Varilla*** *mexicana* A. Gray var. *gypsophila* B.L. Turner, *Henrickson 23018B* (TEX). *Varilla texana* A. Gray, *Carr 13586* (TEX).

Appendix 2. Species and herbarium specimens from which florets were extracted for the morphological studies of anther apical appendages in Tageteae. + strong positive test for lignin presence; +/- weak test for lignin presence; – negative test for lignin presence. *Name not effectively published yet.

Tribe Bahieae

+ *Bahia absinthifolia* Benth., *Makings 1637* (DAV).

Tribe Chaenactideae

+ *Chaenactis douglasii* (Hook.) Hook. & Arn., *Dean 7192* (DAV).

Tribe Eupatorieae

+/- *Carminatia recondita* McVaugh, *Hinojosa-Espinosa 625* (MEXU).

+ /- *Stevia lucida* Lag. var. *bipontinii* B.L. Rob., *Hinojosa-Espinosa 688* (DAV, MEXU).

Tribe Heliantheae

+ *Lasianthaea* cf. *ceanothifolia* (Willd.) K.M. Becker, *Hinojosa-Espinosa 681* (DAV, MEXU).

Tribe Tageteae s.l.

Subtribe **Clappiinae**

+ *Clappia suaedifolia* A. Gray, *Webster 31464* (DAV).

Subtribe **Coulterellinae**

– *Coulterella capitata* Vasey & L. Rose, *Johnson 48* (DAV).

Subtribe **Flaveriinae**

+/- *Flaveria anomala* B.L. Rob., *Hinojosa-Espinosa 753* (DAV, MEXU).

+ *Haploesthes greggii* A. Gray var. *texana* (J.M. Coult.) I.M. Johnst., *Webster 34028* (DAV).

+ *Sartwellia puberula* Rydb., *Stuessy 937* (DAV).

Appendix 2. Continuation.

Subtribe **Jaumeinae**

+ *Jaumea carnosa* (Less.) A. Gray, *Park 196* (DAV).

Subtribe **Tagetinae** (including genera unassigned to a subtribe by Panero 2007)

+ *Adenophyllum cancellatum* (Cass.) Villarreal, *Hinojosa-Espinosa 706* (DAV, CIIDIR, MEXU).

+/- *Adenophyllum glandulosum* (Cav.) Strother, *Hinojosa-Espinosa 630* (MEXU).

+ *Bajacalia tridentata* (Benth.) Loockerman, B.L. Turner & R.K. Jansen, *Webster 19585* (DAV).

+ *Boeberastrum anthemidifolium* (Benth.) Rydb., *Burgess 6505* (MEXU).

+ *Boeberoides grandiflora* (DC.) Strother, *Cruz 7363* (MEXU).

+ *Chrysactinia mexicana* A. Gray, *Webster 32624* (DAV).

+ *Comaclinium montanum* (Benth.) Strother, *Stevens 27746* (MEXU).

+ *Dysodiopsis tagetoides* (Torr. & A. Gray) Rydb., *Webster 33291* (DAV).

+ *Dyssodia pinnata* (Cav.) B.L. Rob, *Hinojosa-Espinosa 749* (DAV, MEXU).

+ *Gymnolaena oaxacana* (Greenm.) Rydb., *Cruz 219* (MEXU).

+/- *Hydropectis aquatica* (S. Watson) Rydb., *Soule 2796* (DAV).

+ *Jamesianthus alabamensis* S.F. Blake & Sherff, *Kral 44062* (TEX).

+ *Leucactinia bracteata* (S. Watson) Rydb., *Poole 2497* (TEX).

+ *Nicolletia edwardsii* A. Gray, *Hinojosa-Espinosa 709* (CIIDIR, DAV, MEXU).

+/- *Oxypappus scaber* Benth., *Sundberg 2944* (TEX).

+/- *Pectis multiflosculosa* (DC.) Sch. Bip., *Webster 17099* (DAV).

+/- *Porophyllum macrocephalum* DC., *Clark s.n.* (DAV).

+ *Porophyllum tagetoides* (Kunth) DC., *Hinojosa-Espinosa 685* (DAV, MEXU).

+ *Pseudoclappia watsonii* A.M. Powell & B.L. Turner, *Powell 6517* (TEX).

+ *Tagetes lucida* Cav., *Hinojosa*-Espinosa *676* (DAV, MEXU).

Appendix 2. Continuation.

+ *Thymophyllastrum aurantiacum* (Brandegee) Hinojosa,* *Hinojosa-Espinosa 691* (DAV, MEXU)

+ *Thymophylla strotheria* (B.L. Turner) *Hinojosa,* Hinojosa-Espinosa 752* (DAV, MEXU).

+ *Thymophylla setifolia* Lag., *Hinojosa-Espinosa 740* (DAV, MEXU, UAT).

Urbinella palmeri Greenm., *Castro 4623* (CIIDIR).

Subtribe **Varillinae**

+ *Varilla mexicana* A. Gray var. *mexicana*, *Turner 15049* (DAV).

FIGURES

Fig. 1. Selected genera of Tageteae, subtribe Tagetinae. From upper left to lower right: *Porophyllum macrocephalum*, leaf with pellucid secretory cavities; *Tagetes nelsonii*, leaf segment with numerous dot-like secretory cavities; **Adenophylloides squamosum* and *Adenophyllum speciosum*, showing gland-like secretory cavities in the involucre; *Boeberoides grandiflora*, used locally as ornamental; *Chrysactinia mexicana* used locally in traditional medicine; *Dyssodia decipiens*, with tight clusters of capitula (here slightly separated) that resemble a single capitulum; *Nicolletia edwardsii*, among the few species with purple rays in the tribe; *Pectis prostrata*, a weedy species in compact soils; *Porophyllum tagetoides*, showing purple discoid capitulum; *Tagetes erecta*, the widely cultivated marigold; **Thymophylla strotheria*, with four disk florets per capitulum only. *Name not effectively published yet.

Fig. 2. Majority-rule consensus tree of Tageteae s.l. from the Bayesian analysis of ITS data after 2 runs of 20 million MCMC cycles conducted in MrBayes 3.2.7. The lineages of the subtribes Clappiinae, Coulterellinae, Jaumeinae, and Varillinae colored in blue, green, brown, and orange, respectively. Dark blue nodes with PP ≥ 0.95 (one node annotated for clarification). Largest genera shown as cartoons (but see Figs. S1-S3 for species relationships in these taxa). Note a paraphyletic Tageteae s.l. due to nested outgroups (highlighted in purple). Also note *Clappia*, *Arnicastrum*, and *Jamesianthus* (highlighted in pink) nested within the subtribe Tagetinae.

Fig. 3. Majority-rule consensus tree of Tageteae s.l. from the Bayesian analysis of ETS data after 2 runs of 20 million MCMC cycles conducted in MrBayes 3.2.7. The lineages of the subtribes Clappiinae, Coulterellinae, Jaumeinae, and Varillinae colored in blue, green, brown, and orange, respectively. Dark blue nodes with PP ≥ 0.95 (one node annotated for clarification). Largest

genera shown as cartoons (but see Figures S1-S3 for species relationships in these taxa). Note a paraphyletic Tageteae s.l. including *Bahia ambrosioides* (highlighted in purple). Also note *Pseudoclappia* and *Oxypappus* (highlighted in pink) nested within the subtribe Tagetinae.

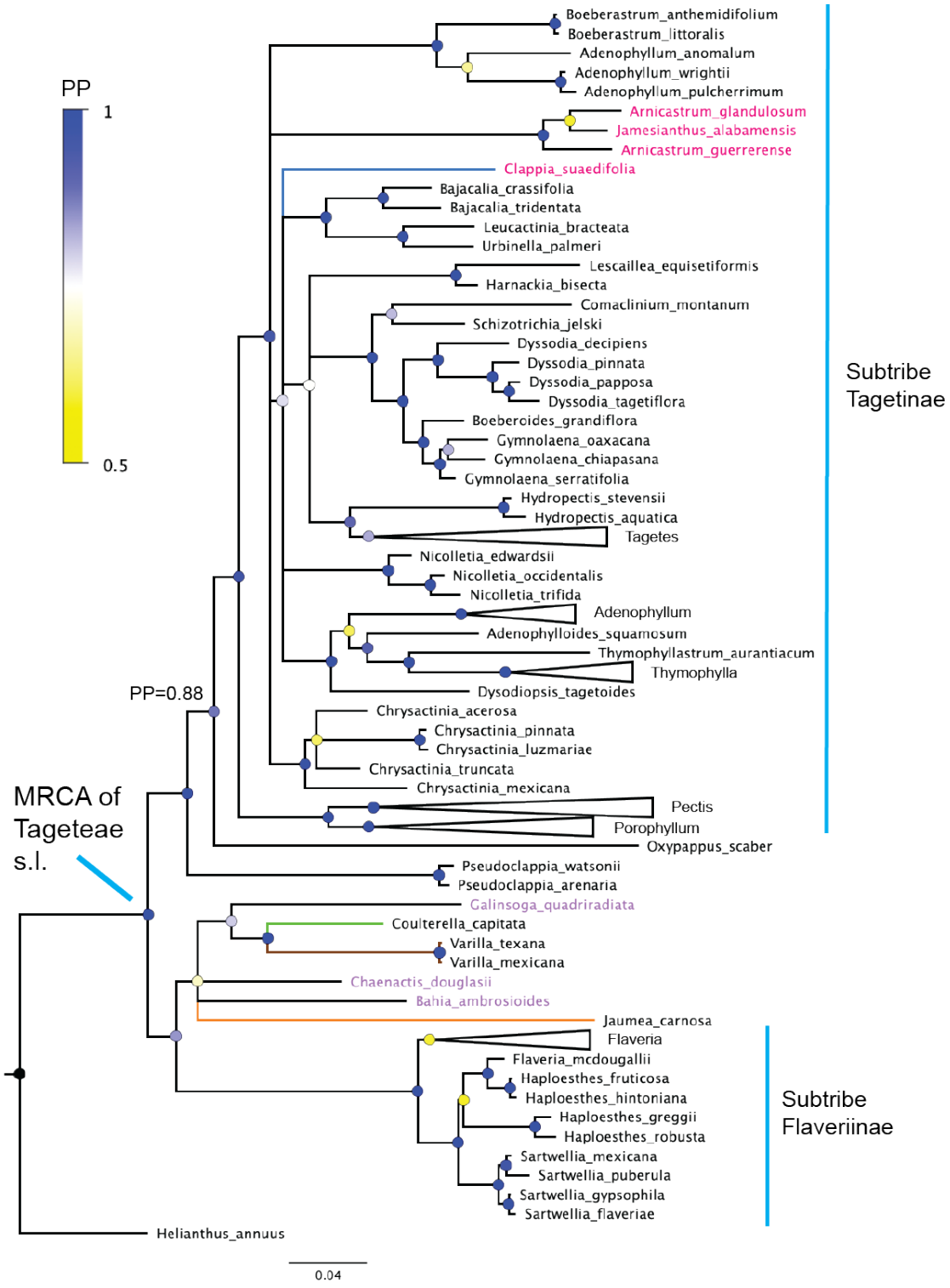
Fig. 4. Majority-rule consensus tree of Tageteae s.l. from the Bayesian analysis of combined ITS plus ETS data after 2 runs of 20 million MCMC cycles conducted in MrBayes 3.2.7. The lineages of the subtribes Clappiinae, Coulterellinae, Jaumeinae, and Varillinae colored in blue, green, brown, and orange, respectively. Dark blue nodes with PP ≥ 0.95. Largest genera shown as cartoons (but see Figures S1-S3 for species relationships in these taxa). Note a paraphyletic Tageteae s.l. due to nested outgroups (highlighted in purple). Also note *Clappia*, *Arnicastrum*, and *Jamesianthus* (highlighted in pink) nested within the subtribe Tagetinae.

Fig. 5. MCC time-calibrated tree from the divergence times analyses of combined ITS and ETS data after two independent runs of 80 million MCMC cycles conducted in BEAST2.6.6. Bars at nodes show the credibility intervals for the mean ages. Only dark brown nodes are strongly supported (PP ≥0.95). Note genera without secretory cavities (highlighted in pink) closely related to or nested within Tagetinae.

Fig. 6. MCC time-calibrated tree showing ancestral state reconstruction of the secretory cavities in the Tageteae s.l. after two independent runs of 1 million MCMC cycles conducted in Bayes Traits as implemented in RASP 4.0. Pies at nodes represent the relative probability for the character states (blue= presence, yellow= absence, red=uncertain) at each node.

Fig. 7. Whole mounts of the anther apical appendages of Tageteae s.l. and selected members of related tribes stained with HCl-Phloroglucinol to test for lignin presence. A-M. Appendages in which the marginal and terminal cells have thick walls and that tested positive for lignin as evidence by the pink-red color. N-R. Appendages with homogenous wall thickness and that tested negative or slightly positive for lignin as evidence by the absence of or only slightly pink-red color. **A**. *Adenophyllum cancellatum* ×40. **B**. *Bajacalia tridentata* ×40. **C**. *Boeberoides grandiflora* ×40. **D**. *Chrysactinia mexicana* ×100. **E**. *Comaclinium montanum* ×100. **F**. *Dysodiopsis tagetoides* ×40. **G**. *Gymnolaena oaxacana* x 25. **H**. *Porophyllum tagetoides* ×100. **I**. *Thymophylla setifolia* ×100. **J**. *Bahia absinthifolia* (Bahieae) ×40, note few glandular trichomes. **K**. *Chaenactis douglassii* (Chaenactideae) ×40, note numerous glandular trichomes. **L**. *Leucactinia bracteata* ×200, showing terminal cells of the appendage. **M**. *Boeberoides grandiflora* ×400, showing detail of the marginal thickened cell walls. **N**. *Adenophyllum glandulosum* ×40, note pollen. **O**. *Coulterella capitata* ×40, note a few glandular trichomes. **P**. *Pectis multiflosculosa* ×100, note emarginate apex. **Q**. *Oxypappus scaber* ×100. **R**. *Stevia lucida* (Eupatorieae) × 100.

Fig. 1


PP
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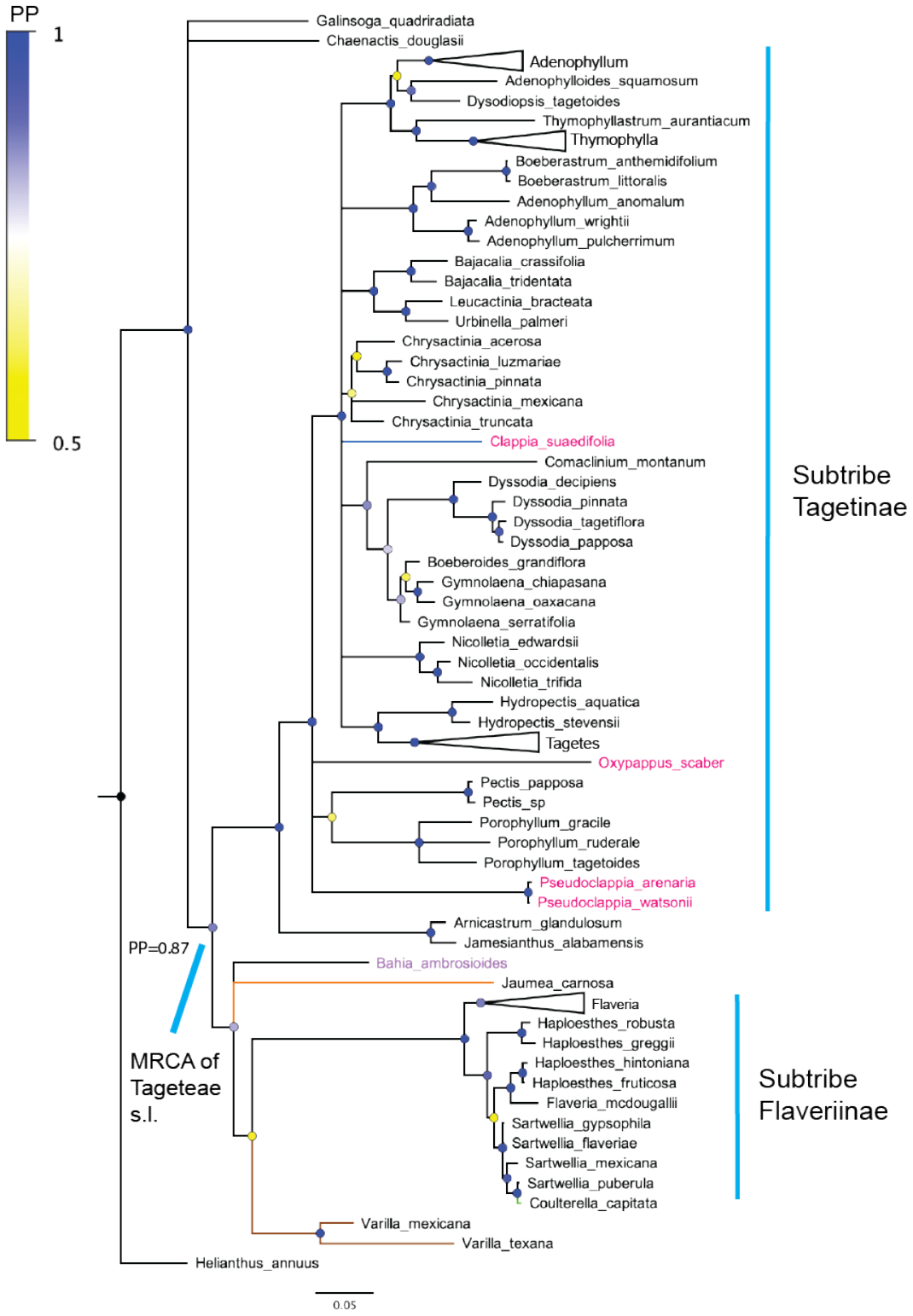
0.5
Boeberastrum_anthemidifolium
Boeberastrum_littoralis
Adenophyllum_anomalum
Adenophyllum_wrightii
Adenophyllum_pulcherrimum
Arnicastrum_glandulosum
Jamesianthus_alabamensis
Arnicastrum_guerrerense
Clappia_suaedifolia
Bajacalia_crassifolia
Bajacalia_tridentata
Leucactinia_bracteata
Urbinella_palmeri
Lescaillea_equisetiformis
Harnackia_bisecta
Comaclinium_montanum
Schizotrichia_jelski
Dyssodia_decipiens
Dyssodia_pinnata
Dyssodia_papposa
Dyssodia_tagetiflora
Boeberoides_grandiflora
Gymnolaena_oaxacana
Gymnolaena_chiapasana
Gymnolaena_serratifolia
Hydropectis_stevensii
Hydropectis_aquatica
Tagetes
Nicolletia_edwardsii
Nicolletia_occidentalis
Nicolletia_trifida
Adenophyllum
Adenophylloides_squamosum
Thymophyllastrum_aurantiacum
Thymophylla
Dysodiopsis_tagetoides
PP=0.88
Chrysactinia_acerosa
Chrysactinia_pinnata
Chrysactinia_luzmariae
Chrysactinia_truncata
Chrysactinia_mexicana
Pectis
Porophyllum
Oxypappus_scaber
Subtribe
Tagetinae
MRCA of
Tageteae
s.l.
Pseudoclappia_watsonii
Pseudoclappia_arenaria
Galinsoga_quadriradiata
Coulterella_capitata
Varilla_texana
Varilla_mexicana
Chaenactis_douglasii
Bahia_ambrosioides
Jaumea_carnosa
Flaveria
Flaveria_mcdougallii
Haploesthes_fruticosa
Haploesthes_hintoniana
Haploesthes_greggii
Haploesthes_robusta
Sartwellia_mexicana
Sartwellia_puberula
Sartwellia_gypsophila
Sartwellia_flaveriae
Subtribe
Flaveriinae
Helianthus_annuus
0.04

Fig. 2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Galinsoga_quadriradiata
Chaenactis_douglasii
Adenophyllum
Adenophylloides_squamosum
Dysodiopsis_tagetoides
Thymophyllastrum_aurantiacum
Thymophylla
Boeberastrum_anthemidifolium
Boeberastrum_littoralis
Adenophyllum_anomalum
Adenophyllum_wrightii
Adenophyllum_pulcherrimum
Bajacalia_crassifolia
Bajacalia_tridentata
Leucactinia_bracteata
Urbinella_palmeri
Chrysactinia_acerosa
Chrysactinia_luzmariae
Chrysactinia_pinnata
Chrysactinia_mexicana
Chrysactinia_truncata
Clappia_suaedifolia
Comaclinium_montanum
Dyssodia_decipiens
Dyssodia_pinnata
Dyssodia_tagetiflora
Dyssodia_papposa
Boeberoides_grandiflora
Gymnolaena_chiapasana
Gymnolaena_oaxacana
Gymnolaena_serratifolia
Nicolletia_edwardsii
Nicolletia_occidentalis
Nicolletia_trifida
Hydropectis_aquatica
Hydropectis_stevensii
Tagetes
Oxypappus_scaber
Pectis_papposa
Pectis_sp
Porophyllum_gracile
Porophyllum_ruderale
Porophyllum_tagetoides
Pseudoclappia_arenaria
Pseudoclappia_watsonii
Arnicastrum_glandulosum
Jamesianthus_alabamensis
Subtribe Tagetinae
PP=0.87
MRCA of Tageteae s.l.
Bahia_ambrosioides
Jaumea_carnosa
Flaveria
Haploesthes_robusta
Haploesthes_greggii
Haploesthes_hintoniana
Haploesthes_fruticosa
Flaveria_mcdougallii
Sartwellia_gypsophila
Sartwellia_flaveriae
Sartwellia_mexicana
Sartwellia_puberula
Coulterella_capitata
Subtribe Flaveriinae
Varilla_mexicana
Varilla_texana
Helianthus_annuus
0.05

Fig. 3

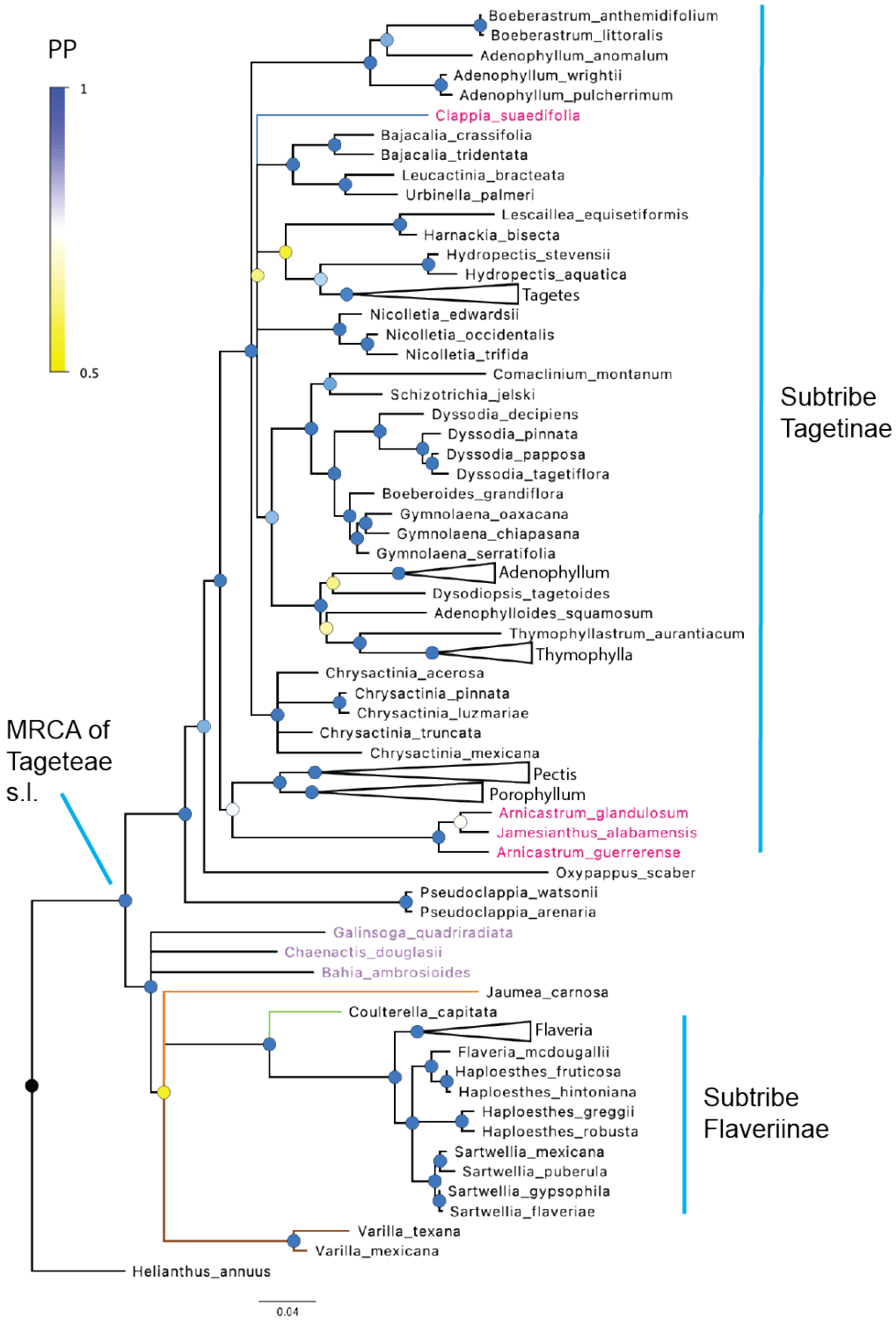
PP
1
0.5
Boeberastrum_anthemidifolium
Boeberastrum_littoralis
Adenophyllum_anomalum
Adenophyllum_wrightii
Adenophyllum_pulcherrimum
Clappia_suaedifolia
Bajacalia_crassifolia
Bajacalia_tridentata
Leucactinia_bracteata
Urbinella_palmeri
Lescaillea_equisetiformis
Harnackia_bisecta
Hydropectis_stevensii
Hydropectis_aquatica
Tagetes
Nicolletia_edwardsii
Nicolletia_occidentalis
Nicolletia_trifida
Comaclinium_montanum
Schizotrichia_jelski
Dyssodia_decipiens
Dyssodia_pinnata
Dyssodia_papposa
Dyssodia_tagetiflora
Boeberoides_grandiflora
Gymnolaena_oaxacana
Gymnolaena_chiapasana
Gymnolaena_serratifolia
Adenophyllum
Dysodiopsis_tagetoides
Adenophylloides_squamosum
Thymophyllastrum_aurantiacum
Thymophylla
Chrysactinia_acerosa
Chrysactinia_pinnata
Chrysactinia_luzmariae
Chrysactinia_truncata
Chrysactinia_mexicana
Pectis
Porophyllum
Arnicastrum_glandulosum
Jamesianthus_alabamensis
Arnicastrum_guerrerense
Oxypappus_scaber
Pseudoclappia_watsonii
Pseudoclappia_arenaria
Galinsoga_quadriradiata
Chaenactis_douglasii
Bahia_ambrosioides
Jaumea_carnosa
Coulterella_capitata
Flaveria
Flaveria_mcdougallii
Haploesthes_fruticosa
Haploesthes_hintoniana
Haploesthes_greggii
Haploesthes_robusta
Sartwellia_mexicana
Sartwellia_puberula
Sartwellia_gypsophila
Sartwellia_flaveriae
Varilla_texana
Varilla_mexicana
Helianthus_annuus
0.04
MRCA of Tageteae s.l.
Subtribe Tagetinae
Subtribe Flaveriinae

Fig. 4

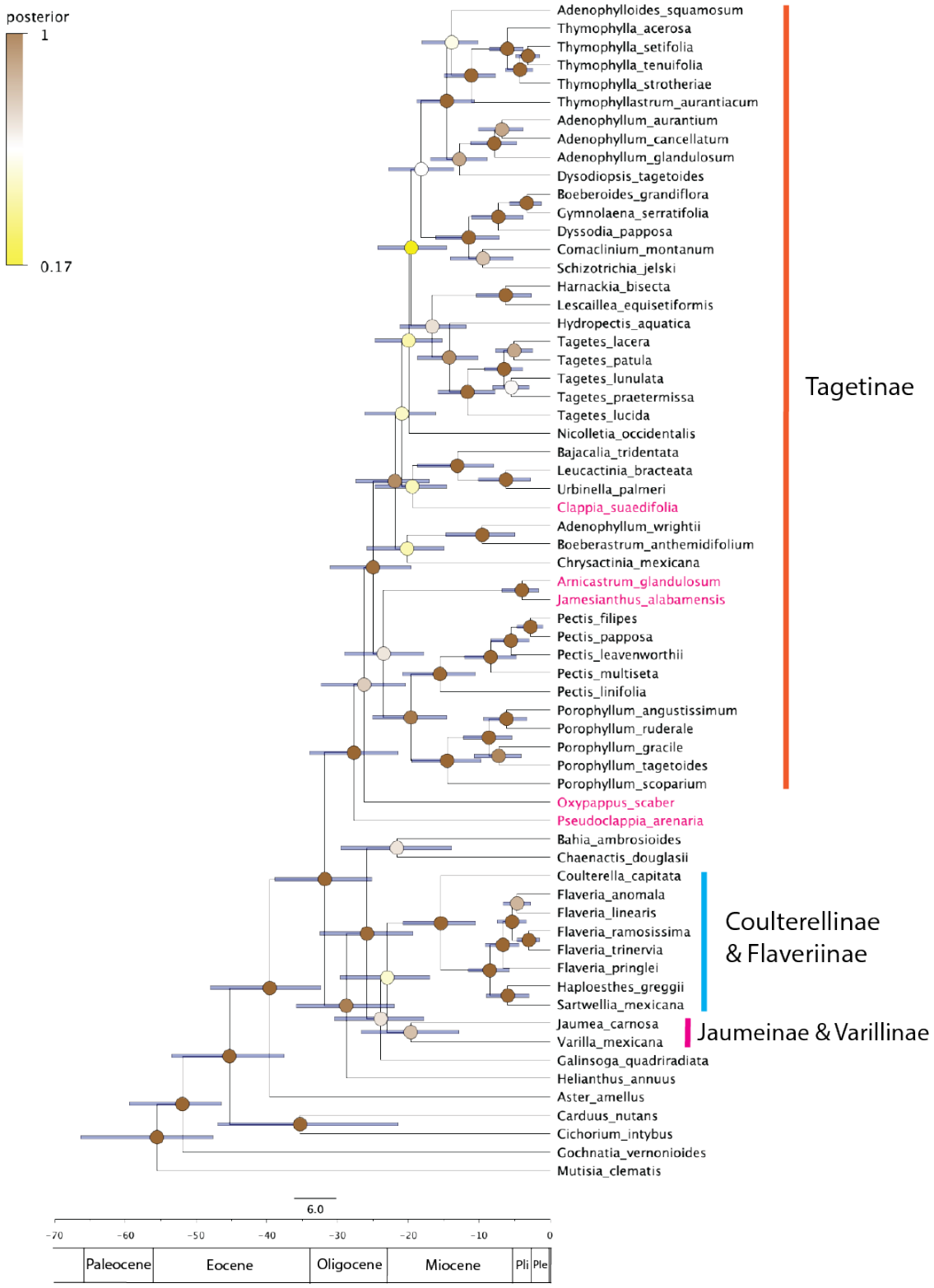
posterior
1
0.17
Adenophylloides_squamosum
Thymophylla_acerosa
Thymophylla_setifolia
Thymophylla_tenuifolia
Thymophylla_strotheriae
Thymophyllastrum_aurantiacum
Adenophyllum_aurantium
Adenophyllum_cancellatum
Adenophyllum_glandulosum
Dysodiopsis_tagetoides
Boeberoides_grandiflora
Gymnolaena_serratifolia
Dyssodia_papposa
Comaclinium_montanum
Schizotrichia_jelski
Harnackia_bisecta
Lescaillea_equisetiformis
Hydropectis_aquatica
Tagetes_lacera
Tagetes_patula
Tagetes_lunulata
Tagetes_praetermissa
Tagetes_lucida
Nicolletia_occidentalis
Bajacalia_tridentata
Leucactinia_bracteata
Urbinella_palmeri
Clappia_suaedifolia
Adenophyllum_wrightii
Boeberastrum_anthemidifolium
Chrysactinia_mexicana
Arnicastrum_glandulosum
Jamesianthus_alabamensis
Pectis_filipes
Pectis_papposa
Pectis_leavenworthii
Pectis_multiseta
Pectis_linifolia
Porophyllum_angustissimum
Porophyllum_ruderale
Porophyllum_gracile
Porophyllum_tagetoides
Porophyllum_scoparium
Oxypappus_scaber
Pseudoclappia_arenaria
Bahia_ambrosioides
Chaenactis_douglasii
Coulterella_capitata
Flaveria_anomala
Flaveria_linearis
Flaveria_ramosissima
Flaveria_trinervia
Flaveria_pringlei
Haploesthes_greggii
Sartwellia_mexicana
Jaumea_carnosa
Varilla_mexicana
Galinsoga_quadriradiata
Helianthus_annuus
Aster_amellus
Carduus_nutans
Cichorium_intybus
Gochnatia_vernonioides
Mutisia_clematis
Tagetinae
Coulterellinae & Flaveriinae
Jaumeinae & Varillinae
6.0
-70
-60
-50
-40
-30
-20
-10
0
Paleocene
Eocene
Oligocene
Miocene
Pli
Ple

Fig. 5

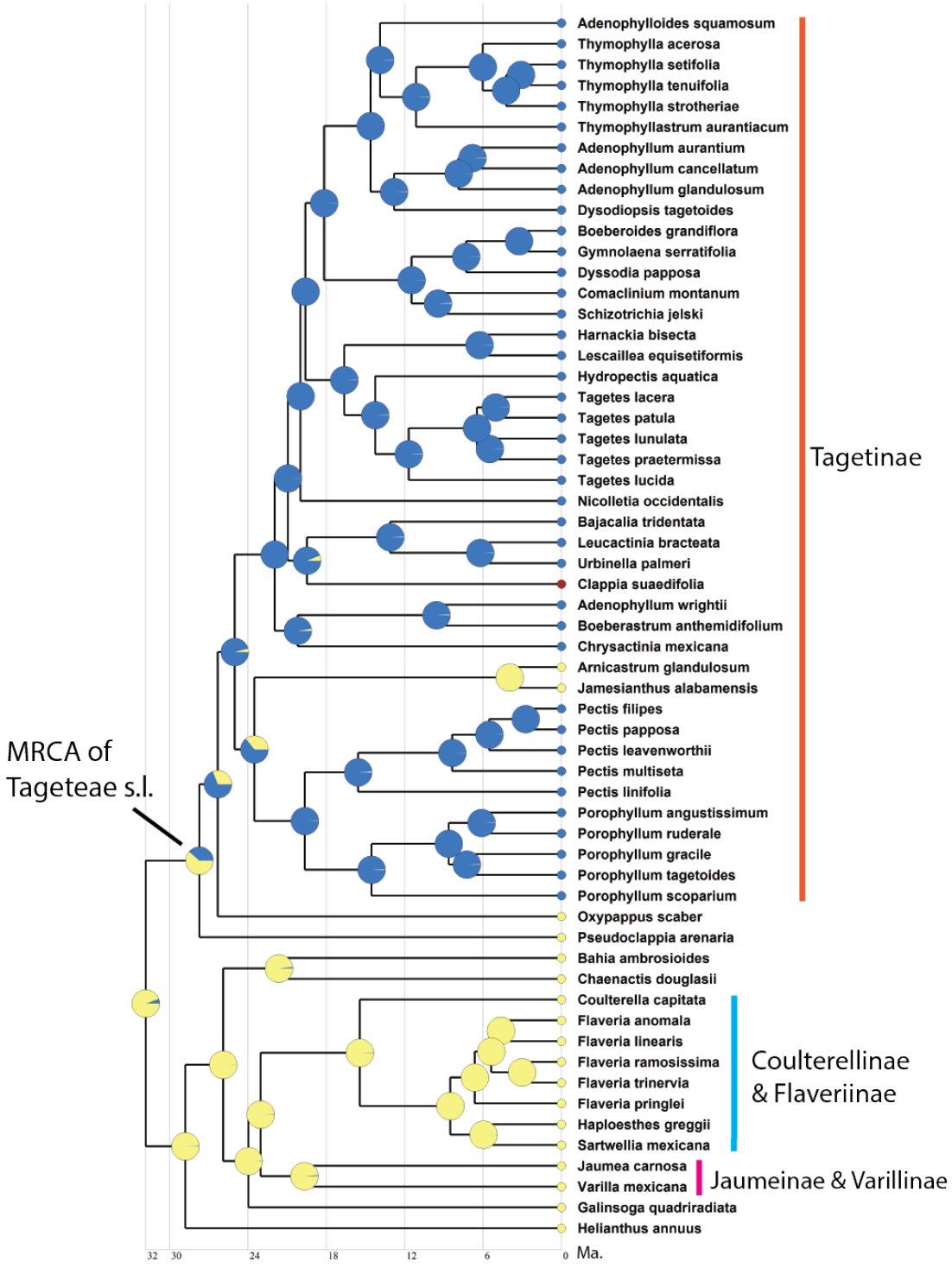
Adenophylloides squamosum
Thymophylla acerosa
Thymophylla setifolia
Thymophylla tenuifolia
Thymophylla strotheriae
Thymophyllastrum aurantiacum
Adenophyllum aurantium
Adenophyllum cancellatum
Adenophyllum glandulosum
Dysodiopsis tagetoides
Boeberoides grandiflora
Gymnolaena serratifolia
Dyssodia papposa
Comaclinium montanum
Schizotrichia jelski
Harnackia bisecta
Lescaillea equisetiformis
Hydropectis aquatica
Tagetes lacera
Tagetes patula
Tagetes lunulata
Tagetes praetermissa
Tagetes lucida
Nicolletia occidentalis
Bajacalia tridentata
Leucactinia bracteata
Urbinella palmeri
Clappia suaedifolia
Adenophyllum wrightii
Boeberastrum anthemidifolium
Chrysactinia mexicana
Arnicastrum glandulosum
Jamesianthus alabamensis
Pectis filipes
Pectis papposa
Pectis leavenworthii
Pectis multiseta
Pectis linifolia
Porophyllum angustissimum
Porophyllum ruderale
Porophyllum gracile
Porophyllum tagetoides
Porophyllum scoparium
Oxypappus scaber
Pseudoclappia arenaria
Bahia ambrosioides
Chaenactis douglasii
Coulterella capitata
Flaveria anomala
Flaveria linearis
Flaveria ramosissima
Flaveria trinervia
Flaveria pringlei
Haploesthes greggii
Sartwellia mexicana
Jaumea carnosa
Varilla mexicana
Galinsoga quadriradiata
Helianthus annuus
32
30
24
18
12
6
0
Ma.
Tagetinae
Coulterellinae & Flaveriinae
Jaumeinae & Varillinae
MRCA of Tageteae s.l.

Fig. 6

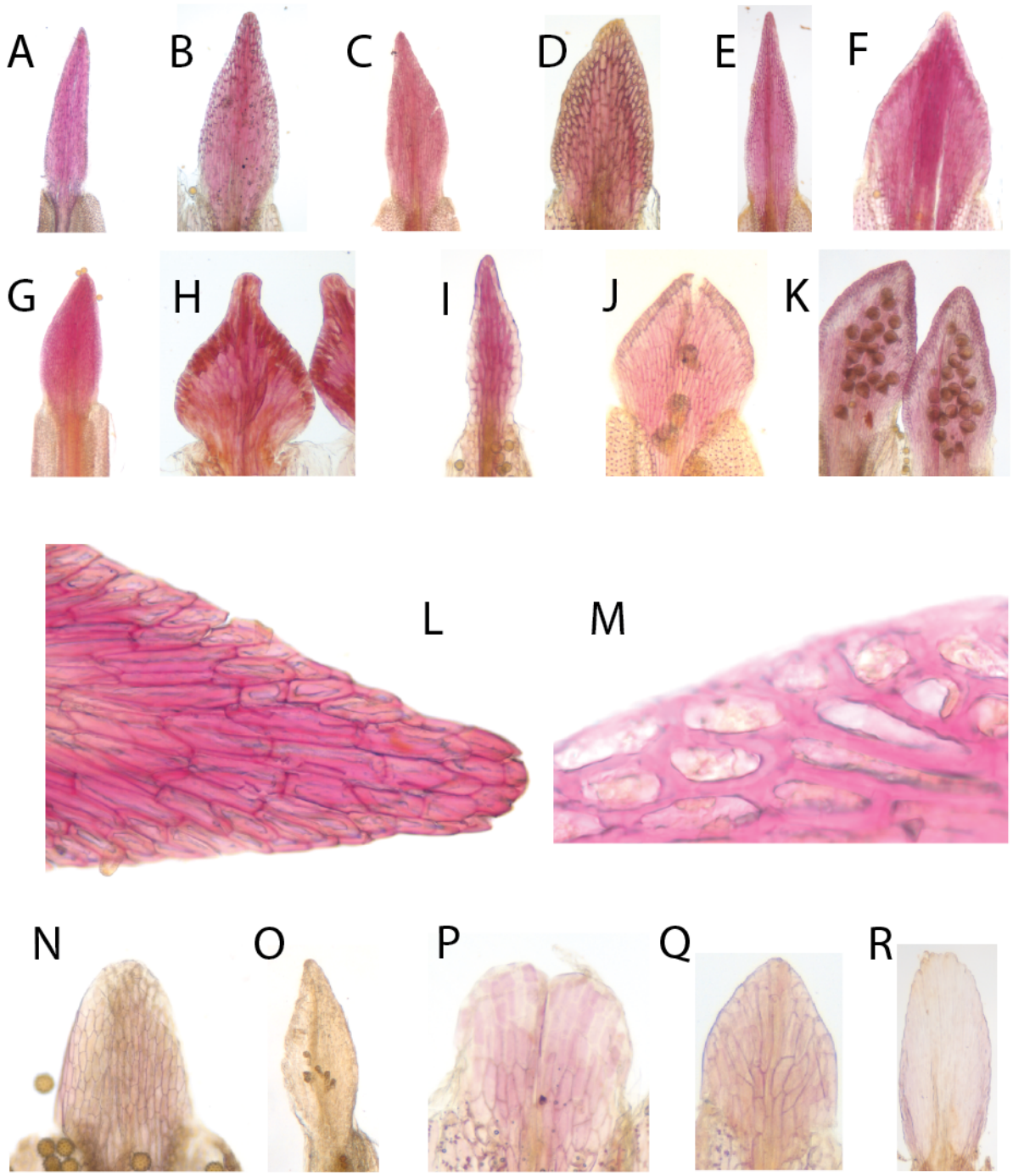

Fig. 7

SUPPLEMENTARY FIGURES

Fig. S1. Majority-rule consensus tree of Tageteae s.l. (focusing on Flaveriinae) from the Bayesian analyses of the concatenated ITS and ETS data after 2 runs of 20 million MCMC cycles conducted in MrBayes 3.2.7. The lineages of the subtribes Clappiinae, Coulterellinae, Jaumeinae, and Varillinae colored in blue, green, brown, and orange, respectively. Dark blue nodes with PP ≥ 0.95. Note a paraphyletic Tageteae s.l. due to nested outgroups (highlighted in purple).

Fig. S2. Majority-rule consensus tree of Tageteae s.l. (focusing on Pectis and Porophyllum clades) from the Bayesian analysis of combined ITS and ETS data after 2 runs of 20 million MCMC cycles conducted in MrBayes 3.2.7. Dark blue nodes with PP ≥ 0.95.

Fig. S3. Majority-rule consensus tree of Tageteae s.l. (focusing on the core Tagetinae clade) from the Bayesian analysis of combined ITS and ETS data after 2 runs of 20 million MCMC cycles conducted in MrBayes 3.2.7. Dark blue nodes with PP ≥ 0.95.

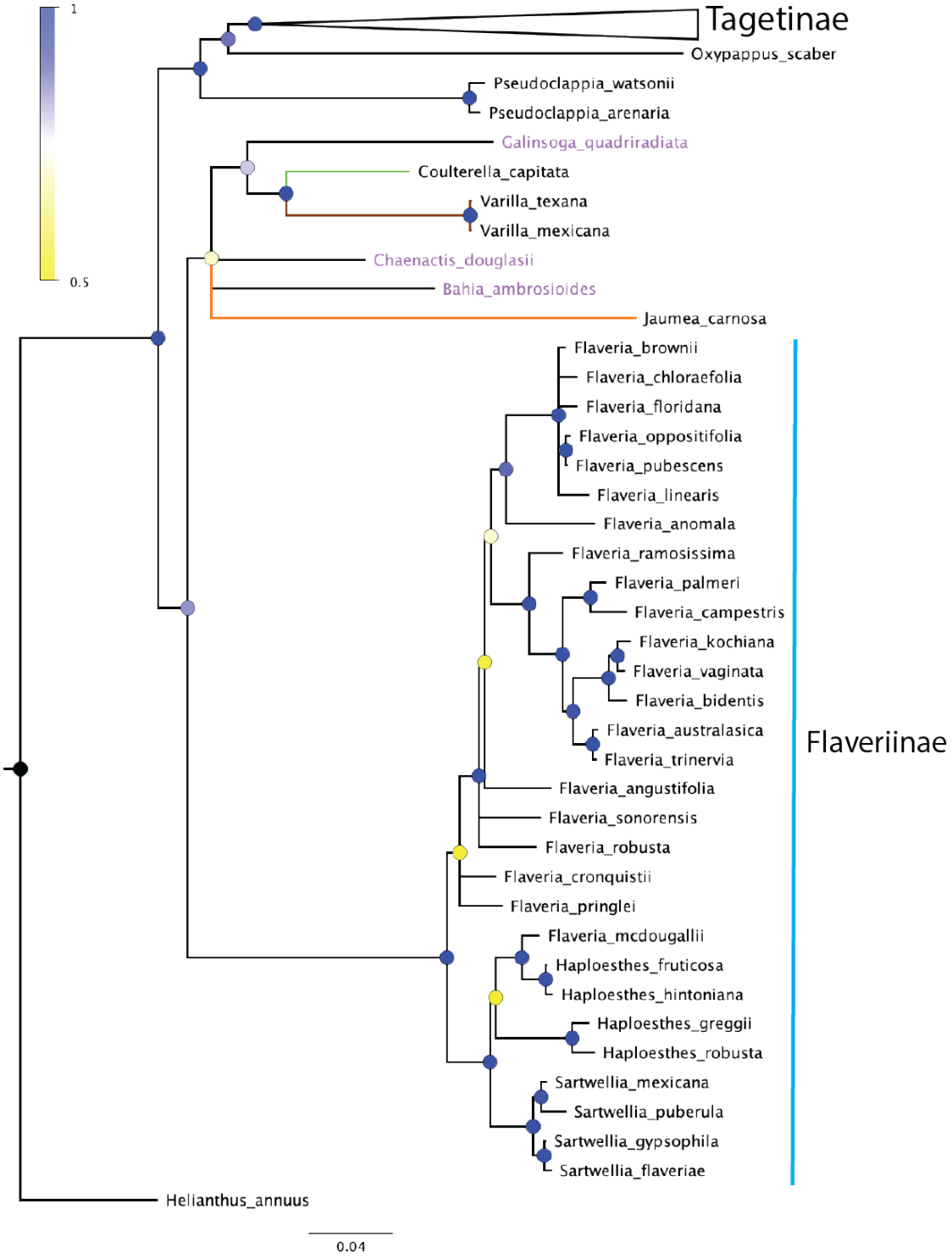
1
0.5
Tagetinae
Oxypappus_scaber
Pseudoclappia_watsonii
Pseudoclappia_arenaria
Galinsoga_quadriradiata
Coulterella_capitata
Varilla_texana
Varilla_mexicana
Chaenactis_douglasii
Bahia_ambrosioides
Jaumea_carnosa
Flaveria_brownii
Flaveria_chloraefolia
Flaveria_floridana
Flaveria_oppositifolia
Flaveria_pubescens
Flaveria_linearis
Flaveria_anomala
Flaveria_ramosissima
Flaveria_palmeri
Flaveria_campestris
Flaveria_kochiana
Flaveria_vaginata
Flaveria_bidentis
Flaveria_australasica
Flaveria_trinervia
Flaveria_angustifolia
Flaveria_sonorensis
Flaveria_robusta
Flaveria_cronquistii
Flaveria_pringlei
Flaveria_mcdougallii
Haploesthes_fruticosa
Haploesthes_hintoniana
Haploesthes_greggii
Haploesthes_robusta
Sartwellia_mexicana
Sartwellia_puberula
Sartwellia_gypsophila
Sartwellia_flaveriae
Flaveriinae
Helianthus_annuus
0.04

Fig. S1.

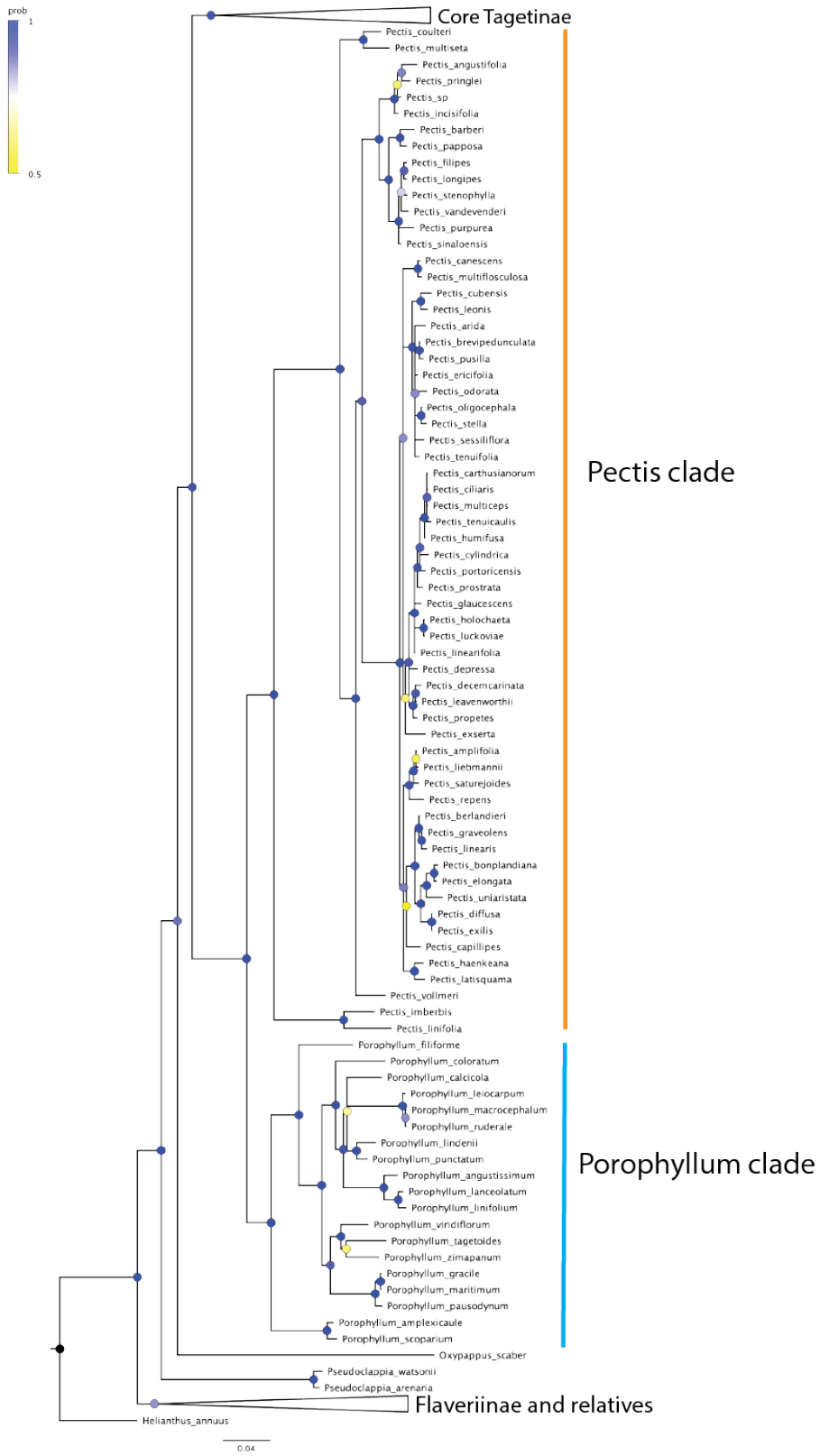
prob
1
0.5
Core Tagetinae
Pectis_coulteri
Pectis_multiseta
Pectis_angustifolia
Pectis_pringlei
Pectis_sp
Pectis_incisifolia
Pectis_barberi
Pectis_papposa
Pectis_filipes
Pectis_longipes
Pectis_stenophylla
Pectis_vandevenderi
Pectis_purpurea
Pectis_sinaloensis
Pectis_canescens
Pectis_multifloculosa
Pectis_cubensis
Pectis_leonis
Pectis_arida
Pectis_brevipedunculata
Pectis_pusilla
Pectis_ericifolia
Pectis_odorata
Pectis_oligocephala
Pectis_stella
Pectis_sessiliflora
Pectis_tenuifolia
Pectis_carthusianorum
Pectis_ciliaris
Pectis_multiceps
Pectis_tenuicaulis
Pectis_humifusa
Pectis_cylindrica
Pectis_portoricensis
Pectis_prostrata
Pectis_glaucescens
Pectis_holochaeta
Pectis_luckoviae
Pectis_linearifolia
Pectis_depressa
Pectis_decemcarinata
Pectis_leavenworthii
Pectis_propetes
Pectis_exserta
Pectis_amplifolia
Pectis_liebmannii
Pectis_saturejoides
Pectis_repens
Pectis_berlandieri
Pectis_graveolens
Pectis_linearis
Pectis_bonplandiana
Pectis_elongata
Pectis_uniaristata
Pectis_diffusa
Pectis_exilis
Pectis_capillipes
Pectis_haenkeana
Pectis_latisquama
Pectis_vollmeri
Pectis_imberbis
Pectis_linifolia
Pectis clade
Porophyllum_filiforme
Porophyllum_coloratum
Porophyllum_calcicola
Porophyllum_leiocarpum
Porophyllum_macrocephalum
Porophyllum_ruderale
Porophyllum_lindenii
Porophyllum_punctatum
Porophyllum_angustissimum
Porophyllum_lanceolatum
Porophyllum_linifolium
Porophyllum_viridiflorum
Porophyllum_tagetoides
Porophyllum_zimapanum
Porophyllum_gracile
Porophyllum_maritimum
Porophyllum_pausodynum
Porophyllum_amplexicaule
Porophyllum_scoparium
Porophyllum clade
Oxypappus_scaber
Pseudoclappia_watsonii
Pseudoclappia_arenaria
Flaveriinae and relatives
Helianthus_annuus
0.04

Fig. S2.

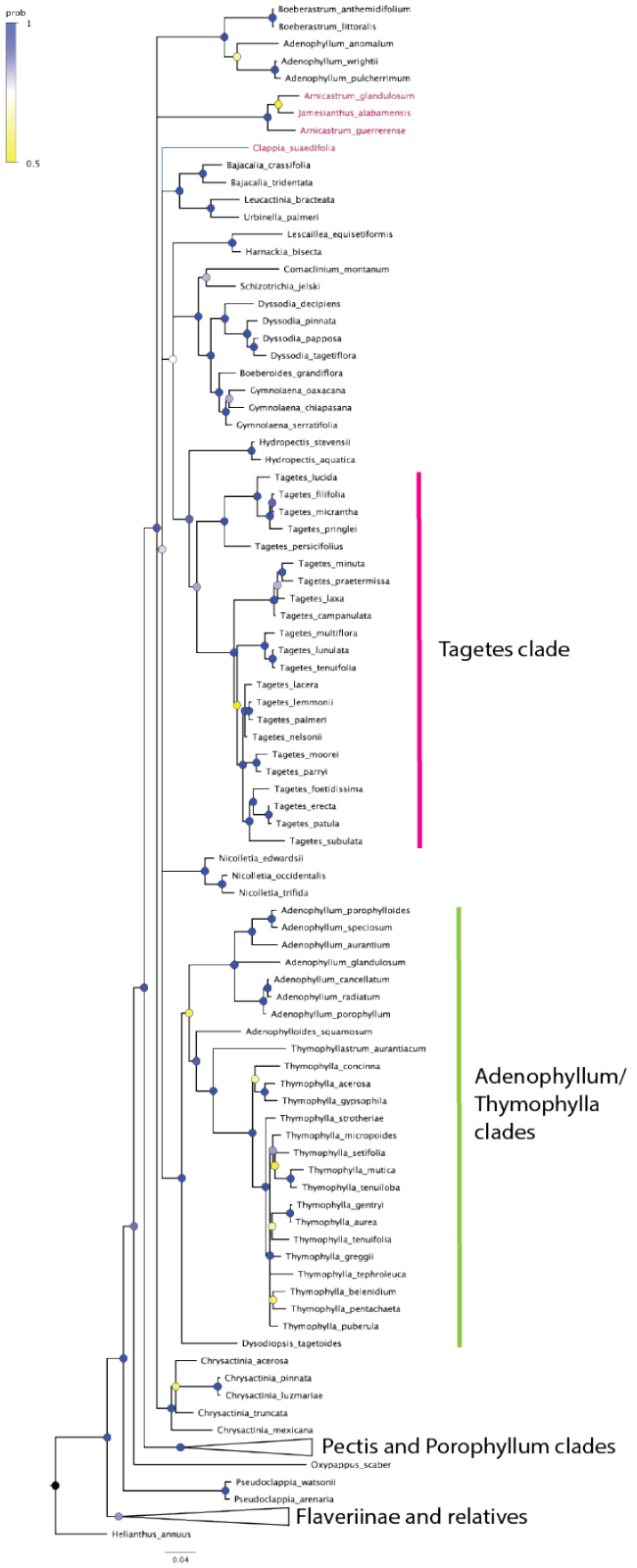
prob
1
0.5
Boeberastrum_anthemidifolium
Boeberastrum_littoralis
Adenophyllum_anomalum
Adenophyllum_wrightii
Adenophyllum_pulcherrimum
Arnicastrum_glandulosum
Jamesianthus_alabamensis
Arnicastrum_guerrerense
Clappia_suaedifolia
Bajacalia_crassifolia
Bajacalia_tridentata
Leucactinia_bracteata
Urbinella_palmeri
Lescaillea_equisetiformis
Harnackia_bisecta
Comaclinium_montanum
Schizotrichia_jelski
Dyssodia_decipiens
Dyssodia_pinnata
Dyssodia_papposa
Dyssodia_tagetiflora
Boeberoides_grandiflora
Gymnolaena_oaxacana
Gymnolaena_chiapasana
Gymnolaena_serratifolia
Hydropectis_stevensii
Hydropectis_aquatica
Tagetes_lucida
Tagetes_filifolia
Tagetes_micrantha
Tagetes_pringlei
Tagetes_persicifolius
Tagetes_minuta
Tagetes_praetermissa
Tagetes_laxa
Tagetes_campanulata
Tagetes_multiflora
Tagetes_lunulata
Tagetes_tenuifolia
Tagetes_lacera
Tagetes_lemmonii
Tagetes_palmeri
Tagetes_nelsonii
Tagetes_moorei
Tagetes_parryi
Tagetes_foetidissima
Tagetes_erecta
Tagetes_patula
Tagetes_subulata
Tagetes clade
Nicolletia_edwardsii
Nicolletia_occidentalis
Nicolletia_trifida
Adenophyllum_porophylloides
Adenophyllum_speciosum
Adenophyllum_aurantium
Adenophyllum_glandulosum
Adenophyllum_cancellatum
Adenophyllum_radiatum
Adenophyllum_porophyllum
Adenophylloides_squamosum
Thymophyllastrum_aurantiacum
Thymophylla_concinna
Thymophylla_acerosa
Thymophylla_gypsophila
Thymophylla_strotheriae
Thymophylla_micropoides
Thymophylla_setifolia
Thymophylla_mutica
Thymophylla_tenuiloba
Thymophylla_gentryi
Thymophylla_aurea
Thymophylla_tenuifolia
Thymophylla_greggii
Thymophylla_tephroleuca
Thymophylla_belenidium
Thymophylla_pentachaeta
Thymophylla_puberula
Adenophyllum/
Thymophylla
clades
Dysodiopsis_tagetoides
Chrysactinia_acerosa
Chrysactinia_pinnata
Chrysactinia_luzmariae
Chrysactinia_truncata
Chrysactinia_mexicana
Pectis and Porophyllum clades
Oxypappus_scaber
Pseudoclappia_watsonii
Pseudoclappia_arenaria
Flaveriinae and relatives
Helianthus_annuus
0.04

Fig. S3.

Chapter 3

Phylogeny of marigolds (*Tagetes* L., Asteraceae) based on ITS sequences

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[3]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), República Argentina

***Abstract*—**The genus *Tagetes* includes about 50 species, all native to the New World. Some species are widely cultivated, notably *T*. *erecta*, and many species are used as medicines, spices, and in rituals. These uses are related to the essential oils that the plants store in secretory cavities in their foliage. Despite several taxonomic contributions, there are still species complexes in need of much work. Moreover, comprehensive phylogenetic studies of *Tagetes* using molecular data have not been conducted yet. Here we present results of phylogenetic analysis of ITS sequences from almost 50% of the species of *Tagetes*. The genus is resolved as a somewhat weakly supported clade, but the clade that includes *Hydropectis* as sister to *Tagetes* is strongly supported. A wild form of *T*. *erecta* is resolved as sister to *T*. *patula*, a cultivated species with smaller heads that is often treated as a synonym of the former. *Tagetes lunulata*, often considered closely related to *T*. *erecta*, is resolved in a different clade. Some other subclades are also strongly supported, such as one composed of mostly subaquatic and riparian species with an anise-like scent. It is necessary to sample species missing from these analyses to obtain a better understanding of the phylogeny of *Tagetes*, which will also allow us to postulate

more robust evolutionary hypotheses, such as divergence times, and character evolution, as well as to guide the search for medicines or other desirable traits from the closest wild relatives of the cultivated species.

Keywords—African marigold, Aztec marigold, French marigold, Heliantheae alliance, secretory cavities.

INTRODUCTION

With approximately 50 species, *Tagetes* L. is the second largest genus in the tribe Tageteae within the Heliantheae alliance clade (Panero 2007, Baldwin 2009). The species are distributed from the southwestern United States to central Argentina and central Chile (Figure 1), with the highest species richness recorded in Mexico and the second highest species diversity in western and northwestern Argentina, Bolivia, Peru, Ecuador, and Colombia (Schiavinato et al. 2017, 2021, Schiavinato & Bartoli 2018). *Tagetes* includes terrestrial or subaquatic annuals or perennials, with entire or pinnately lobed to dissected leaves, radiate heads, a uniseriate connate involucre, and a pappus of short scales, subulate scales, or a combination of both (Figure 2). As most members of tribe Tageteae, the leaves and phyllaries in *Tagetes* have pellucid secretory cavities that contain fragrant essential oils (Figure 2B).

The most widely known species is the cultivated *T*. *erecta* L., often known as French Marigold or African Marigold, although it is native to Mesoamerica (Figure 3A–D). *Tagetes erecta* is also known as Aztec marigold because it was cultivated and used as a medicinal and ritual plant by the Aztecs (Linares & Bye 1997), who noticed that the capitula were inflorescences, not single flowers, as the name in Nahuatl (the Aztec language) is *cempoalxochitl*, which means 20 flowers (Rzedowski 1978). This species is one of the most important ceremonial plants of Mexico (Linares & Bye, 1997), where it is extensively used in Day

of the Dead decorations. On this day, relatives and friends who have passed away are remembered by setting up colorful altars with pictures, candies, fruits, beverages, and dishes (Figure 3C–D). According to tradition, the souls of the departed come back to visit home and feed on the dishes in the altars, and they are guided by the intense orange color of the rays and the large globular capitula of T. erecta.

Tagetes lucida Cav. has also been used in Mesoamerica since pre-Columbian times (Figure 4). This species is known locally as *pericón* or *yerbanís* and is used as a medicinal plant or spice. Also, capitulescences of this anise-scented species are used to make religious crosses that are placed in gates, doors, and windows to repel evil spirits during a religious festivity (*Día de San Miguel Arcángel*) in south central Mexico (Figure 4C). Several other species of *Tagetes* are used as ornamental plants, local medicines, and spices (e.g., *T*. *micrantha* Cav.).

Tagetes was first published by Linnaeus (1753) with three species: *T*. *erecta*, *T*. *minuta* and *T*. *patula*. During the following hundred years, the number of *Tagetes* species was increased with the contributions of different authors (e.g. Cavanilles 1794, Lagasca y Segura 1816, Candolle 1836, etc.), who based their descriptions both on herbarium specimens sent from the American continent by the botanical expeditions of the time, as well as on plants grown in European botanical gardens from seeds collected on these journeys. The first comprehensive taxonomic revision of *Tagetes* was made by Neher (1966), who proposed two subgenera that were not effectively published: '*Tagetes* subgen. *Lucida*' included species with a distinctive anise-like scent, linear to lanceolate leaf laminas, and involucres with punctiform secretory cavities, while the typical subgenus contained species with a pungent odor, deeply pinnately lobed leaves, and involucres with linear secretory cavities. More recently, Soule (1993) conducted a cladistic analysis of *Tagetes* based on morphological data and performed a taxonomic revision. Soule (1993) recognized 55 species and proposed three subgenera, *Hydrotagetes*, *Iya*, and *Tagetes*,

the latter with two sections (*Filifoliae* and *Tagetes*) and 13 series (all of them included within section *Tagetes*). This infrageneric classification was effectively published by Soule (1996).

Despite the contributions of Neher (1966) and Soule (1993, 1996), there are some Mexican species complexes that require additional taxonomic work. One of them includes the cultivated species and several related wild taxa. Neher (1966) and Soule (1993) recognized two cultivated species, *T*. *erecta*, the one with the largest heads and more numerous ray florets (Figure 3B) and *T*. *patula* with smaller heads and fewer ray florets. According to this interpretation, *T*. *erecta* and *T*. *patula* could be derived from wild species such as *T*. *lunulata* Ortega and *T*. *tenuifolia* Cav. On the other hand, Turner (1996) adopted a broader circumscription of *T*. *erecta* that included *T. patula* and a wild form of *T*. *erecta*, which is recognized by its fewer rays and red purplish disk corolla lobes (Figure 3A) and is the most probable source of the cultivated forms. According to Rzedowski (2005), *T*. *lunulata* belongs to this species complex and it is distinguished by its orange-reddish spot at the base of the rays (Figure 3F). In addition, Turner (1996) considered *T*. *tenuifolia* morphologically similar and closely related to *T*. *lunulata*, but it lacks the orange-reddish spot at the base of the rays and the capitulum in bud is round and glabrous (Figure 4D–F), whereas in *T. lunulata* the capitulum in bud is acute and provided with setulae (Figure 3E). However, we have seen populations in central Mexico whose individuals have the orange-reddish spot and round, glabrous capitula in bud (Figures 3–4); these have been either recognized as *T*. *lunulata* (e.g., Rzedowski 2005) or *T*. *tenuifolia* (e.g., Villaseñor 2016).

In addition, the phylogenetic relationships of *Tagetes* have not been investigated using molecular data. The first hypothesis of relationships of the genus were based on morphology. Strother (1977) postulated that the Mexican genera *Gymnolaena* Rydb., *Adenopappus* Benth., and the South American genus *Vilobia* Strother were the closest relatives of *Tagetes*, as all share a uniseriate, connate involucre. However, in the molecular phylogenetic analyses of the tribe

Tageteae (Loockerman et al. 2003), *Adenopappus* and *Vilobia* were nested within *Tagetes* and the small, aquatic genus *Hydropectis* Rydb. (including *Hydrodyssodia* B.L.Turner) was resolved as the sister group of those three genera. Moreover, the phylogenetic relationships within *Tagetes* have not been evaluated using molecular data, and the results of the cladistic analyses based on morphology of Soule (1993) were not published. In this study we estimate the most densely sampled phylogeny to-date of *Tagetes* using ITS sequences aiming to 1) investigate the sister group to *Tagetes*; 2) corroborate the phylogenetic position of *Adenopappus* and *Vilobia* within *Tagetes*; 3) investigate species relationships within *Tagetes*.

Materials and methods

We sampled 22 species of *Tagetes*, including a wild form of *T*. *erecta*, the cultivated *T*. *patula*, *T*. *persicifolia* (Benth.) B.L.Turner (= *Adenopappus persicifolius* Benth.), and *T*. *praetermissa* (Strother) H.Rob. (= *Vilobia praetermissa* Strother). We also sampled the type species of *Adenophyllum* Pers., *Dyssodia* Cav., *Gymnolaena* (DC.) Rydb., and two out of the three species of *Hydropectis*. *Adenophyllum glandulosum* (Cav.) Strother was considered intermediate between *Dyssodia* and *Tagetes* (Strother 1969) and *Gymnolaena* was regarded as closely related to *Tagetes* (Turner 1996). These genera are classified in the subtribe Tagetinae. Moreover, we included two more outgroups, *Flaveria trinervia* (Spreng.) C.Mohr, from the subtribe Flaveriinae (tribe Tageteae), and *Helianthus annuus* L., from the tribe Heliantheae. The latter was used to root the trees. Leaves and voucher specimens were collected during fieldwork in Mexico. The leaves were preserved in silica gel and vouchers were imported to the University of California Davis (DAV) herbarium with the appropriate valid permits. Leaf samples were also removed from herbarium specimens with permission from curators from the following herbaria: CH, DAV,

HCIB, MEXU, SD, TEX. Moreover, additional 15 ITS sequences were extracted from GenBank. A list of voucher specimens and GenBank accession numbers is presented in the Appendix 1.

We used the DNeasy Plant Kit (Qiagen, Valencia, California) for DNA extraction and amplified the ITS region using the ITS5 and ITS4 primers from White et al. (1990). Taq PCR Core Kits (Qiagen, Valencia, California) were used to amplify the ITS region following the protocol of Rivera et al. (2016) with minor modifications. PCR products were separated by agarose gel electrophoresis and submitted for sequencing at the UC Davis College of Biological Sciences DNA Sequencing Facility.

Sequencher 5.4.6 (Gene Codes Corporation) was used to assemble contigs and edit the sequences. MUSCLE (Edgar 2004) implemented in MEGA 7.0 (Kumar et al. 2016) was used to align the sequences followed by minor manual adjustments. Gaps range from 1 to 5 base pairs in length and were treated as missing data. Both Bayesian inference and maximum likelihood approaches were conducted. MrBayes 3.2.7 (Huelsenbeck and Ronquist 2001) was used to perform the Bayesian phylogenetic analyses. The nucleotide substitution models were assessed by implementing Reversible Jump-Markov Chain Monte Carlo (RJ-MCMC). As implemented in MrBayes 3.2.7, all possible time-reversible substitution models (i.e., 203 models) are evaluated during the MCMC. According to the RJ-MCMC analysis, the four-parameter GTR submodel [122341] best fit the data with a posterior probability (PP) of 0.36. Two simultaneous independent runs of 1 million generations using four chains were applied. The runs were compared every 1000 generations and sampled every 100, discarding the first 25% samples as burn-in. Tracer 1.7.1 (Rambaut et al. 2018) was used to assess mixing and convergence. One million generations were more than enough for the two independent runs to converge, as our data set was relatively small. FigTree v1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/) was

used to edit the majority rule consensus tree that MrBayes uses to summarize the sampled phylogenetic trees.

Maximum likelihood and bootstrap analyses were conducted on RaxMLGUI 2.0 (Edler et al. 2020). The GTR substitution model was used since it is closer to the model identified using RJ-MCMC. Ten independent runs and 1000 replications were performed and summarized as a majority rule consensus tree.

RESULTS

The ITS region ranged from 640 base pairs (bp) in *Tagetes campanulata* Griseb. to 651 bp in *T. pringlei* S. Watson, and the aligned data set included 29 species and 678 characters. The majority rule consensus tree from the Bayesian and maximum likelihood analyses were totally congruent (Figure 5). *Tagetes* (including *Adenopappus* and *Vilobia*), was resolved as a monophyletic group with *Hydropectis* as its sister genus (Figure 5). Two main strongly supported subclades were resolved within *Tagetes*. The *Lucida* clade contains *T. persicifolia*, *T. lucida*, *T. pringlei*, *T. filifolia* Lag. and *T. micrantha*. The second subclade is composed of five major subclades (*Lunulata*, *Minuta*, *Moorei*, *Erecta* and *Lemmonii*), which are strongly supported (except the *Lemmonii* subclade, which is weakly supported).

The *Lunulata* subclade comprises *T. lunulata* as sister to *T. tenuifolia*, and with *T. multiflora* Kunth as sister to both. The *Minuta* subclade includes *T. minuta* L., resolved as sister to *T. praetermissa*, with *T. laxa* Cabrera sister to both, and *T. campanulata* Griseb. sister to all three. The *Moorei* subclade is composed of *T. moorei* H.Rob., resolved as sister to *T. parryi* A. Gray. The *Erecta* subclade is composed of the wild form of *T. erecta* and *T. patula* as sister species, with *T. foetidissima* DC. sister to both, and *T. subulata* Cerv. sister to all three. The *Lemmonii* subclade

includes *Tagetes lacera* Brandegee, *T*. *nelsonii* Greenm., and *T*. *lemmonii* A. Gray resolved as sister to *T*. *palmeri* A. Gray.

Discussion

Our results corroborate that *Hydropectis* is the closest relative of *Tagetes* (Loockerman et al. 2003), and further sampling may show that *Hydropectis* is nested within *Tagetes*. The *Tagetes* clade has a slightly low posterior probability value (PP=0.94), as usually a PP value of at least 0.95 is considered statistically robust (Wilcox et al. 2002). However, the *Tagetes* clade is strongly supported by the bootstrap value (0.91). *Hydropectis* is a small genus of three aquatic annuals endemic to Mexico (Turner 1995). It shows similarities to some species of *Tagetes*, such as *T*. *micrantha*, in having small heads with very reduced rays, but it differs from all species of *Tagetes* by having a base chromosome number of *x*=9 (Keil & Stuessy 1977, Zhao & Turner 1993), whereas in *Tagetes* the base chromosome number is *x*=11 or 12 (Soule 1993, Turner 1996). Our results also corroborate that *Adenopappus persicifolius* (=*T. persicifolia*) and *Vilobia praetermissa* (=*T. praetermissa*) belong to *Tagetes*, and that the genera *Dyssodia*, *Gymnolaena*, and *Adenophyllum* are distantly related to *Tagetes* only.

Since *T*. *erecta* and *T*. *patula* were resolved as sister taxa these are perhaps best treated as a single species as proposed by Turner (1996), but it is necessary to sample the cultivated form of *T*. *erecta*. It is also notable that our results suggest that *T*. *foetidissima* and *T*. *subulata* are the nearest relatives of *T*. *erecta*, but not *T*. *lunulata* as previously thought (Soule 1996). Moreover, our results support Rzedowski (2005) treatment of *Tagetes* populations from central Mexico that have an orange-reddish spot near the base of the ray as *T. lunulata*, regardless of the morphology of the head in bud. However, at least the populations from Mexico City and adjacent regions that we have seen in the field have glabrous, round capitula in bud (Figure 4 D–

F), while the populations of *T*. *lunulata* from Western and north-central Mexico have acute, setulaceous capitula in bud (Figure 3 E–F), and perhaps it is best to treat all of these as a single species with two varieties.

Some of the the resolved subclades within *Tagetes* are composed by species that are similar in ecology, morphology or geography. For instance, the *Lucida* subclade is composed of species that are common in wet soils (*T*. *micrantha* and *T*. *filifolia*) or are subaquatic (*T*. *pringlei*). Moreover, the species in this subclade have mostly a sweet anise-like aroma, and appear to correspond to the subgenus *Lucida* proposed by Neher (1966). We would expect that *T*. *epapposa* B.L.Turner, another subaquatic species that is similar in morphology to *T*. *pringlei*, is a member of this clade. The *Minuta* subclade includes only South American species. This group loosely matches Soule's series *Minutae* (1996); however, Soule (1996) placed *T*. *campanulata* in its own series (ser. *Campanulatae*), which is not supported by our preliminary results. The *Lemmonii* subclade woody species that occur mainly in northern Mexico and Southwest USA, except of *T. nelsonii*, which is confined to Chiapas in Southern Mexico and Guatemala.

To estimate phylogenetic relationships more accurately, it is necessary to sample the missing species of *Tagetes* and to use additional molecular markers, such as ETS, plastid markers, and/or low-copy targeted nuclear genes. A more robust phylogeny will be useful to investigate evolutionary processes, such as divergence times and character evolution, and to guide the search for new potential medicines or spices. Furthermore, knowing the closest relatives of the cultivated form of *T. erecta* will facilitate the search for desirable traits in the wild species.

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Appendix 1. Species and vouchers of plant material from which DNA was extracted, together with available GenBank accession numbers within brackets.

Adenophyllum *glandulosum*, *Hinojosa-Espinosa 630*, [ON695767] (MEXU). ***Dyssodia*** *papposa*, *Hinojosa-Espinosa 684* [ON798518] (DAV, MEXU). ***Flaveria*** *trinervia*, **Huffman s.n.**, [ON695768] (DAV). ***Gymnolaena*** *serratifolia*, *Cronquist 11219*, [ON695769] (MEXU). ***Helianthus*** *annuus*, *Schilling 660* [KX671853]. ***Hydropectis*** *aquatica*, *Soule 2796*, [ON695770] (DAV). *Hydropectis stevensii*, *Perez-Calix 4706*, [ON695771] (TEX). ***Tagetes campanulata***, *Soule 3553* (TEX), [AF413574]. ***Tagetes erecta***, *Hansen 126* (TEX), [KJ525046.1]. ***Tagetes filifolia*** [DQ862118.1]. ***Tagetes foetidissima*** [DQ862119.1]. ***Tagetes lacera***, *Medel 2014-03*, [ON695774] (HCIB). ***Tagetes laxa*** [KC800431.1]. ***Tagetes lemmonii***, *Reina 1120*, [ON695775] (HCIB). ***Tagetes lucida***, *Hinojosa-Espinosa 676*, [ON695772] (DAV, MEXU). ***Tagetes lunulata***, *Hinojosa-Espinosa 723* [ON695776], (DAV, MEXU). ***Tagetes micrantha***, *Hinojosa-Espinosa 724*, [ON695773] (DAV, MEXU). ***Tagetes multiflora*** [KC800434.1]. ***Tagetes minuta*** [AF413576]. ***Tagetes moorei*** [KC800433.1]. ***Tagetes nelsonii***, *Hinojosa-Espinosa 731*, [ON695777] (CH, DAV, MEXU). ***Tagetes palmeri***, *Soule 3362* (TEX), [AF413577]. ***Tagetes parryi*** [KC800427.1]. ***Tagetes patula*** [DQ862121.1]. ***Tagetes persicifolia***, *Sundberg 2954* (TEX), [AF413580]. ***Tagetes praetermissa***, *Balls 6183* (UC), [AF413581]. ***Tagetes pringlei***, Soule 2798 (TEX), [AF413578]. ***Tagetes subulata***, *Rebman 30705*, [ON695778] (SD). ***Tagetes tenuifolia*** (*sensu* Turner 1996, *T. lunulata sensu* Rzedowski 2005), *Hinojosa-Espinosa 677* [ON695779] (DAV).

FIGURES

Fig. 1. Native geographic range of *Tagetes* shaded with red (adapted from Soule 1993)

Fig. 2. Selected species of *Tagetes*. A–C. *T*. *lemmonii*. D. *T*. *micrantha*. E. *T*. *foetidissima*. F. *T*. *subulata*. G. *T. nelsonii*. H. *T*. *filifolia*. All pictures by OHE.

Fig. 3. A. Wild form of *Tagetes erecta,* note purplish corolla lobes. B. The cultivated form of *T*. *erecta*. C–D. Use of *T*. *erecta* during the Day of the Dead holiday. E–F. *Tagetes lunulata*, note acute capitulum in bud with apical hairs and red-orange spot at the base of the rays. All pictures by OHE.

Fig. 4. A–C. *Tagetes lucida*, locally known as *pericón* or *yerbanís*, is mostly used as a medicine, and occasionally in religious festivities. D–F. *Tagetes tenuifolia sensu* Turner (1996). Note red-orange spot at the base of the rays and glabrous and round capitulum in bud. All pictures by OHE.

Fig. 5. Majority rule consensus tree of 10,000 sampled trees from the Bayesian analysis of *Tagetes* using ITS performed in MrBayes. Posterior probabilities annotated next to the nodes followed by bootstrap values from the maximum likelihood and bootstrap analyses (1000 replications) of the same data conducted in RaxMLGUI.

FIG. 1.

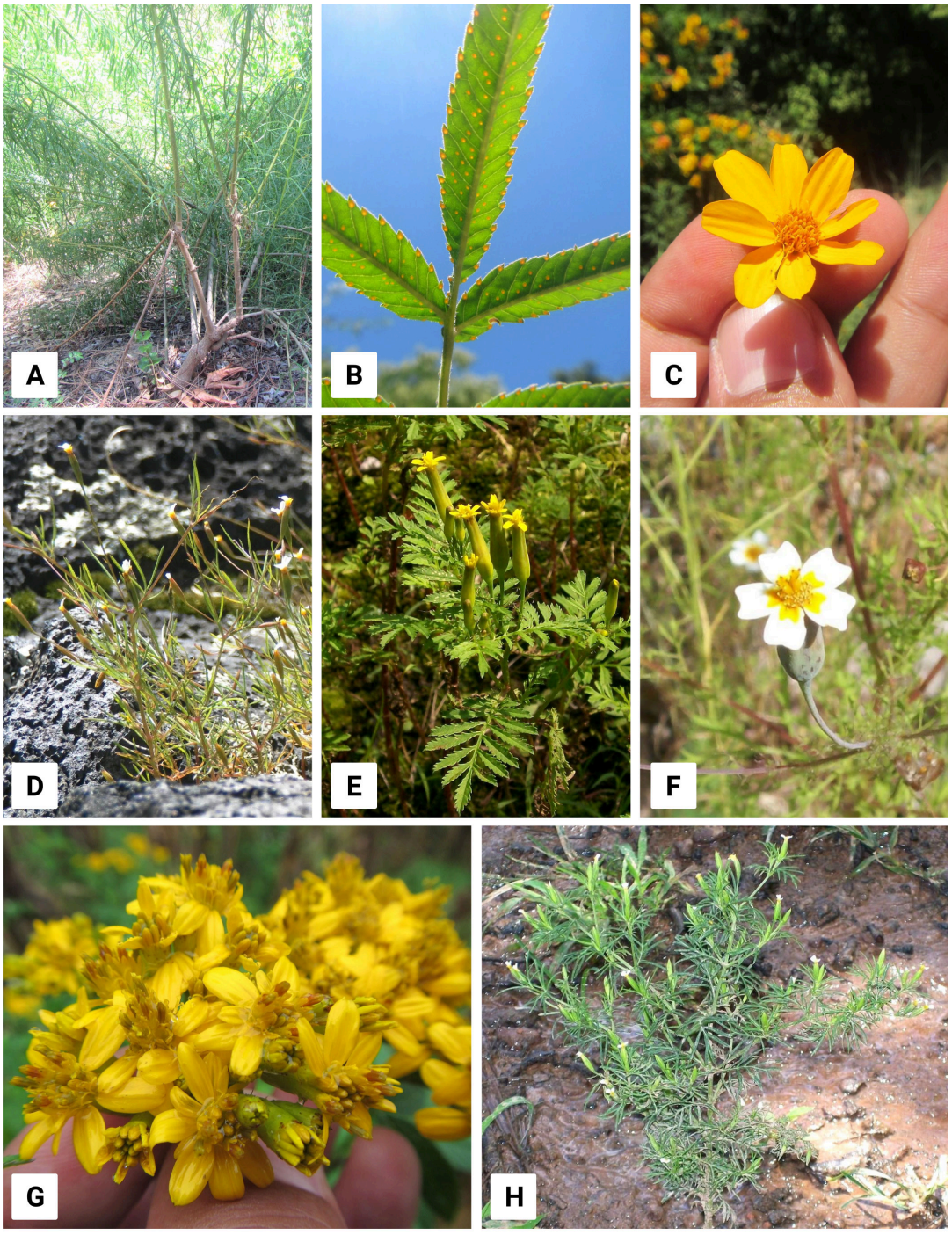

Fig. 2.

Fig. 3.

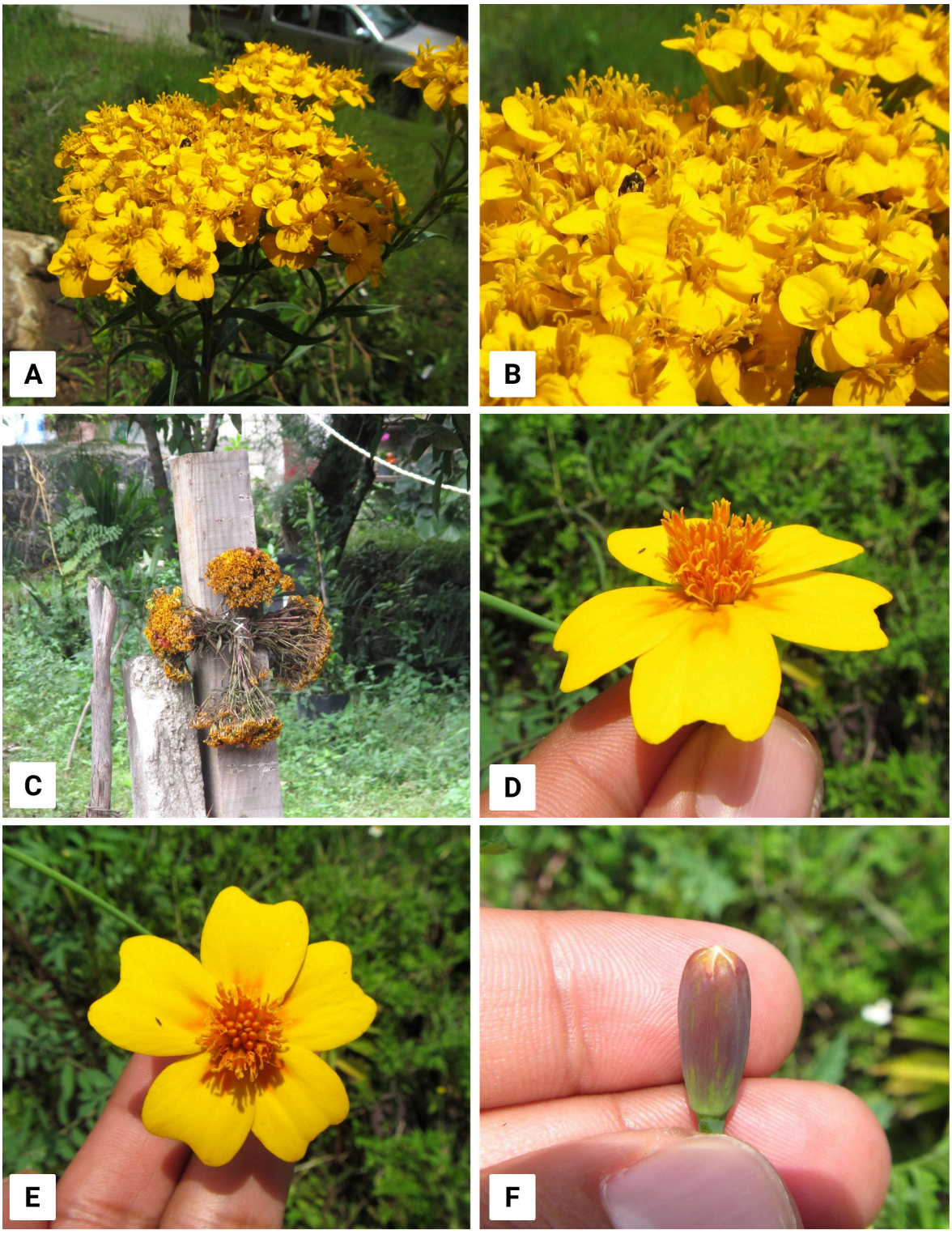

Fig. 4.

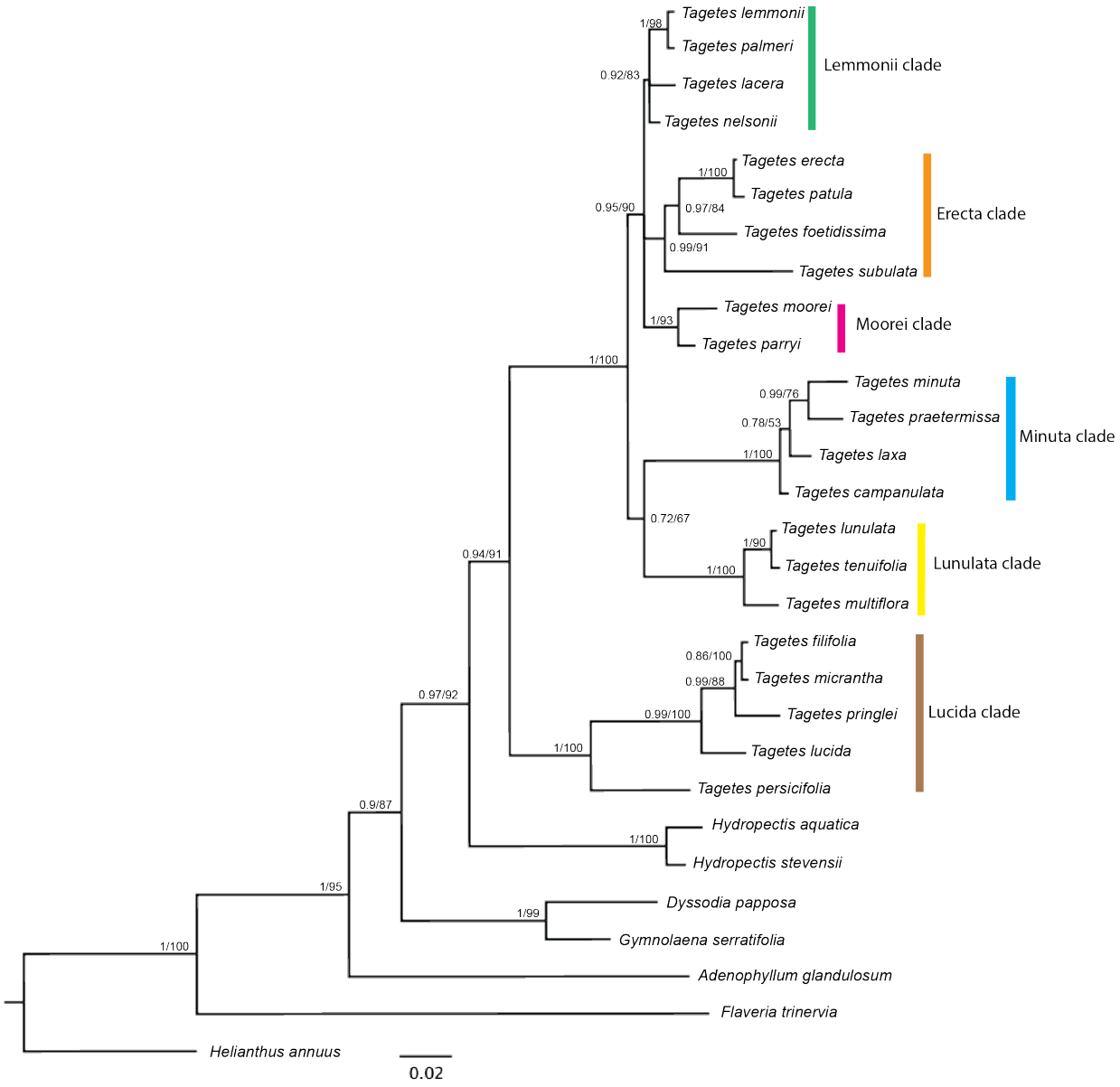
Tagetes lemmonii
Tagetes palmeri
Tagetes lacera
Tagetes nelsonii
Lemmonii clade
Tagetes erecta
Tagetes patula
Tagetes foetidissima
Tagetes subulata
Erecta clade
Tagetes moorei
Tagetes parryi
Moorei clade
Tagetes minuta
Tagetes praetermissa
Tagetes laxa
Tagetes campanulata
Minuta clade
Tagetes lunulata
Tagetes tenuifolia
Tagetes multiflora
Lunulata clade
Tagetes filifolia
Tagetes micrantha
Tagetes pringlei
Tagetes lucida
Tagetes persicifolia
Lucida clade
Hydropectis aquatica
Hydropectis stevensii
Dyssodia papposa
Gymnolaena serratifolia
Adenophyllum glandulosum
Flaveria trinervia
Helianthus annuus
1/98
0.92/83
1/100
0.97/84
0.95/90
0.99/91
1/93
1/100
0.99/76
0.78/53
1/100
0.72/67
1/90
1/100
0.94/91
0.86/100
0.99/88
0.99/100
0.97/92
1/100
0.9/87
1/100
1/95
1/99
1/100
0.02

Fig. 5.

Chapter 4

Systematics of the plumeweeds: The genus *Carminatia* (Eupatorieae, Asteraceae)

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***Abstract*—**The genus *Carminatia* comprises four species of annual herbs with opposite leaves, broadly ovate-deltate to broadly ovate-suborbicular blades with truncate to cordate bases, discoid heads often arranged in spike-like or narrowly raceme-like paniculiform capitulescences, tubular corollas, and prismatic cypselae with a pappus of plumose bristles. *Carminatia* occurs from the southwestern United States to Central America, in scrublands, conifer-oak forests, and tropical deciduous forests. The phylogenetic relationships of *Carminatia* have been unclear, although recent analyses based on molecular data have shown *Brickelliastrum* as its sister genus; however, other analyses supported *Brickellia* as its closest relative. Moreover, the relationships among the species of *Carminatia* have not been fully investigated. In this study, the DNA sequences of the nuclear ITS and ETS, and the plastid psbA-trnH regions, were used to estimate the phylogenetic relationships of *Carminatia* through Bayesian approaches. All analyses performed support the monophyly of *Carminatia* and *Brickelliastrum* as its sister genus, but the relationships with other genera are unclear. *Carminatia alvarezii*, which is

restricted here to the Tehuacan-Cuicatlan region, was resolved as sister to the other species in the genus, *Carminatia recondita* and *C. tenuiflora* were recovered as sister species, and the relationships of *C. papagayana* and a new species described here, *C. balsana*, were equivocal. The new species occurs in the Balsas basin in Mexico, mostly in tropical deciduous forests. It shares the feature of a subapical corolla constriction with *C. alvarezii*, but it is more similar in appearance to *C. recondita*, from which it differs by its shorter heads, corollas, and cypselae, and by its non-secund capitulescences. Additional analyses using more markers, such as phylogenomic approaches, are desirable as they may improve resolution of the relationships within *Carminatia* and among other genera of the Eupatorieae. An updated taxonomic revision including descriptions, keys, distribution maps, and illustrations is provided.

Keywords—Alomiinae, *Brickellia*, *Brickelliastrum*, Compositae, *Dissothrix*, phylogenetics, plumose pappus bristles, taxonomy.

INTRODUCTION

Carminatia Moc. ex DC. is a genus of four species of the tribe Eupatorieae within Asteraceae (Turner 2009). All species are erect annual herbs with decussate, widely ovate-deltate to subcordate leaves and discoid heads, arranged in mostly spike-like or narrow raceme-like paniculiform capitulescences that often constitute more than 2/3 of the shoot (Fig. 1). There is a constant number of 11 flowers per head and their corollas are tubular, whitish, or greenish-white (Hinojosa-Espinosa 2013). The fruits are small prismatic cypselae (often called achenes), which are narrowly obconic, blackish at maturity, and crowned by the characteristic pappus of plumose bristles (Fig. 2). The feathery pappus is one of the main diagnostic characteristics of this genus and the name “plumeweed” has been applied to *C. tenuiflora* DC., the only species

that occurs in the USA (Spellenberg and Zucker 2019). Moreover, a chromosome number of 2n =10 has been found in all species investigated (Turner 1997, 2009).

Carminatia occurs from the southwest United States to Central America. The species occur in tropical deciduous forests, scrublands, and sometimes in juniper, oak, or pine forests. They are often somewhat hidden by the surrounding vegetation and are frequently found in shady and humid places, such as river bluffs, forested or rocky slopes, and canyon slopes, often growing in rock crevices, soil pockets in rock outcrops, stream banks, and sometimes as weeds on trails, in ditches, and along roadsides. The economic importance of *Carminatia* is quite limited. However, according to some herbarium collections (Rangel 1308 MEXU, Lemus 186 MEXU), *C. alvarezii* Rzed. & Calderon is used as fodder and it is sought by goat livestock in the municipality of Santa Maria Ixcatlan, in Oaxaca, Mexico. Other species also might be grazed by farm or even wild animals, such as deer, as evidenced by the finding of plants lacking their main stem near the base. Moreover, they may provide an important source of food to animals in scrublands, woodlands, and tropical forests where they occur.

Ecologically, *Carminatia* is noteworthy for being listed as one of the genera that includes at least one species occurring exclusively or primarily in the tropical deciduous forest of Mexico (Rzedowski and Calderon 2013). As the feathery pappus seems to facilitate the spreading of the small cypselae to disturbed sites, they may have a propensity for proliferating and becoming invasive, warranting caution that the species are not accidentally transported to regions outside their native range or to undesirable places, such as crop lands. Other New World species of tribe Eupatorieae have become troublesome weeds worldwide, such as *Mikania micrantha* Kunth and *Chromolaena odorata* (L.) R.M.King & H.Rob. (Weber 2017). In fact, some populations of *Carminatia recondita* McVaugh in central and eastern Mexico may have been introduced as a result of human activities (Turner 1988).

The genus *Carminatia* was revised by Turner (1988), who rendered a summary of the taxonomic history of the genus. Although the taxonomic revision was not based on the results of a phylogenetic analysis, Turner (1988) considered the genus to comprise extremely closely related species, and their phylogenetic relationships had remained unclear since it was published. However, in agreement with King and Robinson (1987), Turner (1988) placed the genus in the subtribe Alomiinae, which included 22 genera from North and South America. Among them, *Dissothrix* A.Gray, a monotypic South American genus, was regarded as the putative closest relative of *Carminatia* (Turner 1988). On the other hand, *Brickellia* Elliot, also placed in Alomiinae by King and Robinson (1987), was sister to *Carminatia* in a preliminary molecular phylogeny of the Eupatorieae (Robinson et al. 2009), based on a quite limited sampling of genera in which *Dissothrix* was not included. Subsequently, in phylogenetic analyses using morphological data and all putatively closely related genera including *Dissothrix*, Hinojosa-Espinosa (2013) recovered *Carminatia* as a clade with strong support and *Brickellia* as sister to it. There was only one synapomorphy (flat pappus bristles) supporting this sister relationship, and resampling analyses (bootstrap and character removal) did not recover the clade comprising *Carminatia* and *Brickellia*. Most species of *Brickellia* are perennial herbs and shrubs (Schilling et al. 2015), but a few are annuals that resemble those of *Carminatia*. Among the latter, *Brickellia diffusa* (Vahl) A. Gray and *B*. *filipes* B.L. Rob. have leaves that are quite similar to those of *Carminatia* (widely ovate-deltate to subcordate) and they occur in similar shady habitats in tropical deciduous forests. The pappus bristles in the species from the *Kuhnia* group of *Brickellia* (Turner 1989) are subplumose, although otherwise they do not resemble *Carminatia*. Likewise, some species, such as *B*. *odontophylla* A. Gray, have a spike-like or narrowly raceme-like capitulescence as in *Carminatia.*

While investigating the phylogenetic relationships of *Asanthus* R.M.King & H.Rob., a small North American genus segregated from *Brickellia*, Schilling et al. (2013) recovered *Brickelliastrum* R.M.King & H.Rob. as sister to *Carminatia* using ITS and psbA-trnH DNA sequences. In addition, *Steviopsis* R.M.King & H.Rob. was resolved as sister to the *Carminatia*-*Brickelliastrum* clade and *Asanthus* as sister to all three. However, these analyses did not sample all species of *Carminatia* since *Asanthus* was the focus of the research, and for the same reason other putative relatives of *Carminatia*, including *Dissothrix*, were not sampled in their investigation. Hinojosa-Espinosa (2013) also sampled the genera *Flyriella* R.M.King & H.Rob., *Kyrsteniopsis* R.M.King & H.Rob., and Liatris Gaertn. ex Schreb. *Flyriella* and *Kyrsteniopsis* have been classified in the subtribe Alomiinae since King and Robinson (1987) and they share with *Carminatia* the thin, glabrous style base, the prismatic 5-sided cypselae, and the basic chromosome number of x=10 (King & Robinson 1987). Moreover, *Flyriella* species are herbaceous plants with often subcordate leaves like those found in *Carminatia*. In *Liatris*, the pappus is also feathery and the capitulescences are spike-like, but otherwise the genus is quite different morphologically from *Carminatia*.

Relationships among the species of *Carminatia* are also not yet clear. Turner (1988) conceived *C*. *recondita* as the most "generalized" species, and *C*. *tenuiflora* as the most "advanced" one, the latter based on the morphology of the capitulescences and the slim corollas, while *C*. *alvarezii* was considered as "closer" to *C*. *recondita*. Later, when describing *C*. *papagayana*, it was regarded as similar to *C*. *recondita* (Turner 2009). However, Hinojosa-Espinosa (2013), based on phylogenetic analyses of morphological data resolved, *C*. *tenuiflora* as less distantly related to the remaining species in the genus based mainly on the thinner corollas, which resolved as an apomorphy for the species. Hinojosa-Espinosa also found *C*. *papagayana* as sister to *C*. *alvarezii*. In contrast, Schilling et al. (2013) found *C*. *papagayana* as

sister to *Carminatia recondita* and *C. alvarezii* as more distantly related to these two species. The objectives of this study are 1) to investigate further the phylogenetic relationships of *Carminatia* through phylogenetic analyses based on nrDNA and cpDNA sequences that include samples of all species of the genus and all putative closely related genera; 2) to provide an updated taxonomic revision for the genus.

Materials and Methods

Herbarium and field work—The morphology of *Carminatia* and relatives was studied extensively by examining herbarium specimens from CIIDIR, DAV, ENCB, FCME, HUAZ, IBUG, MEXU, QMEX, SLPM, USON, and digital images, especially of type specimens, available from ARIZ, ASC, ASU, DES, GH, MICH, MO, NY, TEX, UC, and US (abbreviations follow Thiers 2021). The identification of each herbarium specimen used for DNA sampling was always corroborated and corrected when necessary. The morphology of the species of *Carminatia* was also studied in the field in 2010–2013 and again in 2019, when fresh leaves were collected and dried in silica gel for the species that were found.

Taxon sampling—A list of taxa and accessions sampled, with associated voucher specimens and GenBank sequence accession numbers, is presented in Appendix 1. The four species of *Carminatia* constituted the ingroup for the phylogenetic analyses. *C. alvarezii* and *C. recondita* exhibit morphological variation across their distributional ranges (Turner 1988, Hinojosa-Espinosa 2013) and thus, samples from different regions were included in the analyses. In the case of *C. alvarezii*, samples 3 and 4 of come from the Tehuacan-Cuicatlan region and the remaining samples come from the Balsas Basin region. *Carminatia tenuiflora* is more homogeneous morphologically and thus fewer samples were used. Only one sample of *C.*

papagayana was available for this study although we also downloaded from GenBank the sequences generated by Schilling et al. (2013).

The outgroup comprised samples from all genera of the tribe Eupatorieae that are putatively closely related to *Carminatia*, i.e., *Asanthus*, *Brickellia*, *Brickelliastrum*, *Dissothrix*, and *Steviopsis* (Robinson 2009; Hinojosa-Espinosa 2013; Schilling et al. 2013). The sampling of 12 species of *Brickellia* was coordinated with that of Hinojosa-Espinosa (2013), in which the species that were considered as the most similar morphologically to *Carminatia* were sampled. Moreover, five additional species of Brickellia were sampled. In addition, 32 GenBank accessions were used in this study (Appendix 1), including ITS and ETS accessions of *Dissothrix imbricata* (Gardner) B.L.Rob., from which herbarium samples were not available to us. Furthermore, although Hinojosa-Espinosa (2013) did not find the genera *Flyriella* R.M.King, *Kyrsteniopsis*, and Liatris being closely related to *Carminatia* in the phylogenetic analyses based on morphology, one species of each of these genera was sampled to explore further their phylogenetic relationships based on DNA sequences.

Likewise, the type species of *Alomia* Kunth, *A*. *ageratoides* Kunth, was also included. This taxon was not sampled by Hinojosa-Espinosa (2013), but it was sampled here as *Alomia* is the type genus of the subtribe Alomiinae. Finally, Stevia lucida Lag. was used to root the trees. *Stevia* Cav. was resolved as one of the most distantly related clades to most Eupatorieae taxa (including the Brickellia-Alomia clade) in the phylogenies by Rivera et al. (2016). Molecular markers—ITS, ETS, and the plastid *psbA-trnH* were selected to estimate phylogenetic relationships. The nrDNA markers have been used to infer phylogenies in the Eupatorieae (Schilling 2011; Schilling 2013, 2015; Tippery et al., 2014, Rivera 2016) and in other groups in the Asteraceae, for example, Baldwin (1992) and Baldwin and Markos (1998). The estimated phylogenies often have a high level of resolution at the genus and species level. Likewise, the

plastid *psbA-trnH* region was also used by Schilling et al. (2013, 2015) to investigate the phylogenetic relationships of *Asanthus* and *Brickellia* and they obtained congruent tree topologies compared to those inferred using the nrDNA markers. Moreover, there are several sequences of these three markers for several Eupatorieae taxa in GenBank that were downloaded to complement our data sets (Appendix 1).

DNA extractions, PCR, and sequencing—For each sample 5–20 mg of dry leaf tissue was used for DNA extractions with the DNeasy Plant Kit (Qiagen, Valencia, California), following the manufacturer's protocols with minor modifications. The concentration of DNA samples was measured with a spectrophotometer. Graphs of UV absorbance and the ratio of absorbance values (A260/A280) were also checked to determine the purity of the extracted DNA.
Taq PCR Core Kits (Qiagen, Valencia, California) in conjunction with Taq DNA polymerase from Thermo Fisher Scientific were used to amplify target genomic regions. The ITS region was amplified and sequenced for all taxa sampled using the forward and reverse primers from White et al. (1990): ITS5 (GGAAGTAAAAGTCGTAACAAGG) and ITS4 (TCCTCCGCTTATTGATATGC). To amplify and sequence the ETS regions, we used the primers employed by Rivera et al. (2016), which were modified versions of those used by Baldwin and Markos (1998): forward primer Ast–1m (CGTAAAGGTGTGTGAGTGGTTT), and reverse primer 18S–Alt (TGAGCCATTCGCAGTTTCACAGTC). The *psbA-trnH* intergenic spacer region was amplified and sequenced using the forward and reverse primers psbAF (GTTATGCATGAACGTAATGCTC) and trnHR (CGCGCATGGTGGATTCACAAATC) as in Sang et al. (1997). Each 50µl PCR reaction contained 41µl of double-distilled water, 5µl of 10× CoralLoad PCR Buffer, 1µl of dNTPs mixed solution (in an equimolar ratio of 10 mM), 0.25µl each of forward and reverse primer solution, 0.5µl of Taq DNA polymerase solution, and 2µl of the template DNA. In each amplification experiment, positive and negative (water) controls were included. The thermocycler was

programmed mostly following Rivera et al. (2016), with an initial denaturation of 4 minutes at 95°C, followed by 34 cycles, each consisting of an initial denaturation of 1 minute at 95°C, an annealing phase of 45 seconds at 50°C, and an extension period of 1 minute at 72°C, with a final extension period of 8 minutes at 72°. PCR products were checked and separated by agarose gel electrophoresis. The amplified DNA was extracted from the gel slices using the QIAquick Gel Extraction kit (Qiagen, Valencia, California), and the manufacturer's protocols with a few minor modifications, such as using 0.25µl of dd-water to elute the DNA instead of 50µl of Buffer TE.

The amplified DNA samples were submitted for sequencing to the College of Biological Sciences UCDNA Sequencing Facility at UC Davis where an ABI Prism 3730 Capillary Electrophoresis Genetic Analyzer and associated software (ABI Prism 3730 Data Collection Software v. 3.0, ABI Prism DNA Sequencing Analysis Software v. 5.2) were used for sequencing and data analyses.

***Sequence analyses and alignment*—**Sequencher 5.4.6 (Gene Codes Corporation) was used to assemble contigs and edit the sequences. Taxon and marker identity for all sequences generated were corroborated by performing BLAST searches. The limits of the ITS, ETS, and psbA-trnH sequences were determined using sequences downloaded from GenBank as guidance. Nucleotides outside these regions were trimmed in MEGA 7.0 (Kumar et al. 2016). In addition, about 20 of the first nucleotides of the 5' end of the ETS sequences were trimmed to get readable sequences. The sequences were aligned using MUSCLE (Edgar 2004) as implemented in MEGA using the default settings (-400 gap open penalty and 0 gap extension penalty). The alignments were checked and slightly edited manually. Finally, the alignments were exported as Fasta files and Mesquite 3.6 (Maddison and Maddison 2018) was used to export them as Nexus data file formats for MrBayes.

Phylogenetic analyses—Bayesian approaches were implemented to estimate phylogenetic relationships. Each data set (ITS, ETS, and psbA-trnH), was analyzed separately and another analysis combining the three data sets was conducted. The phylogenetic analyses were performed in MrBayes 3.2. with two simultaneous runs for 1 million generations each using default settings with the following exceptions: 1) The settings for substitution model were changed using the command lset = mixed, to implement Reversible Jump Markov Chain Monte Carlo (RJ-MCMC) as a method for model selection. As described by Huelsenbeck et al. (2004), this method allows evaluation of all the reversible-time substitution models (203 models in total) as part of the MCMC sampling avoiding the need to specify one substitution model only. A file with the models that have a posterior probability higher than 0.05 is generated. 2) The rate variation across sites parameter was changed from equal to model it as a gamma probability distribution (i.e., lset rates= gamma) following Huelsenbeck et al. (2004). 3) The tree samples from the two simultaneous runs were compared every 1000th generation, and each chain was sampled every 100th generation. The first 25% of the samples was discarded as "burn-in". The combined analysis was set as a 3-partitioned analysis, with a partition for each original data set. Five ETS sequences were not available for the combined analyses (two from *Steviopsis*, two from *Asanthus*, and one for *Carminatia papagayana*) and the plastid *psbA-trnH* sequence of *Dissothrix imbricata* was not available also. The parameters of the partitions were unlinked; this was achieved by entering the following commands: unlink statefreq=(all) revmat=(all) shape=(all) pinvar=(all). The overall rate was allowed to be different across the three partitions typing the commands: prset applyto=(all) ratepr=variable.

After running the analyses, we confirmed that the average standard deviation of the split frequencies between the two simultaneous runs was lower than 0.01, as this is a good indication of convergence (Ronquist et al. 2019). We also confirmed that the effective sample

size (ESS) was larger than 100, as this is an indication of appropriate mixing and convergence of the MCMC (Ronquist et al. 2019). Likewise, the Potential Scale Reduction Factor (PSRF) was inspected and found to fall between 1–1.1, which indicates good quality samples from the posterior probability distributions (Ronquist et al. 2019). Finally, the plot of the generations versus the log probabilities of the data were examined and the plotted values were randomly distributed, indicating stationarity (Ronquist et al. 2019).

***Taxonomic revision*—**The descriptions and keys were elaborated based on detailed examination of herbarium specimens under a stereoscope, previous taxonomic descriptions (protologues and taxonomic treatments), and field observations. Selected cypselae and corollas were removed from herbarium specimens examined under a Zeiss Axio Zoom V16 (Göttingen, Germany) microscope and photographed with a AxioCam MRc5 Zeiss camera (Göttingen, Germany). Ecogeographic data (phenology, habitat, elevational range, geographic coordinates, etc.) were compiled from the collection labels, the literature, and direct observations in the field. The geographic coordinates were corroborated and sometimes estimated or approximated from the information of the collection sites provided in the herbarium specimen labels. Distribution maps were generated using ArcGIS 8.1 (Environmental Systems Research Institute, Redlands, California).

RESULTS

ITS analyses—The length of the unaligned ITS sequences in the samples ranged from 638 base pairs (bp) in *Steviopsis dryophila* to 654 bp in *Brickellia laxiflora*, and from 644 to 646 bp within *Carminatia*. The aligned data set contained 53 taxa and was 689 characters in length. There were 430 uninformative characters. The substitution models with the highest posterior probabilities (pp) that were recovered during the RJ-MCMC were the submodel 123143 (0.213

pp), and the submodel 121341 (0.175 pp). The majority rule consensus tree from the Bayesian analyses is shown in Fig. 3.

ETS analyses—The aligned ETS data set contained 48 samples. ETS sequences were not available for five of the samples (two species of *Asanthus* and two of *Steviopsis*, and sample 8 of *Carminatia recondita*) for which ITS sequences were downloaded from GenBank. The ETS data sequences ranged from 377 bp in *Kyrsteniopsis nelsonii* to 407 bp in *B*. *diffusa*. The aligned data set was 443 characters and had 223 uninformative characters. The substitution models with the highest pp were the submodel 121121 (0.211 pp), and the submodel 121131 (0.167 pp). The majority rule consensus tree from the Bayesian analyses is shown in Fig. 4.

Plastid psbA-trnH analyses—The *psbA-trnH* data set included 52 samples. The sequences varied from 245 bp in *C. papagayana* to 407 bp in *Stevia lucida*. All *Carminatia* sequences were almost identical. They ranged from 245 bp in *C*. *papagayana* to 251 bp in *C*. *recondita*, *C*. *tenuiflora*, and the samples 3 and 4 of *C*. *alvarezii* (the remaining samples of *C*. *alvarezii* were 248 bp). The aligned data set was 499 characters in length and only 46 characters were informative. Notably, all *Carminatia* samples had a conspicuous deletion of 156 bp. This large deletion was first reported by Schilling et al. (2013) in their samples of *Carminatia*. All samples of *C*. *recondita* and *C*. *tenuiflora* were essentially the same. However, more variation was found within *C*. *alvarezii* and *C*. *papagayana*. The substitution models with the highest pp for this data set were the submodel 123222 (0.183 pp), and the submodel 123242 (0.117 pp). The majority rule consensus tree from the Bayesian analyses is shown in Fig. 5.

Combined (ITS, ETS, psbA-trnH) partitioned analyses—The combined data set contained 53 taxa and the aligned data matrix was in 1634 characters in length, with 493 informative characters. The substitution models with the highest pp for each partition, were the same submodels with highest pp that were obtained in the separate analyses of each data set. The

majority rule consensus tree from the Bayesian analyses of the combined data set is shown in Fig. 6.

As shown in Figs. 3–6, the ITS, ETS, *psbA-trnH*, and the combined, partitioned analyses were mostly congruent, but the highest resolution was achieved with the ITS and the combined analyses. Less resolution at the species and infraspecific level was attained with the *psbA-trnH* analyses. *Carminatia* was recovered as monophyletic in all estimated phylogenies (Figs. 3–6). Likewise, *Brickelliastrum* was resolved as sister to *Carminatia* in all analyses, as found by Schilling et al. (2013). However, none of the analyses conducted recovered Steviopsis as sister to the *Carminatia-Brickelliastrum* clade as in the analyses by Schilling et al. (2013). *Asanthus* was monophyletic in all estimated phylogenies, but its relationships with the other taxa were mostly ambiguous. Similarly, *Brickellia* was solved as monophyletic in all analyses, but distantly related to *Carminatia* or as a distinct clade with unclear affinities. *Dissothrix*, the putatively closest genus to *Carminatia* (Turner 1988), was recovered as sister to *Steviopsis* based on ETS sequences and in the combined analyses. Moreover, *Dissothrix* and *Steviopsis* were resolved in a well-supported clade with *Flyriella* and Liatris based on ITS, ETS, and the combined analyses. *Kyrsteniopsis nelsonii* was resolved as most distantly related to the rest of the taxa sampled, except for *Stevia lucida*, which was used to root the trees. The relationships of *Alomia* were also ambiguous in most analyses, but the ETS phylogeny recovered it as the most distantly related to the remaining taxa sampled after *Kyrsteniopsis*.

Within *Carminatia*, the samples of *C. tenuiflora* were resolved as a clade in all the phylogenies (1.0 pp) estimated based on ETS and the combined analyses (Figs. 4 and 6). This clade was also resolved by the ITS analyses (Fig. 3), but with less support (0.85 pp). There was no resolution within the *C. tenuiflora* clade. *Carminatia* recondita was resolved as sister to *C. tenuiflora* in all analyses with 0.99–1.0 of pp, except the *psbA-trnH* analyses, which failed to

resolve the samples of these two species as monophyletic groups (Fig. 8). However, the combined analyses (Fig. 6) resolved the samples of *C*. *recondita* as a clade in all sampled trees (1.0 pp) by the MCMC. The ETS and ITS phylogenies also found this clade but with less frequency (0.95 pp and 0.88 pp, respectively). There were three subclades within *Carminatia recondita* in all analyses except for the plastid one. One of them comprised the samples from Chiapas and Oaxaca (southern Mexico) with the group formed by the samples from Queretaro and Nuevo Leon (central and NE Mexico) as sister, and the third clade comprised the remaining samples. *Carminatia papagayana* was resolved as monophyletic in all analyses. This species was recovered as sister to the *C*. *recondita*-*C*. *tenuiflora* clade in most of the phylogenies based on ITS (0.75 pp), but the ETS analyses recovered *C*. *papagayana* as sister to a clade that comprised the samples of *C*. *alvarezii* from the Balsas Basin in the majority of the phylogenies estimated (0.59 pp). The *psbA*-*trnH* and combined analyses lacked resolution for the sister relationship of *C. papagayana* (Figs. 5 and 6). All the samples of *C*. *alvarezii* were not recovered as monophyletic in any of the analyses (Figs. 3–6). Instead, they formed two distinct but not sister clades, one comprising the samples from the Tehuacan-Cuicatlan Valley and the other comprising the samples from the Balsas Basin. The clade composed of the samples from the Tehuacan Valley was resolved as sister to the rest of the *Carminatia* samples in all analyses, except the one based on psbA-trnH, which lacked resolution. The clade of *C*. *alvarezii* composed of the samples from the Balsas Basin was resolved as two subclades in the ITS, ETS, and combined analyses. One of these two subclades comprised the samples from northeastern Guerrero and Puebla. The second subclade comprised the samples from central Guerrero, east Guerrero, and Michoacan.

Discussion

The phylogenetic analyses implemented in this study, in which all species of *Carminatia* were sampled, corroborate the hypothesis that this genus is monophyletic and that *Brickelliastrum* is its closest relative within the Eupatorieae, as discovered by Schilling et al. (2013). These two genera differ by several morphological characteristics, especially vegetative ones. Brickelliastrum comprises perennial herbs with solid stems and mostly alternate leaves that are gland-dotted on the abaxial surfaces. *Carminatia* comprises annuals with hollow stems and opposite leaves, at least at the base, and the leaf blades lack gland dots. The spike-like or narrowly raceme-like arrangement of heads found in many species of *Carminatia* is absent in *Brickelliastrum*, in which the heads are arranged in corymb-like clusters. Moreover, the pappus bristles are not plumose in *Brickelliastrum* as they are in *Carminatia*. However, the leaves in *Brickelliastrum* are clearly petiolate with deltate to ovate leaf blades that are truncate to subcordate at the base, as is commonly the case in *Carminatia*, and these two genera also share tubular corollas, prismatic, five-sided cypselae, and a chromosome number of x= 10. The relationships of *Carminatia* and *Brickelliastrum* with other Eupatorieae remain unclear, however. Schilling et al. (2013) found *Steviopsis* as sister to these two genera and Asanthus sister to all three, but this was not supported by the results of the analyses conducted here. This is likely due to differences in taxon sampling, mainly the inclusion of *Flyriella*, *Liatris*, and Dissothrix in this study, to which Steviopsis was found to be more closely related (Figs. 3–6). As the close relationship between *Carminatia* and *Brickelliastrum* was not suggested prior to the work of Schilling et al. (2013), further analyses including more genera may help to more firmly establish the closest relatives of these two genera. The use of additional molecular markers and especially phylogenomic approaches may also shed light on this question.

Our results did not support a close relationship between *Carminatia-Brickelliastrum* and *Alomia*, and indicate that *Carminatia* and *Brickelliastrum* may need to be removed from the subtribe Alomiinae. This subtribe as circumscribed by King and Robinson (1987) is polyphyletic as evidenced by the molecular phylogenies of Rivera et al. (2016). The phylogenies estimated here indicate that *Flyriella* also might need to be removed from the subtribe Alomiinae, and perhaps transferred to the Liatrinae (Figs. 3–6). *Flyriella* is a small genus from Texas and northeastern Mexico (Baker and Turner 1988), which had not been sampled in previous molecular phylogenetic studies (Rivera 2016, Robinson et al. 2009, Schilling et al. 2013, 2015). Samples of only the type species were used in this study, and it is desirable to sample the other three species in the genus in investigations that further explore its phylogenetic relationships. Turner (1988) considered Dissothrix as closely related to *Carminatia*, presumably because these two genera within the Alomiinae comprised annuals with opposite leaves, tubular corollas, and prismatic five-sided cypselae. The pappus, however, is quite different in the two genera. In *Dissothrix*, it is composed by five longer bristles and numerous shorter capillary bristles (versus the characteristic plumose bristles of *Carminatia*). Phylogenies estimated using morphological data did not support a close relationship between *Dissothrix* and *Carminatia* (Hinojosa-Espinosa 2013) and, according to the phylogenies estimated in this study, *Dissothrix* is more closely related to the genera *Liatris*, *Flyriella*, and *Steviopsis* than to *Carminatia*. However, as plastid sequences for *Dissothrix* were not available in this study, it will be desirable to use them to investigate further the relationships of this South American genus. According to Rivera et al. (2016), *Dissothrix* is more closely related to South American genera that were not sampled here. Thus, its apparent close relationship to North American Eupatorieae (*Flyriella*, *Liatris*, and *Steviopsis*) may not be corroborated in phylogenies based on denser genus sampling.

The results presented here support the view that *Brickellia* is not closely related to *Carminatia*. Thus, the morphological similarities between these taxa appear to have been acquired independently. Moreover, the results suggest the independent evolution of the feathery pappus in *Carminatia* and *Liatris* within Eupatorieae. Likewise, our results support the findings of Schilling et al. (2013) that *Asanthus*, *Brickelliastrum*, and *Steviopsis* comprise distinct lineages that merit generic recognition as proposed by King and Robinson (1987), but that *Dyscritogyne* R.M. King & H. Rob. (here represented by *Steviopsis adenosperma* and *S*. *dryophila*) is best treated as a synonym of *Steviopsis*. Although there was some resistance to the recognition of these and other small segregates proposed by King and Robinson (1987), for example by Turner (1997), most botanists, such as Schilling et al. (2013), have been recognizing the segregates that have been supported by molecular phylogenies.

Within *Carminatia*, our results did not support *C*. *tenuiflora* as sister to the remaining species of the genus (Hinojosa-Espinosa 2013) nor as the "most advanced" species (Turner 1988). Our findings indicate that this species is most closely related to *C*. *recondita*. For example, in the ITS phylogeny (Fig. 3) all samples of these two species were resolved as a strongly supported clade (pp=1.0) with two subclades, one comprising *C*. *tenuiflora* and the other *C*. *recondita* samples, but these subclades received lower support (pp=0.85 and 0.88, respectively). Likewise, the plastid *psbA-trnH* analyses failed to cluster the samples of these two species in two groupings, likely because their sequences were essentially the same. On the other hand, the ETS (Fig. 4) and the combined analyses (Fig. 6) resolved them as two groups with strong support (pp= 1.0). Moreover, there are several constant morphological differences between these two taxa. *Carminatia tenuiflora* comprises smaller plants with the heads erect to ascending and arranged spirally (Fig. 1), shorter involucres, corollas, and cypselae, and narrower corollas, while *C*. *recondita* are more robust plants with mostly hanging and secund heads (Fig.

1), longer involucres, corollas, and cypselae, and broader corollas. Also, *C*. *recondita* occurs mostly on Pacific slopes and in the Sierra Madre Oriental in Mexico southward to El Salvador and Honduras, while *C*. *tenuiflora* has a more inland distribution, ranging from SW USA to south Central Mexico and recently reported from Guatemala (Pruski and Robinson 2018). For these reasons, we decided to continue treating *C*. *tenuiflora* and *C*. *recondita* as distinct species.

Despite the morphological variation of *Carminatia recondita*, we decided not to recognize any infraspecific taxa as the clusters resolved within the species would be difficult to characterize morphologically. For example, not all samples from specimens with glandular trichomes formed a single clade (i.e., sample Carminatia_recondita3 is glandless). Moreover, we studied few herbarium specimens from central and northeastern Mexico and additional samples from these regions are needed to corroborate they constantly have smaller cypselae.

Our findings support the view of Hinojosa-Espinosa 2013 that the populations of *C*. *alvarezii* from the Balsas Basin warrant treatment as a distinct taxon. Hinojosa-Espinosa (2013) proposed to treat them as a variety of *C*. *alvarezii* as they have stipitate glands on the stems and a subapical corolla constriction, which are diagnostic for the species. However, this was not supported by the estimated phylogenies here, in which the samples from the Tehuacan-Cuicatlan Valley were resolved as a cluster that is less closely related to the remaining species than are representatives of *C*. *alvarezii* from the Balsas Basin. Our results, corroborate those of Schilling et al. (2013), who also found *C*. *alvarezii* from the Tehuacan-Cuicatlan region to be sister to the rest of the species of *Carminatia* they sampled. Based on our results and the morphological and biogeographic differences between the Balsas Basin and Tehuacan-Cuicatlan populations that are discussed further in the taxonomic treatment we decided to describe a new species for the populations of the Balsas Basin.

As for *C. papagayana*, the estimated phylogenies clearly support its recognition as a distinct species (Figs. 3–6). Hinojosa-Espinosa (2013) proposed to treat *C. papagayana* as another variety of *C. alvarezii*, which would be more similar morphologically to the populations from the Balsas Basin. This proposal was congruent with the phylogeny based on ETS, in which *C. papagayana* was resolved as sister to the clade comprising the samples of *C. alvarezii* from the Balsas Basin (Fig. 4). However, this node obtained low support (0.6 pp). On the other hand, the ITS phylogeny (Fig. 3) is more congruent with Schilling et al.'s (2013) findings, in which *C. papagayana* was sister to C. *recondita*. The incongruence between the ITS and ETS analyses with respect to the relationships of *C. papagayana* and the lack of resolution of the psbA-trnH analyses resulted in equivocal relationships for both *C. papagayana* and the newly described *C. balsana* in the combined analyses. Phylogenetic analyses based on data from additional plastid markers and/or broader sampling of the nuclear genome (e.g. Mandel et al. 2015) may yield phylogenies with better resolution that help to understand better the phylogenetic relationships within the genus.

TAXONOMIC TREATMENT

CARMINATIA Moc. ex DC., Prodr. 7: 267. 1838. Type: *C. tenuiflora* DC.

Erect or ascending taprooted annuals. Stems slender, fistulose, terete to polygonal, often unbranched, or occasionally with well-developed axillary branches, green, or sometimes red purplish, slightly sulcate, glabrous or sparsely puberulent to moderately pilose when young, sometimes scarcely to densely stipitate-glandular. Leaves simple, opposite, often confined to the proximal third of the shoot, becoming alternate and reduced or absent at the distal branches, conspicuously petiolate, blades cordate or subcordate to broadly ovate, ovate deltate or broadly ovate-suborbicular, moderately pilose when young, becoming sparsely pilose or

puberulent to glabrous, palmately veined with 3–5 main veins from the base, often membranous upon drying, base obtuse to cordate, margin irregularly dentate or serrate, rarely crenate, apex acute to acuminate. Capitulescence of fasciculately, loosely arranged, or tight cymiform clusters of heads in paniculiform arrays, often spikelike, mostly naked or rarely bracteate, and comprising the distal half or more of the shoot. Heads discoid, oblong, ascending or often pendulous, mostly short-pedunculate, rarely conspicuously pedunculate, peduncles wiry, puberulent, sometimes scarcely to densely stipitate-glandular; involucre cylindric, turbinate or subcampanulate, rarely narrowly ovoid when fresh, phyllaries imbricate, spirally arranged in 3–4 series, graduate, with the outermost phyllaries shorter and broader, middle phyllaries lanceolate, and innermost phyllaries lanceolate-oblong to linear-oblong, as long as the florets, conduplicate and embracing the marginal florets, all phyllaries green and frequently stained red-purplish, striate with three blackish veins, glabrous to pubescent, sometimes sparsely to rarely densely stipitate-glandular; receptacle naked, alveolate, glabrous. Florets 11 per head, perfect, fertile, corollas white or greenish-white, often stained red-purplish, narrowly tubular, sometimes filiform, with five small deltate apical lobes, often moderately to conspicuously constricted below apex, slightly attenuate towards the swollen base, rarely attenuate distally, vascular veins swollen towards base; anthers hyaline, inserted, with ovate, flat, apical appendages, thecae bases obtuse; style branches filiform to linear, barely to notably exserted, yellowish-green or white, with clavate to tapering apical appendages, style slender and glabrous, throughout. Cypselae prismatic, with five ribs, narrowly turbinate, or linear-turbinate to linear-fusiform, greyish to blackish, smooth to corrugate at maturity, minutely spiculate to sparsely puberulent or glandular, often arcuate at base, contracted below the pappus to a short pilose neck or truncate and glabrous below the pappus, carpopodium cartilaginous; pappus of white, plumose bristles, connate at the very base, straight to

conspicuously sinuous, dilated towards the base, persistent, but sometimes brittle and falling as a ring, in clusters or individually. Chromosome number 2*n*=20.

Carminatia is a genus of five species, including one newly described here. It occurs from SW United States to Honduras and El Salvador. All species occur in Mexico and three are endemic there.

KEY TO THE SPECIES OF *CARMINATIA*

1. Heads loosely arranged in a broad paniculiform capitulescence, or in tight cymiform clusters……2
    2. Stems, peduncles, and involucral bracts moderately to densely stipitate-glandular; all corollas conspicuously constricted below apex; heads short pedunculate, the peduncles up to 5 mm long; endemic to the Tehuacan-Cuicatlan region in Oaxaca and Puebla, Mexico……*C. alvarezii*
    2. Stems, peduncles, and involucral bracts glabrous to sparsely pubescent; few or none of the corollas constricted below apex; at least some heads conspicuously pedunculate, the peduncles up to 52 mm long; endemic to the Papagayo River area, near Acapulco, Guerrero, Mexico……*C. papagayana*
1. Heads fasciculately arranged in a spikelike paniculiform capitulescence, or not in tight cymiform clusters……3
    3. Corollas filiform, 0.3–0.4 mm wide when pressed; anthers 0.6–0.8 mm long; cypselae truncate and glabrous below the pappus; pappus bristles 10……*C. tenuiflora*
    3. Corollas tubular, 0.6–1.0 mm wide when pressed; anthers 1–2.3 mm long; cypselae contracted below the pappus to a short pilose neck; pappus bristles 13–18……4
        4. Involucre (11–) 13–17.5 mm long, corollas 7–10 mm long, none or few corollas constricted below apex; cypselae linear-turbinate to linear-subfusiform (3.8–) 5–8

mm long, Pacific slopes from Sinaloa, Mexico, to Honduras and El Salvador, and in the slopes of the Sierra Madre Oriental, in eastern Mexico……*C. recondita*

4. Involucre 9–12 mm long; corollas 5–6 mm long, all constricted below apex; cypselae narrowly turbinate, 2.9–3.7 (–4.3) mm long; Guerrero, Mexico State, Michoacan, (Morelos?) and eastern Puebla, in the Balsas Basin, Mexico……*C. balsana*

CARMINATIA ALVAREZII Rzed. & Calderón, Anales Esc. Nac. Ci. Biol. 31: 9. 1987—Type: Mexico. Oaxaca, 5 km al E de Teotitlán del Camino, por el camino a Huautla, 1350 m, laderas gneissicas con bosque tropical caducifolio, 25 Oct 1980, *Rzedowski 37075* (holotype: ENCB!, isotypes: MEXU!, NY [photo!]). *Carminatia anomala* B.L. Turner, Pl. Syst. Evol. 160: 173. 1988.— Type: Mexico. Puebla, along Hwy. 190 to Oaxaca, 3.6 mi N of the Oaxaca border, between Izucar de Matamoros and Huajuapan de Leon, limestone soil, with fan palms, Oct 6 1984, *Sundberg 3032* (holotype: TEX [photo!], isotypes: MEXU!, MO [photo!], NY [photo!]).

Stems 20–80 cm high, moderately to densely stipitate-glandular. Leaf blades 2.3–6.0 cm long, 2.6–7.3 cm wide, petiole 2.5–7.0 cm long. Capitulescence paniculiform, bracteate, comprising the distal third of the shoot, the heads in tight cymiform clusters, spreading to pendulous, not secund, short pedunculate, peduncles 1.7–5 mm long, moderately to densely stipitate-glandular; involucre turbinate to subcampanulate, 9–12 (–13) mm long, moderately to densely stipitate-glandular. Corollas narrowly tubular, 7–9 mm long, 0.8–1.1 mm wide when pressed, conspicuously constricted below apex, apical lobes 0.3–0.4 mm long, sparsely glandular, anthers 1.7–2.5 mm long, style branches linear, apical appendages clavate, 0.2–0.3 mm wide. Cypselae narrowly turbinate, 3.0–4.8 mm long, 0.8–1.0 mm wide, sparsely glandular, contracted below the pappus to a short pilose neck; pappus bristles 10–20, 3–5 mm long, conspicuously sinuous, persistent. Chromosome number 2n=20.

Distribution and Habitat—*Carminatia alvarezii* as circumscribed here is only known from the Tehuacan-Cuicatlan Valley, in the states of Oaxaca and Puebla in south-central Mexico (Fig. 7). This area is well known for its high floristic richness and endemism (Dávila et al. 2002). *Carminatia alvarezii* occurs at the bases of forested hills, mostly on limestone or sometimes gneissic-derived soils, in dry scrublands, *Quercus* forests, and tropical deciduous forests at 1350–2420 m altitude. It is associated with *Ferocactus*, *Beaucarnea*, *Bursera*, *Krameria*, *Yucca*, and *Brahea*. Flowering occurs from September to December.

Notes—*Carminatia alvarezii* as treated here is easily distinguished by many characteristics, such as the stipitate-glandular indument, which is usually dense in the stem, branches, peduncles, and involucre, the corollas, which are conspicuously constricted below the apex, and the glandular cypselae with sinuous pappus bristles. In addition, in this species the mostly naked spike-like paniculiform capitulescence that usually comprises more than half of the plant is lacking. Instead, the heads are clustered in tight terminal cymiform arrays. The main branches of these clusters are subtended by usually well-developed leaves. It is used as fodder in Santa María Ixcatlán, Oaxaca.

Additional specimens examined—Mexico. —Oaxaca. *Hinojosa-Espinosa 698* (DAV, MEXU); *Lemus 186* (MEXU); *Panero 6776* (CIIDIR, MEXU, TEX); *Rangel 1308* (MEXU); *Salinas 6485* (MEXU); *Tenorio 17622* (MEXU); s.c. *377* (MEXU); *Tenorio 21343* (MEXU). Puebla. *Mota 107* (MEXU); *Ripley 14709* (NY); *Rosas 240* (MEXU); *Tenorio 7250C* (MEXU); *Tenorio 7624* (MEXU, TEX); *Tenorio 7693* (MEXU); *Tenorio 7788* (ENCB, MEXU); *Tenorio 7924* (MEXU); *Tenorio 15311* (MEXU); *Tenorio 17711* (FCME, MEXU, SLPM); *Tenorio 21101* (MEXU).

CARMINATIA PAPAGAYANA B.L.Turner, Phytologia, 91: 88. 2009— Type: Mexico. Guerrero, Acapulco. Autopista del Sol Mexico-Acapulco, zona rocosa a unos metros del puente sobre el Rio

Papagayo, 280 m, 17° 08’ 2.9” N, 99° 33’ 24.2” W, Oct 9 1995, *Panero 6193* (holotype: TEX [photo!], isotype: MEXU!).

Stems 20–170 cm high, glabrous to sparsely pubescent. Leaf blades 3.4–10 cm long, 3.7–14 cm wide, petiole 3.4–11 cm long. Capitulescence paniculiform, naked, comprising the distal half of the shoot, the heads loosely arranged in the broad paniculiform array, spreading to ascendent, not secund, short to long pedunculate, the peduncles 2–52 mm long, puberulent to glabrous; involucre cylindric, 10–13 mm long; glabrous or sparsely puberulent. Corollas narrowly tubular, 6.5–8.4 mm long, 0.7–1.1 mm wide when pressed, few or none of the corollas constricted below apex, apical lobes 0.2–0.3 mm long, glabrous or occasionally sparsely pubescent, anthers 1.7–2.3 mm long, style branches linear, apical appendages clavate, 0.2–0.3 mm wide. Cypselae narrowly turbinate to linear-turbinate, 4.5–7 mm long, 0.7 mm wide, glabrous to sparsely puberulent, contracted below pappus to a short pilose neck; pappus bristles 15–18, 5–8 mm long, straight, rarely slightly sinuous, persistent. Chromosome number 2n=20. Figs.

Distribution and Habitat—*Carminatia papagayana* is only known from the Papagayo River area near Acapulco, Guerrero, in southwestern Mexico (Fig. 7), where it is found in tropical deciduous forests at 157–280 m elevation on steep slopes, along roadsides and riverbanks, and in limestone crevices. It occurs with species of *Anthurium*, *Cecropia*, *Ipomoea*, *Hectia*, and *Hofmeisteria*. Flowering occurs from September to November.

Notes—*Carminatia papagayana* is easily distinguished by its mostly glabrous condition, long-pedunculated heads, and narrow geographic distribution. Occasionally, some corollas have a subapical constriction, as in *C. alvarezii* and *C. balsana*. Turner (1988) cited one specimen collected almost at the type locality (*Kruse 1220*) as *C. alvarezii*. For these reasons Hinojosa-Espinosa (2013) proposed that the taxon here designated as *C. papagayana* should be treated

as a long-pedunculate variety of *C. alvarezii* and the specimens from the Balsas Basin, here treated as *Carminatia balsana*, as another variety of *C. alvarezii*, but the phylogenetic analyses conducted here clearly support treatment of these three taxa as distinct species (Figs. 3–6).

Additional specimens examined—Mexico. —Guerrero. *Hinojosa-Espinosa 578* (MEXU); *Kruse 1220* (ENCB, FCME, MEXU); *Villaseñor 1857* (MEXU).

CARMINATIA TENUIFLORA DC., Prodr. 7: 267. 1838— Type: Mexico. Prov. *Leoninâ ad occid. urbis Guanaxuato*, *Mendez 3* (holotype: G-DC [photo!]). *Brickellia tenuiflora* (DC.) Keil & Pinkava, Amer. J. Bot. 63: 1393. 1976.

Stems 20–120 cm high, moderately pilose to mostly glabrous. Leaf blades 1–10 cm long, 1.2–15 cm wide, petiole 1–7 cm long. Capitulescence paniculiform, spike-like, naked, comprising 2/3 or more of the shoot, the heads in fasciculate clusters, ascendent, not secund (may appear so in herbarium specimens), short-pedunculate, peduncles 1.2–7 mm long, glabrous to puberulent, involucre narrowly turbinate to cylindric, 11–14 mm long, glabrous to puberulent. Corollas filiform (5-) 6-7 (-8) mm long, 0.3–0.4 mm wide when pressed, not constricted below apex, apical lobes 0.1 mm long, sparsely pubescent, anthers 0.6–0.8 mm long, style branches filiform, apical appendages acute to clavate, 0.1 mm wide or less. Cypselae linear-turbinate to linear-subfusiform, 4–5 mm long, 0.5–0.7 mm wide, sparsely puberulent, truncate and glabrous below the pappus; pappus bristles 10, 5.5–7.5 mm long, straight, often brittle and falling as a ring or in clusters. Chromosome number 2*n*=20. Figs.

Distribution and Habitat—*Carminatia tenuiflora* is native to the southwestern USA to south-central Mexico (Fig. 8), where it is found in grasslands, scrublands, Juniper and/or *Quercus* forests, and tropical deciduous forest at elevations from 994–2312 m on canyon slopes, rock outcrops, road banks and trail-sides. Flowering occurs from September to November.

Notes—*Carminatia tenuiflora* is unique in the genus for its narrow corollas and ascendent heads arranged in a spike-like paniculiform capitulescence. Moreover, the pilose neck between the cypselae apex and the pappus present in all other species is absent in *C. tenuiflora*. Spellenberg and Zucker (2019) used the common name plumeweed for this species, but here that name is used for the whole genus.

Additional specimens examined—. Mexico. Aguascalientes. *De la* Cerda *7043* (SLPM), *Hinojosa-Espinosa 718* (MEXU); *Garcia 4485* (MEXU); *Ramirez 946* (SLPM). Baja California Sur. *Carter 3474* (MEXU); *León 741* (MEXU). Coahuila. *Henrickson 15044* (MEXU); *Wendt 1722* (MEXU, TEX). Chihuahua. *Gentry 2874* (MEXU); *Laferriere 2105* (MEXU, TEX); *Lebgue 2520* (MEXU); *Palmer 239* (MEXU, NY); *Pringle 1017* (MEXU, NY); *Spellenberg 12100* (MEXU); *Spencer 1135* (MEXU); *Sundberg 2778* (MEXU, TEX); *Weber 8375* (MEXU). Durango. *Gonzalez 3019* (MEXU); *Patoni 267* (ENCB, MEXU). Guanajuato. *Aguilera 288* (IBUG); *Flores 19* (ENCB, MICH, MEXU); *Rzedowski 40588* (ENCB, MEXU); *Zamudio 4673* (MEXU). Guerrero. *Moore 5564* (MEXU). Hidalgo. *Moore 5423* (MEXU). Jalisco. *Barrie 1153* (MEXU); *Breedlove 61654* (MEXU, NY); *Carrillo 3495* (IBUG); *Harker 1860* (IBUG); *Keil 15604* (MEXU); *Lot 1351* (MEXU); *Santana 7577* (IBUG); *Villarreal de Puga 1140* (IBUG); *Villarreal de Puga 2098* (IBUG); *Villarreal de Puga 3559* (IBUG); *Villarreal de Puga 8081* (IBUG); *Villarreal de Puga 8152* (IBUG); *Villarreal de Puga 10887* (IBUG); *Villaseñor 724* (MEXU). Mexico City. *Cespedes 671* (MEXU); *Hinojosa-Espinosa 573* (MEXU); *Hinojosa-Espinosa 574* (MEXU); *Lyonnet 800* (MEXU); *Lyonnet 1679* (MEXU, NY); *Urbina 8807* (MEXU). Mexico State. *Hinojosa-Espinosa 628* (MEXU); *Hinton 4871* (HINTON); *Vibrans 5622* (MEXU); *Vibrans 6227* (MEXU). *Michoacan*. *Diaz 2952* (ENCB, IBUG); *Diaz 3132* (ENCB, IBUG); *Diaz 3188* (MEXU); *Escobedo 2189* (MEXU); *Espinosa 2242* (ENCB, SLPM); *Grimaldo 551* (MEXU); *Ramamoorthy 4326* (MEXU); *Rzedowski 39108* (ENCB, IBUG, MEXU); *Santos 1852* (ENCB, IBUG, MEXU); *Silva 447* (MEXU); *Tejero 5270* (MEXU). Morelos. *Cabrera*

12124 (MEXU); *Castro 127* (IBUG); *Cerros 356* (FCME, IBUG). Nayarit. *Flores 1464* (MEXU); *Flores 1586* (MEXU); *Flores 1646* (IBUG, MEXU); *Flores 4028* (MEXU); *Harker 822* (IBUG); *Téllez 11059* (MEXU); *Tenorio 16530* (MEXU). Nuevo Leon. *Hinton 22524* (HINTON, TEX). Oaxaca. *Lopez 1516* (MEXU); *Sundberg 3071* (TEX). Queretaro. *Hernandez 4765* (QMEX). Sinaloa. *Vega 1754* (IBUG, MEXU, SLPM). Sonora. *Burquez 1244* (MEXU); *Flores 5057* (MEXU); *Joyal 1857* (MEXU); *Sanchez 110* (USON); *Thurber 1017* (NY); *Van Devender 506* (MEXU, TEX). VERACRUZ. *Müller 671* (NY). Zacatecas. *Balleza 9636* (HUAZ, IBUG, MEXU); *Balleza 1301* (HUAZ, MEXU); *Balleza 7269* (HUAZ, MEXU); *Balleza 10080* (HUAZ, MEXU); *Balleza 11799* (HUAZ, MEXU); *Gonzalez 29* (IBUG). USA: Arizona. *Carnahan 2010* (ARIZ); *Coburn 2810* (ASU); *Hodgson 20754* (DES); *Reif 10624* (ARIZ); *Reeves R1791* (ARIZ); *Smith 111* (ASC). New Mexico. *Worthington 15101* (NY); *Worthington 20087* (NY); *Worthington 21586* (NY). Texas: *Carr 16976* (TEX); *Carr 23500* (TEX); *Correll 33677* (ENCB); *Lott 5291* (TEX).

CARMINATIA RECONDITA McVaugh, Contr. Univ. Michigan Herb. 9: 384. 1972.—Type: Mexico. Nayarit, Mountains 10 miles southeast of Ahuacatlan, on the road to Barranca del Oro and Amatlan, precipitous rocky south facing slopes, elevation 1100-1300 m, wooded ravine near water, above the road, weedy in an old road, Nov 17–18 1959, *McVaugh 804* (holotype: MICH [photo!], isotypes: ENCB!, NY [photo!], TEX [photo!], US [photo!]). *Brickellia recondita* (McVaugh) Keil & Pinkava, Amer. J. Bot. 63: 1393. 1976.

Stems 20–200 cm high, glabrous to sparsely pilous, sometimes scarcely to moderately stipitate-glandular. Leaf blades 2–15 cm long, 2–17 cm wide, petiole 1–15 cm long. Capitulescence paniculiform, spike-like, naked, comprising 2/3 or more of the shoot, the heads in fasciculate clusters, pendulous, secund, short pedunculate, peduncles 0.5–3.7 mm long, glabrous to puberulent, rarely stipitate-glandular; involucre cylindric, (11–) 13–17.5 mm long,

glabrous to puberulent, rarely stipitate-glandular. Corollas narrowly tubular, 7–10 mm long, 0.6–1.0 mm wide when pressed, none or few constricted below apex, apical lobes 0.2–0.4 mm long, glabrous or occasionally sparsely pubescent, anthers 1.0–1.5 mm long, style branches filiform or linear, apical appendages acute to clavate, 0.1–0.2 mm wide. Cypselae linear-turbinate to linear-subfusiform, (3.8–) 5–8 mm long, 0.5–0.7 mm wide, glabrous to sparsely puberulent, contracted below the pappus to a short pilose neck; pappus bristles 11–17, 5.5–7.5 mm long, straight, mostly persistent, sometimes brittle and falling as a ring or in clusters. Chromosome number 2*n*=20.

Distribution and Habitat—*Carminatia recondita* occurs on Pacific slopes from Sinaloa, Mexico south to Honduras and El Salvador, and it is also found on slopes of the Sierra Madre Oriental, from Nuevo Leon to Hidalgo and Veracruz (Fig. 9). This species occurs mostly in canyons, open hillsides, steep slopes, roadsides, road banks, and stream banks of tropical deciduous forests and scrublands, and sometimes in *Quercus* or *Quercus-Pinus* forests, at elevations of 10–2200 m. Flowering occurs from October to November.

Notes—*Carminatia recondita* is distinguished by its long, pendulous, heads, which are directed to one side (Fig. 1); however, this latter feature may not be evident in some herbarium specimens. This species also has the longest corollas and cypselae in the genus. However, in some specimens, especially from Oaxaca and Queretaro, the involucre and cypselae are shorter than usual (about 11–12 mm and 4–5 mm long, respectively) and these specimens are sometimes identified as *C. alvarezii*, which is confined to the Tehuacan-Cuicatlan region. This kind of specimens of *C*. *recondita* can also be confused with the new species described here (and discussed under the new species below). When the cypselae of *C*. *recondita* are shorter (4–5 mm long), they are usually linear-turbinate and resemble those of *C*. *tenuiflora* (Fig. 2). In both, *C*. *balsana* and *C*. *alvarezii*, the cypselae are clearly broader at apex and attenuate to base

(Fig. 2). The more common type of cypselae shape in *Carminatia recondita*, here described as linear-subfusiform.

Representative Specimens— El Salvador. Ataco. *Linares 3780* (MEXU); *Sandoval 1501* (MEXU). Candelaria de la Frontera. *Linares 952* (MEXU), *Linares 3948* (MEXU). Guatemala. Guatemala. Prusky 4506 (MO). Huehuetenango. *Standley 81239* (F), *Stuessy 4320* (MEXU). Honduras. Copan. *Pruski 4535* (MO). Mexico. Colima. *Cuevas 5402* (IBUG); *Orcutt 4655* (MEXU); *Ramirez 2412* (IBUG); *Reyes* s.n. (IBUG). Chiapas. *Bachem 216* (MEXU); *Breedlove 13498* (NY); *Breedlove 13826* (ENCB, NY); *Breedlove 29091* (ENCB, NY); *Breedlove 40599* (MEXU); *Breedlove 46151* (ENCB); *Breedlove 46691* (MEXU); *Breedlove 46769* (MEXU, NY); *Breedlove 54306* (NY); *Breedlove 54482* (MEXU, NY); *Breedlove 70835* (MEXU); *Cronquist 9667* (MEXU, NY, TEX); *Davidse 29732* (MEXU); *Fryxell 3238* (MEXU); *Fryxell 3317* (ENCB, MEXU, NY); *Panero 6228* (MEXU); *Reyes 1146* (MEXU); *Soto 13501* (MEXU). Guerrero. *Barrie 492* (MEXU); *Calonico 12031* (FCME, MEXU); *Calonico 12962* (FCME); *Calonico 17868* (FCME); *Calonico 18123* (FCME, MEXU); *Calonico 19011* (FCME); *Campos 1774* (FCME); *Cruz 1572* (FCME, MEXU); *Garcia 294* (FCME); *Hinton 14949* (HINTON, NY); *Martinez 1969* (FCME, MEXU); *Martinez 4976* (MEXU); *Reyes 247* (FCME); *Soto 6778* (MEXU); *Tenorio 4848* (MEXU); *Valencia 4466* (FCME); *Villaseñor 1071* (MEXU); *Villaseñor 1819* (MEXU); *Villaseñor 1870* (MEXU); *Villaseñor 1873* (MEXU); *Villaseñor 1885* (MEXU); *Yahara 296* (MEXU); *Yahara 1942* (MEXU). Hidalgo. *Coulter 242* (GH); *Cruz 1424* (ENCB); *Moore 1685* (GH); *Rzedowski 23328* (ENCB); *Villarreal de Puga 15112* (IBUG). Jalisco. *Barrie 1153* (MEXU, NY); *Díaz 4483* (MEXU); *Guerrero 1004* (IBUG); *Harker 479* (IBUG); *Harker 1946* (IBUG); *Harker 1972* (IBUG); *Michel 37* (IBUG); *Ornelas 15* (IBUG); *Ramirez 1787* (MEXU); *Santana 108* (IBUG); *Santana 4852* (IBUG); *Urbina s.n.* (MEXU); *Velasco s.n.* (IBUG); *Villarreal de Puga 15026* (IBUG); *Webster 15985* (IBUG). Mexico State. *Hinojosa-Espinosa 627* (MEXU); *Hinton 2228* (HINTON, MEXU, NY); *Matuda 27035* (MEXU, NY); *Matuda 30116* (NY); *Matuda*

31771 (MEXU, NY); *Paray 2776* (ENCB); *Vibrans 6359* (MEXU). Michoacan. *Arsene 2550* (MEXU); *Barrie 567* (MEXU, NY); *Hinton 15228* (ENCB, HINTON, NY); *King 4600* (MEXU, NY); *Labat 1130* (MEXU); *Martinez 5313* (MEXU); *Martinez 5360* (MEXU); *Martinez 5396* (MEXU); *Moore 5603* (MEXU); *Soto 10699* (MEXU); *Soto 11015* (MEXU); *Soto 11070* (MEXU); *Soto 11117* (MEXU); *Steinmann 2113* (MEXU); *Steinmann 4754* (MEXU); *Torres 13128* (MEXU); *Torres 13479* (MEXU); *Ventura 2499* (ENCB); *Villaseñor 301* (FCME, MEXU). Morelos. *Garcia 4* (MEXU); *Miranda 4803* (MEXU); *Ripley 14558* (NY). NAYARIT. *Campos 5027* (MEXU); *Cronquist 9601* (MEXU); *Harker 974* (IBUG); *Tellez 9897* (MEXU). Nuevo Leon. *Hinojosa-Espinosa 745* (DAV, MEXU); *Yahara 1453* (MEXU); *Yahara 1752* (MEXU). Oaxaca. *Anderson 12987* (MEXU); *Bartholomew 3055* (MEXU); *Breedlove 13703* (MEXU, NY); *Calzada 19370* (MEXU); *Conzatti 1900* (MEXU); *Koch 78433* (ENCB, MEXU, NY); *Koch 79358* (ENCB, MEXU, NY); *Koch 79443* (ENCB, MEXU, NY); *Linares 4453* (MEXU); *Martínez 33110* (MEXU); *Pascual 1672* (MEXU); *Reyes 667* (MEXU); *Rivera 1996* (MEXU); *Salinas 6250* (MEXU); *Sanchez 52* (MEXU); *Sundberg 3038* (NY); *Sundberg 3045* (ENCB, MEXU); *Tenorio 3556* (MEXU); *Velasco 569* (MEXU); *Villaseñor 331* (MEXU); *Warnock 2557* (MEXU); *Zamudio 10964* (MEXU). Queretaro. *Carranza 2850* (MEXU, QMEX); *Fernandez 2083* (ENCB, MEXU, NY); *Hernandez 5977* (MEXU, QMEX); *Hernandez 9301* (MEXU); *Rubio 326* (MEXU, QMEX); *Rzedowski 47517* (MEXU, QMEX); *Smith 842* (TEX); *Treviño 367* (QMEX); *Webster 16357* (TEX); *Zamudio 5874* (QMEX). San Luis Potosi. *Purpus 4812* (F, MO, UC); *Rzedowski 6811* (ENCB); *Rzedowski 8214* (ENCB, MEXU, TEX). Sinaloa. *Breedlove 35662* (MO). Veracruz. *Purpus 2192* (NY).

CARMINATIA BALSANA Hinojosa, sp. nov. Type: Mexico. Guerrero. Atenango del Río, 2.84 km al N de Tuzantlán, 18° 12’ 58” N, 99° 11’ 48” O, cañadas y base de laderas en bosque tropical

caducifolio, con Acmella radicans, Alvaradoa sp., Selaginella sp., Scleropappus papposus, 1107 m, Oct 08 2011, Hinojosa-Espinosa 576 (holotype: MEXU).

Carminatia recondita similis sed involucrum, *corollis*, *cypselae*, *et pappi brevioribus*, *et cypselae turbinatae et capitula non secund differt*.

Stems 30–170 cm high, sparsely pilose to glabrous and scarcely to moderately stipitate-glandular. Leaf blades 2.4–9 cm long, 2.4–9.5 cm wide, petiole 1–8 cm long. Capitulescence paniculiform, spike-like, naked, comprising 2/3 of the shoot or more, the heads in fasciculate clusters, spreading or ascendent to pendulous, not secund, short pedunculate, peduncles 0.5–8.0 mm long, sparsely puberulent and moderately to scarcely stipitate-glandular; involucre cylindric to narrowly turbinate, 9–12 mm long, glabrous to puberulent or scarcely stipitate-glandular. Corollas narrowly tubular, 5–6 mm long, 0.7–0.9 mm wide when pressed, all constricted below apex, apical lobes 0.2–0.3 mm long, glabrous or sparsely glandular, anthers 1.5–2.3 mm long, style branches linear, apical appendages clavate, 0.1–0.2 mm wide. Cypselae narrowly turbinate, 2.9–3.2 (–4.3) mm long, 0.7–0.8 mm wide, glabrous to sparsely puberulent, contracted below apex to a short pilose neck; pappus bristles 13–16, 4–6.5 mm long, straight to slightly sinuous, persistent. Fig. 10.

Distribution and Habitat—*Carminatia balsana* is endemic to the Balsas Basin, currently known from Michoacan to eastern Puebla (Fig. 3), where it occurs mostly in tropical deciduous forests and sometimes in *Juniperus* forest. It is found in canyons, steep slopes, stream banks, trailsides, and roadsides at elevations of 300-1780 m, and it is associated with species of *Alvaradoa*, *Bursera*, *Ficus*, and *Lysiloma*. Flowering occurs from late October to late November.

Etymology—The specific epithet refers to the Balsas Basin in Mexico, where the species may be confined.

Notes—Turner (1988, 1997) treated all specimens from the Balsas Basin as *Carminatia alvarezii* based on the presence of a constriction below the corolla apex and congruent geographic distribution. However, *C*. *balsana*, as treated here is not closely related to *C. alvarezii*. It is similar to *C*. *papagayana* in having ascendent heads at anthesis and in the size of the involucre, but *C*. *papagayana* has longer peduncles, corollas, cypselae, and pappus bristles, and it lacks stipitate glands and a spike-like inflorescence. *C. balsana* is more likely to be confused with *C*. *recondita*, especially in herbarium specimens. The heads in *C*. *balsana* are not secund and they are not all pendulous as in *C*. *recondita*, but these characters sometimes are not well preserved in mounted dry specimens. Because of its long heads, corollas, and fruits, *C*. *recondita* is usually easy to distinguish from *C*. *balsana*. However, some plants from Oaxaca, Chiapas, and Queretaro, have shorter heads and fruits and may also have some stipitate glands on the stems and even a constriction underneath the corolla apex in some florets. In this case, the shape of the cypselae (or ovaries if immature) may help to discern between these species; as in *C*. *balsana* the cypselae are smaller (2.9–4.3 mm long) and narrowly turbinate (Fig. 10), whereas in *C*. *recondita* the cypselae are as short as 4 mm long (but mostly 5–8 mm long) and are linear-turbinate, resembling the cypselae of *C*. *tenuiflora* (Fig. 2). Moreover, *C*. *balsana* does not occur in Queretaro or Hidalgo and has not been found in Oaxaca so far. It may occur in northeast Oaxaca and Morelos.

Additional Specimens Examined—Mexico. Guerrero. *Cruz 999* (FCME); *Cruz 1901* (FCME, MEXU); *Cruz 6797* (FCME, MEXU); *Delgado 982* (FCME, MEXU); *Hinojosa-Espinosa 17* (FCME); *Hinojosa-Espinosa 70* (FCME); *Hinojosa-Espinosa 214* (FCME, MEXU); *Hinojosa-Espinosa 215* (FCME, MEXU); *Hinojosa-Espinosa 226* (FCME, MEXU); *Hinojosa-Espinosa 373* (MEXU); *Hinton 6870* (MICH, MO, NY); *Martinez 49976* (MEXU); *Moreno 1134* (FCME); *Ramos s.n.* (MEXU); *Reyes 169* (FCME); *Rojas 302* (MEXU); *Soto 3340* (ENCB, MEXU); *Soto 19873* (MEXU); *Soto 19988*

(MEXU); *Valencia 2618* (FCME). Mexico State. *Matuda 27499* (MEXU, MO, US). Michoacan. *Soto 10683* (MEXU); *Soto 17408* (MEXU); *Soto 21887* (MEXU). Puebla. *Gaiser 74* (GH); *Guizar 1787* (SLPM), *Hinojosa-Espinosa 679* (DAV, MEXU); *Miranda 2320* (MEXU); *Miranda 2442* (MEXU).

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AUTHOR CONTRIBUTIONS

OHE conceive and executed most of the research design, did all the lab work and most of the field work, studied all the herbaria collections, gathered and analyzed most of the data, and wrote most of the manuscript. JLV curated the herbarium collection at MEXU, compiled and

cleaned the database, performed field work, conceived the earliest stages of the research study, and helped analyze the data and write the manuscript. DP provided all lab reagents, gave feedback on the research design, helped with herbaria loans and analyzing the data and writing the manuscript.

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Appendix 1. Taxa and vouchers of plant material from which DNA was extracted for sequence analyses, together with GenBank accession numbers. [ITS; ETS; *psbA-trnH*], —sequence not available. *These samples belong to the new species described here, *Carminatia balsana*.

Ingroup: Carminatia alvarezii1, *Hinojosa-Espinosa 576** (MEXU) [OM060471; OM058347 ; OM058387]; C. alvarezii2, *Hinojosa-Espinosa 679** (DAV, MEXU) [OM060472; OM058348; OM058388]; C. alvarezii3, *Hinojosa-Espinosa 698* (DAV, MEXU) [OM060473 ; OM058349; OM058389]; C. alvarezii4, *Mota 107* (MEXU), [OM060470 ; OM058350; OM058390]; C. alvarezii5, *Ramos s.n.** (MEXU), [OM060474 ; OM058351; OM058391]; C. alvarezii6, *Redonda 786** (MEXU) [OM060475 ; OM058352; OM058392]; C. alvarezii7, *Soto 21887** (MEXU), [OM060476 ; OM058353; OM058393]. Carminatia papagayana1, *Panero 6193* (TEX/LL), [JQ737025.1; KM669061.1; JQ737055.1]; C. papagayana2, *Villaseñor 1857* (MEXU), [OM060477; OM058354; OM058394]. Carminatia recondita1, *Alvarado 541* (MEXU), [OM060481; OM058355; OM058395]; C. recondita2, *Alvarado 722* (MEXU), [OM060482; OM058356; OM058396]; C. recondita3, *Aparicio 214* (MEXU), [OM060478; OM058357; OM058397]; C. recondita4, *Carranza 2850* (MEXU), [OM060479; OM058358; OM058398]; C. recondita5, *Hinojosa-Espinosa 627* (MEXU); [OM060484; OM058359; OM058399]; C. recondita6, *Hinojosa-Espinosa 745* (DAV, MEXU); [OM060485; OM058360; OM058400]; C. recondita7, *Mirón 575* (MEXU); [OM060483; OM058361; OM058401]; C. recondita8, *Panero 8833* (TEX/LL), [JQ737026.1; —; JQ737056.1]; C. recondita9, *Villasenor 1870* (MEXU), [OM060480; OM058362; OM058402]. Carminatia tenuiflora1, *Balleza 19576* (MEXU), [OM060486; OM058363; OM058403]; C. tenuiflora2, *Hinojosa-Espinosa 572* (MEXU), [OM060487; OM058364; OM058404]; C. tenuiflora3, *López 1516* (MEXU), [OM060488; OM058365; OM058405]; C. tenuiflora4, *Tejero 5270* (MEXU), [OM060489; OM058366; OM058406].

Appendix 1. Continuation.

Outgroups: *Alomia ageratoides*, *Webster 16172* (DAV), [OM060459; OM058336; OM058376]. *Asanthus solidaginifolius*, *Sundberg 2736* (TEX/LL); [JQ737014.1; —; JQ737044.1]. *Asanthus squamosus*, *Bye 4077* (TEX/LL), [JQ737016.1; —; JQ737046.1]. *Asanthus thyrsiflorus*, *Tejero 6037* (MEXU) [OM060460; OM058337; OM058377]. *Brickellia adenolepis*, *Panero 8824* (TENN), [KM668944.1; KM669042.1; KM669128.1]. *Brickellia coixtlahuaca*, *Hinojosa-Espinosa 696* (DAV, MEXU); [OM060462; OM058339; OM058379]. *Brickellia diffusa*, *López 523* (MEXU), [OM060463; OM058340; OM058380]. *Brickellia filipes*, *Martínez 32885* (MEXU) [OM060464; OM058341; OM058381]. *Brickellia laxiflora*, *Sundberg 3070* (TEX/LL), [KM668895.1; KM668992.1; KM669080.1]. *Brickellia odontophylla*, *Miller 3418* (TENN), [KM668906.1; KM669004.1; KM669091]. *Brickellia oreithales*, *Bye 8193* (TEX/LL), [KM668945.1; KM669043.1; KM669129.1]. *Brickellia pavonii*, *Hinojosa-Espinosa 683* (DAV, MEXU) [OM060465; OM058342; OM058382]. *Brickellia problematica*, *Hinojosa-Espinosa 694* (DAV, MEXU), [OM060466; OM058343; OM058383]. *Brickellia scoparia*, *Balleza 19863* (MEXU) [OM060467; OM058344; OM058384]. *Brickellia sonorana*, *Steinmann 94-101* (MEXU), [OM060468; OM058345; OM058385]. *Brickellia subuligera*, *Hinojosa-Espinosa 744* (DAV, MEXU), [OM060469; OM058346; OM058386]. *Brickelliastrum fendleri*, *Schilling 2012* (TENN) [JQ737023.1; KM669060.1; JQ737053.1]. *Brickelliastrum nesomii*, *Hinton 24031* (MEXU), [OM060461; OM058338; OM058378]. *Dissothrix imbricata*, *Araujo 1467* (HUEFS), [KP454347.1, KP454498.1; KP454652]. *Flyriella parryi1*, *Johnston 10611* (MEXU), [OM060490; OM058367; OM058407]; F. parryi2, Mayfield 1385 (MEXU), [OM060491; OM058368; OM058408]; F. parryi3, *Nesom 7416* (MEXU), [OM060492; OM058369; OM058409]; F. parryi4, *Paterson 7400* (MEXU), [OM060493; OM058370; OM058410]. *Kyrsteniopsis nelsonii*, *Calzada 23715* (MEXU), [OM060494; OM058371; OM058411]. *Liatris microcephala*, *Schilling s.n.*, (TENN), [HQ416370.1;

Appendix 1. Continuation.

HQ416403.1; HQ416189.1]. *Stevia lucida*, *Hinojosa-Espinosa 688* (MEXU), [OM060495; OM058372; OM058412]. *Steviopsis adenosperma*, *Machaca 7591* (TEX/LL), [JQ737021; —; JQ737052.1]. *Steviopsis amblyolepis*, *Panero 6166* (TEX/LL), [JQ737019; —; JQ737049.1]. *Steviopsis dryophila*, *Yahara 796* (MEXU), [OM060496; OM058373; OM058413]. *Steviopsis rapunculoides*, *Tenorio 16514* (MEXU), [OM060497; OM058374; OM058414]. *Steviopsis vigintiseta*, *Carrillo 6528* (MEXU), [OM060498; OM058375; OM058415].

FIGURES

Fig. 1. Species of *Carminatia* **A-B**, *C. alvarezii.* **C-D**, *C. recondita*. **E-F**. *C. papagayana*. **G-I**. *C. tenuiflora*.

Fig. 2. Cypselae of species of *Carminatia.* Note plumose bristles. **A.** *C. alvarezii* (from *Tenorio 7693* (MEXU)). **B.** *C. papagayana* (from *Hinojosa-Espinosa 578* (MEXU)). **C**. *C. recondita* (from *Flores 1646* (MEXU). **D.** *C. tenuiflora* (from *Rzedowski 40588* (MEXU)).

Fig. 3. Majority rule consensus tree from the Bayesian analyses of ITS sequences. Posterior probabilities shown above branches.

Fig. 4. Majority rule consensus tree from the Bayesian analyses of ETS sequences. Posterior probabilities shown above branches.

Fig. 5. Majority rule consensus tree from the Bayesian analyses of *psbA-trnH* sequences. Posterior probabilities shown above branches.

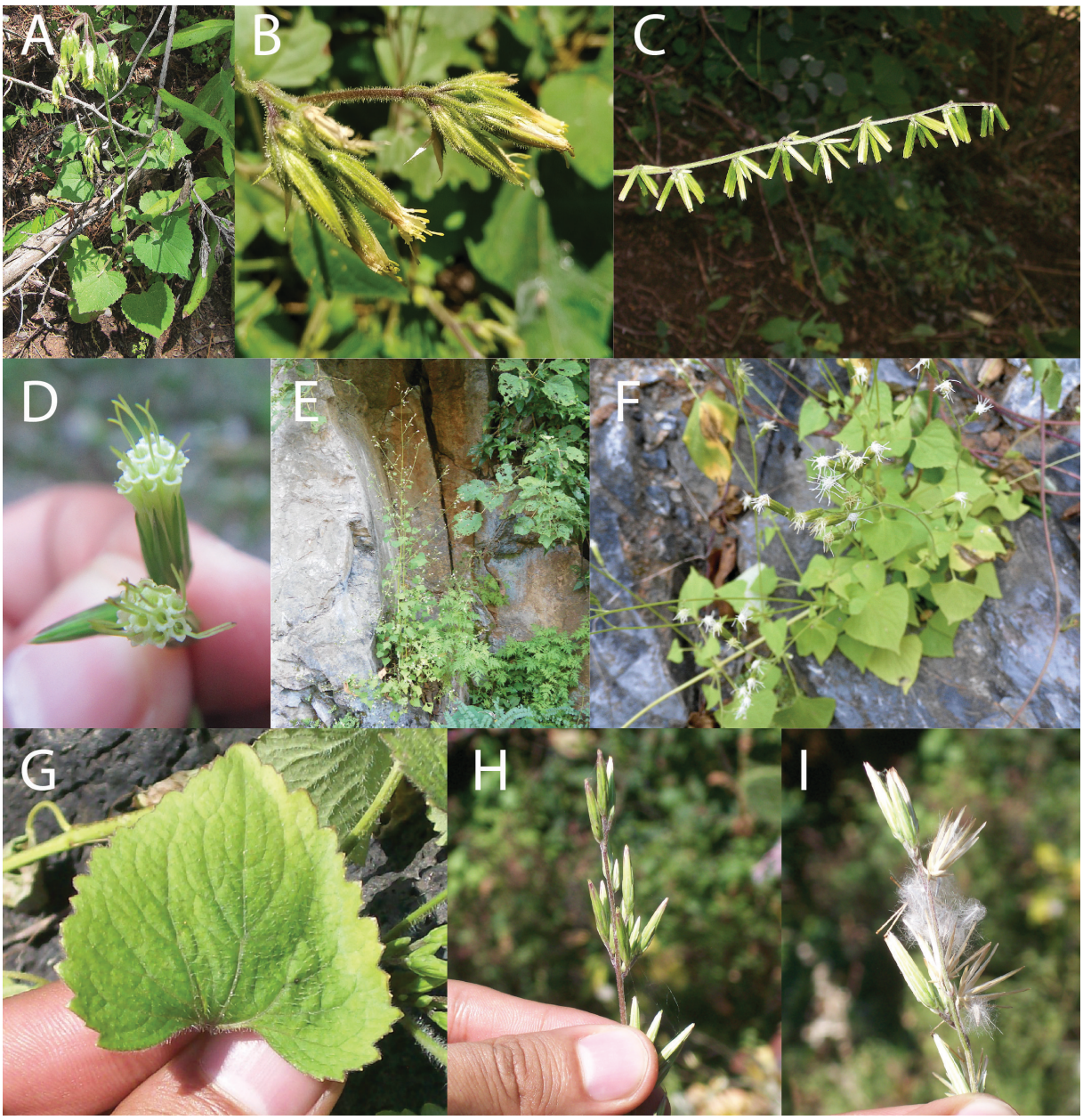
Fig. 6. Majority rule consensus tree from the Bayesian analyses of the combined data set (ITS, ETS, and *psbA-trnH* sequences). Posterior probabilities shown above branches.

Fig. 7. Geographic distribution of the three species of *Carminatia* endemic to Mexico. Blue circles, *C. alvarezii*; red triangles, *C. papagayana*; green rectangles, *C. balsana*.

Fig. 8. Geographic distribution of *Carminatia tenuiflora*.

Fig. 9. Geographic distribution of *Carminatia recondita*.

Fig. 10. *Carminatia balsana* at the type locality. **A**. Leaves. **B-D**. Capitulescence. **E**. Cypsela with plumose bristles (from *Matuda 27499* (MEXU)).



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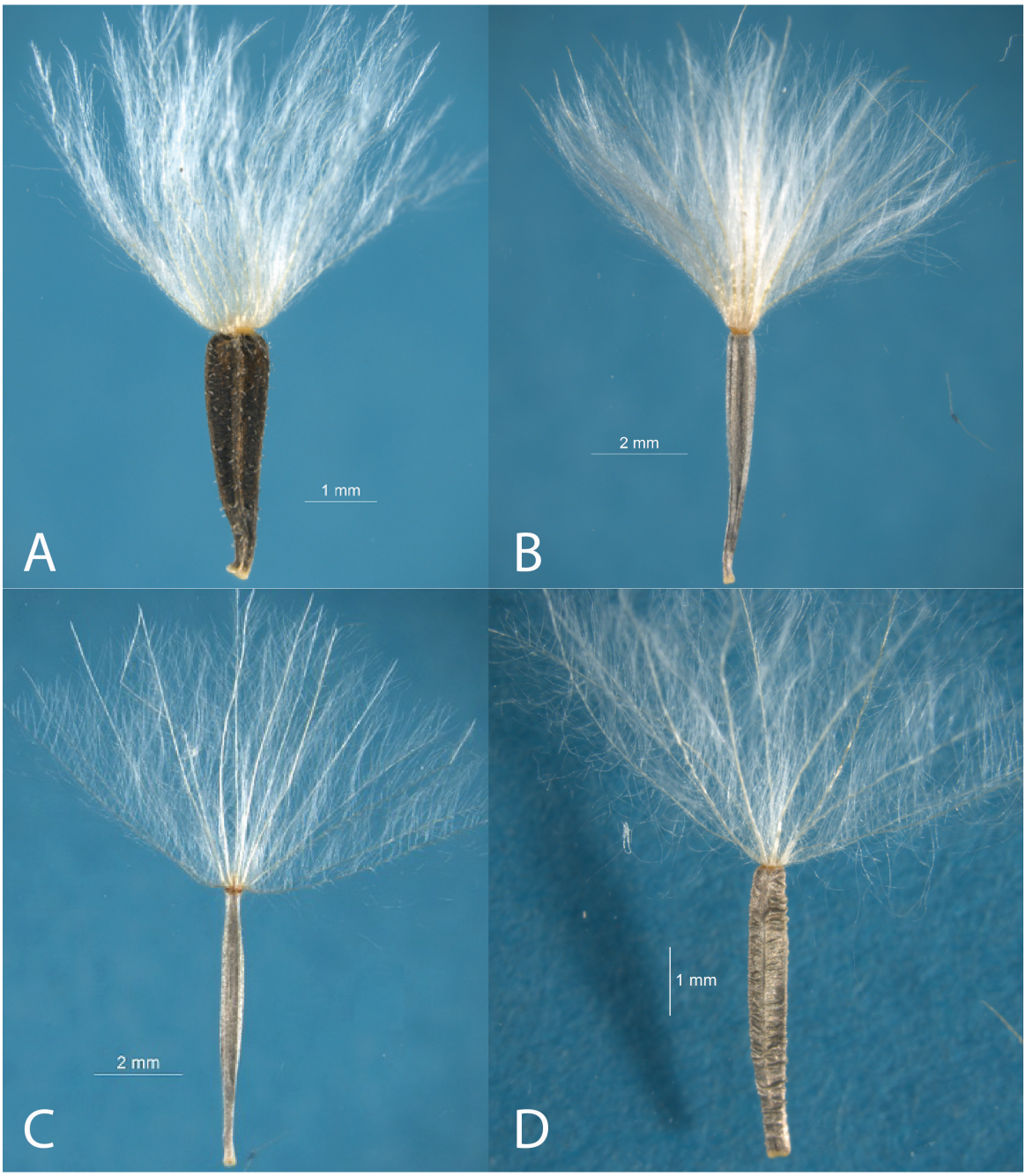

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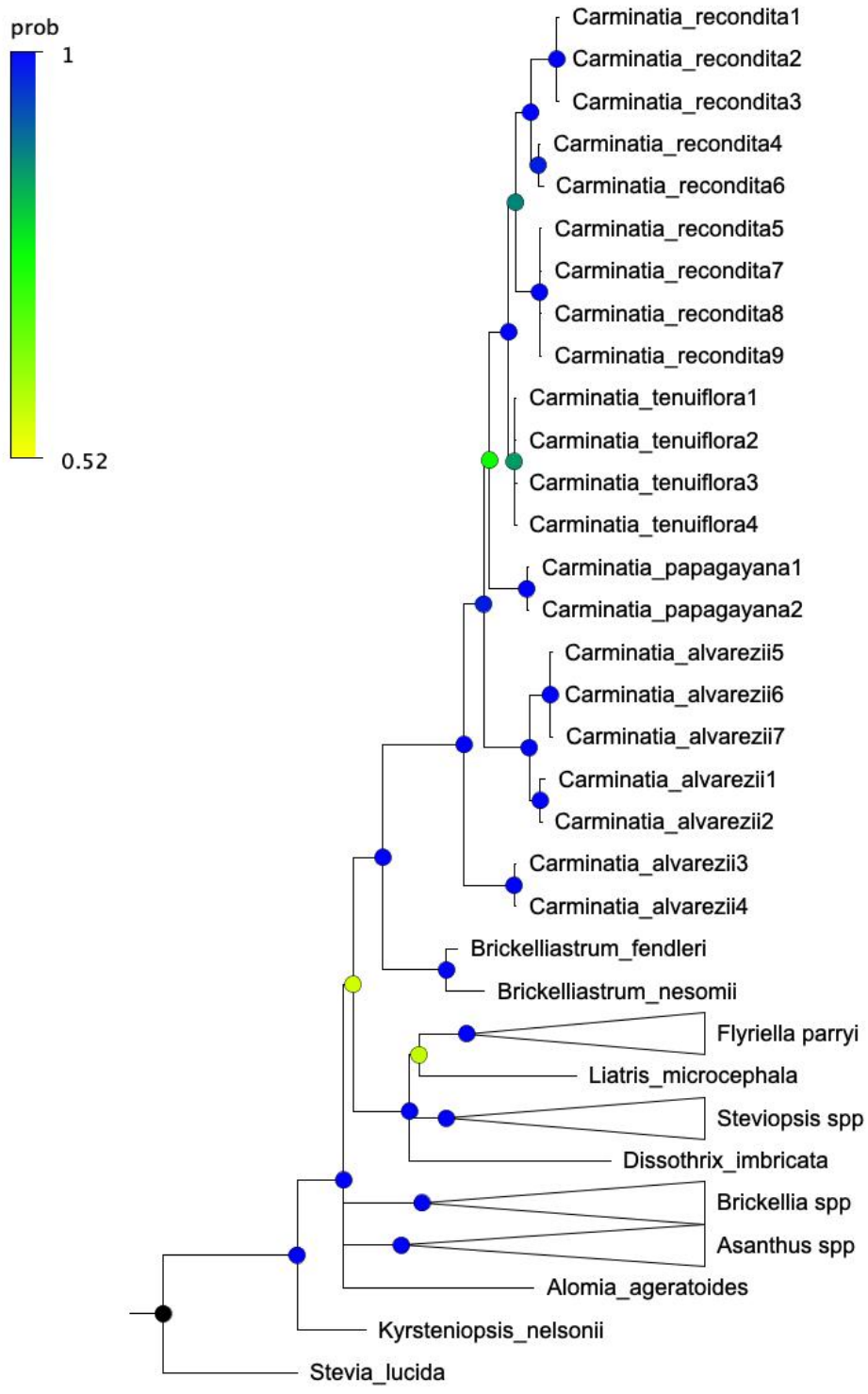
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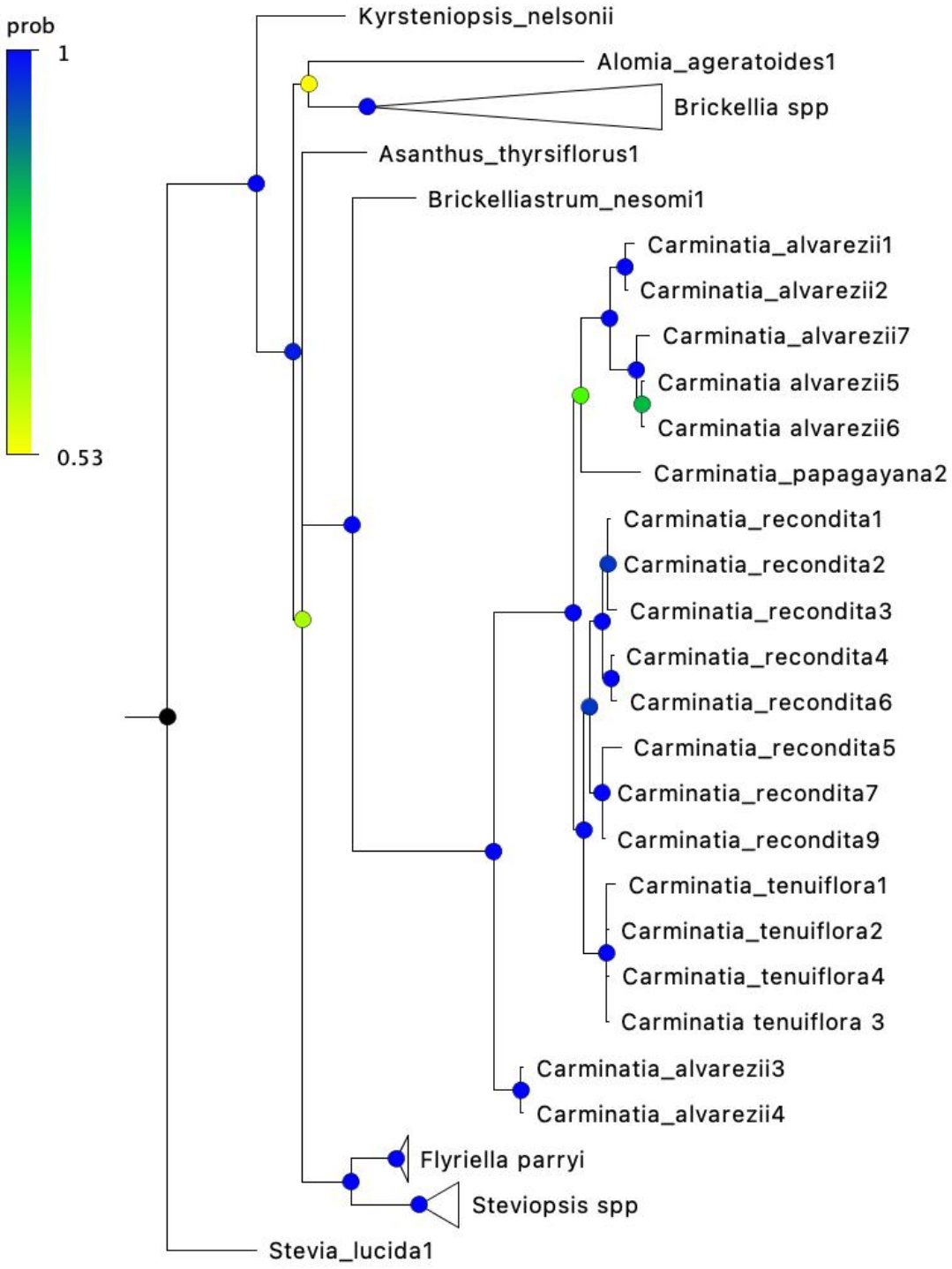
Fig. 3.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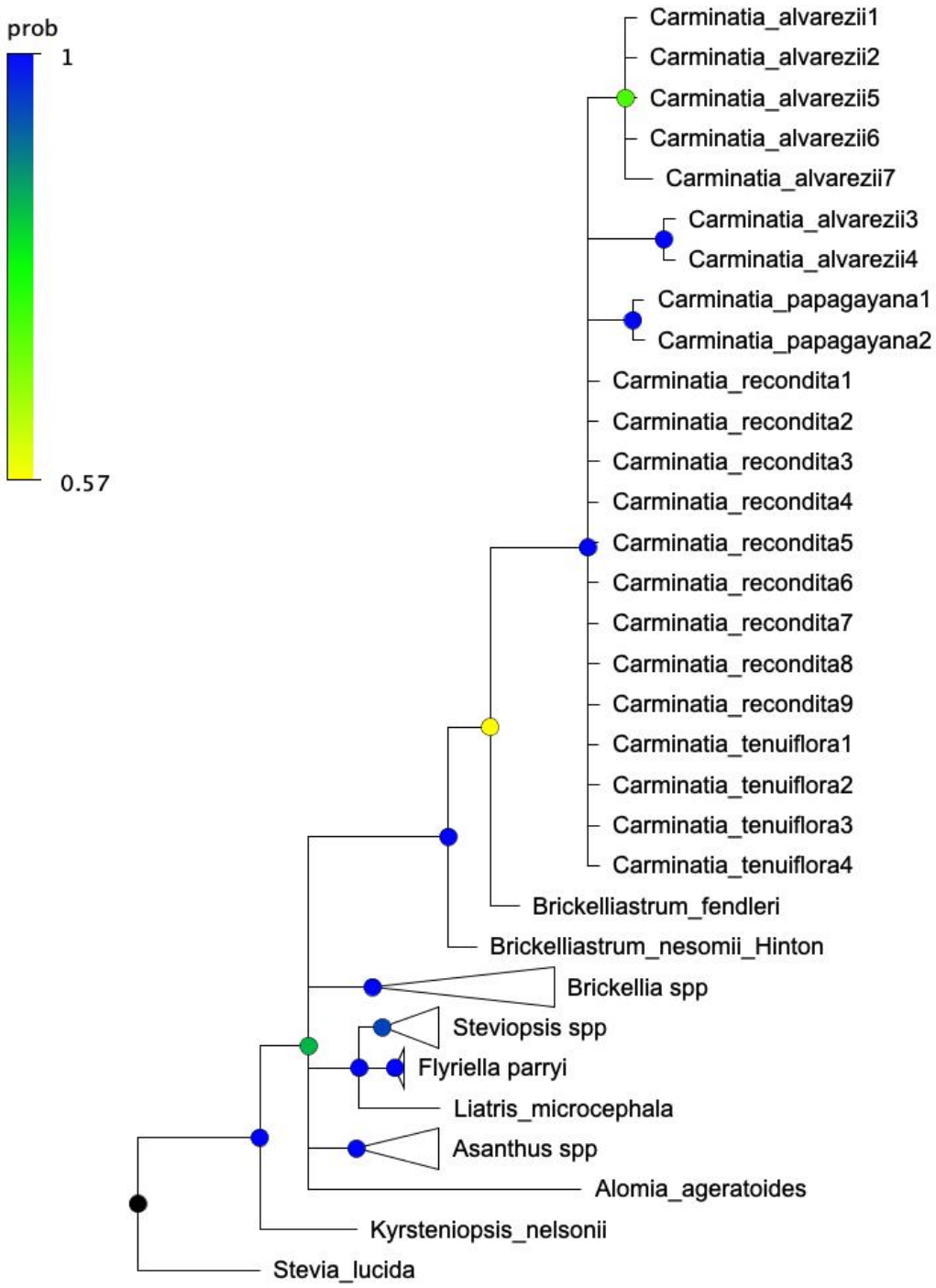
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Fig. 5.

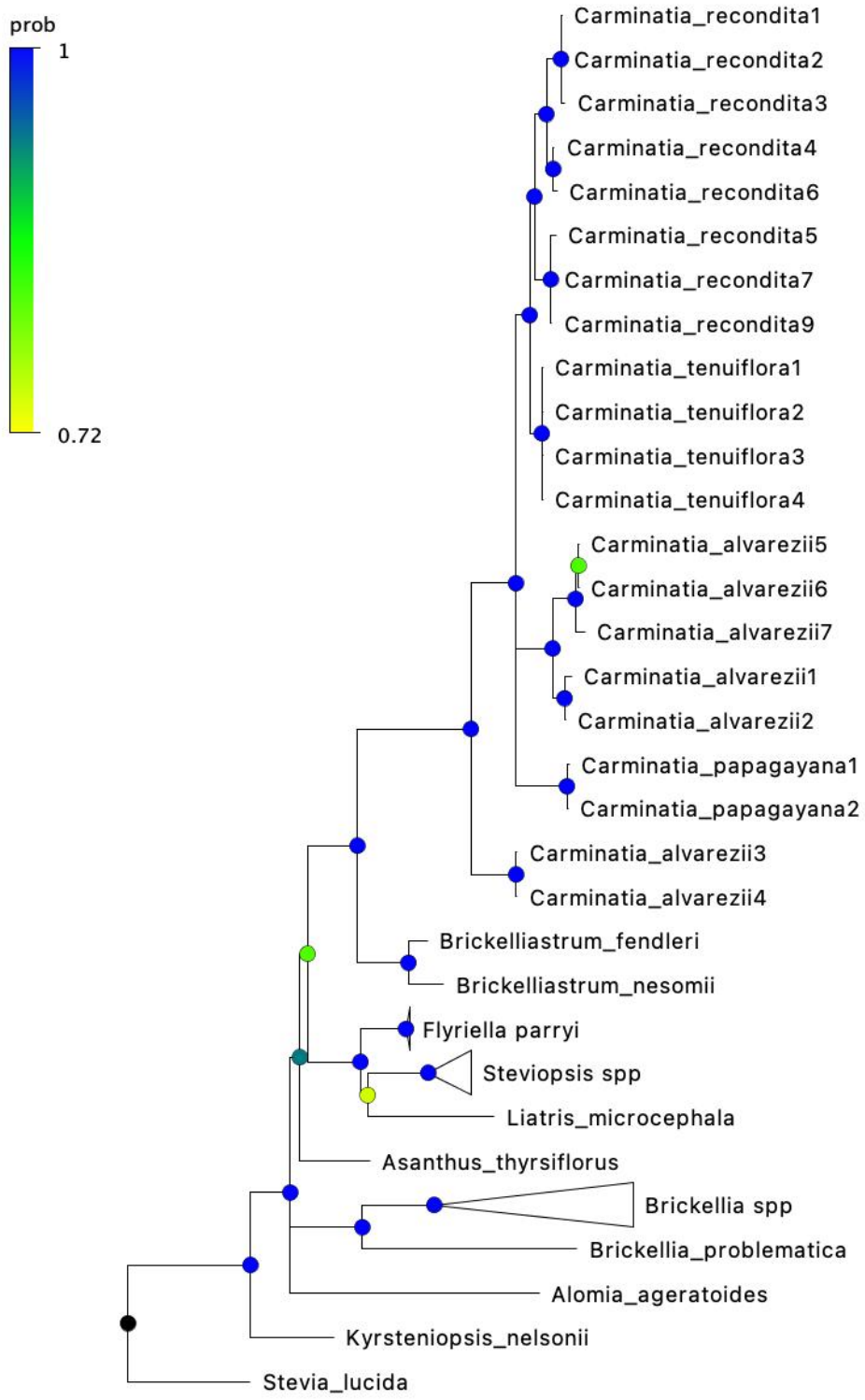
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Fig. 6.

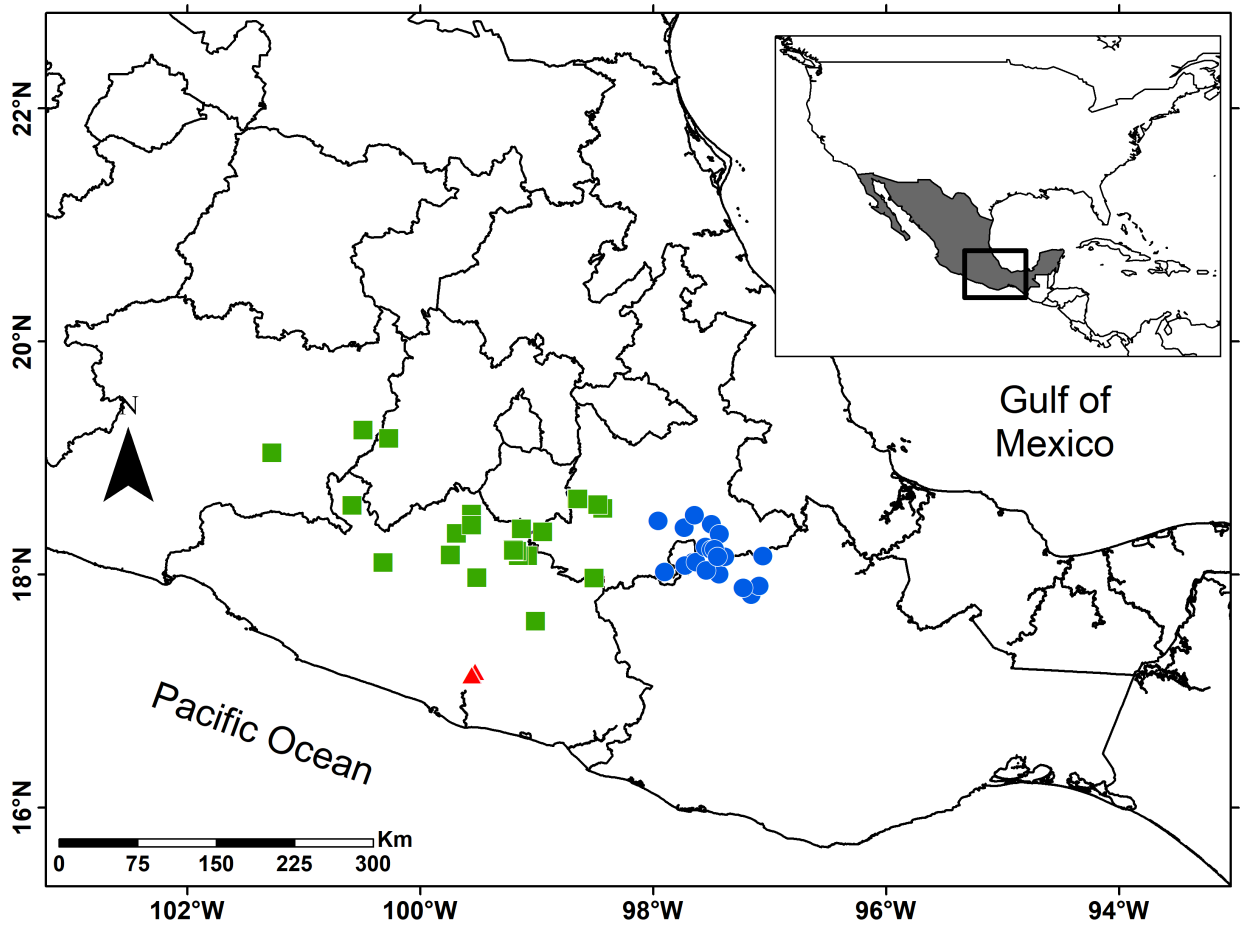
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Fig. 7.

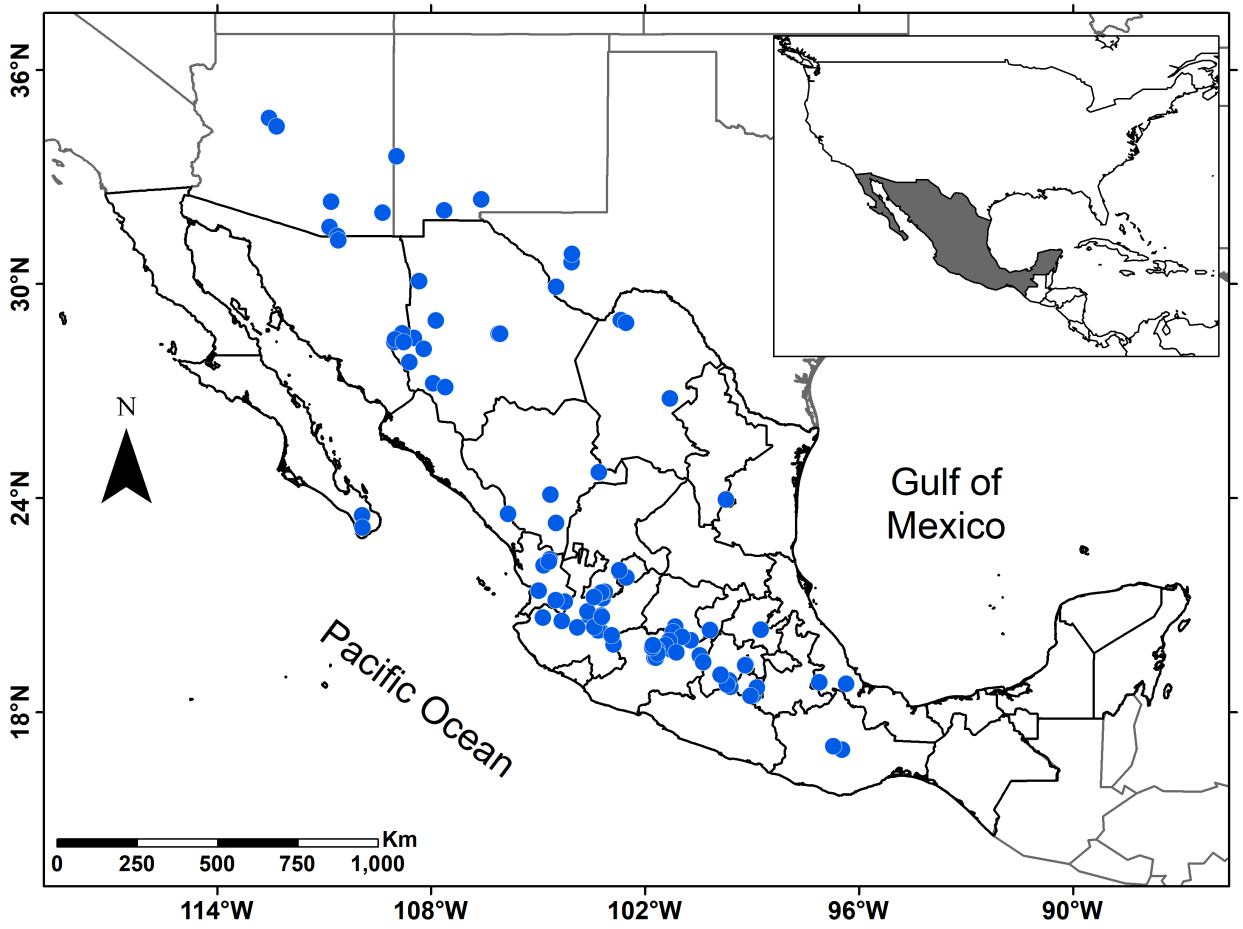
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Fig. 8.

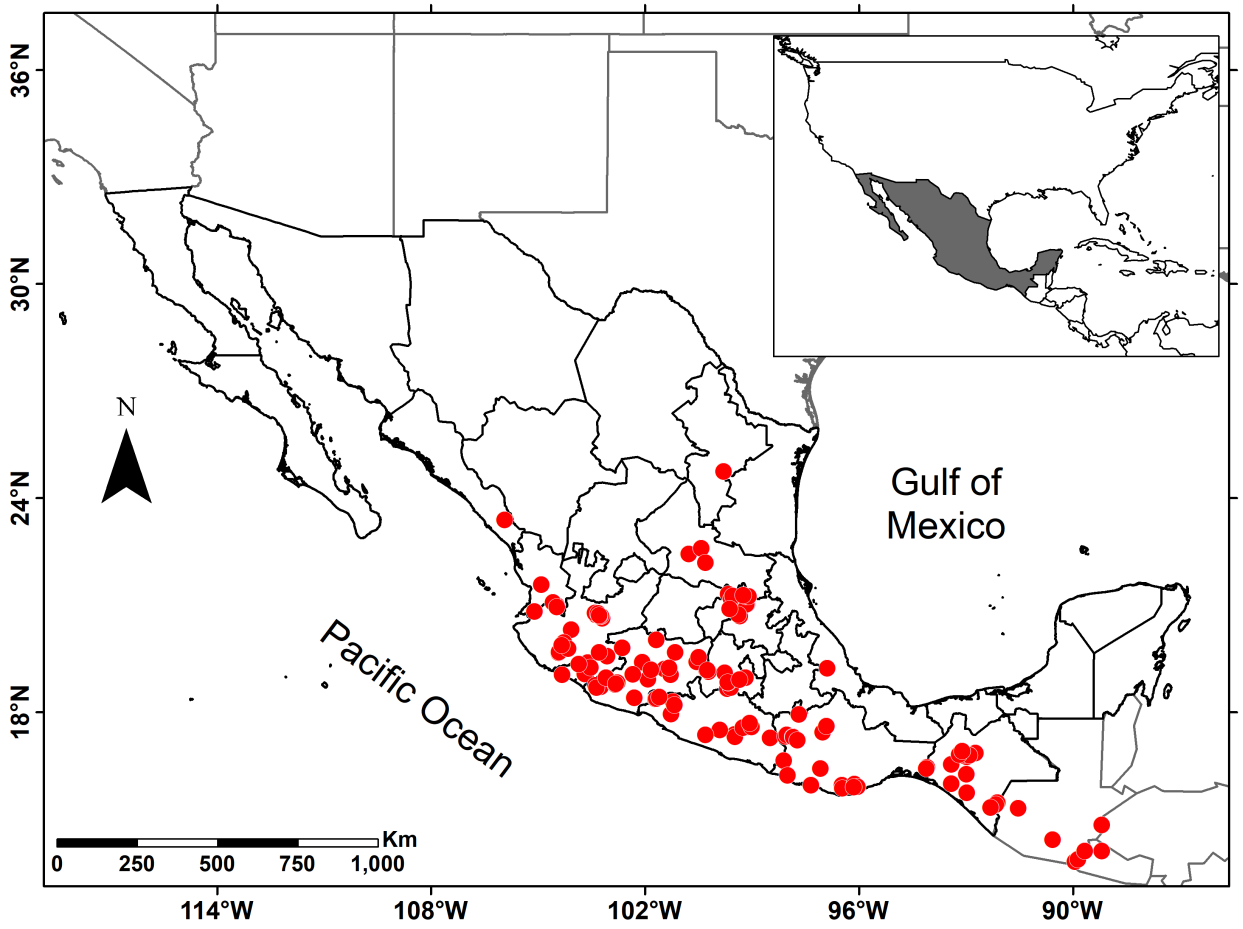
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Fig. 9.

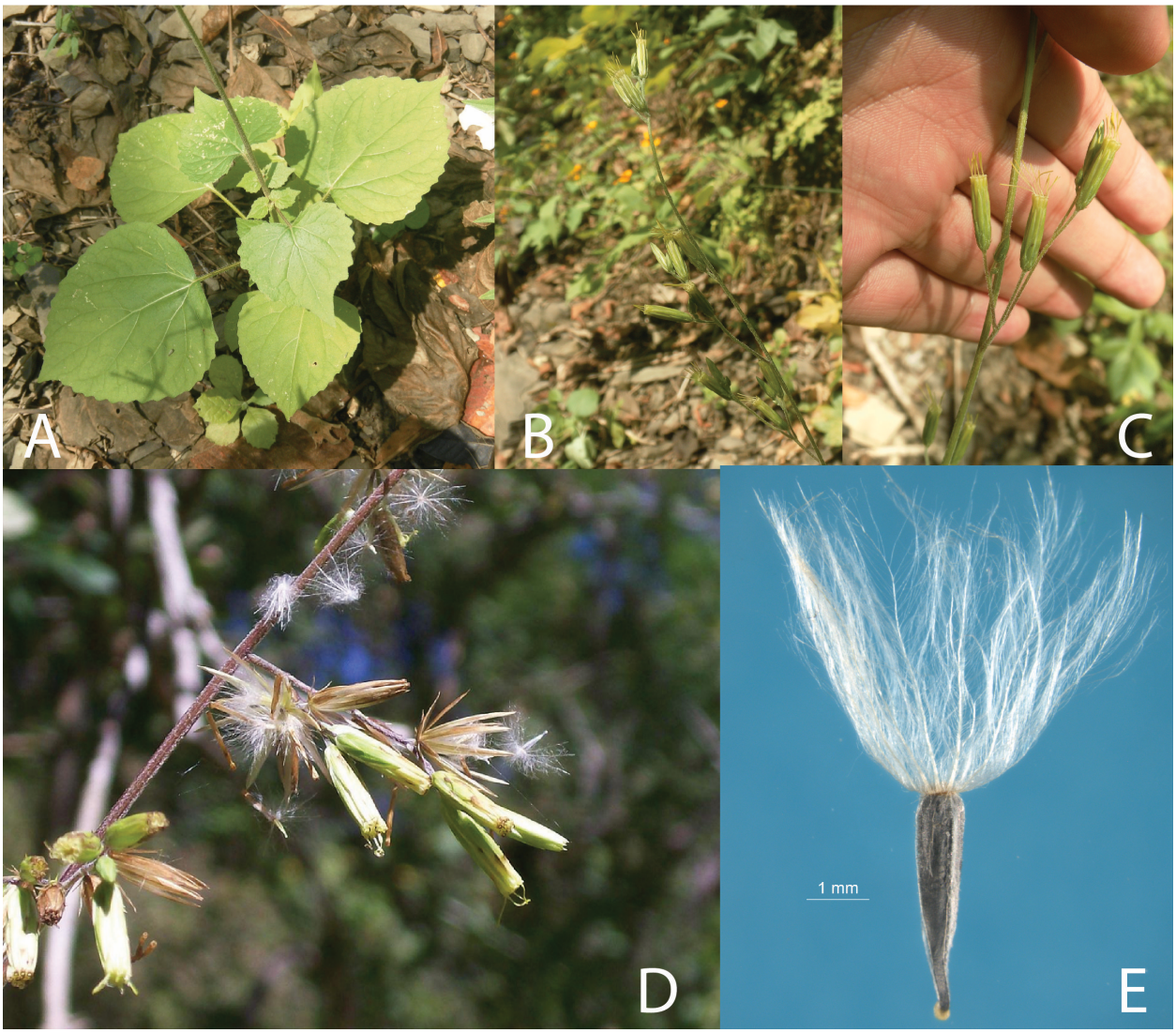

Fig. 10.