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

Oscar Hinojosa-Espinosa[1] & **Darío Javier Schiavinato**[2,3]

[1] Plant Biology Graduate Group, University of California Davis, Shields Avenue, Davis, California 95616, USA; *ohinojosa@ucdavis.edu*
[2] Cátedra de Botánica Sistemática, Facultad de Agronomía, Universidad de Buenos Aires, Av. San Martín 4453, C1417DSE, Ciudad Autónoma de Buenos Aires, Argentina; *schiavi@agro.uba.ar*
[3] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), República Argentina

DOI: http://dx.doi.org/10.53875/capitulum.02.1.03

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 monophyletic and *Hydropectis* as sister to *Tagetes*. 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 clades are also strongly supported, such as Lucida clade, 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 Cass. 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, uniseriate connate involucres, and a pappus of short and truncated scales, long and subulate scales, or a combination of both (Figure 2). As most members of tribe Tageteae, the leaves and phyllaries in *Tagetes* have pellucid glands (secretory cavities) that contain fragrant essential oils (Figure 2B).

The most widely known species is the cultivated *Tagetes erecta* L., often known as French Marigold or African Marigold, although it is native to Mesoamerica (Figure 3A–C). *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 interestingly noticed that the capitula were inflorescences, not single flowers,

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

as *cempoalxochitl,* Aztec's Nahuatl name meaning 20 flowers, clearly implies (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 3B–C). 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 4B). 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: *Tagetes erecta, T. minuta* L., and *T. patula* L. During the following hundred years, the number of recognized *Tagetes* species increased with the contributions of different authors (e.g. Cavanilles, 1794; Lagasca, 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, *Tagetes* subgen. *Hydrotagetes* Soule, *Tagetes* subgen. *Iya* Soule, and *Tagetes* subgen. *Tagetes*, the latter with two sections (i.e. *Tagetes* sect. *Filifoliae* Soule and *T.* sect. *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, *Tagetes erecta*, the one with the largest heads and more numerous ray florets (Figure 3C) 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

Figure 2. Diversity of *Tagetes* L. **A-C.** *Tagetes lemmonii*, one of the shrubby species. Also note secretory cavities filled with ethereal oils. **D.** *Tagetes micrantha*, an anise-like scented species with reduced capitula, which is used as spice. **E.** *Tagetes foetidissima*, a strongly pungent species. **F.** *Tagetes subulata*, a white-ray form (rays are usually yellow in this species). **G.** *Tagetes nelsonii*, used in traditional medicine in its native range (Chiapas, Mexico and adjacent Guatemala). **H.** *Tagetes filifolia*, another anise-like scented species, which is often found in wet soils. *Photos by Oscar Hinojosa-Espinosa*

Figure 3. A. Wild form of *Tagetes erecta* L., note purplish corolla lobes. **B–C.** Use of *T. erecta* during the Day of the Dead holiday. **D–E.** *Tagetes lunulata*, note acute capitulum in bud with apical hairs and red-orange spot at the base of the rays. *Photos by Oscar Hinojosa-Espinosa*.

Figure 4. A–B. *Tagetes lucida*, locally known as *pericón* or *yerbanís*, is mostly used as a medicine, and occasionally in religious festivities. **C–E.** *Tagetes tenuifolia* sensu Turner (1996). Note red-orange spot at the base of the rays and glabrous and round capitulum in bud. *Photos by Oscar Hinojosa-Espinosa.*

Tagetes fields, Forever!

Every year plants of cempasúchil or flor de muerto (*Tagetes erecta*) are grown in many places in Mexico to use them during the holiday of Día de Muertos (Day of the Dead, usually, November 1-2). Seeds are planted in June so the blooming plants are ready to harvest in October. It is thought that the intense orange color of the rays guides the souls of the dead who, according to the tradition, come back to visit home in the night of Día de muertos.

Crop of *Tagetes erecta* in Oaxaca, México
Photo by O. Hinojosa-Espinosa

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 3E). 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 4E), whereas in *T. lunulata* the capitulum in bud is acute and provided with setulae (Figure 3D). However, we have seen populations in central Mexico whose individuals have the orange-reddish spot and round, glabrous capitula in bud (Figure 4C-E); 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 Dumort. Moreover, we included two more outgroups, *Flaveria trinervia* (Spreng.) C.Mohr, from the subtribe Flaveriinae (tribe Tageteae), and *Helianthus annuus* L., from 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 (Thiers, 2022). Moreover, additional 15 ITS sequences were extracted from GenBank. A list of voucher specimens and GenBank accession numbers is presented in the Appendix.

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 checked and separated by agarose gel electrophoresis. The amplified ITS was extracted from gel slices using the QIAquick Gel Extraction Kit (Qiagen, Valencia, California) 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 & 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

PHYLOGENY OF TAGETES

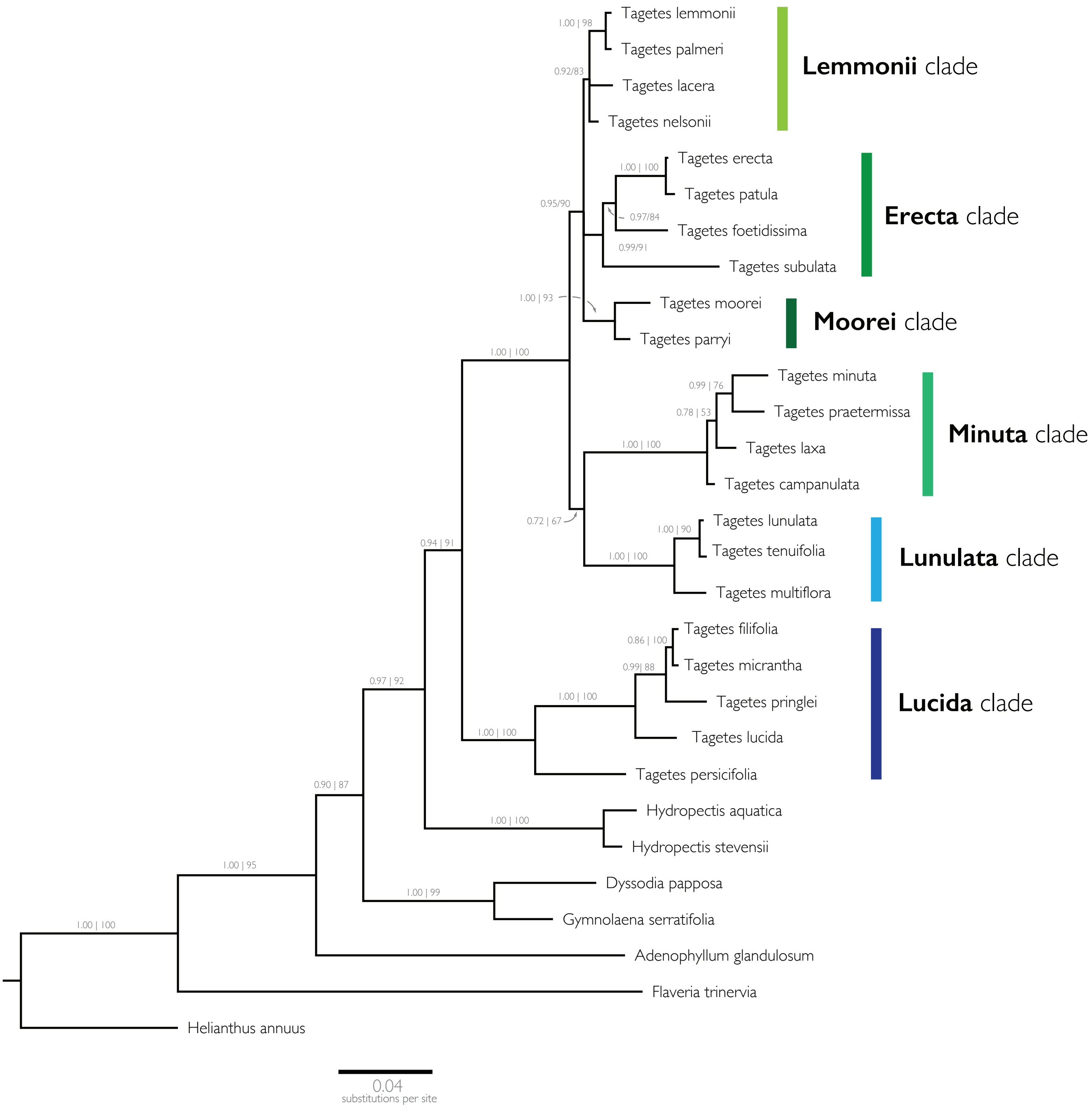

Figure 5. Majority rule consensus tree of 10,000 sampled trees from the Bayesian analysis of *Tagetes* using ITS performed in MrBayes. Posterior probabilities annotated next to the nodes followed by bootstrap values from the maximum likelihood and bootstrap analyses (1000 replications) of the same data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

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 bootstrap 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 (PP=0.94, bootstrap=91) with *Hydropectis* as its sister genus (PP=0.97, bootstrap=92) (Figure 5). Two strongly supported clades were resolved within *Tagetes*: 1) the Lucida clade, which is composed of *T. persicifolia*, *T. lucida*, *T. pringlei*, *T. micrantha*, and *T. filifolia* Lag.; and 2) a larger clade that is composed of five smaller clades (Lemmonii, Erecta, Moorei, Minuta, and Lunulata). The Lemmonii clade includes *T. lacera* Brandegee, *T. nelsonii* Greenm., and the sister species *T. lemmonii* A. Gray and *T. palmeri* A. Gray. The Erecta clade contains *T. erecta* resolved as sister to *T. patula*, with *T. foetidissima* as sister to both, and *T. subulata* as sister to all three. The Moorei clade includes *T. moorei* H.Rob. and *T. parryi* A. Gray. The Minuta clade contains *T. minuta* L., resolved as sister to *T. praetermissa,* with *T. laxa* Cabrera sister to both, and *T. campanulata* sister to all three. Finally, the Lunulata clade includes *T. lunulata* resolved as sister to *T. tenuifolia*, with *T. multiflora* as sister to both.

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 is weakly supported (PP=0.94), as usually a posterior probability value of at least 0.95 is considered statistically robust (Wilcox et al., 2002), however, the *Tagetes* clade is strongly supported by the bootstrap analyses (Figure 5). The sister relationship of *Tagetes* and *Hydropectis* is strongly supported (PP=0.97, bootstrap=92). *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* (=*Tagetes persicifolia*) and *Vilobia praetermissa* (=*Tagetes praetermissa*) belong to *Tagetes*, and that the genera *Dyssodia*, *Gymnolaena*, and *Adenophyllum* are distantly related to *Tagetes* only.

Since *Tagetes 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 4C-E), while the populations of *T. lunulata* from Western and north-central Mexico have acute, setulaceous capitula in bud (Figure 3D-E), and perhaps it is best to treat all of these as a single species with two varieties.

Some of the the resolved clades are composed of species that are similar in ecology, morphology or geography. For instance, the Lucida clade includes *Tagetes persicifolia*, a riparian species, and it is also composed of species that are common in wet soils (*T. micrantha* and *T. filifolia*) or are subaquatic (*T. pringlei*). Moreover, the species in this clade 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. Another example is the Minuta clade, which in this case is composed of South American species only. 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. Another example is the Lemmonii clade, which is composed of species that are suffruticose and occur 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 *Tagetes erecta* will facilitate the search for desirable traits in the wild species.

ACKNOWLEDGMENTS

We thank the curators of the consulted herbaria for allowing us to study their collections and for permission to remove leaf fragments for DNA extraction. Mario Ishiki, Henry Castaneda, Alfonso Luna, Marco Constante, Jose Luis Villaseñor, and Rafael Torres helped during field work. We thank Daniel Potter, Bruce Baldwin, and two anonymous reviewers who carefully read the manuscript and provided valuable feedback. We also thank Jeniffer Mandel for inviting us to write this contribution and Mauricio Bonifacino for his help editing figures. This paper represents a side project of the PhD thesis of OHE in Plant Systematics at UC Davis, for which funding was provided by the UC-MEXUS scholarship, Jastro-Shields Graduate Research Award, and Davis Botanical Society, and a systematic study of *Tagetes* is the PhD thesis of DJS at Universidad de Buenos Aires, Argentina.

LITERATURE CITED

Baldwin, B.G. 2009. Heliantheae Alliance. Pp. 689–711 in: V.A.Funk, A. Susanna, T.F. Stuessy & R.J. Bayer (eds), *Systematics, evolution, and biogeography of Compositae*. Vienna: IAPT, 965 pp.

Candolle, A.P. de. 1836. *Prodromus systematis naturalis regni vegetabilis* 5. Treuttel & Würtz, Paris, 706 pp.

Cavanilles, A.J. 1794. *Icones et descriptiones plantarum, quae aut sponte in Hispania crescunt, aut in hortis hospitantur* 3 (2). Madrid: Regia Typographia, pp. 31–52, plates 261–300.

Edgar, R. C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res.* 32: 1792–1797.

Edler, D., Klein, J., Antonelli, A. & Silvestro, D. 2020. raxmlGUI 2.0: A graphical interface and toolkit for phylogenetic analyses using RAxML. *Methods Ecol. Evol.*, doi: http://dx.doi.org/10.1111/2041-210X.13512

Kumar, S., Stecher, G. & Tamura, K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Mol. Biol. Evol.* 33: 1870–1874.

Huelsenbeck, J.P., & Ronquist, F. 2001. MRBAYES: Bayesian inference of phylogeny. *Bioinformatics* 17: 754–755.

Huelsenbeck, J.P., Larget, B. & Alfaro, M.E. 2004. Bayesian Phylogenetic Model Selection Using Reversible Jump Markov Chain Monte Carlo, *Mol. Biol. Evol.* 21: 1123–1133, https://doi.org/10.1093/molbev/msh123

Keil, D.J. & Stuessy, T.F. 1975. Chromosome counts of Compositae from Mexico and the United States. *Amer. J. Bot.* 64(6): 791–798.

Lagasca, M. 1816. *Genera et species plantarum, quae aut novae sunt aut nondum recte cognoscuntur*. Madrid: Typographia Regia, 35 pp.

Linares, E. & Bye, R. 1997. Mexican Ceremonial Flowers. *Voices of Mexico* 41: 97–99.

Loockerman, D.J., Turner, B.L. & Jansen, R.J. 2003. Phylogenetic relationships within the Tageteae (Asteraceae) based on nuclear ribosomal ITS and chloroplast ndhF gene sequences. *Syst. Bot.* 28: 191–207.

Neher, R.T. 1966. *Monograph of the genus Tagetes*. Unpublished Ph.D. thesis, Indiana University, Bloomington, Indiana, 306 pp.

Panero, J.L. 2007. Tribe Tageteae. Pp. 420-431 in: Kadereit, J.W. & Jeffrey, C. (eds.) *The Families and Genera of Vascular Plants*, vol. 8. Berlin, Springer.

Rambaut, A., Drummond, A.J, Xie, D., Baele, G. & Suchard, M.A. 2018. Posterior summarisation in Bayesian phylogenetics using Tracer 1.7. *Syst. Bot.* syy032. doi:10.1093/sysbio/syy032

Rivera, V.L., Panero, J.L., Schilling, E.E., Crozier, B.S. & Dias Moraes, M. 2016. Origins and recent radiation of Brazilian Eupatorieae (Asteraceae) in the eastern Cerrado and Atlantic Forest, *Mol. Phylogenet. Evol.* 97: 90–170.

Rzedowski, J. 1978. Claves para la identificación de los géneros de la familia Compositae en México. Editorial Universitaria Potosina, Universidad Autónoma de San Luis Potosí, México, 143 pp.

Rzedowski, J. 2005. *Tagetes*. Pp. 921-925 in: Acosta Castellanos, S., Aguilar Rodríguez, S., Aguilar Santelices, R., Akcelrad Lerner, L., Andrade Malfavon, J.A., Arreguín Sánchez, M.DelaL., Arroyo Frías, H., Azcárraga, M.DelR., Barrios Rodríguez, M.A., Beaman, J.H., Brizuela Venegas, F., Calderón De Rzedowski, G., Calderón Díaz Barriga, G., Campos Díaz, J.L., Cárdenas Flores, M.E., Carranza González, E., Cervantes Cervantes, M., Cruz Peralta, B.L., Denton, M.F., Díaz Betancourt, M.E., Díaz Osorno, A., Dunn, D.D., Espejo Serna, A., Espinosa Garduño, J., Fernández Nava, R., Flores Martínez, G., Fryxell, P.A., Galván Villanueva, R., García Pérez, J., García Romero, L.M., García Sánchez, F., García Zúñiga, A., González Elizondo, M.DelS., González Ledesma, M., Hernández Reyna, A., Herrera Arrieta, Y., Ishiki Ishihara, M., López Ferrari, A.R., Koch, S.D., Lot Helgueras, A., Lott, E.J., Marroquín De La Fuente, J.S., Medina Cota, J.M., Montesinos Rodríguez, M.D., Morelos Ochoa, S., Novelo Retana, A., Núñez Tancredi, I., Ochoa Gaona, S., Ortega Rubio, A., Patiño Siciliano, A., Peña, M., Pérez Hernández, S., Pérez Ramírez, H.E., Ramamoorthy, T.P., Rico Rodríguez, L., Rodríguez Jiménez, C., Romero Rangel, S., Rzedowski Rotter, J., Salas Navarro, A., Sánchez Colón, S., Schubert, B.G., Stevens, W.D., Valdés Reyna, J., Vargas Nicasio, A.A., Vega Ochoa, R.M., Vélez Marín, R., Victoria Hernández, A. & Wendt, T. Flora fanerogámica del Valle de México. 2a. ed., 1a reimp., Instituto de Ecología, A.C. y Comisión Nacional para el Conocimiento y Uso de la Biodiversidad, Pátzcuaro, Michoacán, México, 1406 pp.

Schiavinato, D.J. & Bartoli, A. 2018. Una nueva cita para la Flora Argentina: *Tagetes praetermissa* (Asteraceae, Tageteae). *El Bol. Soc. Argent. Bot.* 53: 465–468. https://doi.org/10.31055/1851.2372.v53.n3.21319

Schiavinato, D.J., Gutiérrez, D.G. & Bartoli, A. 2017. Typifications and nomenclatural clarifications in South American *Tagetes* (Asteraceae, Tageteae). *Phytotaxa* 326: 175–188. https://doi.org/10.11646/phytotaxa.326.3.2

Schiavinato, D.J., Gutiérrez, D.G. & Bartoli, A. 2021. Typifications and taxonomical rearrangements in North and Central American *Tagetes* (Asteraceae, Tageteae). *Phytotaxa* 507: 81–97. https://doi.org/10.11646/phytotaxa.507.1.4

Soule, J.A. 1993. *Biosystematics of Tagetes*. Ph.D. thesis, University of Texas, Austin, 780 pp.

Soule, J.A. 1996. Infrageneric systematics of *Tagetes*. Pp. 435-443 in: D.J.N. Hind & H.J. Beentje (eds.). Compositae: Systematics. Proceedings of the International Compositae Conference, Kew, 1994, vol. 1. Kew: Royal Botanic Gardens. 689 pp.

Strother, J.L. 1977. Tageteae—systematic review. Pp. 769-783 in: V.H. Heywood, J.B.Harborne & B.L. Turner (eds.), The Biology and Chemistry of the Compositae, vol. 2. London: Academic Press. 1189 pp.

Thiers, B.M. 2022. Index Herbariorum. http://sweetgum.nybg.org/science/ih/

Turner, B.L. 1995. Resubmergence of *Hydrodyssodia* B.L.Turner into *Hydropectis* McVaugh (Asteraceae, Tageteae), with description of a new species, *Hydropectis estradii*, from Chihuahua, Mexico. *Phytologia* 78(3): 211–213.

Turner, B.L. 1996. The Comps of Mexico: A systematic account of the family Asteraceae, vol. 6. Tageteae and Anthemideae. *Phytologia Mem.* 10, i–ii: 1–22, 43–93.

Villaseñor, J.L. 2016. Checklist of the native vascular plants of Mexico. *Rev. Mex. Biodivers.* 87: 559–902.

White, T.J., Brims, T. , Lee, S. & Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pp, 315-322 in: M. Innis, D. Gelfand, J. Sninsky, & T.J. White (eds.). PCR Protocols: A Guide to Methods and Applications. San Diego: Academic Press, 482 pp.

Wilcox, T.P., Zwickl, D.J., Heath, T.A. & Hillis, D.M. 2002. Phylogenetic relationships of the dwarf boas and a comparison of Bayesian and bootstrap measures of phylogenetic support. *Molec. Phylogenet. Evol*. 25: 361–371.

Zhao, Z. & Turner, B.L. 1993. Documented chromosome numbers 1993: 3. Miscellaneous U.S.A. and Mexican species, mostly Asteraceae. *Sida* 15(4): 649–653.

APPENDIX

Species, vouchers of plant material from which DNA was extracted and GenBank accession numbers.

Species	Voucher	GenBank accession #
Adenophyllum glandulosum (Cav.) Strother	Hinojosa-Espinosa 630 (MEXU)	ON695767
Dyssodia papposa (Vent.) Hitchc.	Hinojosa-Espinosa 684 (DAV, MEXU)	ON798518
Flaveria trinervia (Spreng.) C.Mohr	Huffman s.n. (DAV)	ON695768
Gymnolaena serratifolia Rydb.	Cronquist 11219 (MEXU)	ON695769
Helianthus annuus L.	Schilling 660	KX671853
Hydropectis aquatica Rydb.	Soule 2796 (DAV)	ON695770
Hydropectis stevensii McVaugh	Perez-Calix 4706 (TEX)	ON695771
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)	ON695774
Tagetes laxa Cabrera	-------**	KC800431.1
Tagetes lemmonii A.Gray	Reina 1120 (HCIB)	ON695775
Tagetes lucida Cav.	Hinojosa-Espinosa 676 (DAV, MEXU)	ON695772
Tagetes lunulata Ortega	Hinojosa-Espinosa 723 (DAV, MEXU)	ON695776
Tagetes micrantha Cav.	Hinojosa-Espinosa 724 (DAV, MEXU)	ON695773
Tagetes multiflora Kunth	-------**	KC800434.1
Tagetes minuta L.	-------**	AF413576
Tagetes moorei H.Rob.	-------**	KC800433.1
Tagetes nelsonii Greenm,	Hinojosa-Espinosa 731 (CH, DAV, MEXU)	ON695777
Tagetes palmeri A.Gray	Soule 3362 (TEX)	AF413577
Tagetes parryi A.Gray	-------**	KC800427.1
Tagetes patula L.	-------**	DQ862121.1
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)	ON695778
Tagetes tenuifolia Cav.*	Hinojosa-Espinosa 677 (DAV)	ON695779

* (sensu Turner 1996, *T. lunulata* Ortega sensu Rzedowski 2005)
** Information not available in GenBank