

A MORPHOLOGICAL ANALYSIS OF THE *TRIFOLIUM AMABILE* KUNTH SPECIES COMPLEX IN NORTH AMERICA

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ABSTRACT

The *Trifolium amabile* species complex (Fabaceae) is a group of New World clovers ranging from the mountains of southern Arizona of the USA, throughout the mountainous regions of Central America, and continuing southward to northern-central Argentina. As currently circumscribed in Zohary and Heller's monograph (1984), this group comprises a single species, *T. amabile*, and five varieties. Specimens from herbaria worldwide have been utilized for a morphometric examination to determine specific boundaries for the North American members of this complex. In this paper, seven previously described species, *Trifolium amabile*, *T. cognatum*, *T. goniocarpum*, *T. hickeyi*, *T. lozani*, *T. rhombeum*, and *T. sonorensis*, are recognized as distinct, and two new species are described: ***Trifolium laciae*** T.K. Ahlquist & Vincent, **sp. nov.**, and ***Trifolium madreense*** T.K. Ahlquist & Vincent, **sp. nov.**

The *Trifolium amabile* species complex is a very widespread group, ranging from the mountains of southern Arizona, through the mountainous regions of Central America, and continuing southward to the province of Córdoba in north-central Argentina. In addition to its extensive geographical distribution, members of this species complex vary greatly in their size, habit, and morphological features. *Trifolium amabile* Kunth was described in 1824 from material collected in Mexico by Humboldt and Bonpland. Since then, over thirty names have been published for members of this complex. In Zohary and Heller's (1984) monograph, the most recent treatment of this group, the authors recognize five varieties of a single species, *T. amabile*, based on pubescence characters, calyx lobe shapes, leaflet shape, leaflet size, leaflet apex shape, and inflorescence size: var. *amabile*, var. *hemsleyi* (Lojac.) Heller & Zoh., var. *pentlandii* Ball, var. *mexicanum* (Hemsl.) Heller & Zoh., and var. *longifoliolum* Hemsl. However, it has been noted that this treatment is outdated and inadequate. For example, McVaugh (1987) called for a revision of the Mexican species, especially commenting on differences between *T. amabile* and *T. goniocarpum* Lojac., included in var. *longifoliolum*. Even the monographers themselves noted that the distinctions among the varieties are not clear and difficult to distinguish. A reexamination of these organisms was undertaken to clarify the delimitation of taxa, their distributions, and correct nomenclature for the taxa described in this study.

MATERIALS AND METHODS

Specimens spanning the morphological diversity and North American range of this species complex were obtained from herbaria worldwide, a total of 948 specimens from 24 herbaria. Exemplar specimens, representing the morphological diversity and geographic range of this group, were selected for measurements. Flowers and fruits were removed from the specimens and rehydrated using a 1% v/v dilution of Aerosol OT solution in distilled water (Ayensu 1967).

Analyses were performed using the Numerical Taxonomy System (NTSYSpc) (Rohlf 2000) to first standardize the data, and then perform a similarity matrix, using a sequential, agglomerative, hierarchical, non-overlapping (SAHN) clustering analysis, and a principal component analysis (PCA). Preliminary species groups from these analyses were subjected to refinement and further testing in R 2.11.0 to calculate a variety of descriptive statistics, Shapiro-Wilk Normality Tests, and two-sided Student's t-Tests between pairings of the groups of specimens. In addition, the “car” (Fox and Weisberg 2010) and “lattice” (Sarkar 2010) packages in R were used to create comparative box and whisker plots between pairings of the groups. Adjustments were made as needed to the groups, and this process was repeated until the groups were composed of specimens whose data were analogous and had significant differences from the other groups that could be used to key out each individual group.

Using the statistically significant characters between each group pairing, data sets were made and final PCAs were run in R for each group pairing. Both two- and three-dimensional plots were constructed with the first two and three principal components, respectively. The “scatterplot3d” (Ligges & Mächler 2011) package in R was used to make the three-dimensional plots. These results were congruent with the previous results from the t-tests of nine different groups which will be presented here as nine different species.

Illustrations were created in Adobe Photoshop 20.0.8 and Adobe Illustrator 23.1.1. Distribution maps were created in Esri ArcGIS 10.0 and Adobe Illustrator using coordinates from Google Earth 6.0 derived from locality data on specimen labels.

RESULTS

Examination of specimens and analyses indicates that the *Trifolium amabile* species complex is composed of nine morphologically distinct species (see Ahlquist 2012). Five of these species had previously been described in the literature: *T. amabile* Kunth, *T. cognatum* House, *T. goniocarpum* Lojac., *T. lozani* House, and *T. rhombeum* S. Schauer. Two new species in the complex have subsequently been published: *T. hickeyi* T.K. Ahlquist & Vincent and *T. sonorensis* T.K. Ahlquist & Vincent. The remaining two species are described here as new species.

TAXONOMIC TREATMENT

KEY TO THE NORTH AMERICAN SPECIES OF THE TRIFOLIUM AMABILE SPECIES COMPLEX

1. Stipule length >19 mm; peduncles >76 mm in length; ovaries glabrous **T. hickeyi**
1. Stipule length <19 mm; peduncles <76 mm in length; ovaries pubescent, especially distally.
 2. Leaf texture thick and leathery; sepals serrate, becoming curled in fruit; petals yellow; 3 ovules per ovary and 3 seeds per fruit **T. laciae**
 2. Leaf texture thin and delicate; sepals entire, not curled in fruit; petals not yellow; 2 ovules per ovary and 2 seeds per fruit.
 3. Terminal leaflet lamina moderately to densely pubescent abaxially, at least distally; fruits inflated **T. madrense**
 3. Terminal leaflet lamina glabrous abaxially; fruits laterally compressed.
 4. Wing petals not dilated distally, the margins parallel, <0.8 mm at the widest point **T. goniocarpum**
 4. Wing petals dilated distally, the margins not parallel, >0.8 mm at the widest point.
 5. Keel petal lamina length >2.8 mm; inflorescences >16 mm in diameter.
 6. Terminal leaflets <15 mm in length and <8 mm in width; internodes <20 mm in length; flowers pinkish purple to brownish purple; plants prostrate or creeping **T. cognatum**

6. Terminal leaflets >15 mm in length and >8 mm in width; internodes >20 mm in length; flowers white or cream; plants prostrate to ascending **T. rhombeum**
5. Keel petal lamina length <2.8 mm; inflorescences <16 mm in diameter.
7. Sepal lobes >3 mm in length; keel petal claws >1.7 mm in length; connate filaments >3.6 mm in length **T. sonorensis**
7. Sepal lobes <3 mm in length; keel petal claws <1.7 mm in length; connate filaments <3.6 mm in length.
8. Stems densely pubescent; sepals <3 mm in length; banner petals <4.2 mm or length; wing petal claws <1.1 mm in length; keel petals <3.1 mm in length; keel petal claws <1.3 mm in length; ovaries <1.3 mm in length **T. lozani**
8. Stems glabrous to moderately pubescent; sepals >3 mm in length; banner petals >4.2 mm in length; wing petal claws >1.1 mm in length; keel petals >3.1 mm in length; keel petal claws >1.3 mm in length; ovaries >1.3 mm in length **T. amabile**

1. TRIFOLIUM AMABILE Kunth, Nov. Gen. Sp. (quarto ed.) 6: 503, pl. 593. 1823[1824]. *Lupinaster amabilis* (Kunth) C. Presl, Symb. Bot. 1(3): 47. 1830. **LECTOTYPE** (designated here): **MEXICO. México.** Toluca, in pratis, *Humboldt & Bonpland* (P00659966!). Holotype: B (destroyed), photos GH!-Fig. 1, NY!; isotype: P00659966!). Figure 2.

Trifolium schiedeana S. Wats., Proc. Amer. Acad. Arts 17: 339. 1882. **TYPE: MEXICO. Coahuila.** Lirios, 45 mi E of Saltillo, Jul 1880, *E. Palmer 201* (holotype: GH!; isotypes: NY!, VT!).

Plants perennial. **Roots** large, woody, 2–10 mm in diameter. **Stems** prostrate, ascending, 5–49+ cm in length, 0.5–2 mm in diameter, internodes glabrous–moderately pubescent, nodes sparsely–densely pubescent. **Leaves** trifoliate, ± uniform in size throughout. **Stipules** narrowly triangular–triangular, 4–12 × 1–7.5 mm, glabrous adaxially, glabrous–moderately pubescent abaxially, margins entire–weakly serrate, sparsely pubescent, apices acute. **Petioles** 4–45 × 0.2–0.5 mm, sparsely–densely pubescent. **Petiolules** 0.3–1.2 × ≤0.5 mm, sparsely–densely pubescent. **Leaflets** obovate–obcordate, glabrous adaxially, sparsely–densely pubescent along midribs abaxially, sparsely–moderately pubescent abaxially on outer halves of lateral leaflet, bases acute, margins weakly serrate–serrate, often with short, straight teeth alternating with larger, antrorsely curved teeth, sparsely pubescent, apices rounded–emarginate, weakly mucronulate, terminal leaflets 3–15 × 2.5–2.5 mm. **Peduncles** 9.5–50.5 × <1 mm, sparsely–densely pubescent. **Inflorescences** axillary, racemose–umbellate, 4.5–15.5 × 0.9–15 mm. **Flowers** 3–25, each subtended by a linear triangular bract, in 1–3 whorls, terminal sterile projection sometimes present. **Pedicels** 0.5–2.5 × <0.5 mm, sparsely–densely pubescent. **Calyx** 2.5–5 mm long, tubes 1.9–3.1 mm in circumference, glabrous–densely pubescent, lobes narrowly triangular, 1.4–4 × <1 mm, glabrous–moderately pubescent abaxially, margins entire, sparsely–densely pubescent, apices attenuate. **Petals** pink–purple. **Banner petals** obovate, 3–7.5 × 2.2–5.6 mm. **Wing petals** 2.8–7.2 mm long, asymmetrically clawed, claws 0.7–2.7 × ≤0.3 mm, laminae spatulate, 2–4.5 mm long, 0.8–2 mm wide at widest point, <1 mm wide at narrowest point, bases shortly auriculate, auricles acute–rounded, margins entire, apices rounded. **Keel petals** 2.7–4.3 mm long, asymmetrically clawed, claws 0.9–1.6 × <0.5 mm, laminae spatulate, 1.8–3.5 mm long, 0.9–1.4 mm wide at widest point, 0.3–1.2 mm wide at narrowest point, bases obtuse, margins entire, apices rounded. **Stamens** diadelphous, connate filaments 2.2–3.6 × 0.9–1.5 mm, fused for 40–72% of their length, free filaments 1–2.7 × ≤0.2 mm. **Pistils** sessile, ovaries 0.9–3.1 × 0.4–1.5 mm, sparsely–densely pubescent distally and occasionally along suture, styles 0.6–1.3 × ≤0.2 mm, glabrous, ovules 2. **Legumes** laterally compressed, 3–5.4 × 1.7–2.8 mm. **Seeds** 2, globular–mitten-shaped, brownish orange–reddish brown, occasionally dark-speckled, 1.2–1.9 × 1–1.5 mm.

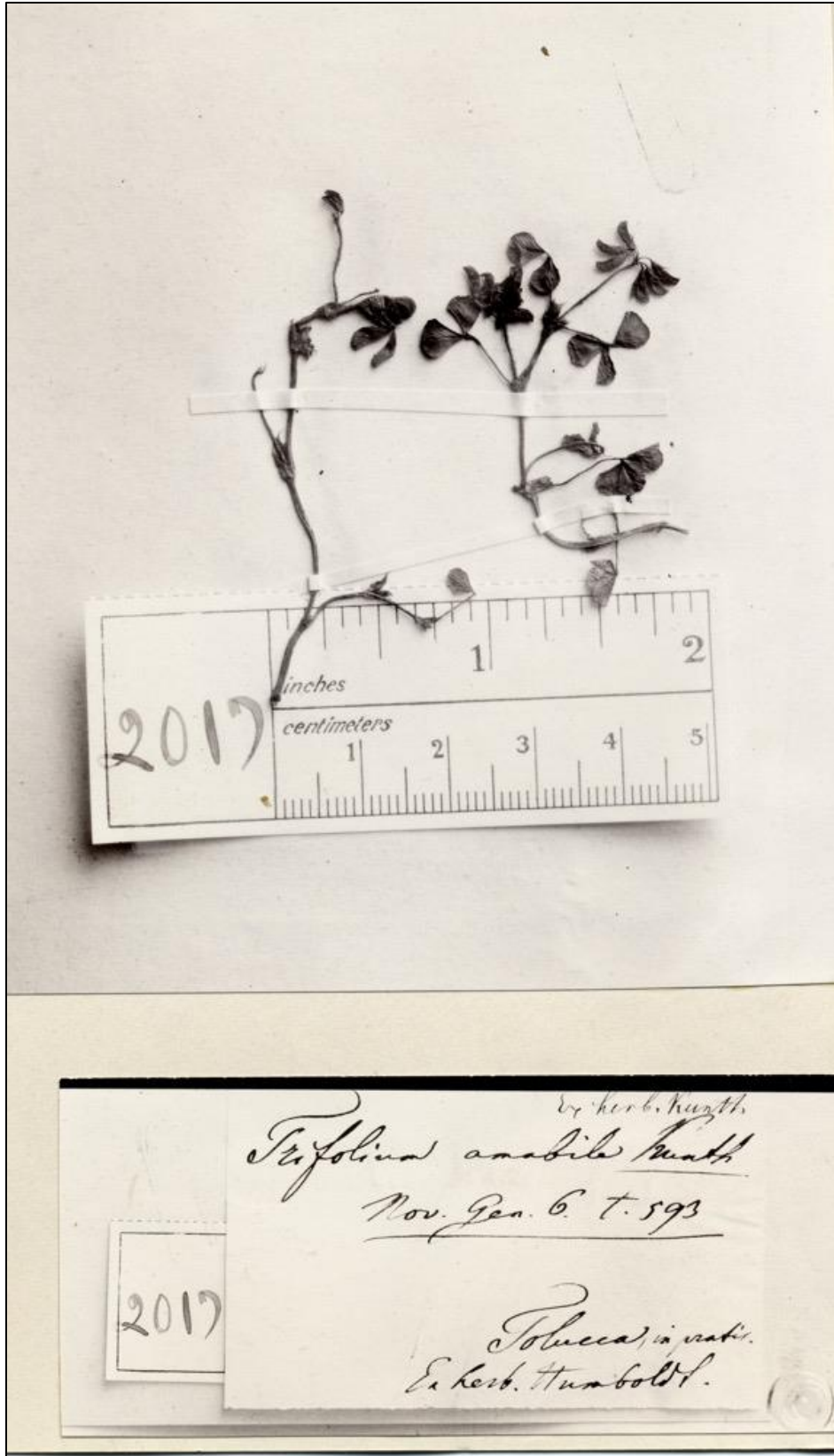


Figure 1. Photograph of the holotype specimen of *Trifolium amabile*. Originally housed at the Berlin herbarium (B), it is no longer extant. Photo from Harvard University (GH).

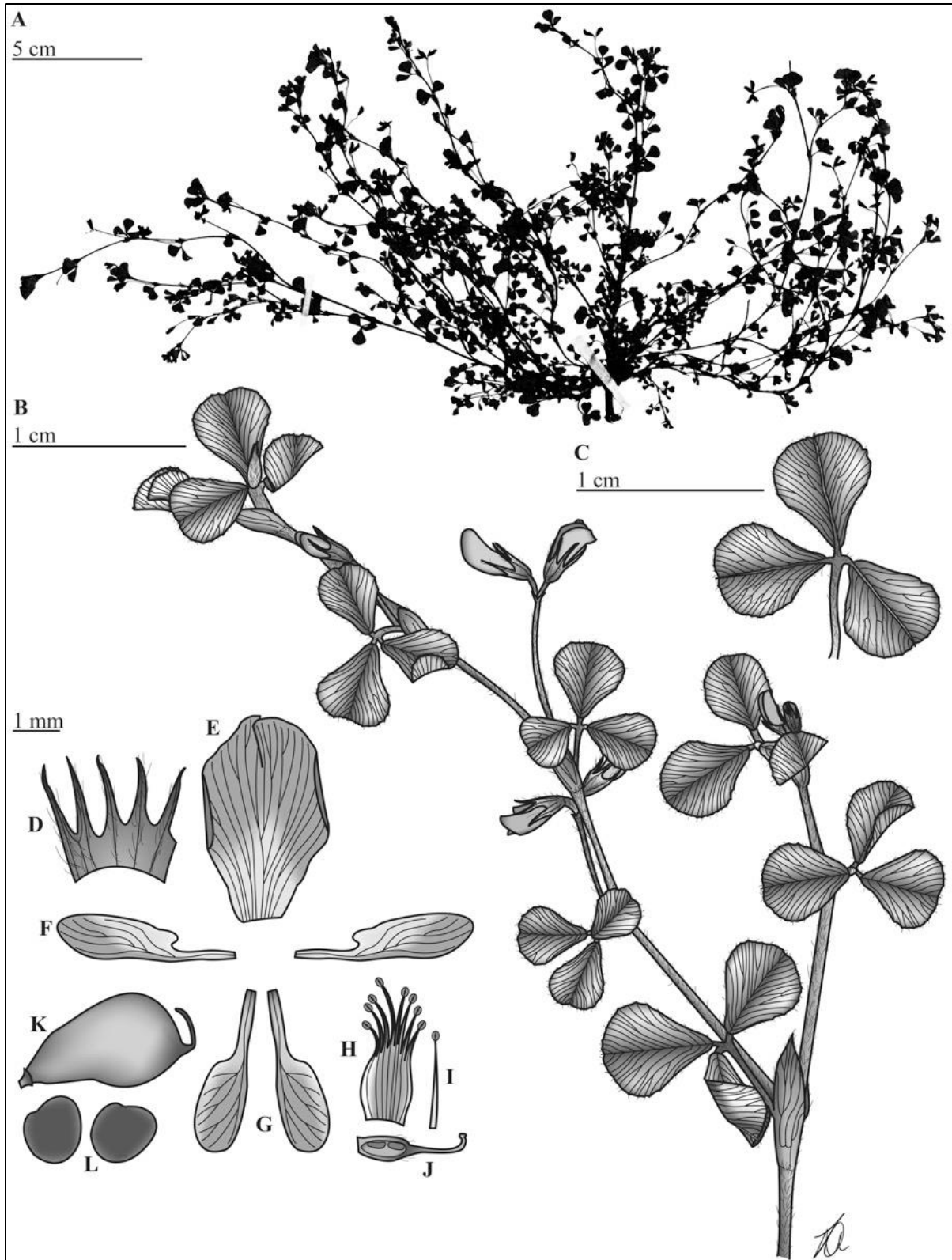


Figure 2. *Trifolium amabile* (from Pringle 10278 at Harvard University (GH)). A. Habit silhouette. B. Fertile branch. C. Abaxial leaf surface. D. Sepals. E. Banner petal. F. Wing petals. G. Keel petals. H. Connate stamens. I. Free stamen. J. Ovary. K. Legume. L. Seeds.

Additional collections examined. COSTA RICA. Alajuela-Heredia. Along the cart-road from Vara Blanca (between Poás and Barva Volcanoes) to La Concordia, 10.1°, -84.1°, 1950 m, 23 Jul 1923, *Maxon 8440 & Harvey* (US); Finca La Giralda, above Heredia, in very fertile pasture with kikuyu grass, CR-L25, 10.13°, -84.16°, 2000 m, 1 Sep 1959, *Semple s.n.* (US). **Cartago.** Volcán Irazú, 9.9°, -83.8°, 2133 m, 24 Jun 1874, *Kuntze 2317* (NY); along the Río Reventado, north of Cartago, moist meadow, 9.83°, -83.9°, 1460 m, 26 Feb 1926, *Standley 49409 & Valerio* (US). **San José.** Potrereros between Guayabillos and Cabeza de Vaca, 9.967°, -83.892°, 2350 m, 4 Nov 1929, *Dodge 4931 & Thomas* (F); prairies et bords des chemins à San José, 9.9°, -84.1°, 1100 m, Nov 1894, *Pittier 1536* (US); San Francisco de Guadalupe, 9.93°, -84.09°, 1150 m, Oct 1894, *Pittier s.n.* (US); vicinity of Santa María de Dota, in potrero, 9.92°, -84°, 1800 m, 14 Dec 1925, *Standley 42429* (F, US); chemin près de la savane de San José, [prairies of San José], 9.9°, -84.1°, 1150 m, Nov 1892, *Tonduz 1536 & Barbey* (P, US). **GUATEMALA. Chimaltenango.** San Rafael, 14.878°, -90.817°, 1900 m, 7 Jul 1936, *Lewis 309* (F, P). **El Quiché.** Nebaj, in clearing, 12 km north of the village on Cotzal Road, 15.4194, -91.1641, 2000 m, 1 Jul 1964, *Contreras 5152* (MEXU). **Guatemala.** La Cumbre, Socorro, 14.5°, -90.2°, 1860 m, 8 Nov 1930, *Rodriguez 2901* (P). **Huehuetenango.** Estación Ovino, Aldea San Nicolás, Chiantla, 15.4°, -91.5°, 3130 m, 10 Oct 1975, *Rushin s.n.* (F). **Jalapa.** Between Jalapa and Montaña Miramundo, llanos on top of ridge, 14.633°, -89.989°, 1500 m, 7 Dec 1939, *Steyermark 32898* (F). **Quezaltenango.** Volcán Santa Maria; near summit of mountain, in open somewhat weedy and disturbed meadow above timberline, 14.75°, -91.55°, 3770 m, 12 Aug 1960, *Beaman 4122* (NY). **Sacatepéquez.** San Lucas Sacatepéquez, 14.612°, -90.655°, 2088 m, 9 Aug 2003, *Ponce 032 & García* (MO). **San Marcos.** Volcán Tacaná, vicinity of Sibinal, open, dry white clay slopes, 15.147°, -92.054°, 2589 m, 18 Feb 1940, *Steyermark 35952* (F). **Sololá.** Volcán Santa Clara, on summit, 14.65°, -91.28°, 3000 m, 5 Jun 1942, *Steyermark 47011* (F, NY). **Totonicapán.** Open pine woodland, 11 mi SE of Totonicapán, along roadside, 14.78°, -91.27°, 3048 m, 9 Aug 1962, *Webster 12863 et al.* (DAV, F); ravines in Sierra Madre Mountains near Villa Las Cruces, about 8 km southwest of Totonicapán, in ravine, 14.8°, -91.4°, 2800 m, 13 Dec 1963, *Williams 22952 et al.* (F, NY, US). **MEXICO. Chiapas.** Dorfanger..., 16.196°, -93.136°, 808 m, 4 Mar 1896, *Seler 2092* (GH); aergahhang oberh, Huitzlan, 16.195722°, -93.135730°, 808 m, 10 Mar 1896, *Seler 2143* (GH). **Chihuahua.** 2 km SE of Cocheño on road to Mena Tarahumara, pine-oak woods, 28.308333°, -108.208333°, 2200 m, 28 Aug 1986, *Martin et al. s.n.* (ARIZ). **Ciudad de México.** Villa de Obregon, 19.5°, -99.13°, 2270 m, 18 Jul 1935, *Fisher 35236* (ARIZ, NY, P, US). **Durango.** Mpio. de El Salto, 11 km del Entronque de la Brecha a Sn. Miguel de Cruces con Carr. Durango-Mazatlán, 24.042°, -104.654°, 1900 m, 06 Jul 1982, *Tenorio L. & Romero de T. 842* (MEXU). **Guanajuato.** Puerto Jondo, 26 km de Tarimoro, entre los cerros La Bufa y La Pucha, ladera de cerro, potrero, 20.44°, -100.61°, 1900 m, 14 Oct 1974, *Flores 174* (XAL). **Guerrero.** La Hoya, 18.7°, -100.7°, 700 m, Jul 1841, *Liebmann 4932* (C). **Hidalgo.** San Vicente, 20.15°, -98.31°, 2377 m, 16 Aug 1937, *Fisher 3757* (US, NY). **Jalisco.** Northern slopes of the Nevado de Colima, west of summit of the northern ridge, near junction of the old pack road to Zapotlán with Atenquique-Jazmín road, steep mountain sides in pine forest, 19.56°, -103.6°, 2200 m, 15 Oct 1952, *McVaugh 13526* (MICH, US). **México.** Paso de Cortes, slopes of Popocatepetl and Iztaccihuatl, 1.4 m W of Paso de Cortes near km. 21, pine forest with grass tussocks, along road, 19.120°, -98.766°, 3300 m, 6 Jul 1972, *Denton 1750* (MICH). **Michoacán.** 9 km NE of Patzcuaro, within 10 meters of the road in disturbed area, 19.601°, -101.525°, 2144 m, 9 Jul 1981, *LaSalle 810709-18 et al.* (ARIZ). **Morelos.** Cuernavaca, Moore lawn, 18.93°, -99.23°, 1500 m, Jun 1953, *Ross 36* (US); banks, Tres Marías, 19°, -99.2°, 2743m, 11 Aug 1906, *Pringle 10278* (ARIZ, ASU, DAV, GH, MICH, RSA, VT). **Nayarit.** Acaponeta, in pine and oak forest at La Cinenga on ridge about 10 mi NW of Mesa del Nayar, 22.793°, -105.193°, 1187 m, 30 Jul 1970, *Norris & Taranto 14554* (MICH). **Nuevo León.** Sierra Madre Oriental, Mt. "El Infernillo", Pablillo, SE of Galeana, rocky summit, 24.6°, -99.7°, 3000 m, 29 Jun 1934, *Pennell 17119* (US). **Oaxaca.** 110 km by road S of Teotitlán on road to Oaxaca, oak forest, 16.2°, -96.6°, 2180 m, 11 Oct 1983, *Anderson 13009* (MICH, NY). **Puebla.** Laguna de San Baltasar, 19°, -98.2°, 2135 m, 7 Jul 1907, *Arsène 1308* (US). **San Luis Potosí.** Catorce, Sierra de Catorce, Sierra Madre Oriental, grassy, andesitic slopes, SE of Catorce, 23.6°, -100.87°, 2900 m, 24 Jul 1934, *Pennell*

17530 (GH, NY, US). **Tamaulipas.** Southern Tamaulipas, top of Sierra Madre Oriental (Sierra de Guatemala), rugged, wooded, karstic limestone plateau with sink holes and big boulders, ca 6 km NW of Rancho del Cielo (Harrison Ranch of Martin & Harrell), (ca 4 km NW of Julio) on road to La Joya de Salas, ca 12 km NW of Gomez Farias, 108 km SSW of Ciudad Victoria, very open, sunny secondary growth *Pinus-Quercus* woodlands (canopy 8 m), with rugged white limestone outcrops, bright red soil (hence local name "Tierra Colorado"), at fork of road to Las Canoas, 23.2°, -99.283333°, 2000 m, 12 Aug 1991, *Iltis & Simon 30702* (MU). **Tlaxcala.** Nanacamilpa, agua buena, bosque de pino, 19.491°, -98.537°, 2750 m, 24 Oct 1986, *Fontes C. s.n.* (XAL). **Veracruz.** Lomogrande, Mt. Orizaba, on open slopes and edges of cultivation, 19°, -97.2°, 3100 m, 29 Apr 1938, *Balls 4401* (MICH, US); antes de Acultzingo limite de los edos de Puebla y Veracruz, bosque de *Quercus*, ruderal junto al pavimento, 18.715°, -97.309°, 2300 m, 28 Jul 1971, *Nevling I. 2105 Gomez-Pompa* (GH). **Zacatecas.** Sierra Madre Oriental, Mt. "El Temeroso", N of Aranzazu, andesitic gravelly slopes, 4.65°, -101.47°, 3000 m, 19 Jul 1934, *Pennell 17470* (GH, MICH, NY, US).

Trifolium amabile is by far the most widely distributed species in the *T. amabile* species complex, ranging throughout Mexico, Guatemala, and Costa Rica (Fig. 3). In South America, the range of *T. amabile* continues in Colombia, Ecuador, Peru, Bolivia, and Argentina (Hendy 2013). This species is most often found in sunny, moist, open areas of secondary pine and oak forests on gentle to steep slopes and ravines. It has also been collected in meadows and pastures, including cultivated cornfields. Other habitats include disturbed areas along riverbanks and roadsides. While these are the most common habitats, other locales include xeric hillsides, mountain and volcano summits, old lava flows, and areas of karst topography and limestone outcrops. *Trifolium amabile* has been found growing on a variety of substrates. Loam to clay-loam soils are the most common along with gravelly and sandy soils which are brown, red, or black in color. Other types of soils include andisols and andesitic soils. It has been collected at elevations from below 100 m to 5000 m. In South America, it grows in the same variety of habitats as the North American specimens (Hendy 2013).

Trifolium amabile is most similar to *T. madrense* but can be easily distinguished by lacking characters such as abaxial terminal leaf pubescence and inflated fruits. Instead, the abaxial surface of the terminal leaflet is glabrous and the fruits are laterally compressed in *T. amabile*. Another similar species is *T. lozani*, but, *T. amabile* is less densely pubescent and has larger floral characters including sepals, ovaries, and banner, wing, and keel petals than *T. lozani*. T-tests ($\alpha=0.05$) revealed that *T. amabile* significantly differs in 50% and 45% of the 50 characters analyzed in this study between *T. madrense* and *T. lozani*, respectively (Ahlquist 2012).

Disjunct populations of *Trifolium amabile* in Costa Rica, at first look, appear to have some differences from *T. amabile* specimens from Mexico and Guatemala, but these were never confirmed in analyses. Nonetheless, this group warrants a closer look, and possibly a molecular phylogenetic analysis.

2. TRIFOLIUM COGNATUM House, Bot. Gaz. 41: 345. 1906. **TYPE: MEXICO. Hidalgo.** Bare hills above Pachuca, 8500–9500 ft (2590–2895 m), 30 Jul 1898, *C.G. Pringle 6933* (holotype: US; isotypes: GH!, MICH!, NY!, P!, POM!, VT!). Figure 4.

Plants perennial. **Roots** large, woody, 1.9–11.7 mm in diameter. **Stems** prostrate–creeping, 11–25+ cm in length, 1–2 mm in diameter, internodes and nodes sparsely pubescent. **Leaves** trifoliate, ± uniform in size throughout. **Stipules** narrowly triangular–triangular, 7.3–12 × 2.5–4.8 mm, glabrous adaxially, glabrous abaxially, margins entire, glabrous–sparsely pubescent, apices acute. **Petioles** 9.5–32 × <1 mm, glabrous–sparsely pubescent. **Petiolules** 0.5–1 × <0.5 mm, sparsely pubescent. **Leaflets** obovate–rhombic, glabrous adaxially, sparsely pubescent along midribs abaxially, glabrous–sparsely

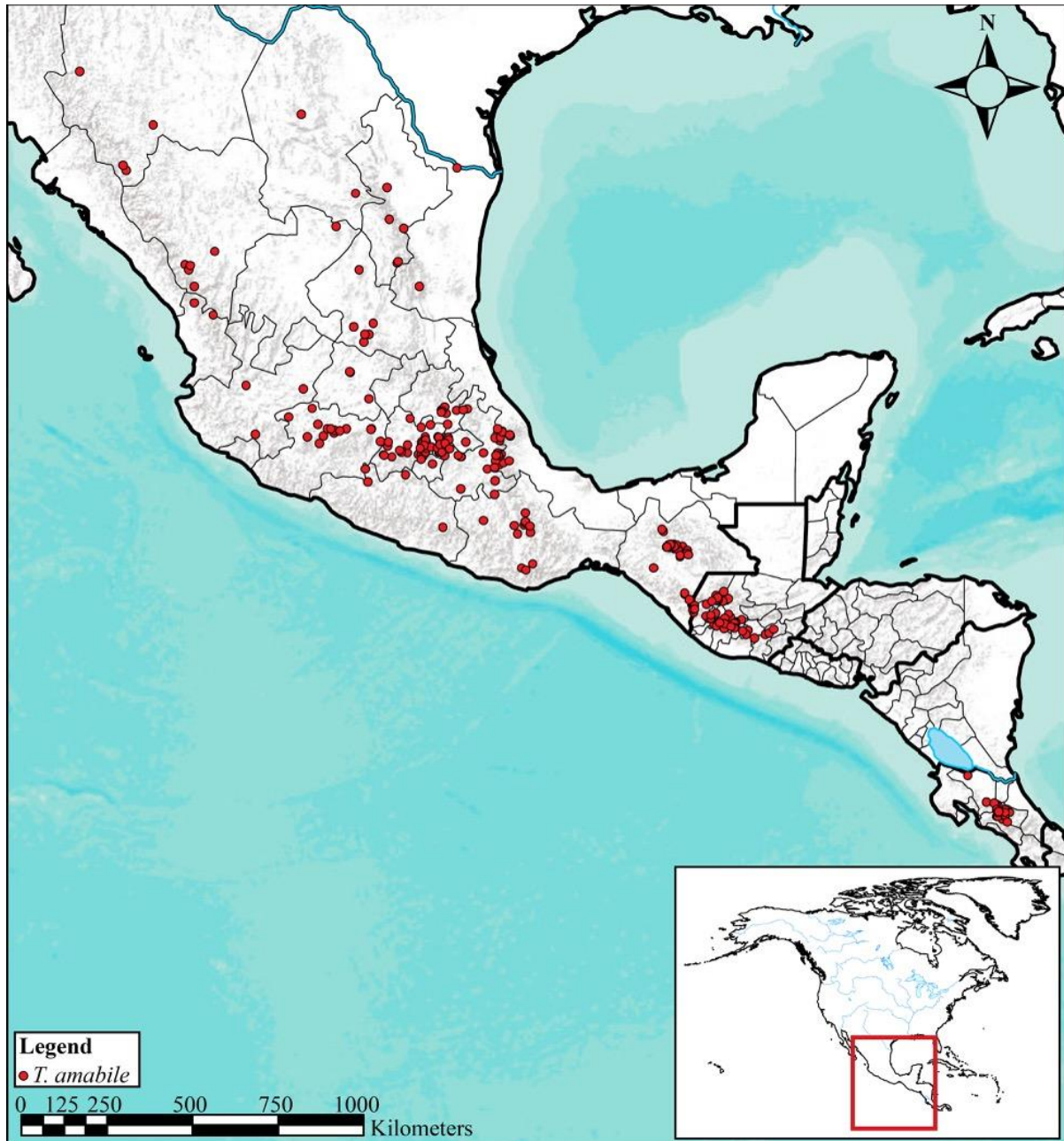


Figure 3. Distribution of *Trifolium amabile* in North America.

pubescent abaxially on outer halves of lateral leaflets, bases acute, margins serrate, often with short, straight teeth alternating with larger, antrorsely curved teeth, sparsely pubescent, apices rounded–acute, weakly mucronulate, terminal leaflets $9\text{--}15 \times 5\text{--}8$ mm. **Peduncles** $27\text{--}70 \times \leq 1$ mm, moderately pubescent. **Inflorescences** axillary, racemose–umbellate, $9\text{--}20.5 \times 16\text{--}20$ mm. **Flowers** 12–30, each subtended by a linear triangular bract, in 2–4 whorls. **Pedicels** $1.8\text{--}3.2 \times < 0.5$ mm, sparsely pubescent. **Calyx** 3.6–4.5 mm long, tubes 2.4–3.3 mm in circumference, glabrous–sparsely pubescent, lobes narrowly triangular, $2.5\text{--}3.3 \times \leq 0.7$ mm, glabrous–sparsely pubescent abaxially, margins entire, sparsely–moderately pubescent, apices attenuate. **Petals** pinkish purple–brownish purple. **Banner petals** obovate–elliptic, $4.7\text{--}7.6 \times 3.4\text{--}4.5$ mm. **Wing petals** 4.5–6.7 mm long, asymmetrically clawed, claws $0.9\text{--}1.7 \times \leq 0.4$ mm, laminae spatulate, 3.6–5.1 mm long, 1.4–2.7 mm wide at widest

point, ≤ 1.2 mm wide at narrowest point, bases shortly auriculate, auricles acute, margins entire, apices rounded. **Keel petals** 3.7–5 mm long, asymmetrically clawed, claws $0.9\text{--}1.7 \times < 0.5$ mm, laminae spatulate, 2.8–3.3 mm long, 1.3–1.7 mm wide at widest point, 1–1.3 mm wide at narrowest point, bases truncate, margins entire, apices rounded. **Stamens** diadelphous, connate filaments $2.9\text{--}4.5 \times 1.3\text{--}1.6$ mm, fused for 45–55% of their length, free filaments $1.6\text{--}3 \times \leq 0.2$ mm. **Pistils** sessile, ovaries $1.4\text{--}2.5 \times 0.5\text{--}1.1$ mm, glabrous–compressed, $5.3\text{--}5.7 \times 3\text{--}3.3$ mm. **Seeds** 2, mitten-shaped, burnt orange–olive brown, occasionally moderately pubescent distally, styles $1.7\text{--}2 \times \leq 0.2$ mm, glabrous, ovules 2. **Legumes** laterally dark-speckled, $1.2\text{--}1.7 \times 1.2\text{--}1.5$ mm.

Additional specimens examined. **MEXICO.** México. Cerro Ahumada, cerca del Rancho Nuevo, 4 km al NE de Huehuetoca, ladera andesítica con vegetación de matorral xerófilo, 19.872° , -99.219° , 2350 m, 11 Aug 1971, *Rzedowski 28414* (ARIZ). Hidalgo. El Bordo, 4 km al N de Pachuca, encinar perturbado, orilla de arroyo, 20.151389° , -98.739444° , 2600 m, 27 Aug 1967, *Rzedowski 24287* (MICH); Cerro Ventoso, entre Pachuca y Real del Monte, bosque abierto de *Juniperus* y *Quercus*, planta rastrera, 20.134° , -98.698° , 2700 m, 29 Aug 1965, *Rzedowski 20602* (MICH); 4 km al NE de Pachuca, sobre la carretera a Real del Monte, matorral xerófilo, 20.134° , -98.698° , 2650 m, 14 Sep 1975, *Rzedowski 33557* (MICH).

Trifolium cognatum is found primarily in the Sierra Madre Oriental mountain range in the Mexican states of México and Hidalgo (Figure 5). It has been collected in hilly regions with disturbed pine-oak forests between 2350 m and 2750 m in elevation.

3. TRIFOLIUM GONIOCARPUM Lojac., Nuov. Giorn. Bot. Ital. 15: 145. 1883. **LECTOTYPE** (designated here): **MEXICO.** San Luis de Potosí. Chiefly in the region of San Luis Potosi, 22° N, 6000–8000 ft (1830–2440 m), 1878, *E. Palmer & C.C. Parry 134*. (K; isoelectotypes: GH!, NY!). Figure 6.

Trifolium amabile Kunth var. *longifoliolum* Hemsl., Biol. Centr. Am. Bot. 1: 232. 1879. *Trifolium longifoliolum* (Hemsl.) House (as “*longifolium*”), Bot. Gaz. 41: 342. 1906. **LECTOTYPE** (designated here): **MEXICO.** San Luis de Potosí. Chiefly in the region of San Luis Potosi, 22° N, 6000–8000 ft (1830–2440 m), 1878, *E. Palmer & C.C. Parry 134* (K; isoelectotypes: GH!, NY!). Syntype: **Mexico.** Orizaba, *Botteri 703* (K, NY!, P!).

Plants perennial. **Roots** large, woody, 3.3–10 mm in diameter. **Stems** creeping, 13–53+ cm in length, 0.7–3.1 mm in diameter, internodes sparsely–moderately pubescent, nodes sparsely–densely pubescent. **Leaves** trifoliate, \pm uniform in size throughout; stipules narrowly triangular, $4.8\text{--}19 \times 1\text{--}4.5$ mm, glabrous adaxially and abaxially, margins entire–weakly serrate, glabrous–sparsely pubescent, apices acute. **Petioles** $1.3\text{--}29 \times \leq 2$ mm, sparsely–moderately pubescent. **Petioliules** $0.4\text{--}1.1 \times \leq 0.5$ mm, sparsely–moderately pubescent. **Leaflets** obcordate–elliptic, glabrous adaxially, sparsely–moderately pubescent along midribs abaxially, glabrous–sparsely pubescent abaxially on outer halves of lateral leaflets, bases acute, margins weakly serrate–serrate, often with short, straight teeth alternating with larger, antrorsely curved teeth, glabrous–sparsely pubescent, apices rounded–emarginated, weakly mucronulate, terminal leaflets $8.9\text{--}28.5 \times 2.6\text{--}9$ mm. **Peduncles** $30\text{--}77 \times < 1$ mm, sparsely–densely pubescent. **Inflorescences** axillary, racemose–umbellate, $6\text{--}14 \times 8\text{--}16.5$ mm. **Flowers** 11–23, each subtended by a linear triangular bract, in 1–3 whorls. **Pedicels** $0.9\text{--}3 \times < 0.5$ mm, sparsely–densely pubescent. **Calyx** 2.5–5.2 mm long, tubes 1.7–3.4 mm in circumference, glabrous–sparsely pubescent, lobes narrowly triangular–linear triangular, $1.8\text{--}4.4 \times \leq 0.5$ mm, sparsely–moderately pubescent abaxially, margins entire, sparsely–moderately pubescent, apices acuminate. **Petals** white. **Banner petals** obovate, $3.6\text{--}5.4 \times 2\text{--}3$ mm. **Wing petals** 3–4.5 mm long, asymmetrically clawed, claws 1–1.4

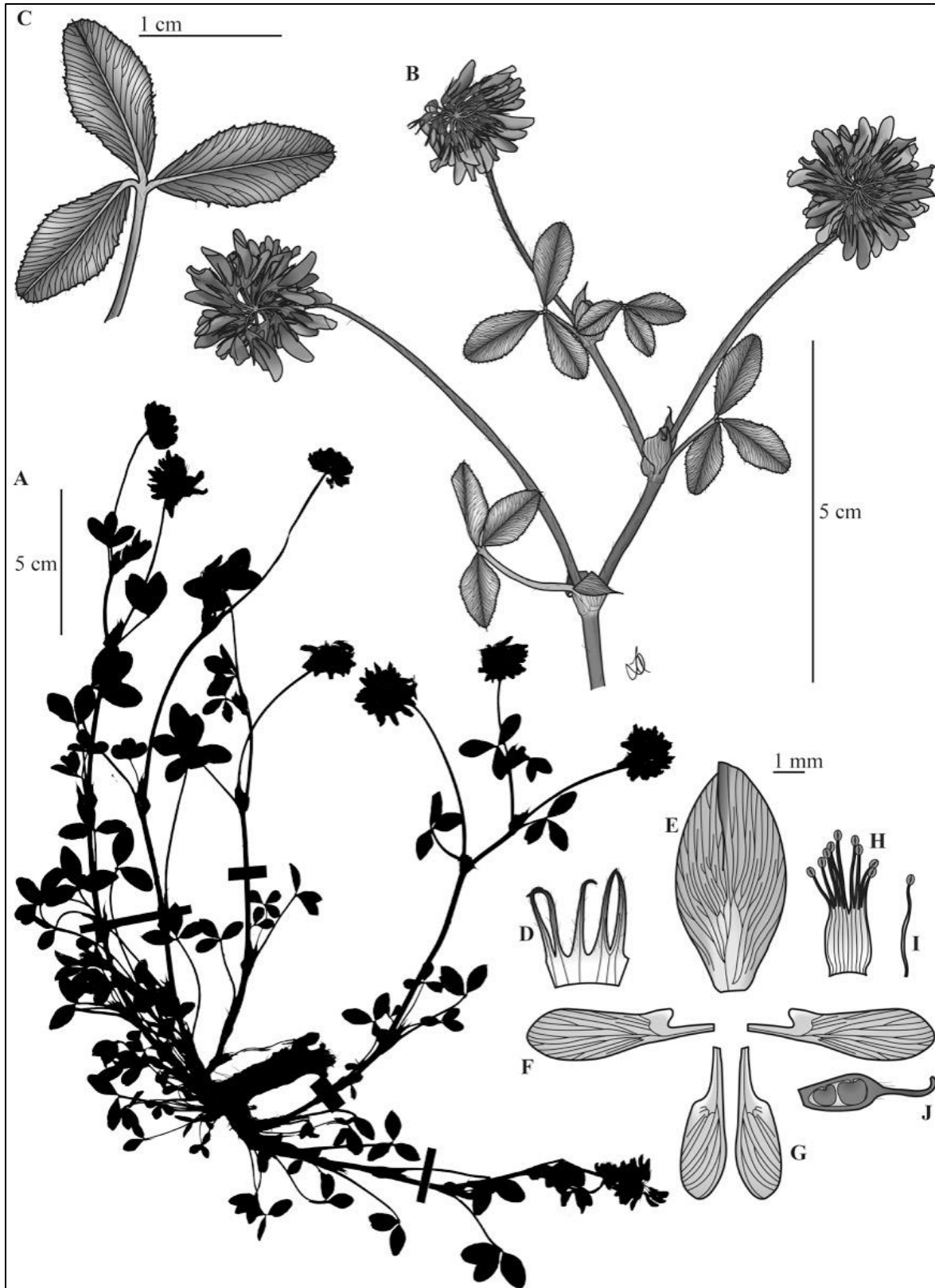


Figure 4. *Trifolium cognatum* (from Pringle 6933 (GH)). A. Habit silhouette. B. Fertile branch. C. Abaxial leaf surface. D. Sepals. E. Banner petal. F. Wing petals. G. Keel petals. H. Connate stamens. I. Free stamen. J. Ovary.

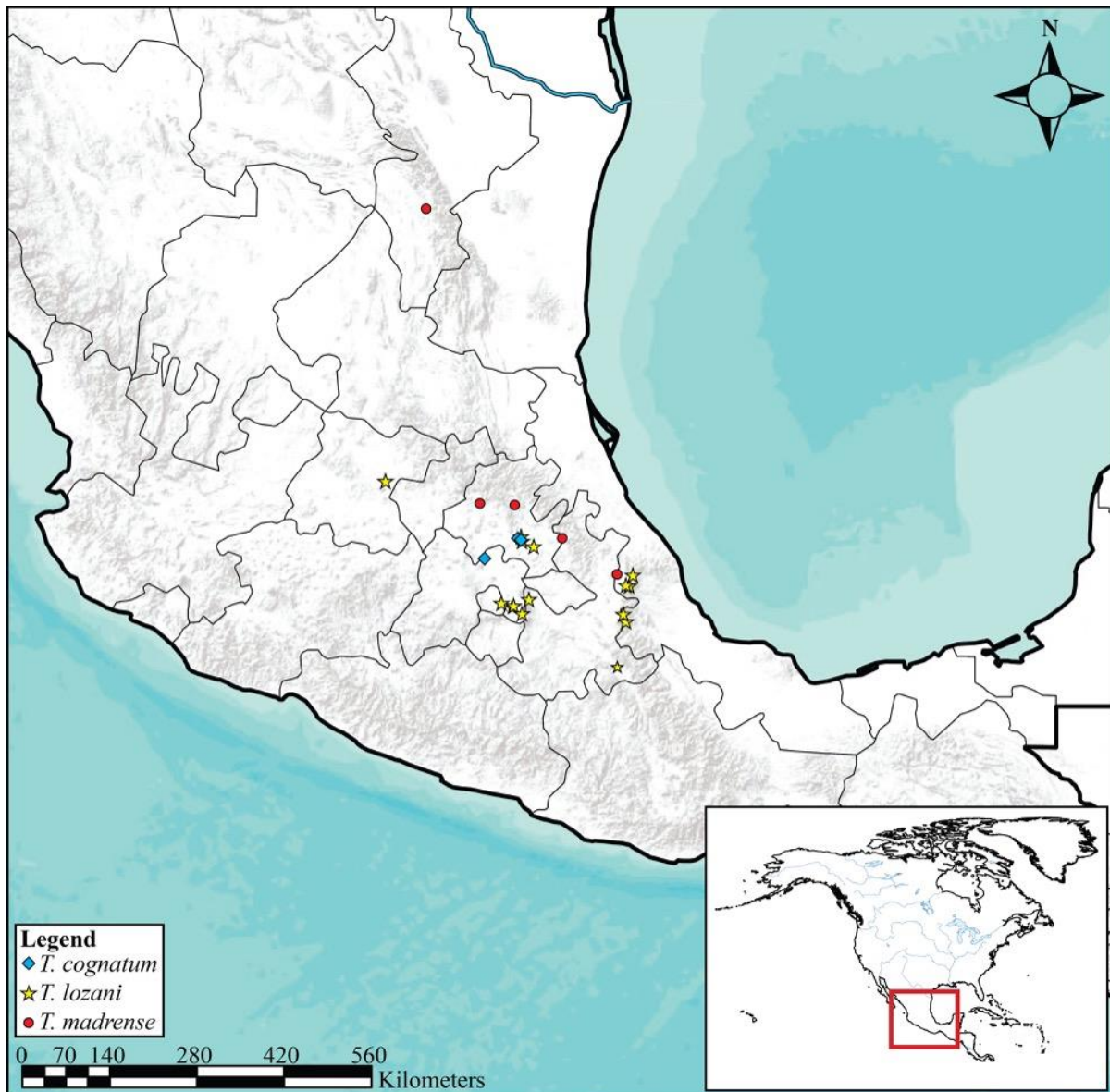


Figure 5. Distribution of *Trifolium cognatum*, *T. lozani*, and *T. madrense*.

$\times \leq 0.2$ mm, laminae spatulate, 1.9–3 mm long, 0.5–0.8 mm wide at widest point, ≤ 0.7 mm wide at narrowest point, bases shortly auriculate–truncate, if auriculate, auricles acute, margins entire, apices rounded. **Keel petals** 2.8–3.5 mm long, asymmetrically clawed, claws 1.1–1.6 $\times < 0.5$ mm, laminae elliptic–spatulate, 1.5–2 mm long, 0.7–1 mm wide at widest point, 0.6–0.8 mm wide at narrowest point, bases truncate, margins entire, apices rounded. **Stamens** diadelphous, connate filaments 2.4–3 \times 0.8–1.5 mm, fused for 44–60% of their length, free filaments 1.5–1.9 $\times < 0.2$ mm. **Pistils** sessile, ovaries 1.2–3.6 \times 0.4–1.3 mm, sparsely–densely pubescent distally and occasionally along suture, styles 0.3–1.3 $\times \leq 0.2$ mm, glabrous, ovules 2. **Legumes** laterally compressed, 4.2–5.8 \times 2.3–3 mm. **Seeds** 2, globular–mitten-shaped, brownish orange–dark brown, occasionally dark-speckled, 1.5–2 \times 1.1–1.6 mm.

Additional specimens examined. MEXICO. Aguascalientes. Shrub-covered, nearly treeless mountain sides ca 20 km E of Rincón de Romos, road to Asientos, between Cerro Altamira and Cerro

San Juan, limestone areas with *Ceanothus*, *Ephedra*, *Mimosa*, areas of rhyolitic rock with *Opuntia*, *Mimosa*, *Eupatorium*, *Eriogonum*, in flat land 3 km E of Asientos, seepage area near a small watercourse, 22.2303°, -102.0568°, 2100 m, 4 Sep 1967, *McVaugh 23781* (MICH). **Chihuahua**. Plains near Guerrero, 28.56°, -107.49°, 2000 m, 8 Sep 1887, *Pringle 1208* (GH, MICH, NY, RSA, US, VT). **Cd. de México**. Valley of Mexico, 19.25°, -98.99°, 2316 m, 28 Sep 1909, *Pringle 15645* (GH, MICH, US, VT). **Durango**. City of Durango, 24.02°, -104.6°, 1900 m, 1 Aug 1898, *Nelson 4586* (GH, US). **Guanajuato**. San Luis de la Paz, Cerro El Quijay, camino hacia Pozos, matorral arbustivo, ladera de cerro, planta herbácea rastrera, flores blancas, escasa, 21.25°, -100.507°, 1900 m, 31 Jul 1991, *Ventura 9380 & López* (XAL). **Hidalgo**. Tula, 20.052259°, -99.344429°, 2048 m, Jul 1905, *Rose et al. 8307* (GH). **Jalisco**. Guadalajara, 20.67°, -103.34°, 1550 m, Jul 1886, *Palmer 236* (GH, MICH, NY, P). **México**. Lecheria, railroad station, 19.611°, -99.186°, 1917 m, 2 Jul 1904, *Pringle 13264* (ARIZ, ASU, GH, MICH, VT). **Michoacán**. Morelia, Loma del Zapota, 16.7°, -101.2°, 1950 m, Sep 1911, *Arsène 6011* (GH, US). **Morelos**. Cuernavaca, 18.934°, -99.232°, 1700 m, 13 Aug 1906, *Pringle 10282* (ARIZ, ASU, DAV, GH, MICH, RSA, VT, XAL). **Nayarit**. Sierra Madre, near Santa Teresa, Territorio de Tepic, 22.5°, -104.7°, 2100 m, 9 Aug 1897, *Rose s.n.* (US). **Oaxaca**. Miahuatlán, San Juan Mixtepec, edge, PMZ #103, 16.301667°, -96.3000278°, 1897 m, 26 Oct 1996, *Hunn OAX-416* (MO, XAL). **Puebla-Veracruz**. Carretera 140, ruderal, secundaria a los lados de la Carretera, 19.5°, -97.4°, 2250 m, 12 Jul 1971, *Nevling 1611 & Chiang* (GH). **Sonora**. 6.6 km W of Yécora on Mex. 16, pine-oak forest, 28.361667°, -108.985°, 1760 m, 5 Sep 1996, *Burquez M. 96-844 et al.* (NY). **Tlaxcala**. Jardín Botánico de Tizatlán, bosque secundario de *Alnus*, ruderal, arenoso, 19.3308°, -98.2168°, 2300 m, 18 Aug 1988, *Acosta P. & Rizo B. 2267* (XAL). **Zacatecas**. 9 mi NW of Sombrerete on eastern bajada of Sierra Papanton, oak-juniper grassland, clay loams from igneous or sedimentary rocks, 23.657°, -103.663°, 2400 m, 26 Sep 1948, *Gentry 8487* (ARIZ, MICH, POM).

Trifolium goniocarpum is distinguished by the narrow wing and keel petals in the flowers and the long and narrow leaflets. *Trifolium goniocarpum* is most similar to *T. laciae*; however, they differ in the aforementioned characters in addition to sepal circumference and lobe width. *Trifolium goniocarpum* also has relatively small inflorescences, which sets it apart from most other members of this species complex. Comparative T-tests ($\alpha=.05$) of these characters supported these differences (Ahlquist, 2012).

Trifolium goniocarpum is fairly widespread throughout Mexico, growing in the states of Aguascalientes, Chihuahua, México, Durango, Guanajuato, Hidalgo, Jalisco, México, Michoacán, Morelos, Nayarit, Oaxaca, Puebla, San Luis Potosí, Sonora, Tlaxcala, Veracruz, and Zacatecas (Figure 7). It has been collected in a variety of habitats including secondary pine-oak forests, along roadsides and other disturbed areas, hillsides, along streams or damp depressions in grasslands, and riparian areas. In these locations, the soil varies from very sandy and rocky to moist clay loams. *Trifolium goniocarpum* occurs across a broad range in elevation from 1100 m to 4500 m.

4. TRIFOLIUM HICKEYI T.K. Ahlquist & Vincent, *Phytoneuron* 2020-19: 1. 2020. **TYPE: MEXICO.** **Michoacán.** Morelia, Rincón prise d'eau, 16.698°, -101.187°, 1950 m, 14 Apr 1910, *G. Arsène 5483* (holotype: ILL!; isotypes: BM!, GH!, NY!).

This species was described and discussed in Ahlquist & Vincent (2020).

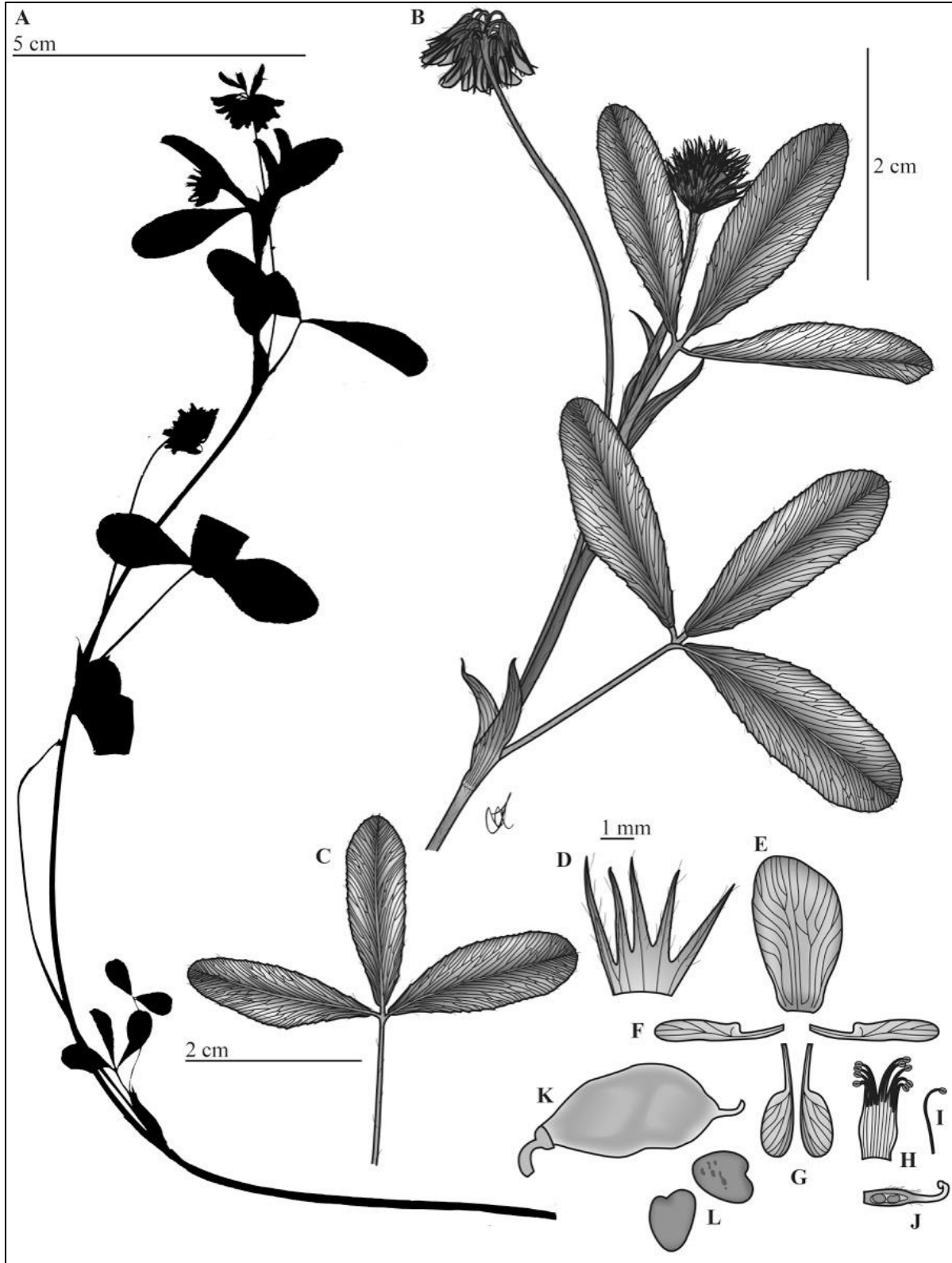


Figure 6. *Trifolium goniocarpum* (from Palmer & Parry 134 (NY)). A. Habit silhouette. B. Fertile branch. C. Abaxial leaf surface. D. Sepals. E. Banner petal. F. Wing petals. G. Keel petals. H. Connate stamens. I. Free stamen. J. Ovary. K. Legume. L. Seeds.

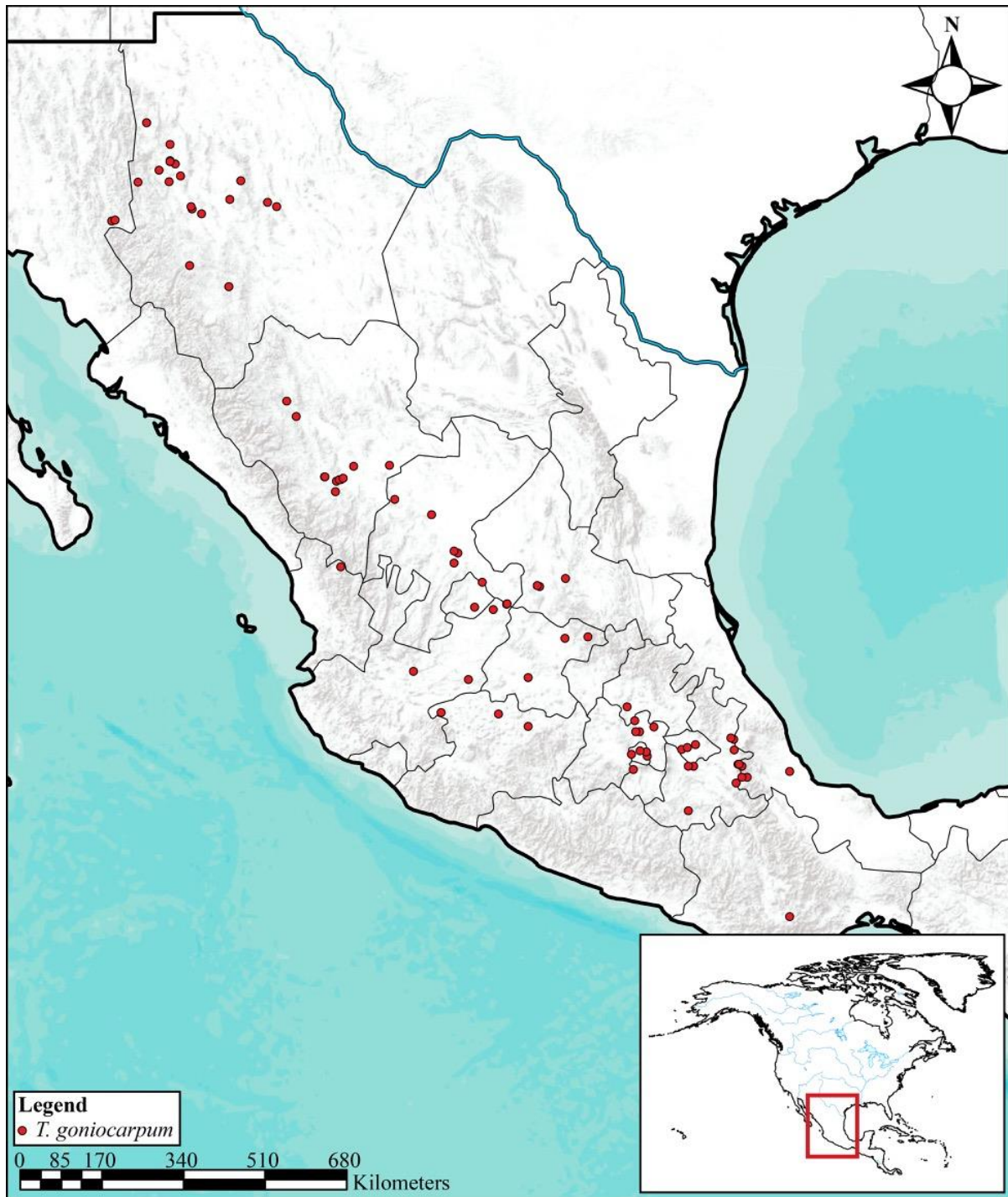


Figure 7. Distribution of *Trifolium goniocarpum*.

5. TRIFOLIUM LACIAE T.K. Ahlquist & Vincent, **sp. nov.** **TYPE: MEXICO. Chihuahua.** Mesa W of Hop Valley, Sierra Madre Mountains, 7000 ft (2133 m), 17 Sep 1903, *M.E. Jones 7501A* (holotype: POM27959!; isotypes: MICH!, POM! 27942 & 27943, US!). Figure 8.

Trifolium laciae has several characters that distinguish it from other members of this species complex, most of which are floral and reproductive characters. While other species may have a sterile projection of the inflorescence, it is a prominent character in *T. laciae*. The serrate sepals, yellow petals, and three ovules per ovary and three seeds per fruit, are not shared with any other members of this species complex. Other observations worth noting not found in any other species in this complex are that the leaves seem to be thicker and have a leathery texture, and the remnant sepals become curled in fruit.

Plants perennial. **Roots** large, woody, 4.3–7.9 mm in diameter. **Stems** 15–30+ cm in length, 1.1–1.9 mm in diameter, internodes sparsely–moderately pubescent, nodes moderately–densely pubescent. **Leaves** trifoliate, \pm uniform in size throughout. **Stipules** narrowly triangular–triangular, 10–16.5 \times 2.7–5 mm, glabrous adaxially and abaxially, margins entire–weakly serrate, sparsely–moderately pubescent, apices acute. **Petioles** 6–19 \times <1 mm, moderately–densely pubescent. **Petiolules** $\leq 1 \times \leq 0.5$ mm, sparsely–densely pubescent. **Leaflets** obovate–rhombic, glabrous adaxially, sparsely–moderately pubescent along midribs abaxially, sparsely–moderately pubescent abaxially on outer halves of lateral leaflets, bases acute, margins weakly serrate–serrate, often with short, straight teeth alternating with larger, antrorsely curved teeth, sparsely–moderately pubescent, apices rounded–acute, weakly mucronulate, terminal leaflets 14–21.5 \times 7.5–9 mm. **Peduncles** 13–50 \times <1 mm, moderately–densely pubescent. **Inflorescences** axillary, racemose–umbellate, 6.5–15.5 \times 11.5–18 mm. **Flowers** 10–20, each subtended by a linear triangular bract, in 1–3 whorls, terminal sterile projection present. **Pedicels** 1.6–4.5 \times <0.5 mm, sparsely–moderately pubescent. **Calyx** 3.3–5.7 mm long, tubes 2.5–3.5 mm in circumference, sparsely–densely pubescent, lobes narrowly triangular, 2.6–4.6 \times ≤ 0.6 mm, sparsely–densely pubescent abaxially, margins usually serrate, sparsely–densely pubescent, apices acuminate. **Petals** yellow. **Banner petals** obovate, 3.6–5.7 \times 2.4–4.2 mm. **Wing petals** 3.4–5 mm long, asymmetrically clawed, claws 0.9–1.2 \times ≤ 0.3 mm, laminae spatulate, 2.6–3.7 mm long, 0.8–1.2 mm wide at widest point, <1 mm wide at narrowest point, bases shortly auriculate, auricles acute, margins entire, apices rounded. **Keel petals** 2.8–3.8 mm long, asymmetrically clawed, claws 0.8–1.4 \times <0.5 mm, laminae spatulate, 2–2.5 mm long, 0.9–1.3 mm wide at widest point, 0.6–1 mm wide at narrowest point, bases truncate, margins entire, apices rounded–obtuse. **Stamens** diadelphous, connate filaments 2.2–3.4 \times 1–1.6 mm, fused for 47–55% of their length, free filaments 1.2–2.3 \times ≤ 0.1 mm. **Pistils** sessile, ovaries 1.4–2.7 \times 0.5–1.3 mm, sparsely–densely pubescent distally and occasionally along suture, styles 0.6–1.1 \times ≤ 0.2 mm, glabrous, ovules 3. **Legumes** laterally compressed, 5–5.3 \times 2.6–2.9 mm. **Seeds** 3, globular–mitten-shaped, brownish orange–dark brown, occasionally dark-speckled, 1.4–1.8 \times 1.2–1.5 mm.

Additional collections examined. MEXICO. Chihuahua. Near Colonia García in the Sierra Madres, 29.97°, -108.34°, 2286 m, 27 Jul 1899, *Townsend 177* (GH, ILL, NY, P, POM, US, VT); just NE of La Zaragosa, about 19.4 mi SW of San Buenaventura, cornfields at base of hill with Mexican pine-oak woodland, flat area near cornfield, under *Pinus engelmannii*, 29.620°, -107.899°, 2500 m, 22 Oct 1984, *Van Devender 84-523 et al.* (ARIZ); Madera, Laguna de Babícora, Arroyo El Jara, bosque de pino-encino, 29.3°, -107.8°, 2300 m, 10 Sep 1994, *Quintana 3602* (NY). **Durango.** Otinapa, 24.05°, -105°, 2367 m, Jul 1906, *Palmer 417* (GH, NY).

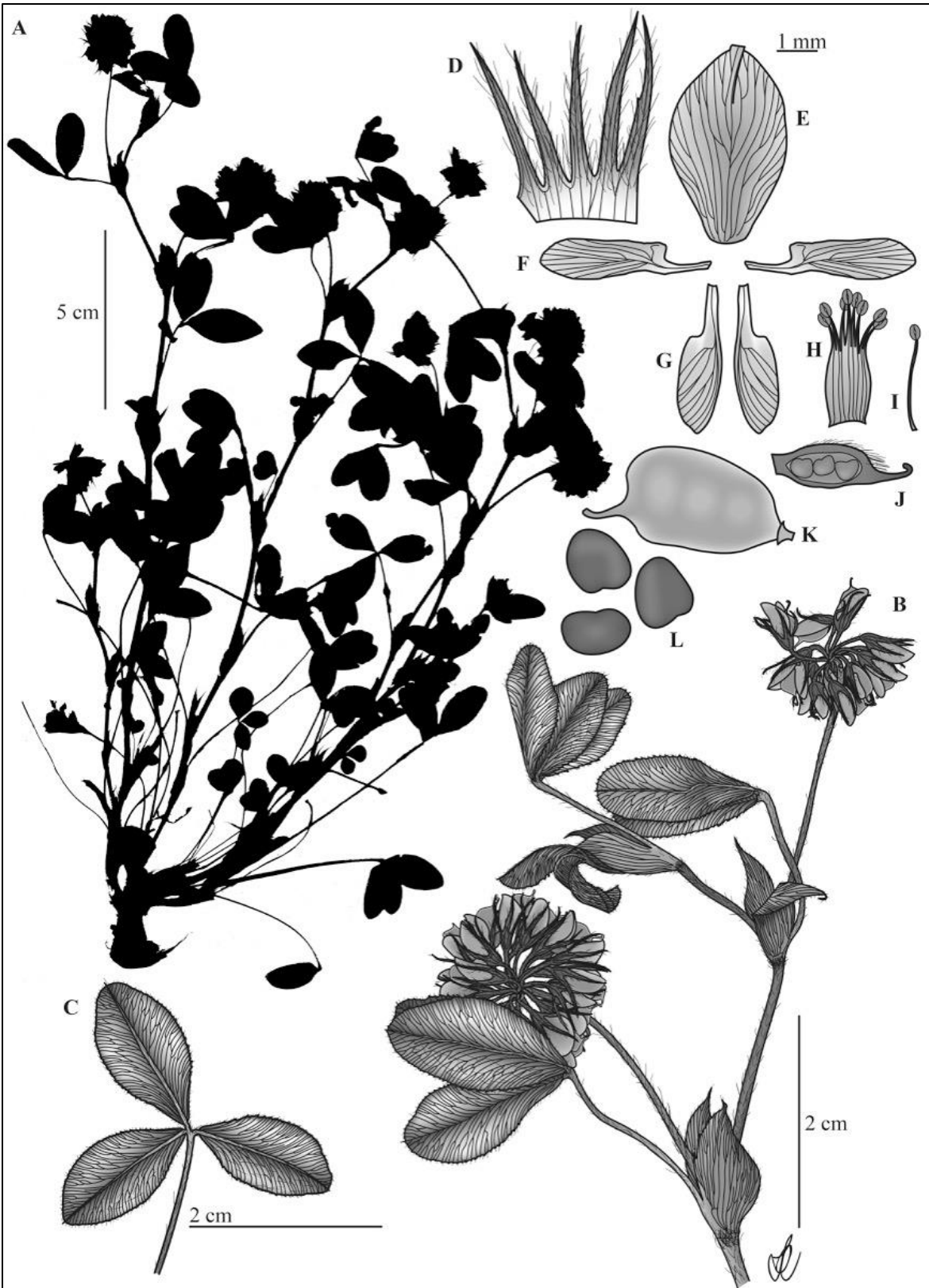


Figure 8. *Trifolium laciae* (from the holotype). A. Habit silhouette. B. Fertile branch. C. Abaxial leaf surface. D. Sepals. E. Banner petal. F. Wing petals. G. Keel petals. H. Connate stamens. I. Free stamen. J. Ovary. K. Legume. L. Seeds.

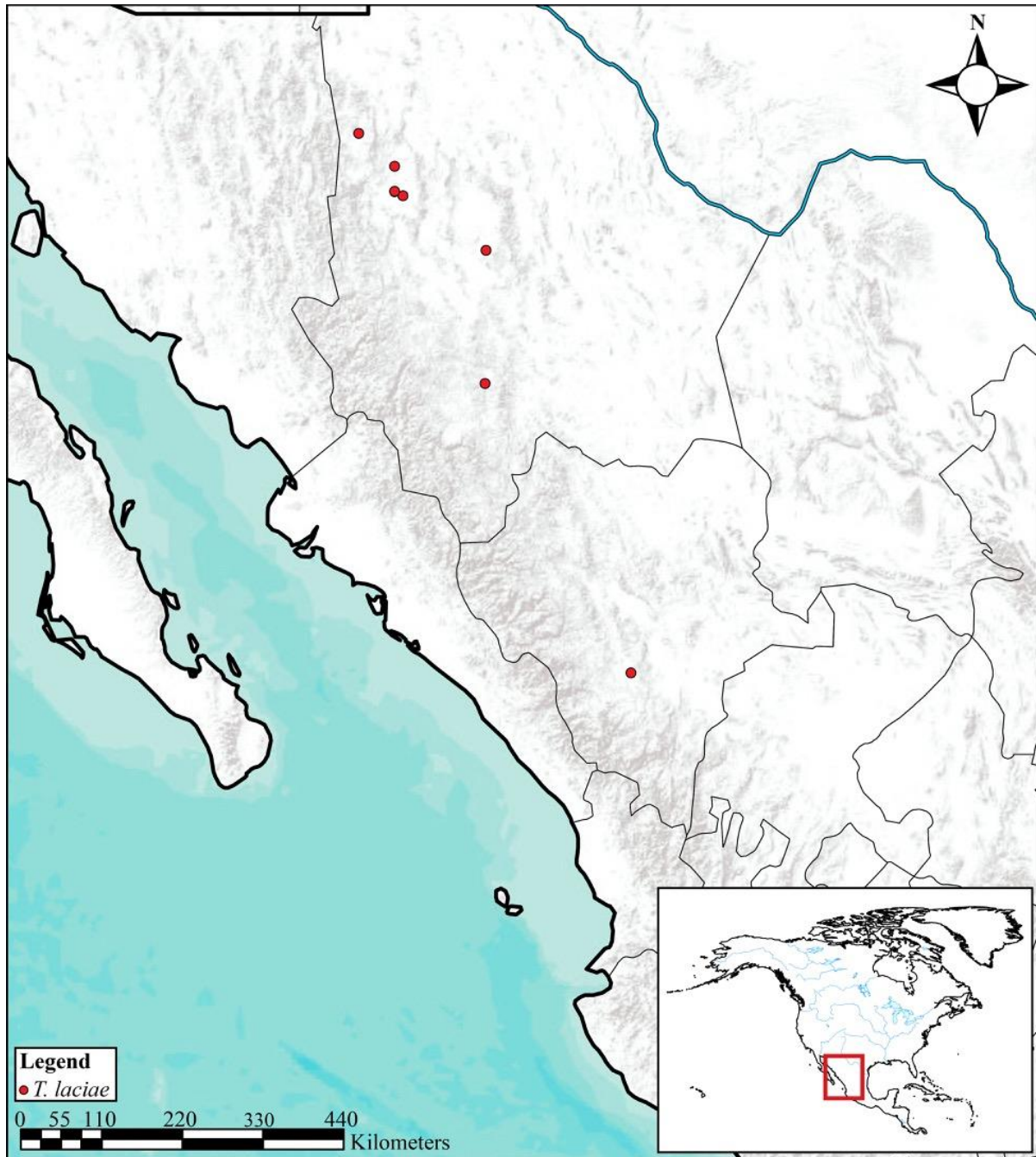


Figure 9. Distribution of *Trifolium laciae*.

Trifolium laciae is found in the Sierra Madre Occidental mountain range, primarily in the Mexican states of Chihuahua and Durango (Figure 9). It has been collected in pine-oak woodlands and occasionally near lakes between 1750 m and 2400 m in elevation. *Trifolium laciae* is named in honor of Laci Ahlquist, sister of the first author, for her ongoing love and support during this research.

6. TRIFOLIUM LOZANI House, Bot. Gaz. 41: 342. 1906. **TYPE: MEXICO. México.** Eslava, Valley of Mexico, 7600 ft (2316 m), 15 Jun 1901, C.G. Pringle 9512 (holotype: US; isotypes: GH!, VT!-Figure 10).

Plants perennial. **Roots** large, woody, 1.2–5.1 mm in diameter. **Stems** prostrate, 6–26+ cm in length, 0.4–1.3 mm in diameter, internodes and nodes moderately–densely pubescent. **Leaves** trifoliate, \pm uniform in size throughout. **Stipules** elliptic, 5–7.4 \times 1.5–3.5 mm, glabrous adaxially, moderately pubescent abaxially, margins entire–weakly serrate, sparsely–moderately pubescent, apices acute. **Petioles** 7–14 \times \leq 0.5 mm, moderately–densely pubescent. **Petioliules** \leq 0.6 \times \leq 0.5 mm, moderately–densely pubescent. **Leaflets** obovate–elliptic, glabrous adaxially, sparsely–moderately pubescent along midribs abaxially, sparsely–moderately pubescent abaxially on outer halves of lateral leaflets, bases acute, margins weakly serrate–serrate, often with short, straight teeth alternating with larger, antrorsely curved teeth, sparsely–moderately pubescent, apices rounded, weakly mucronulate, terminal leaflets 6.1–11.2 \times 4–7.9 mm. **Peduncles** 15–31 \times $<$ 1 mm, moderately–densely pubescent. **Inflorescences** axillary, racemose–umbellate, 4.5–12 \times 7–13 mm. **Flowers** 13–28, each subtended by a linear triangular bract, in 1–3 whorls, terminal sterile projection sometimes present. **Pedicels** 0.8–2.4 \times \leq 0.2 mm, sparsely–densely pubescent. **Calyx** 2.2–3.3 mm long, tubes 1.9–2.6 mm in circumference, sparsely–densely pubescent, lobes narrowly triangular, 1.7–2.4 \times \leq 0.5 mm, moderately–densely pubescent abaxially, margins entire, moderately–densely pubescent, apices attenuate. **Petals** light pink–pink. **Banner petals** obovate, 2.5–4.2 \times 2.1–3.2 mm. **Wing petals** 2.3–3.8 mm long, asymmetrically clawed, claws 0.5–1.1 \times \leq 0.2 mm, laminae spatulate, 1.8–2.7 mm long, 0.8–1.2 mm wide at widest point, \leq 0.6 mm wide at narrowest point, bases shortly auriculate–truncate, auricles acute, margins entire, apices rounded. **Keel petals** 2.2–3.1 mm long, asymmetrically clawed, claws 0.8–1.3 \times \leq 0.3 mm, laminae spatulate, 1.4–2 mm long, 0.8–1.1 mm wide at widest point, 0.7–0.8 mm wide at narrowest point, bases truncate, margins entire, apices rounded. **Stamens** diadelphous, connate filaments 1.6–2.7 \times 0.8–1.2 mm, fused for 37–55% of their length, free filaments 0.7–1.9 \times $<$ 0.2 mm. **Pistils** sessile, ovaries 0.7–1.3 \times 0.3–0.6 mm, sparsely–densely pubescent distally, styles 0.8–1.2 \times $<$ 0.2 mm, glabrous, ovules 2. **Legumes** laterally compressed, 3.4 \times 2.1–2.8 mm. **Seeds** 2, mitten-shaped, brown, 1.3 \times 1.1 mm.

Additional collections examined. MEXICO. Guanajuato. 5 mi E of San Miguel Allende, 20.916565°, -100.667001°, 2032 m, 18 Aug 1947, *Kenoyer 2425* (A). **Hidalgo.** Pachuca, open meadow and rocks in fir forest above Pueblo Nuevo and below Parque Nacional El Chico on road from Real del Monte to El Chico, 20.163°, -98.710°, 3000 m, 6 Jul 1948, *Moore Jr. 3693* (A). **México.** Pine woods, open field, Llano Grande, 19.219°, -98.817°, 2800 m, 9 Jul 1950, *Matuda 19222* (NY). **Puebla.** Boca del Monte, moist meadows, 18.4°, -97.3°, 1200 m, Aug 1908, *Purpus 3053* (GH). **Veracruz.** Mpio. de Perote, NW slopes of Cofre de Perote, 6.5 km (by road) SE of town of Perote, along road to television towers on summit of Cofre de Perote, bosque de pinos, forest of *Pinus teocote*, *P. pseudostrabus*, *Quercus crassifolia*, *Quercus spp.*, steep slopes and now dry canyon, 19.4871°, -97.1503°, 2700 m, 8 Jul 1980, *Hansen 7682* (POM, NY); Las Vigas de Ramírez, San Juan del Monte, Predio San Juan del Monte, bosque de pino, pradera inundable, 19.633333°, -97.1°, 2450 m, 5 Aug 1985, *Marín 89* (XAL).

Trifolium lozani is found in the Sierra Madre Oriental mountain range and the Valley of Mexico, in the Mexican states of Ciudad de México, Guanajuato, Hidalgo, México, Puebla, San Luis Potosí, and Veracruz (Figure 5). It has been collected in open meadows and pine forests between 1200 m and 3250 m in elevation.

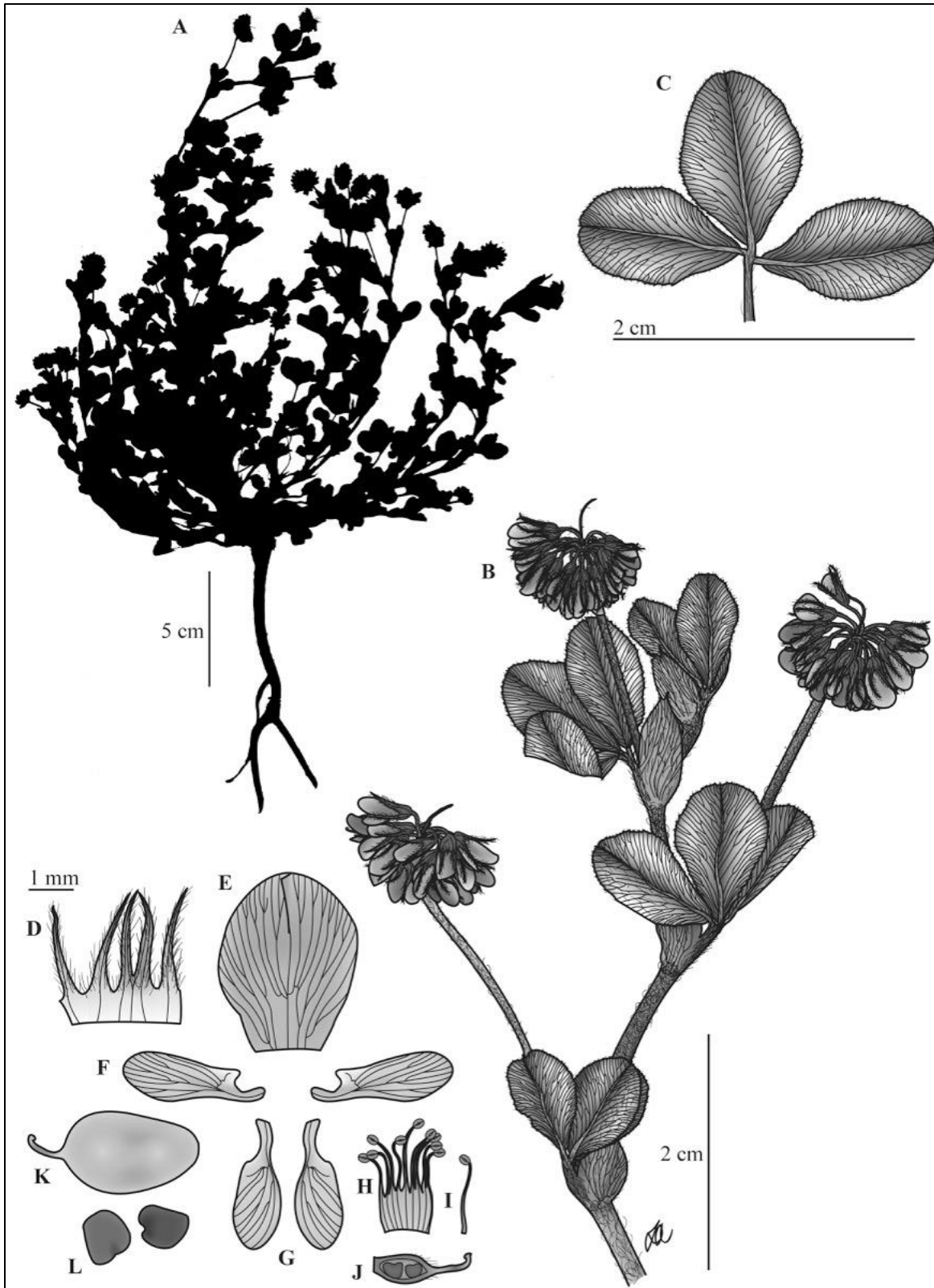


Figure 10. *Trifolium lozani* (from C.G. Pringle 9512 (VT)). A. Habit silhouette. B. Fertile branch. C. Abaxial leaf surface. D. Sepals. E. Banner petal. F. Wing petal. G. Keel petals. H. Connate stamens. I. Free stamen. J. Ovary. K. Legume. L. Seeds.

7. TRIFOLIUM MADRENSE T.K. Ahlquist & Vincent, **sp. nov.** TYPE: MEXICO. Nuevo León.

Pablillo on Hwy 68, on grazed grassy slopes of road grade, abundant and short because of dryness and grazing, plants a little larger in damp soil patches, 20 Aug 1975, *J.M. Gillett 17092* (holotype: NY!). Figure 11.

Trifolium madrense one of the most densely pubescent members of this complex, the other being *T. lozani*. *Trifolium madrense* differs from *T. lozani* most notably by its inflated fruits, which are laterally compressed in *T. lozani*, and its less dense inflorescences with 5–13 flowers, while those of *T. lozani* are denser with 13–28 flowers.

Plants perennial. **Roots** woody, 3–6.7 mm in diameter. **Stems** prostrate or creeping, 5.5–36+ cm in length, 0.4–1.2 mm in diameter, internodes moderately–densely pubescent, nodes moderately–densely pubescent. **Leaves** trifoliate, ± uniform in size throughout. **Stipules** narrowly triangular–triangular, 4.3–8.8 × 1.5–3.2 mm, glabrous–sparsely pubescent adaxially, sparsely–moderately pubescent abaxially, margins entire–weakly serrate, moderately–densely pubescent, apices acute. **Petioles** 3.5–21 × 0.2–0.5 mm, sparsely–moderately pubescent. **Petiolules** <1 × ≤0.3 mm, sparsely–densely pubescent. **Leaflets** obcordate–rhombic, glabrous adaxially, moderately–densely pubescent along midribs abaxially, moderately–densely pubescent abaxially on distal portion of terminal leaflet and outer halves of lateral leaflets, bases acute, margins weakly serrate–serrate, often with short, straight teeth alternating with larger, antrorsely curved teeth, sparsely–moderately pubescent, apices emarginate–acute, weakly mucronulate, terminal leaflets 4.2–12.5 × 2.8–7.7 mm. **Peduncles** 13–40 × ≤0.3 mm, moderately–densely pubescent. **Inflorescences** axillary, racemose–umbellate, 5.2–12 × 8.4–11.5 mm. **Flowers** 5–13, each subtended by a linear triangular bract, in 1–2 whorls; pedicels 1–2 × ≤0.1 mm, sparsely–moderately pubescent. **Calyx** 2.5–3.4 mm long, tubes 2.1–2.7 mm in circumference, sparsely–densely pubescent, lobes narrowly triangular, 1.5–2.5 × ≤0.6 mm, sparsely–densely pubescent abaxially, margins entire, moderately–densely pubescent, apices attenuate. **Petals** white–pinkish. **Banner petals** elliptic–obovate, 3.3–4.9 × 2.1–3 mm. **Wing petals** 2.9–4 mm long, asymmetrically clawed, claws 0.8–1.2 × ≤0.2 mm, laminae spatulate, 2–2.8 mm long, 0.8–1.1 mm wide at widest point, ≤0.8 mm wide at narrowest point, bases shortly auriculate, auricles acute, margins entire, apices rounded. **Keel petals** 2.3–3.4 mm long, asymmetrically clawed, claws 1–1.4 × <0.5 mm, laminae spatulate–elliptic, 1.3–2.2 mm long, 0.9–1.2 mm wide at widest point, ≤1 mm wide at narrowest point, bases truncate, margins entire, apices rounded. **Stamens** diadelphous, connate filaments 2.2–2.7 × 0.9–1.4 mm, fused for 40–55% of their length, free filaments 0.9–1.6 × ≤0.1 mm. **Pistils** sessile, ovaries 1–1.5 × <1 mm, densely pubescent distally, styles ≤1 × <0.2 mm, glabrous, ovules 2. **Legumes** inflated, 2.3–3.8 × 1.8–2.4 mm. **Seeds** 2, globular–mitten-shaped, brownish yellow–light brown, occasionally dark-speckled, 1.2–2.1 × 0.9–1.5 mm.

Additional specimens examined. MEXICO. **Hidalgo.** Las Emes, 20 km al N de Ixmiquilpan, hacia la Pechuga, 20.667°, -99.216°, 2200 m, 18 Nov 1981, *Hernández M. 6666* (MEXU, XAL); Mpio. de Zacualtipan, Loc. Zoquizoquipan, 5 km al N de Zacualtipan, bosque perturbado de encinos y pinos principalmente, 20.6484°, -98.7101°, 2200 m, 21 Dec 1981, *Hernández M. 6927* (MEXU). **Puebla.** In grass beside railroad, 1 km NW of Huachinango, Puebla, 20.191916°, -98.028480°, 1450 m, 18 Mar 1949, *Clausen 7465* (MEXU). **Veracruz.** Pastizal arriba de Santiago, Veracruz, suelo volcanico, 19.7°, -97.24°, 1980 m, 20 Jul 1971, *Nevling 1859* (GH).

Trifolium madrense is found in the Sierra Madre Oriental mountains of eastern Mexico from the states of Hidalgo, Nuevo León, Puebla, and Veracruz (Figure 5). It has been collected in grassy pastures, disturbed pine and oak forests, and along roadsides and railroads between 1450 m to 2200 m in elevation. *Trifolium madrense* has been named after the Sierra Madre Oriental mountain range of eastern Mexico where it grows.

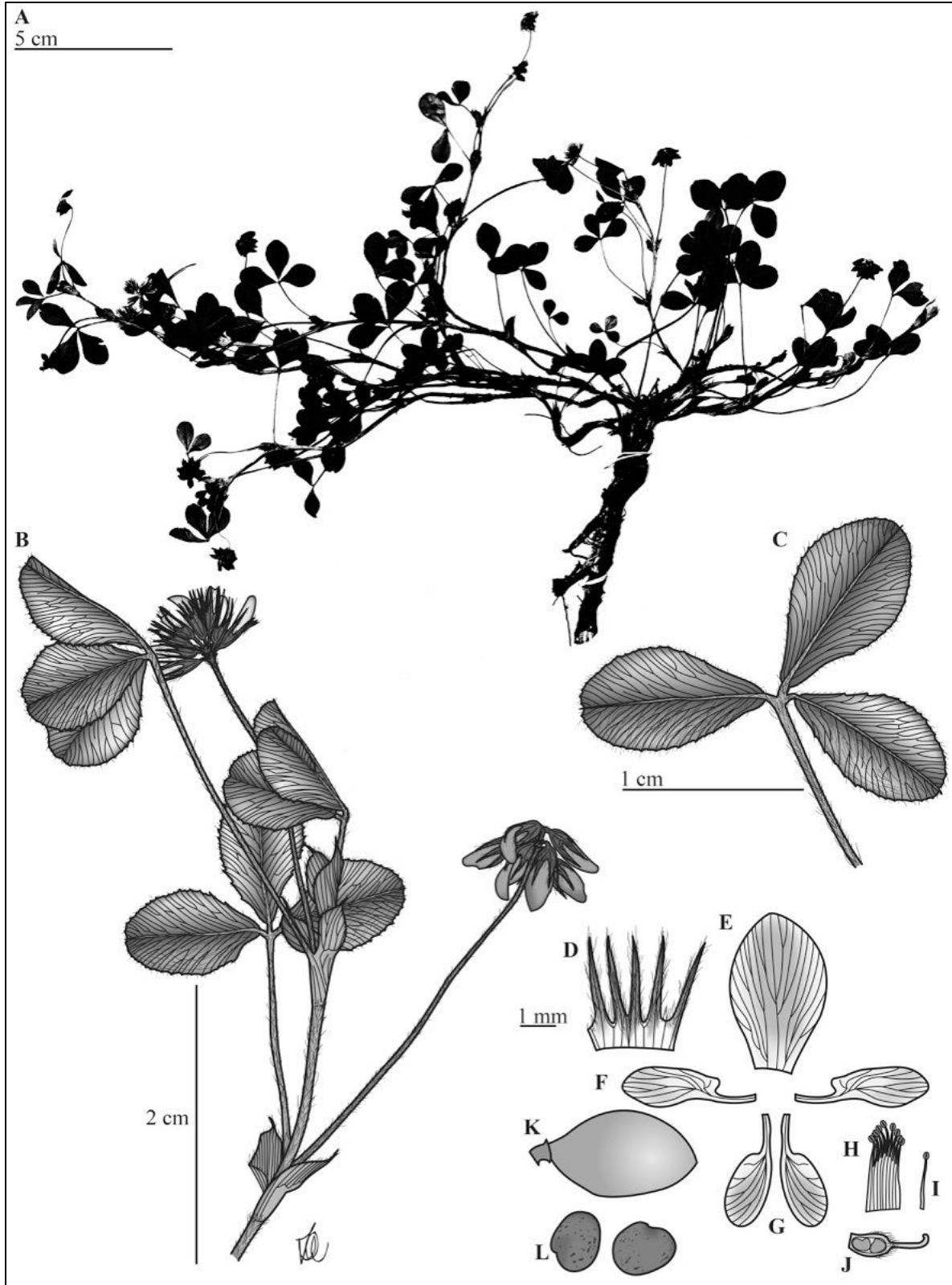


Figure 11. *Trifolium madrense* (from the holotype). A. Habit silhouette. B. Fertile branch. C. Abaxial leaf surface. D. Sepals. E. Banner petal. F. Wing petals. G. Keel petals. H. Connate stamens. I. Free stamen. J. Ovary. K. Legume. L. Seeds.

8. **TRIFOLIUM RHOMBEUM** S. Schauer, *Linnaea* 20: 740. 1847. **TYPE: MEXICO.** *A. Aschenborn 164* (not located). **NEOTYPE** (designated here): **MEXICO.** *A. Aschenborn 106* (W).

Searches for *Aschenborn 164* were unsuccessful. The specimen at W, *Aschenborn 106*, may indeed be the original material, as indicated by annotations on the specimen, if the original number was lost in remounting. However, it seems best to take the label at face value and designate the specimen as neotype until such time as a specimen labeled "164" can be located. It may be that a specimen labeled as such could be found in FR, JE, KIEL, or P. Specimens at JE and P labeled *Aschenborn 106* are types of *Ageratina pichinchensis* (Kunth) King & Rob.

Trifolium mexicanum Hemsl., *Biol. Centr. Am. Bot.* 1: 233. 1879. *Trifolium amabile* Kunth var. *mexicanum* (Hemsl.) Heller & Zoh., *Gen. Trifolium* 128. 1984. **TYPE: MEXICO. San Luis de Potosí.** Chiefly in the region of San Luis Potosí. 22° N, 6000–8000 ft (1830–2440 m), 1878, *E. Palmer & C.C. Parry 137* (holotype: K; isotypes: GH! [Figure 12], NY!, P!).

Trifolium potosanum Lojac., *Nuov. Giorn. Bot. Ital.* 15: 144. 1883. **TYPE: MEXICO. San Luis de Potosí.** Chiefly in the region of San Luis Potosí. 22° N, 6000–8000 ft (1830–2440 m), 1878, *E. Palmer & C.C. Parry 137* (holotype: K; isotypes: GH!, NY!, P!).

Trifolium nelsonii House, *Bot. Gaz.* 41: 344. 1906. **TYPE: MEXICO. Oaxaca.** Vicinity of La Parada, 19 Aug 1894. *E.W. Nelson 1016* (holotype: US).

Plants perennial. **Roots** large, woody, 3.5–9.2 mm in diameter. **Stems** prostrate–ascending, 14–70+ cm in length, 1–2.5 mm in diameter, internodes sparsely–moderately pubescent, nodes moderately–densely pubescent. **Leaves** trifoliolate, ± uniform in size throughout. **Stipules** narrowly triangular–triangular, 8.4–17 × 2.5–8 mm, glabrous adaxially, glabrous–basally moderately pubescent abaxially, margins entire–serrate, sparsely–moderately pubescent, apices acute. **Petioles** 5–60.5 × ≤1 mm, sparsely–densely pubescent. **Petiolules** 0.6–1.2 × <1 mm, moderately–densely pubescent. **Leaflets** rhombic–obovate, glabrous adaxially, sparsely–moderately pubescent along midribs abaxially, sparsely pubescent abaxially on outer halves of lateral leaflets, bases acute, margins serrate, often with short, straight teeth alternating with larger, antrorsely curved teeth, sparsely pubescent, apices rounded–acute, weakly mucronulate, terminal leaflets 9.7–26.5 × 5.9–18 mm. **Peduncles** 28–78.5 × ≤1 mm, moderately–densely pubescent. **Inflorescences** axillary, racemose–umbellate, 10–24 × 15–24 mm. **Flowers** 20–50, each subtended by a linear triangular bract, in 2–5 whorls, terminal sterile projection sometimes present. **Pedicels** 1–4.2 × <0.5 mm, sparsely–moderately pubescent. **Calyx** 3.4–6.1 mm long, tubes 2.3–4 mm in circumference, sparsely–moderately pubescent, lobes narrowly triangular–linear triangular, 2.3–4.8 × ≤0.7 mm, sparsely–moderately pubescent abaxially, margins entire, sparsely–moderately pubescent, apices attenuate. **Petals** white–cream. **Banner petals** obovate, 5.7–8.5 × 3.5–5.5 mm. **Wing petals** 5.1–7.3 mm long, asymmetrically clawed, claws 1.4–2.2 × <0.5 mm, laminae spatulate, 3.7–5.2 mm long, 1.3–2.3 mm wide at widest point, ≤1.2 mm wide at narrowest point, bases shortly auriculate, auricles acute, margins entire, apices rounded. **Keel petals** 4.4–5.9 mm long, asymmetrically clawed, claws 1.6–2.4 × <0.5 mm, laminae spatulate, 2.8–3.9 mm long, 1.2–1.7 mm wide at widest point, ≤1.3 mm wide at narrowest point, bases truncate, margins entire, apices rounded. **Stamens** diadelphous, connate filaments 3.4–5.1 × 0.4–1.6 mm, fused for 45–61% of their length, free filaments 2.1–3.5 × ≤0.2 mm. **Pistils** sessile, ovaries 1.5–4.5 × 0.5–2.1 mm, sparsely–densely pubescent distally and occasionally along suture, styles 1.4–2.5 × ≤0.2 mm, glabrous, ovules 2. **Legumes** laterally compressed, 3.5–3.6 × 2.1–2.2 mm. **Seeds** 2, globular–mitten-shaped, brownish orange–dark brown, occasionally dark-speckled, 1.2–1.5 × 1–1.2 mm.

Additional collections examined. **MEXICO. Durango.** Súchil, San Juan de Michis, rumbo al Cerro "Chihuahuailla", flores de color crema, aprox. 35 cm de altura, 23.626°, -103.929°, 2000 m, 14 Aug 1984, *Chávez 54* (TEX); upper slopes of Mimbres Canyon, 26 mi W of Durango, Rte 40, plants repent, flowers white, 24.337°, -104.306°, 2020 m, 24 Jul 1958, *Correll 20122* (ASU, NY); Súchil, al S de San Juan de Michis, bosque de pino-encino, común, 23.43°, -104.12°, 2370 m, 6 Aug 1981,

González 1810 (XAL); Mpio. de El Salto, 9 km al E de Llano Grande, planta herbácea de 10–20 cm de alto, abundante, flores blancas, veg: bosque de pino-encino principalmente, suelo pardo o casi blanco, 23.77°, -105.347°, 2200 m, 12 Jul 1982, *Hernández M. 7877 et al.* (RSA). **Guanajuato.** Cañada de La Virgen, bosque de encino colorado, ladera de cerro, planta de 60 cm de alto., flor roja, abundante, 21.15°, -101.183°, 2500 m, 13 Jun 1994, *Cano Mares s.n. & Cano Mares* (XAL). **Guerrero.** Omiltemi, tipo de vegetación es encinar-pinar, flor blanca, hierba perenne postrada, 17.554373°, -99.516250°, 2020 m, 3 Aug 1967, *Sousa 3144* (GH). **Hidalgo.** Hills, Cuylamaloya station, 20.033°, -98.533°, 2575 m, 9 Aug 1942, *Pringle 10274* (ARIZ, ASU, DAV, GH, MICH, MU, RSA, VT). **Jalisco.** Bolaños, Las Banderitas, crucero Tuxpan de Bolaños-Las Banderitas, flora de la Sierra de Bolaños, bosque de *Quercus* con *Alnus*, hierba 0.3 m, semiprostrada, flores blancas, 21.922222°, -103.867778°, 2450 m, 4 Jul 1996, *Calónico-Soto 2488* (MO); pine forest 1-2 mi E of Tapalpa, rocky soil on broken hills, abundant, prostrate, flowers white, 19.9455°, -103.7376°, 2100 m, 1 Nov 1960, *McVaugh 20588* (MICH). **México.** Amecameca, 19.11°, -98.75°, 2773 m, 29 Jul 1924, *Fisher 243* (US); calcareous bluffs, Flor de Maria, large heads, 19.34°, -99.20°, 2317 m, 4 Sep 1890, *Pringle 3238* (GH, MICH, MU, NY, P, RSA, VT); partially grazed, moist *Quercus* forest with epiphytic ferns, steep hillsides with many moist cliffs covered with mosses, *Pinus-Quercus* zone 6 km S of Temascaltepec on Hwy 130, white flowers, 18.95°, -100.083333°, 2000 m, 3 Sep 1965, *Roe 1696 et al.* (MICH, NY). **México-Puebla.** Ixtaccíhuatl, 19.2°, -98.6°, 4600 m, Nov 1905, *Purpus 1753* (GH, NY). **Michoacán.** Mpio. de Morelia, lado sureste del Cerro El Águila subiendo por Huatzanguio, bosque de *Quercus*, hierba con flores blancas, 19.610278°, -101.379167°, 2484 m, 16 Nov 2007, *García 388 et al.* (MO). **Morelos.** Hacia el Valle del Tepeite, canal Mt. Zempoala, Sta. Maria, 19.0607°, -99.3203°, 3000 m, Aug 1932, *Lyonnet 1029* (MEXU). **Nayarit.** Mpio. de Acaponeta, in pine and oak forest al La Ciénaga on ridge about 10 mi NW of Mesa del Nayar, flowers white, 22.328°, -104.744°, 2600 m, 30 Jul 1970, *Norris 14579* (MICH). **Nuevo León.** Zaragoza, Cerro El Viejo, oak and pine woods, flowers purple, scattered plants, 23.97°, -99.769°, 2465 m, 29 Jul 1992, *Hinton 22213 et al.* (TEX). **Oaxaca.** San Miguel, Nochixtlán, Centro, 17.458°, -97.224°, 2500 m, 19 Jun 1907, *Hugo 1872* (MEXU). **Puebla.** Pico de Orizaba, 19.03°, -97.27°, 3048 m, *Botteri 349367* (GH). **Querétaro.** Aprox. 3–4 km al ENE de Pinal de Amoles, bosque de pino y encino, ladera de cerro, plant herbácea, flores blanco-verdosas, abundante, 21.153°, -99.596°, 2450 m, 25 Oct 1988, *Carranza 1090* (XAL). **San Luis Potosí.** F.C. Potosi & Rio Verde, Sierra de Alvarez, Sierra Madre Oriental, 21.9°, -100°, 2150 m, 1 Aug 1934, *Pennell 17763* (GH, US). **Tamaulipas.** Southern Tamaulipas, top of Sierra Madre Oriental (Sierra de Guatemala), rugged, wooded, karstic limestone plateau with sink holes and big boulders, ca 6 km NW of Rancho del Cielo (Harrison Ranch of Martin & Harrell), (ca 3 km NW of Julio) on road to La Joya de Salas, ca 11 km NW of Gomez Farias, 108 km SSW of Ciudad Victoria, dry, open *Pinus-Quercus* forest with rich herbaceous cover on top of ridge, 23.2°, -99.266667°, 2060 m, 12 Aug 1991, *Iltis 30682* (MU). **Veracruz.** Huayacocotle, Jarillos, bosque de pino-encino, suelo aroillos, hierba, annual, 0.7 m, regular, flor blanca, bracteas rosadas, 20.5°, -98.5°, 2490 m, 11 Aug 1984, *Cabrera-R. 171* (XAL).

Trifolium rhombeum is most similar to *T. cognatum* but differs in its denser pubescence, larger leaves, lacking a sterile projection at the apex of the inflorescence, larger fruits, and most distinctly is purple flowered. In total, nearly 25% of the characters studied were significantly different as shown in T-tests ($\alpha=.05$) (Ahlquist 2012). *Trifolium rhombeum* is a very robust species that is similar to *T. hickeyi*. They are most readily differentiated by the moderate to dense pubescence and shorter peduncles of *T. rhombeum*. Of the characters observed in this study, over 50% were significantly different between *T. rhombeum* and *T. hickeyi* as shown by T-tests ($\alpha=.05$) (Ahlquist 2012).



Figure 12. *Trifolium rhombum* (from E. Palmer & C.C. Parry 137 (GH)). A. Habit silhouette. B. Fertile branch. C. Abaxial leaf surface. D. Sepals. E. Banner petal. F. Wing petals. G. Keel petals. H. Connate stamens. I. Free stamen. J. Ovary.

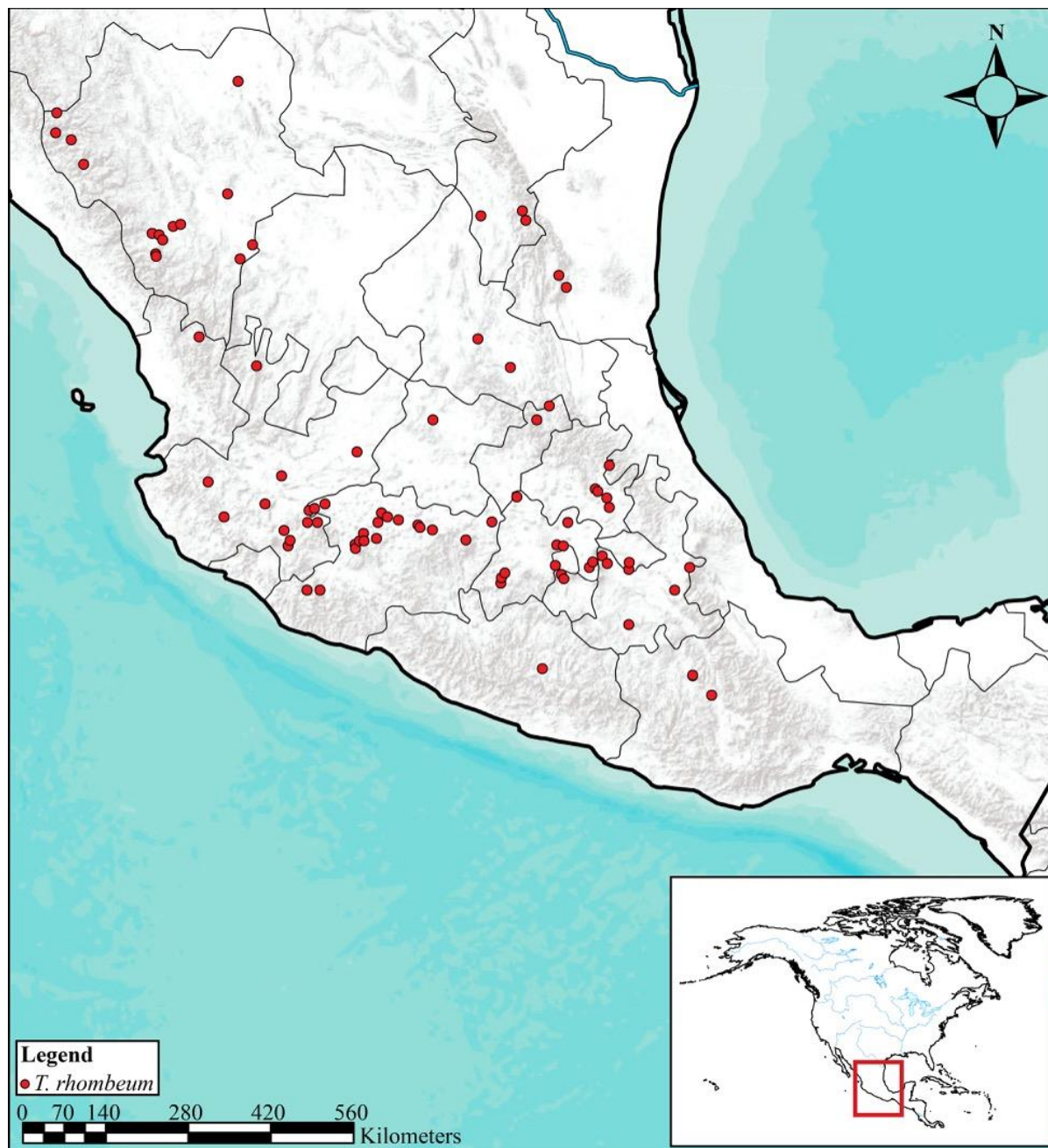


Figure 13. Distribution of *Trifolium rhombeum*.

Trifolium rhombeum is widely distributed throughout Mexico in the states of Durango, Guanajuato, Guerrero, Hidalgo, Jalisco, México, Michoacán, Morelos, Nayarit, Nuevo León, Oaxaca, Puebla, Querétaro, San Luis Potosí, Tamaulipas, and Veracruz (Figure 13). It has been collected in a variety of habitats including open pine-oak woodland, hillsides and slopes, grasslands, and along roadsides. In these locations the soil varies from sandy, calcareous, rocky, volcanic, to clay loams. *Trifolium rhombeum* is found from 1100 m to 4600 m in elevation.

- 9. TRIFOLIUM SONORENSE** T.K. Ahlquist & Vincent, *Phytoneuron* 2018-1: 1. 2018. **TYPE: USA. Arizona.** Cochise Co.: Huachuca Mountains, Garden Canyon. 31.49°, -110.32°, 1500 m, 3 Sep 1928, *G.J. Harrison 5768 with T.H. Kearney* (holotype: ARIZ 92895!; isotypes: US 1435169!, US 1435168!).

This species was described and discussed in Ahlquist & Vincent (2018).

ACKNOWLEDGEMENTS

We thank the herbarium of Miami University (MU) for providing the support and resources necessary for this endeavor. We would also like to thank the curators of the following herbaria for loans of specimens for this study: A, AAU, AHUC, ARIZ, ASU, B, C, DAV, F, GH, ILL, L, LPB, MEXU, MICH, MO, MU, NY, P, POM, RSA, SI, TEX, U, UAT, US, VT, XAL.

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