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A morphological analysis of the *Mammillaria fittkaui* species complex (Cactaceae) reveals a new species from Jalisco, Mexico

El análisis morfológico del complejo de especies *Mammillaria fittkaui* (Cactaceae) revela una especie nueva de Jalisco, México

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Abstract
Background: The *Mammillaria* series *Stylothelae* (Cactaceae) includes 16 taxa distributed mostly among the limits of the Chihuahuan Desert (CD) with the Sierra Madre Oriental and the Transmexican Volcanic Belt (TVB). In Jalisco, four pink flowered species occur at the southwestern end of the CD where it meets the TVB. The boundaries and recognition of three species related with *M. fittkaui* (*M. fittkaui* complex) are debatable.
Question: Should the taxa of the *Mammillaria fittkaui* species complex be considered as species?
Studied species: Four pink flowered *Mammillaria* series *Stylothelae* (*M. fittkaui* species complex) and *M. zeilmanniana* as comparative species.
Study site: Western Mexico.
Methods: Six morphological characters were sampled in specimens from the type locations of three pink flowered species, one locality of *Mammillaria* sp., and *M. zeilmanniana*. We used a permutational multivariate analysis of variance on six characters of each of the groups and a discriminant analysis to evaluate the morphological variation among species and to determine the correct classification of each individual.
Results: The analyses identified four well defined morphological groups with statistical significance within the *Mammillaria fittkaui* complex. We recognized *M. fittkaui*, *M. limonensis*, and *M. manana* as species and here describe *M. arreolae* as a new species from Jalisco.
Conclusions: In Cactaceae, morphometry has been useful to establish species boundaries. The *Mammillaria fittkaui* complex includes four species. *Mammillaria arreolae* differs from the other species by the shorter tubercles, flowers, and fruits. In addition, it develops more than one hooked central spine per areole.
Key Words: cacti, morphometry, *Stylothelae*

Resumen
Antecedentes: *Mammillaria* serie *Stylothelae* (Cactaceae) incluye 16 taxones distribuidos principalmente entre los límites del Desierto Chihuahuense (DC), la Sierra Madre Oriental y el Eje Volcánico Transmexicano (EVT). En Jalisco, cuatro taxones de flores rosas ocurren en el extremo suroeste del DC que confluye con el EVT. Los límites y el reconocimiento de tres especies relacionadas con *M. fittkaui* (complejo *M. fittkaui*) son debatibles.
Pregunta: ¿Los taxones del complejo de especies *Mammillaria fittkaui* deberían ser considerados como especies diferentes?
Especies estudiadas: Cuatro especies de *Mammillaria* serie *Stylothelae* de flores rosas (complejo *M. fittkaui*) y *M. zeilmanniana* como grupo comparativo.
Sitio de estudio: Occidente de México.
Métodos: Seis caracteres morfológicos fueron muestreados en las localidades tipo de las tres especies de flores rosas, una localidad de *Mammillaria* sp. y *M. zeilmanniana*. Se utilizó un análisis multivariado de varianza basado en permutaciones en seis caracteres entre los cinco grupos y un análisis discriminante para evaluar la variación morfológica entre especies y determinar la correcta clasificación de cada individuo.
Resultados: Los análisis identificaron cuatro grupos bien definidos con significancia estadística dentro del complejo *Mammillaria fittkaui*. Reconocemos a *M. fittkaui*, *M. limonensis* y *M. manana* y describimos a *M. arreolae* como una especie nueva de Jalisco.
Conclusiones: En Cactaceae, la morfometría es útil para establecer límites entre especies. El complejo *Mammillaria fittkaui* incluye cuatro especies. *Mammillaria arreolae* difiere de las otras especies por sus tubérculos, flores y frutos más cortos. Además, desarrolla más de una espina central uncinada por aréola.
Palabras clave: cactáceas, morfometría, *Stylothelae*

Mammillaria Haw. (Cactaceae) includes globose or subglobose plants with non-grooved mammilloid tubercles. These are arranged into spiraled series and develop dimorphic areoles (Anderson 2001). It is one of the largest genera of Cactaceae with 143-200 recognized species (Hunt 2006, Hunt 2016, Korotkova *et al*. 2021). The discrepancy between the number of recognized species associates with the high morphological variation among these plants. Depending on the author, the variation is used to separate or aggregate taxa. Also, the lack of morphological and geographical information plus taxonomic changes contributes to the different number of recognized species (Sánchez *et al.* 2020, Breslin *et al*. 2021).

Based on morphological characters, *Mammillaria* is divided into eight subgenera (Hunt 2006). *Mammillaria* subg. *Mammillaria* comprises most of the species and it is further divided into 13 series. The *Mammillaria* series *Stylothelae* Pfeiff. differs from the others by the presence of axillary bristles, flowers and fruits slightly embedded within the stem, and a usually central uncinated spine. The number of recognized taxa for the series (including species and subspecies) ranges from 13 to 16 (Fitz-Maurice & Fitz-Maurice 2006a, Hunt 2016, Zamudio & Guzmán 2017).

The series *Stylothelae* ranges mainly among the Chihuahuan Desert and the adjacent slopes of the Sierra Madre Oriental and the Transmexican Volcanic Belt biogeographic provinces (Hernández & Gómez-Hinostrosa 2015, Morrone *et al.* 2017). With the exception of *Mammillaria crinita* DC., all the species of the series that occur in western Mexico (Aguascalientes, Colima, Guanajuato, Jalisco, Michoacán, and Nayarit) develop pink perianth segments.

Several debates exist about the taxonomic recognition of the members of the *Mammillaria fittkaui* species complex. *Mammillaria fittkaui* Glass & R.A. Foster was described from the proximity of Lake Chapala in Jalisco (Glass & Foster 1971). Then, Reppenhagen (1985) described *M. limonensis* Repp. from El Limón, in southern Jalisco. According to the author, *M. limonensis* differs from *M. fittkaui* mainly in having axillary bristles (*vs.* axils glabrous), 14-20 radial spines (*vs.* 5-9), and 4-7 central spines (*vs.* 1-4). In addition, *M. limonensis* has brown central spines, and bright-red fruits while *M. fittkaui* has black central spines and reddish-orange fruits. However, Lüthy (1995), Hunt (2006), Arreola-Nava & Ramírez-Ulloa (2017), and Korotkova *et al.* (2021) treated it as *M. fittkaui* subsp. *limonensis* (Repp.) Lüthy.

Another population of a pink flowered *Stylothelae* from central Jalisco was described as *Mammillaria manana* W. A. & B. Fitz-Maurice (Fitz-Maurice & Fitz-Maurice 2006b). According to the authors, this taxon is related to *M. marcosii* Fitz Maurice, B. Fitz Maur. & Glass, *M. anniana* Glass & R.A. Foster, *M. schwarzii* Shurly, and *M. crinita*. Even though *M. manana* develops more and larger radial spines (16 to 24, 8-15 mm long) than *M. limonensis* (14 to 20, 4-8 mm long) and shorter pink fruits (6-8 mm vs red, 6-20 mm), Hunt (2016) and Korotkova *et al*. (2021) considered *M. manana* to be a synonym of *M. fittkaui* subsp. *limonensis*.

During an exploration trip to the municipality of San Cristóbal de la Barranca in central Jalisco, another pink flowered population of *Mammillaria* series *Stylothelae* was found (hereafter *Mammillaria* sp.). After a detailed literature revision and morphological comparisons, we concluded that it differs from all the pink flowered species known for the series. We suggest that there are four well defined morphological groups among the *M. fittkaui* species complex.

Mallet (1995) defines a species as a group of individuals with shared common characters that allow separating them from another group by some morphological discontinuities. However, closely related species with similar morphological characters form a species complex and sometimes it is difficult to set the species boundaries among them (Pinheiro *et al.* 2018). There are many approaches to study this phenomenon (Sites & Marshall 2003). Morphometry plus multivariate methods have been used successfully to solve some cases (Rohlf & Marcus 1993). For example, multivariate analyses of variance uncover differences among group means, while ordination analyses allow to state the magnitude of the differences of *a priori* defined groups in a multiple set of dimensions (De-Luna 2020). In cacti, morphometric analyzes were useful to delimitate species of *Neobuxbaumia* Backeb. (Tapia *et al.* 2016), *Epithelantha* F.A.C. Weber ex Britton & Rose (Aquino *et al.* 2019), *Echinocereus* Engelm. (Sánchez *et al.* 2020), and *Melocactus* Link & Otto (Barrios *et al.* 2022). Here, we used morphological characters to delimit and recognize the species of the *M. fittkaui* complex. Also, we provide a key for the *Mammillaria* series *Stylothelae* from western Mexico.

Materials and Methods

Sampling and morphological characters selection. First, we reviewed the Mexican herbaria IBUG and MEXU (acronyms according to Thiers 2022) and did an extensive revision of literature. With the exception of *Mammillaria fittkaui*, which is known from two localities (Fitz-Maurice & Fitz-Maurice 1992), all taxa in the *M. fittkaui* complex are known only from the type locality. We visited the type localities of *M. fittkaui* (*Brunel 824*¸ IBUG), *M. limonensis* (*Brunel 871*¸ IBUG), *M. manana* (*Brunel 862*¸ IBUG), and the first discovered population of *Mammillaria* sp. (*Brunel 887*¸ IBUG). As an external comparative species in the statistical analyses, we used a recently discovered population of *M. zeilmanniana* Boed from western Mexico (*Brunel 1032*¸ IBUG). It represents the population of a pink flowered *Stylothelae* located closest to the area of distribution of all species in the *M. fittkaui* complex. One plant from each locality was collected and processed to serve as herbarium vouchers.

With the morphological data from the field, herbaria, and literature, we elaborated a data set to facilitate the comparison of quantitative and qualitative morphological characters (Table 1). We selected the most informative quantitative characters based on the observations by Reppenhagen (1991) and Fitz-Maurice & Fitz-Maurice (1993, 2006a). Central spine length, radial spine number, radial spine length, radial spine width, flower length, and fruit length were evaluated. This combination includes vegetative and reproductive characters and facilitates the recognition of the taxa at any time of the year. Most taxa of the *Mammillaria fittkaui* complex grow on vertical rock walls or hard-to-reach places. Thus, we randomly selected and measured ten adult individuals from each taxon to have an equilibrated design. Photographs of the plants and areoles were taken with a metric scale reference. Flowers in anthesis and fully developed fruits were extracted from the plants and then photographed with the same metric scale reference. All the measurements from the photographs were calculated with the software ImageJ (Schneider *et al*. 2012). From each individual, we took five measurements of each character and then averaged them. The final database contained 50 averaged (10 per taxon) values for each character.

Table 1. Morphological comparison among the *Mammillaria fittkaui* species complex.

Character	*M. arreolae*	*M. limonensis*	*M. manana*	*M. fittkaui*
Stem	3-6 × 2.5-3 cm	4-12 × 3-5 cm	5 × 10 cm	5-10 × 3-5 cm
Tubercles disposition	5 and 8 spiraled series	8 and 13 or 13 and 21 spiraled series	8 and 13 or 13 and 21 spiraled series	8 and 13 or 13 and 21 spiraled series
Axils	with white bristles	with white bristles	with white bristles	glabrous
Radial spines	11-15, flexible, soft, 5-8 mm long	14-20, flexible, soft, 4-8 mm long	16-24, flexible, 8-15 mm long	6-10, rigid, 4-7 mm long
Central spines	4-5, acicular, reddish, 1-4 hooked, 6-9 mm long	4-8, acicular, light yellow to black, 1 hooked, 7-20 mm long	4-8, subulate, light brown to yellow, 1 or none hooked, 6-20 mm long	1-4, acicular, brown to black, 1 hooked, 8-11 mm long
Flower	Campanulate-infundibuliform, 11-14 mm long	Campanulate-infundibuliform, 15-16 mm long	Campanulate-infundibuliform, 15 mm long	Campanulate, 15-18 mm long
Fruit	Ovoid, 4-5 × 4 mm, purple reddish	Ellipsoidal to claviform, 6-20 × 4-7 mm, bright red	Ovoid 6-8 × 4-5 mm, pale pink to whitish	Ellipsoidal 5-10 × 3-5 mm, pale brown to light red

Statistical analyses. The analyses included the four groups that we identified for the *Mammillaria fittkaui* species complex and *M. zeilmanniana* as a comparative group. The final database was transformed to square root values to standardize the scale of the variables and then to a resemblance matrix based on Euclidean distances. We performed a one-way permutational multivariate analysis of variance (PERMANOVA) to test if there were statistically significant differences among the selected characters of all the groups. This analysis is flexible and robust even when the data do not meet the parametric assumptions, when variables have different nature, or when variables exceed the number of samples (Anderson *et al.* 2008). We used a type I model with the morphological group as a factor. The statistical significance of the PERMANOVA was tested with 10,000 permutations based on a type III sum of squares. If the test revealed overall significance, then we ran the pair-wise comparisons between the four groups. The analyses were carried out in Primer 6 + PERMANOVA (Anderson *et al*. 2008). Boxplots of each character for the five groups were executed in the software R (R Core Team 2018).

We did a Shapiro-Wilk's test with the morphological variables to corroborate that the data met the assumptions of normality and a Pearson's product-moment correlation to discard collinearity. Lastly, a linear discriminant analysis (LDA), in Past v. 4.02, evaluated the morphological variation among the five groups of pink flowered *Mammillaria* series *Stylothelae* and tested the correct classification of each individual (Hammer *et al.* 2001).

Taxonomic treatment. The key to the *Mammillaria* series *Stylothelae* of western Mexico was elaborated with the information from the literature, herbaria specimens, and the morphological comparisons (Table 1). For the distribution map, occurrence points were obtained from the herbaria vouchers and complemented with fieldwork data. The map was elaborated on QGIS 3.12.2 (QGIS Development Team 2018). We used the biogeographic provinces of Morrone *et al.* (2017) to contrast the distribution of the species.

For the conservation status assessment of the new species, we estimated the extent of occurrence (EOO) and area of occupancy (AOO) indices in the GeoCAT tool (Bachman *et al*. 2011) and based on the IUCN Red List Categories and Criteria (IUCN 2022).

Results

Statistical analyses. The overall PERMANOVA test showed statistically significant differences ($P = 0.0001$). Likewise, in the pair-wise test, the five groups showed statistically significant differences ($P < 0.001$) between each other (Figure 1). The LDA obtained statistically significant differences ($P < 0.001$; Table 2). The LDA displayed five well-defined groups and the first two canonical functions explained 80 % of the total variation (Figure 2). The first function explained 45.73 % of the variation. The radial spines number (-0.715), the radial spines width (0.468), and the flower length (0.616) had the highest discriminatory power. Meanwhile, the second function explained 34.38 % of the variation and the central spine length (0.746) and the fruit length (0.619) were the most powerful discriminatory variables. The confusion matrix classified 100 % of the individuals correctly into their *a priori* species group.

Taxonomic treatment. Based on PERMANOVA and LDA results on quantitative characters plus the qualitative data, we propose and discuss the recognition of three previously described species and describe a new species within the *Mammillaria fittkaui* species complex.

Mammillaria arreolae P. Carrillo & Ortiz-Brunel, sp. nov. (Figures 3, 4, 5). *Type*. México, Jalisco, municipio San Cristóbal de la Barranca, Arroyo Los Cuartos, 1,282 m, 19 August 2020, *Brunel & P. Carrillo-Reyes 887* (Holotype: IBUG; Isotype: MEXU).

Diagnosis. Mammillaria arreolae is similar to *M. manana* and *M. limonensis* but it produces shorter tubercles [4-5(-7) mm], 1-4 uncinated central spines, shorter flowers (11-14 mm), and shorter ovoid fruits (4-5 mm). Also, it could be confused with *M. fittkaui*, from which it differs by the presence of axillary bristles, the noticeable thinner radial spines, and the fruit size and shape (ovoid 4-5 mm long in *M. arreolae* vs ellipsoidal 5-10 mm long in *M. fittkaui*).

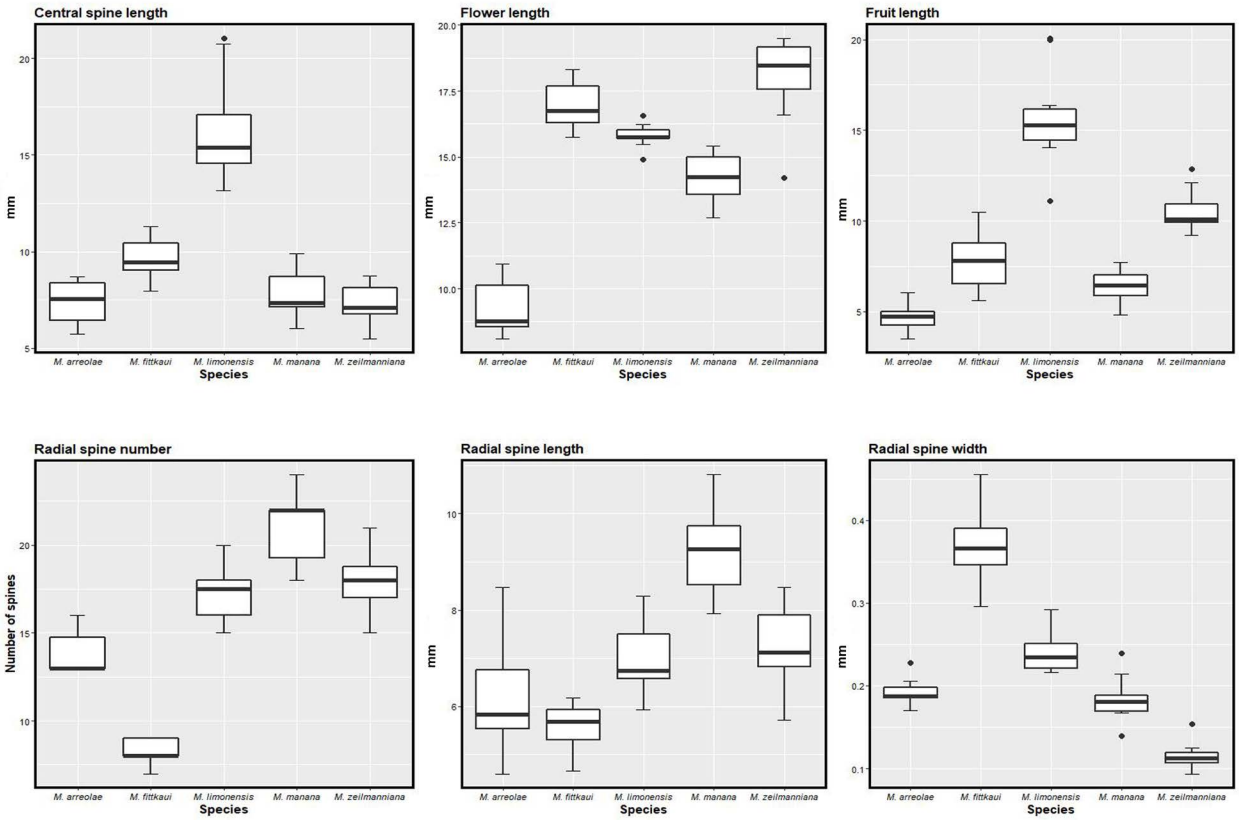

Figure 1. Morphological characters evaluated of the *Mammillaria fittkaui* species complex. The six characters had statistically significant differences among the five species ($P < 0.001$). Boxes represent the upper and the lower quartiles, the middle lines indicate the median, the exterior lines indicate the range of the data, and the dots indicate outliers.

Table 2. Linear discriminant analysis results. Bold numbers represent statistically significant results.

Function	Eigenvalue	%	Canonical correlation	Lambda	P value
1	21.3895	45.73	0.97741	< 0.001	**< 0.001**
2	16.0784	34.38	0.97028	0.00206398	**< 0.001**
3	6.53925	13.98	0.93132	0.0352494	**< 0.001**
4	2.76288	5.91	0.85688	0.265754	**< 0.001**

Description. Perennial plants, solitary when young, then caespitose, growth form globose to cylindrical, apex slightly depressed, stems 3-6 × 2.5-3 cm, dark green. Roots fibrous. Tubercles disposed in 5 or 8 spiral series, shortly cylindrical, apex rounded, 4-7 × 3.5-5 mm, sap watery. Axils with short white trichomes when young, and white bristles 3-9(-12) mm long when full grown. Areoles circular, diameter 1 mm, with short deciduous white trichomes. Radial spines 11-15, divergent, acicular, straight, flexible, white, 5-8 mm long, 0.1-0.2 mm width. Central spines 4-5, divergent, acicular, (1-)2-4 uncinated, reddish at the tip and yellow to white at the base, 6-9 mm long. Flowers campanulate-infundibuliform, close to the apex, 11-14 × 4-8 mm; perianth segments lanceolate, acuminate, entire; the external ones pink with a purple mid-stripe, 4 × 1-1.5 mm; the internal segments pale pink with a darker pink mid-stripe, 6-7 × 3-4 mm; pistil purple, 6 mm long; stigma lobes 4, pale pink, 1 mm long; filaments purple, 4 mm long; anthers pale yellow. Fruit ovoid, 4-5 × 4 mm, purplish-red, dry perianth persisting. Seeds ovoid, black, 1-1.2 × 0.7-0.8 mm, lateral hilum deltoid, with the micropyle included, testa cells isodiametric, periclinal walls concave.

Distribution and ecology. *Mammillaria arreolae* is known from a reduced area in four locations, all of them within the Santiago River Basin in the San Cristóbal de la Barranca, Tonalá, and Zapopan municipalities in Jalisco (Figure 3). It

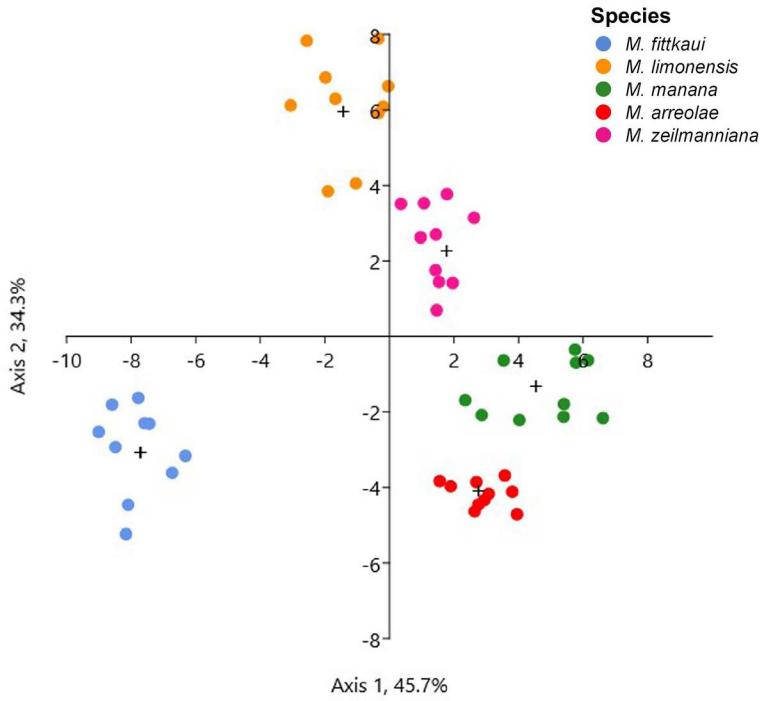

Figure 2. Scatter plot of the linear discriminant analysis. Black crosses represent the centroids for each group.

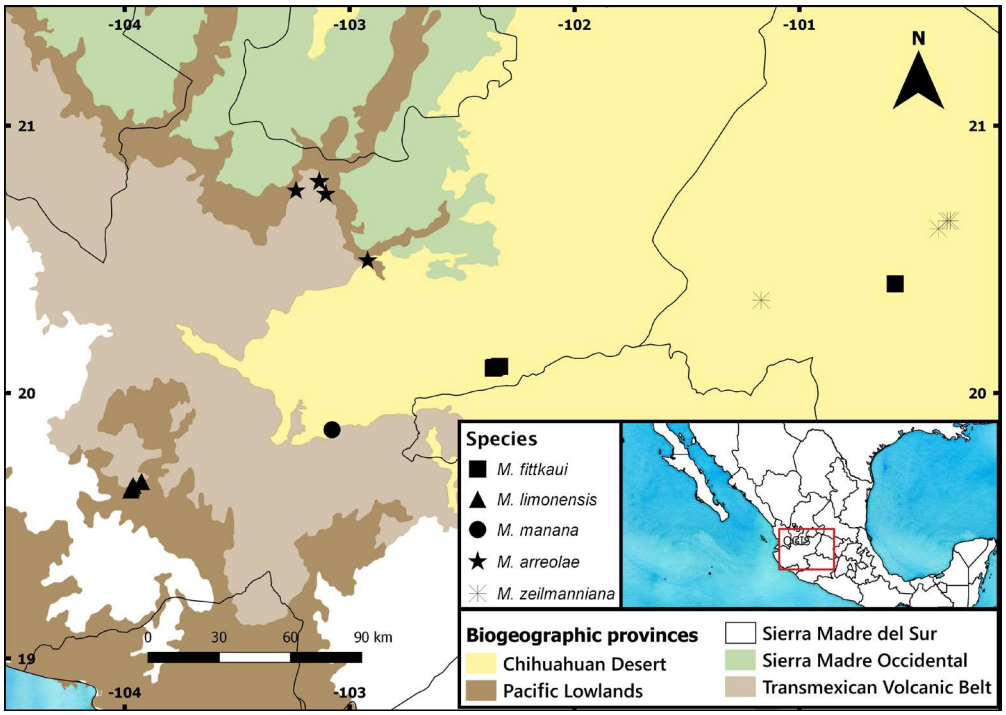

Figure 3. Distribution map of the species of the *Mammillaria fittkaui* species complex.

grows in humid areas of the tropical deciduous forest or the ecotone with the oak forest and forms colonies in vertical walls of igneous rocks between 1,200 and 1,500 m altitude. In the type locality, we counted 25 adult individuals. However, the population density and structure in the three other locations remains unknown due to the inaccessibility of the places. *Mammillaria arreolae* shares the habitat with *Oncidium cebolleta* (Jacq.) Sw. (Orchidaceae), *Bletia coccinea* Lex. (Orchidaceae), *Sedum jaliscanum* S. Watson (Crassulaceae), and *Tillandsia capitata* Griseb. (Bromeliaceae). Also, this species shares the type locality with *Arachnothryx jaliscensis* Borhidi & E. Martínez (Rubiaceae), another micro endemic species in the zone (Borhidi & Martínez-Salas 2013).

Conservation status. *Mammillaria arreolae* is known from four sites within the Santiago River Basin. GeoCAT estimated an EOO of 198.9 km^2 and an AOO of 16 km^2. According to the IUCN criteria (CR/B1a and B2a), a preliminary category of Endangered is proposed. Further exploration is needed since there are many ravines in the area with the optimal conditions for the development of the plants. This could change the proposed conservation status. Finally, the populations are not under any external pressures.

Phenology. *Mammillaria arreolae* flowers from April to August and fruits from May to September.

Etymology. The specific epithet honors Hilda Julieta Arreola Nava. She was a professor at the Universidad de Guadalajara, an outstanding cactologist, and an extraordinary human being. She died in September 26 of 2019.

Additional specimens examined. México, Jalisco, San Cristóbal de la Barranca: Arroyo Los Cuartos (El Escalón), 1,420 m, 17/07/1999, *P. Carrillo-Reyes & R. Bello 818* (IBUG); Arroyo Los Cuartos, 1,282 m, 19/07/2020, *Brunel & P. Carrillo 887* (IBUG); Zapopan: cerca de San Lorenzo, 1,325 m, 3/07/2000, *M. A. Macías-Rodríguez et al. 964a* (GUADA); Rancho Mesas de Nahuatlán, Cañón del Arroyo Agua Blanca, antes de la cascada, 1,489 m, 13/04/2021, *Brunel 1103* (IBUG); Tonalá: Barranca de Colimilla, cerca de Los Monos, 1,233 m, 4/05/2022, *Brunel 1468* (IBUG).

Mammillaria fittkaui Glass & R.A. Foster, Cactus and Succulent Journal 43:115–117, 1971. Type: Mexico, Jalisco, rocks near the north shore of Lake Chapala, *AbG 69-1169* deposited at Pomona Colleague Herbarium, collected by Father Hans Fittkau *s.n.* (holotype POM).

Additional specimens examined. México, Jalisco, Jamay, Carretera Ocotlán-Jamay, 1,620 m, 05/06/2017, *Brunel & A. Rodríguez 143* (IBUG); Carretera Ocotlán-Jamay, 1,624 m, 23/07/2020, *Brunel 824* (IBUG).

Mammillaria limonensis Repp., Kakteen und andere Sukkulenten 36: 44-46, 1985. Type: Mexico, Jalisco, El Limón, cultivated in the Reppenhagen collection, 2/03/1980, *Reppenhagen 1620* (holotype: K). *Mammillaria fittkaui* subsp. *limonensis* (Repp.) Lüthy, Taxon. Untersuch. Gattung *Mammillaria* 162. 1995.

Additional specimens examined. México, Jalisco, El Limón, 8.7 km Brecha desde El Grullo vía la Capilla, 2 km a pie, 1,570 m, 15/02/2018, *Brunel & A. Rodríguez 409* (IBUG); Cerro de Las Piedras Blancas, 1,843 m, 10/08/2020, *Brunel & J. Aragón-Parada 871* (IBUG).

Mammillaria manana W. A. & B. Fitz-Maurice, Cactus World 24: 7-11, 2006. Type: Mexico, Jalisco, 1,550 m, on near-vertical portions of brown volcanic rock, 7/11/2004, *A. Machuca 9619* (holotype IBUG, lost), lectotype (designated here): Mexico, Jalisco, Teocuitatlán, 1,500 m, south of Teocuitatlán, 5/12/1998, *W. Fitz-Maurice & B. Fitz-Maurice 2404* (MEXU!).

Additional specimens examined. México, Jalisco, Teocuitatlán de Corona, Cañón de Teocuitatlán, 1,576 m, 05/03/2020, *Brunel, A. Machuca & K. Machuca 765* (IBUG); Barranca de Teocuitatlán, 1,576 m, 02/08/2020, *Brunel & A. Flores-Argüelles 862* (IBUG).

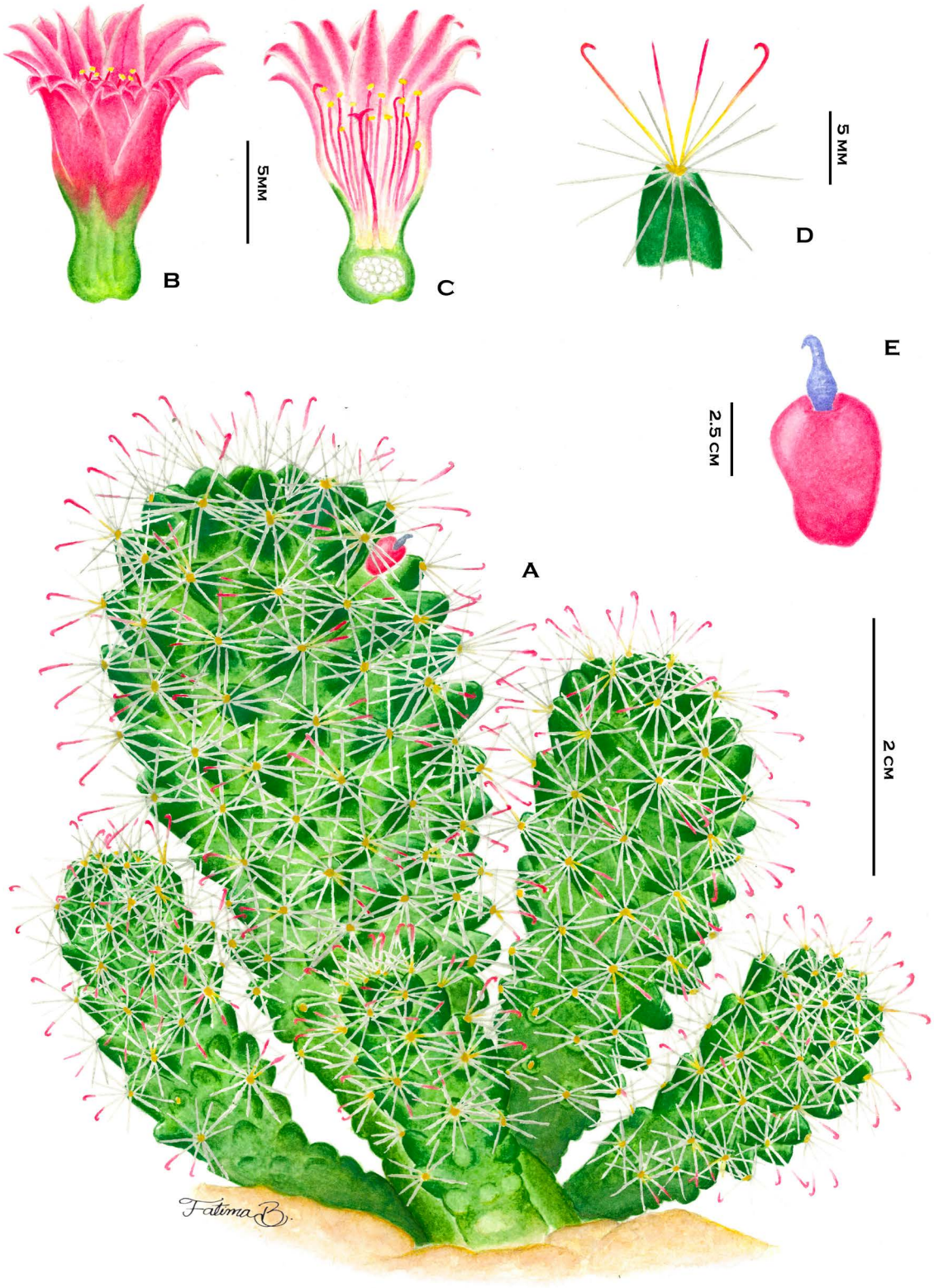

Figure 4. *Mammillaria arreolae* P. Carrillo & Ortiz-Brunel; A) habit; B) external face of the flower C) inner face of the flower; D) areole and spines; E) fruit. Drawn by Fátima Bracamontes based on the type material (*Brunel & P. Carrillo 887*).

Key to the species of the *Mammillaria* series *Stylothelae* from western Mexico

1. Plants globose or globose depressed, 1-4 × 3-6 cm; perianth segments white, yellow, or cream ……... *M. crinita*
1. Plants slightly globose to cylindrical, 4-12 × 2.5-5 cm; perianth segments pink or pale pink ……..... 2
2. Axils usually without bristles; radial spines 6-10, rigid, more than 0.3 mm wide ……..……. *M. fittkaui*
2. Axils with white bristles; radial spines more than 11, semi-rigid or flexible, less than 0.3 mm wide…….. 3
3. Perianth segments purple-pink, 16-20 mm long, more than 1 cm in diameter at anthesis; fruit light green; plants from Guanajuato ……..... *M. zeilmanniana*
3. Perianth segments light pink, 11-16 mm long, less than 1 cm in diameter at anthesis; fruit red, pink or whitish pink; plants from Jalisco ……..……..... 4
4. Tubercles 4-5(-7) × 3.5-5 mm; central spines 4-5, (1-)2-4 uncinated, 6-9 mm long; flowers 11-14 mm long; fruit ovoid-elliptic, 4-5 × 4 mm ..... *M. arreolae*
4. Tubercles 6-10 × 5-7 mm; central spines 4-8, 1 or none uncinated, 6-20 mm long; flowers 15-16 mm long; fruit ellipsoidal to claviform, 6-20 × 4-7 mm ..……..... 5
5. Radial spines 14-20, 4-8 mm long; fruit bright red, claviform, 6-20 mm long …… *M. limonensis*
5. Radial spines 16-24, 8-15 mm long; fruit pale pink to whitish, ovoid, 6-8 mm long ……..…… *M. manana*

Discussion

Statistical analyses. In Cactaceae, discriminant analyses of morphological characters are powerful tools that help to clarify the limits in species complexes (Tapia *et al*. 2016, Aquino *et al*. 2019, Sánchez *et al.* 2020, Barrios *et al.* 2022). The PERMANOVA analyses found statistically significant differences in all the selected characters among the five groups (Figure 1). Moreover, the LDA displayed five well-defined groups and the confusion matrix assigned correctly the 100 % of the samples to their given group (Figure 2). Both analyses recovered five well statistically supported groups, which correspond to *Mammillaria arreolae*, *M. fittkaui*, *M. limonensis*, *M. manana*, and *M. zeilmanniana*, the last represented the external comparative group.

Taxonomic treatment. Mammillaria series *Stylothelae* includes plants with flexible spines or bristles in the axils, flowers and fruits slightly embedded in the stem, pink or red fruits, and generally one uncinated central spine (Hunt 2006). However, some species lack uncinated central spines or axillary bristles, which makes their recognition within the series difficult (Glass & Foster 1971). *Mammillaria arreolae* is easily classified in this series by the presence of all the characters mentioned above. Within the series, the new species is related to *Mammillaria fittkaui* species complex, which includes *M. fittkaui*, *M. limonensis*, and *M. manana*. It differs from these taxa by its noticeably shorter tubercles, flowers, fruits, and the presence of more than one uncinated spine per areole. Morphologically, the LDA showed *M. manana* and *M. arreolae* close together. However, both species differ in the number of radial spines and their length, the number of hooked central spines, flower and fruit length, and fruit color (Table 1).

The taxonomic recognition of some members of the *Mammillaria fittkaui* species complex was difficult. *Mammillaria fittkaui* has been widely accepted as a valid species (Bravo-Hollis & Sánchez-Mejorada 1991, Hunt 2006, 2016, Korotkova *et al*. 2021). However, after the description of *M. limonensis* (Reppenhagen 1985), either it was recognized as a valid species (Reppenhagen 1991, Fitz-Maurice & Fitz-Maurice 1993) or considered as *M. fittkaui* subsp. *limonensis* (Lüthy 1995, Hunt 2006, 2016, Arreola-Nava & Ramírez-Ulloa 2017, Korotkova *et al*. 2021). Our results supported Fitz-Maurice & Fitz-Maurice (1993) observations regarding the number of radial spines, their width, the length of the central spine, and the length and color of the fruit, which are distinctive characters. All of them help to delimitate these species (Table 1, Figures 1, 2). Therefore, we recognized two distinct species.

On the other hand, *Mammillaria manana* was described (Fitz-Maurice & Fitz-Maurice 2006b) and recognized only by Fitz-Maurice & Fitz-Maurice (2006a) and Arreola-Nava & Ramírez-Ulloa (2017). Hunt (2016) considered it to be a form of *M. fittkaui* subsp. *limonensis* and Korotkova *et al.* (2021) supported this opinion. Fitz-Maurice & Fitz-Maurice (2006b) related *M. manana* with *M. marcosii*, *M. anniana*, *M. schwarzii*, and *M. crinita*. We disagree

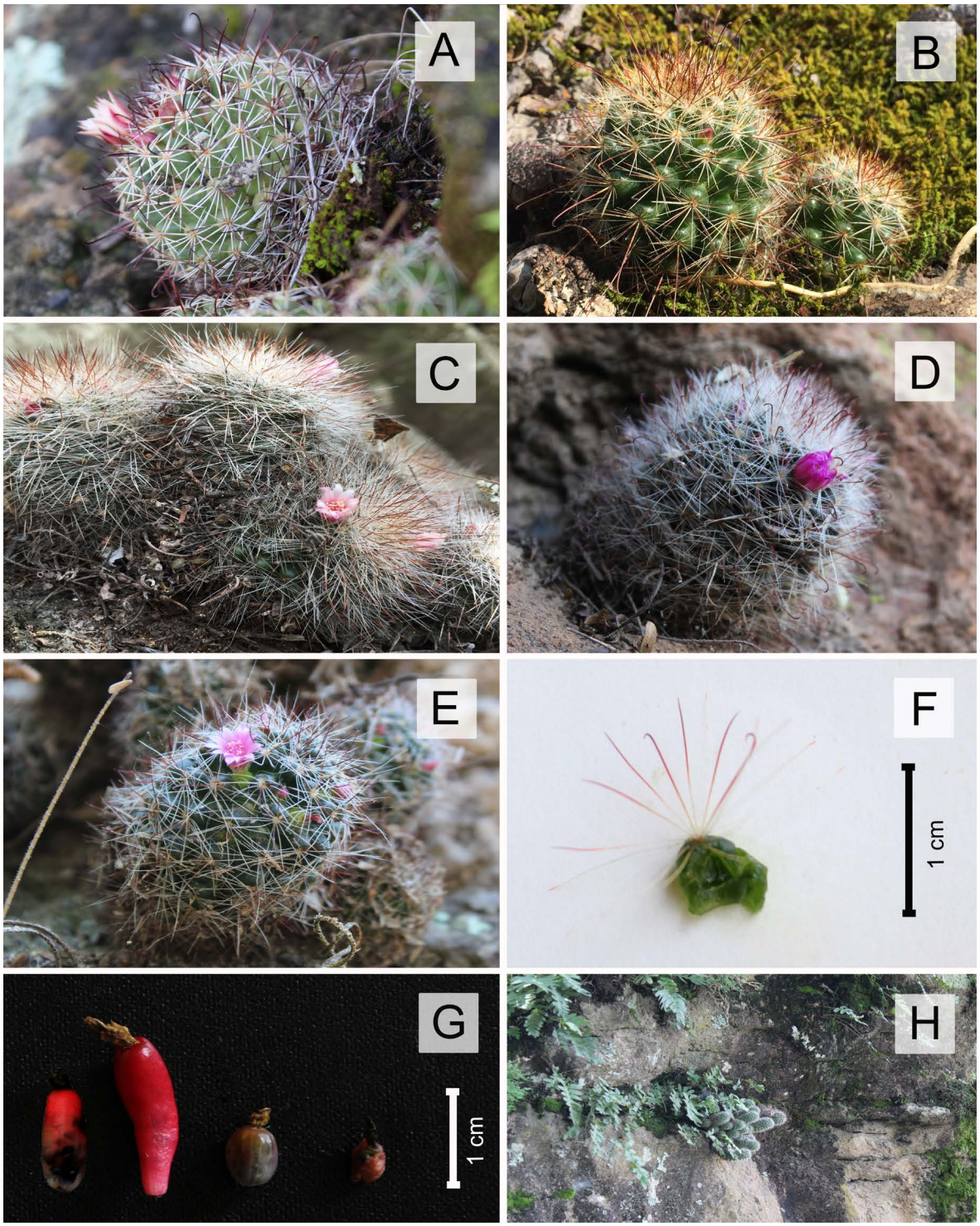

Figure 5. Pink flowered *Mammillaria* series *Stylothelae* of western Mexico; A) *M. fittkaui*; B) *M. limonensis*; C) *M. manana*; D) *M. zeilmanniana*; E) *M. arreolae*; F) tubercle and spines of *M. arreolae*; G) fruit comparison among the *Mammillaria fittkaui* species complex. From left to right: *M. fittkaui*, *M. limonensis*, *M. manana*, *M. arreolae*; H) *M. arreolae* in habitat.

with this mainly because *M. anniana* and *M. schwarzii* lack the uncinated central spine and their radial spines are noticeably shorter (6-11 mm and 6 mm, respectively). Also, the longer central spines and the different size, shape, and color of the flower and fruit in *M. manana* set it apart from *M. crinita*. Our data supported a close relationship between *M. manana* and the *M. fittkaui* species complex.

Furthermore, all species of the *Mammillaria fittkaui* complex are allopatric (Figure 3). First, *M. arreolae* lies in the uppermost portion of the Santiago River basin, which corresponds to the Transmexican Volcanic Belt in its contact zone with the Pacific Lowlands and the Sierra Madre Occidental biogeographical provinces. Second, *M. fittkaui* is known from two locations, both in tropical deciduous forest in the Chihuahuan Desert. Third, *M. manana* inhabits a very steep canyon with tropical deciduous forest and xerophytic elements such as *Myrtillocactus geometrizans* (Mart. ex Pfeiff.) Console and *Dasylirion* sp. on the limits of the Chihuahuan Desert with the Transmexican Volcanic Belt. Finally, *M. limonensis* occurs in an oak forest in the limits of the Pacific Lowlands with the Transmexican Volcanic Belt. Meanwhile, *Mammillaria zeilmanniana* was only known from three collections near San Miguel de Allende (Fitz-Maurice & Fitz-Maurice 1988). We report a new locality situated approximately 100 km southwest (México, Guanajuato, Pénjamo, Sierra de Pénjamo, 1,816 m, 28/11/2020, *Brunel & M. F. Hernández 1032*, IBUG). All populations of the *M. fittkaui* species complex are isolated due to the presence of mountain chains, lakes, lagoons, and rivers, so gene flow seems very unlikely.

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