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Reproductive biology and potential distribution of *Ficus pringlei* (Moraceae), a microendemic New World species

Biología reproductiva y distribución potencial de *Ficus pringlei* (Moraceae), una especie microendémica del Nuevo Mundo

Ángela P. Rojas-Cortés^{1,2} , Ángela P. Cuervo-Robayo³ , Guadalupe Cornejo-Tenorio¹ , Guillermo Ibarra-Manríquez^{1,4} 

Abstract:

Background and Aims: One of the most diverse and ecologically important tree genera in the tropics is *Ficus*. The characterization of developmental phases and morphological changes of its syconium is essential for facilitating pollination and dispersal studies, but only a few species in the Americas have been characterized. *Ficus pringlei* is endemic to Mexico for which detailed information on the potential distribution and reproductive aspects are lacking. The aims of this study were to describe syconia development, determine the type of pollination, and detect areas of potentially suitable environments for establishing *F. pringlei*.

Methods: Syconia were collected to describe their development phases. The syconia and its pollinating wasps were fixed in alcohol for processing and described using scanning electron microscopy. Ecological niche models were used to predict areas with suitable environments to locate *F. pringlei*.

Key results: The syconium changed in size, color, consistency, and shape of the ostiole over the course of its developmental phases, particularly in stages that were most critical for their interaction with pollinators and dispersers. Syconia development was asynchronous at both the within- and between-individual level in the sampled population. *Ficus pringlei* is actively pollinated. The predicted suitable distribution area for this species was concentrated mainly in the tropical dry forests of the states of Jalisco and Michoacán. The most important variable to explain the potential distribution model for *F. pringlei* was the seasonality of temperature, which had higher values in areas north of the Trans-Mexican Volcanic Belt.

Conclusions: The developmental phases of this species are consistent with those described previously for other monoecious species. *Ficus pringlei* is only distributed in western Mexico, mainly in tropical dry forests, and is barely registered in Protected Areas. Therefore, it is essential to establish strategies for its conservation.

Key words: active pollination, ecological niche model, *Ficus* section *Americanae*, *Pegoscapus*, seasonally dry tropical forest, syconium development.

Resumen:

Antecedentes y Objetivos: Uno de los géneros de árboles más diversos y ecológicamente importantes de los trópicos es *Ficus*. La caracterización de las fases de desarrollo y cambios morfológicos de su sicono es fundamental para facilitar los estudios de polinización y dispersión, pero estos aspectos solo se han caracterizado en pocas especies en América. *Ficus pringlei* es endémico de México y no se dispone de información detallada sobre su distribución potencial y aspectos reproductivos. Este estudio tuvo como objetivo describir las fases del desarrollo del sicono de *F. pringlei*, determinar su tipo de polinización, y detectar áreas con ambientes potencialmente adecuados para localizarlo.

Métodos: Se recolectaron siconos para describir las diferentes fases de su desarrollo. Los siconos y sus avispas polinizadoras se fijaron en alcohol para ser procesadas y descritas mediante microscopía electrónica de barrido. Se usaron modelos de nicho ecológico para predecir áreas con ambientes adecuados para localizar a *F. pringlei*.

Resultados clave: El sicono cambió de tamaño, color, consistencia y forma del ostiolo durante sus fases de desarrollo; particularmente en las etapas más críticas de su interacción con polinizadores o dispersores. Se registró un desarrollo asincrónico, tanto a nivel individual, como poblacional, de los siconos. *Ficus pringlei* presenta una polinización activa. El área potencialmente adecuada de distribución se concentró principalmente en los bosques tropicales secos de los estados de Jalisco y Michoacán. La variable más importante para explicar el modelo de distribución de *F. pringlei* fue la estacionalidad de la temperatura, con valores mayores en las zonas al norte de la Faja Volcánica Transmexicana.

Conclusiones: Las diferentes fases de desarrollo de esta especie son consistentes con las descritas previamente para otras especies monoicas. *Ficus pringlei* solo se distribuye en el occidente de México, principalmente en bosques tropicales secos y se encuentra minoritariamente en Áreas Protegidas. Por lo tanto, es importante establecer estrategias que aseguren su conservación.

Palabras clave: bosques tropicales estacionalmente secos, desarrollo de sicono, *Ficus* sección *Americanae*, modelo de nicho ecológico, *Pegoscapus*, polinización activa.

¹Universidad Nacional Autónoma de México, Instituto de Investigaciones en Ecosistemas y Sustentabilidad, Antigua carretera a Pátzcuaro 8701, 58190 Morelia, Michoacán, México.

²Universidad Nacional Autónoma de México, Posgrado en Ciencias Biológicas, Avenida Universidad 3000, Ciudad Universitaria, 04510 Cd. Mx., México.

³Comisión Nacional para el Conocimiento y Uso de la Biodiversidad (CONABIO), Periférico-Insurgentes Sur 4903, 14010 Cd. Mx., México.

⁴Author for correspondence: gbarra@iies.unam.mx

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Introduction

One of the most diverse and ecologically important tree genera in the tropics is *Ficus* L. (Moraceae). Within *Ficus*, there are ca. 876 species (POWO, 2021), with different growth forms and breeding systems, as well as a wide variety of shapes, sizes, anatomical wall structures, and colors of syconia (Berg, 1989; Kravtsova and Carvajal, 1995; Berg and Corner, 2005; Lomáscolo et al., 2008). Species of this genus are considered a keystone resource in many tropical ecosystems (Janzen, 1979; Herre et al., 2008), since they provide food for a diverse assemblage of mammals, birds, and other fig-eating animals, which also disperse their seeds (Shanahan et al., 2001).

This genus is characterized by having an urn-shaped and nearly completely closed syconium, with an apical entrance called the ostiole (Berg, 1989; Verkerke, 1989). The syconium initially functions as an inflorescence, which contains pistillate and/or staminate flowers; with the development of the fruits, the syconium then becomes an infructescence (Galil and Eisikowitch, 1968; Berg, 1989; Verkerke, 1989). *Ficus* species are closely dependent on wasps of the family Agaonidae because they are the figs' sole pollinators (Ramírez, 1974; Janzen, 1979). In turn, the wasps depend on the fig for reproduction because their larvae feed only on gall flowers (Ramírez, 1970a; Jusselin and Kjellberg, 2001). This interaction is considered a classic example of coevolution (Ramírez, 1974; Hembry and Althoff, 2016).

The complex development of syconia reflects adaptation to pollination by agaonid wasps and has been divided into different developmental phases (Galil and Eisikowitch, 1968; Smith and Bronstein, 1996; Piedra-Malagón et al., 2019). However, there is considerable diversity in this obligate mutualism, and fig-pollinator wasp interactions vary. For instance, half of all *Ficus* species are monoecious, with both wasps and seeds produced in the same syconium (Verkerke, 1989), while the remaining *Ficus* are gynodioecious, where the syconia of female trees give rise only to seeds, and the functionally male trees solely to wasps (Berg, 1989). Recently, gynomonoecy was discovered in *F. umbrae* Ezedin & Weiblen; seed syconia have pistillate flowers and non-functional male flowers, while gall syconia have fully functional male and female flowers (Ezedin and Weiblen, 2019).

Five to eight phases of syconium development have been described in monoecious species; phases B (female phase) and D (male phases) are the phases during which the wasps interact most closely with the different structures inside of the syconium (Galil and Eisikowitch, 1968; Verkerke, 1989; Smith and Bronstein, 1996; Piedra-Malagón et al., 2019; Delgado-Pérez et al., 2020). During Phase B, a limited number of female wasps enter the syconium through the ostiole, depending on the size of the syconium. Small syconia have few flowers which are often small (Murray, 1985; Liu et al., 2011), which are probably visited by fewer foundress wasps, and have smaller pollen-transfer genetic neighborhoods than large syconia visited by larger wasps (Borges, 2021).

In addition, there are different pollination behaviors among agaonids; some pollinate actively and others passively (Jusselin and Kjellberg, 2001). In active pollination, female wasps collect pollen from the anthers during the male phase (Phase D) before leaving their natal figs and place it in special thoracic structures called corbiculae and coxal combs to transport them to another syconium with receptive pistillate flowers (Phase B) (Ramírez, 1969). Once inside a receptive fig, the wasp puts a bit of the pollen from its corbiculae on the stigmatic surface each time it lays an egg (Ramírez, 1970a). In the case of passive pollination, the pollinator does not show any pollen collection and deposition behavior and lacks corbiculae (Galil and Neeman, 1977; Kjellberg et al., 2001). Active pollination has probably evolved to improve progeny nourishment and larval survivorship, as fertilized ovules may provide a better feeding substrate for the developing larvae (Jusselin et al., 2003). Also, this type of pollination behavior is likely to be more efficient at transferring pollen than passive pollination (Kjellberg et al., 2001).

Distinctive morphological adaptations in both wasps and figs have been associated with pollination behaviors. In most species with active pollination, the stigmas can be cohesive, forming a common surface for pollen tube germination (the synstigma), which may help ensure seed production in flowers when pollinating wasps do not deposit pollen directly in the pistillate flower (Jusselin and Kjellberg, 2001; Teixeira et al., 2018, 2021). Furthermore, the pollen of actively pollinated fig species is ellipsoid,



and the anther-to-ovule ratio is low ($A/O < 0.16$), since they produce less pollen, because wasps can only carry a limited amount of pollen in their corbiculae (Jousselin and Kjellberg, 2001; Wang et al., 2014). Active pollinator wasps present sternal corbiculae, coxal corbiculae, and coxal combs (Kjellberg et al., 2001). However, there can be passive pollinator species within genera that are mostly active pollinators; these species may have reduced sternal corbiculae and lack coxal corbiculae and combs. Examples include *Pegoscapus carlosi* Ramírez, 1970, *P. mariae* Ramírez, 1970, and *Ceratosolen galili* Wiebes, 1964. In the case of passively pollinated *Ficus* species, the synstigma is absent, the pollen is spherical or cylindrical, and the anther-to-ovule ratio varies from 0.29 to 0.92; their pollinator wasps lack corbiculae and coxal combs (Kjellberg et al., 2001; Teixeira et al., 2018).

Phase E of syconium development is particularly relevant in the interaction of *Ficus* species with their seed dispersers, since the size and coloration of ripe syconia are directly related to the type of frugivore that consumes them, and the distance seeds are dispersed (Shanahan et al., 2001). For example, small syconia, which are often red, are probably consumed by small birds with short dispersion distances, contributing to more local gene movement (Borges, 2021). Meanwhile, larger birds and bats and other mammals prefer large, dull-colored fruits and disperse seeds at longer distances (Shanahan et al., 2001). Nevertheless, the dispersal distance depends on foraging behavior, since bats of the family Phyllostomidae fly to temporary feeding roosts near the fruiting tree, which limits their effective dispersal distance (Heer et al., 2015).

In several monoecious *Ficus* species, the development of syconia in an individual tree is usually highly synchronized (Janzen, 1979; Cook and Power, 1996). However, in highly seasonal environments where tree reproduction and wasp dispersal are likely to be reduced during cold and/or dry periods, *Ficus* populations can break within-tree synchrony in order to allow the persistence of small pollinator populations year-round (Ramírez, 1970a; Janzen, 1979; Smith and Bronstein, 1996). In small populations, rather than promoting selfing, it appears that within-tree flowering asynchrony enhances reproductive assurance by increasing opportunities to contribute pollen to and receive

pollen from other trees, thus enhancing both the male and female components of fitness (Gates and Nason, 2012). Also, the presence of syconia throughout the year may be an important resource in periods of low fruit availability in seasonal tropical and subtropical forest ecosystems (Shanahan et al., 2001; Bianchini et al., 2015; but see Compton and Greeff, 2020).

The developmental phases and morphological changes of the syconium have been characterized in only a few fig species in the Americas (e.g., Hernández Sosa and Saralegui Boza, 2001; Piedra-Malagón et al., 2019; Delgado-Pérez et al., 2020; Cervantes-Pasqualli and Laborde, 2021). This characterization is essential to identify the critical phases involved in the pollination and dispersion of *Ficus* species. There are three endemic species of *Ficus* in Mexico, one of which is *F. pringlei* S. Watson (Quintana and Carvajal, 2001; Ibarra-Manríquez et al., 2012). Unlike most species of the subgenus *Spherosuke* Raf., which have a hemiepiphytic or strangler habit (Berg and Corner, 2005), *F. pringlei* is a rupicolous tree up to 12 m high that grows on rocky outcrops or cliffs in the Mexican states of Colima, Guerrero, Jalisco, Michoacán, Nayarit, and Zacatecas (Serrato et al., 2004; Durán-Ramírez et al., 2010; Carvajal, 2012; Ibarra-Manríquez et al., 2012). The syconia of *F. pringlei* have a diverse associated community of wasps, but all are undescribed, including its pollinator (*Pegoscapus* sp.) and several non-pollinating genera of Chalcidoidea. Furthermore, detailed ecological information about the potential distribution and reproductive aspects of *F. pringlei* (e.g., description of developmental phases of syconia and morphological adaptations related to wasp pollination behavior) is unavailable.

This study therefore aimed to i) describe and illustrate the main changes over the course of the developmental phases of *F. pringlei* syconia, ii) determine the type of pollination of this *Ficus* species, and iii) detect areas of suitable environments to locate *F. pringlei* using ecological niche modeling (ENM). Achieving these objectives is relevant to understanding the reproductive biology of these rare species, particularly their relationships with their pollinators and potential frugivores in the tropical dry forest (TDF) and discovering new locations where *F. pringlei* has not yet been recorded.



Material and Methods

Sampling and preparation of material

Branches with syconia in the different development phases were collected from five individuals of *F. pringlei* in August 2021 from three locations: Colima (19.3466°N, 103.8412°W, one individual), Jalisco (21.0308°N, 103.4607°W, two individuals), and Zacatecas, Mexico (21.1783°N, 103.5332°W, two individuals).

The syconia were preserved in 70% ethanol. The characterization of Smith and Bronstein (1996) and Piedra-Malagón et al. (2019) were used to establish typical phases of development. Five figs of each phase of development were cut in half and fixed in 70% ethanol, dehydrated in an ethanol series, and processed in the laboratory of Escuela Nacional de Estudios Superiores (ENES), Universidad Nacional Autónoma de México (UNAM) in Morelia, Mexico.

More than ten mature stamens from two figs were collected to measure pollen size (20 grains), pollen shape based on Reitsma (1970), and detailed exine ornamentation according to Wang et al. (2014). The wasps were collected from closed syconia in Phase D and stored in 70% ethanol. The fore coxae and the ventral and lateral parts of the female thorax were examined to establish whether the fore coxa bore a comb (a line of setae) and corbiculae were absent or present (either fully developed or reduced).

The external morphology of the stigma, pollen, and wasps was assessed using a Jeol JSM-IT300LV scanning electron microscope (SEM; JEOL, Peabody, USA). Previously fixed samples were dehydrated in an ethanol series, critical point dried in an Autosamdri-815 Series A critical point dryer (Tousimis, Rockville, USA), adhered to metal sample holders, and sputter-coated with gold in a Denton Desk V instrument (Denton Vacuum, Moorestown, USA). The A/O ratio was measured by counting male and female flowers in five Phase C figs from five trees.

Ecological niche modeling

Ecological niche models were used to examine the potential distribution of *F. pringlei*. All analyses were performed with the software R v. 4.0.3. (R Core Team, 2020). Data on the presence of this species were obtained from a critical review of the material deposited in the National Herbarium of Mexico (MEXU, 2019), Global Biodiversity Information

Facility (GBIF, 2020), and new records in the field (Appendix). The initial data set was reduced by removing duplicates and records that were not georeferenced or whose coordinates were inconsistent with the locality or municipality. The spThin package (Aiello-Lammens et al., 2015) was used to minimize the spatial autocorrelation of records, leaving only one record within a 10 km range in the final data set.

Nineteen bioclimatic variables available from WorldClim v. 1.4 (Hijmans et al., 2005; WorldClim, 2020) were initially included as predictor variables for the niche model, with a spatial resolution of 2.5 arc-min. Highly correlated variables ($r > 0.85$) were subsequently detected with the function `correlation_finder` of the package `nichetoolbox` (Osorio-Olvera et al., 2020), which resulted in the selection of the following nine bioclimatic variables: annual mean temperature, annual mean diurnal range, isothermality, temperature seasonality, annual precipitation, precipitation of driest month, precipitation seasonality, precipitation of warmest quarter, and precipitation of the coldest quarter. The union between 12 physiographic subprovinces (INEGI, 2001) and a buffer area of 200 km around the presence records were used for the calibration area.

Model calibration, evaluation, and ensemble were performed with the package `biomod2` (Thuiller et al., 2020), which combines multiple algorithms to reduce uncertainty (Araújo and Guisan, 2006; Thuiller et al., 2009). Five algorithms were used: artificial neural network (ANN), generalized additive model (GAM), generalized boosted model (GBM), generalized linear model (GLM), and maximum entropy model (MaxEnt). For each algorithm, except for MaxEnt, default configurations were used (obtained with the `kuenm` package; Cobos et al., 2019). The combinations of four parameters (FC: linear, quadratic, product, and hinge) were used to determine MaxEnt configurations, which adjust the flexibility in the response of the model and the regularization multipliers (RM: 0.5-2.5 (intervals of 0.5) and 3-6 (intervals of 1, 8, and 10), which penalize the complexity of the model.

The best model was selected based on three criteria: 1) statistical significance (based on partial ROC analysis), 2) predictive capacity (5% omission rate, OR), and 3) complexity of the model (evaluated with AIC). Since the occurrence data set consisted only of presence data, a set of 2000 pseudo-absence data were generated. To calibrate



the model developed in biomod2, ten repetitions of each algorithm were used with different calibration and evaluation data; 80% of all data was for calibration. The models with the ROC metric were tested, assembling only those with a ROC value ≥ 0.8 .

The ensemble model was used to project the potential distribution under current climatic conditions. The importance of the variables in the model assembled with three permutations was also determined. This model was validated with statistical significance of the partial ROC (≤ 0.05) and the dataset of new records that were not used in the calibration process in nichetoolbox. The continuous maps of climatic suitability were transformed into a binary map of presence and absence using the threshold of the probability of occurrence that maximizes the ROC metric (Thuiller et al., 2009). The predicted area was compared with the vector data set of land use and vegetation at a scale of 1:250000, Series VII (INEGI, 2018). Finally, the percentage of the predicted and available area to locate *F. pringlei* within Natural Protected Areas was evaluated using vector data (CONANP, 2022).

Results

Development of syconia and pollination mode traits

Phase A1. The syconia begin their development as two buds, in pairs, in the axil of a leaf, each protected by a pubescent basal bract (Figs. 1A, 2A, 3A). The first structure to differentiate at the apex of each syconium is the ostiole, which is composed of a series of overlapping bracts. The bracts are divided in three categories: i) external (or superficial), ii) transitional (located in the middle part of the ostiole and horizontally intertwined), and iii) internal or wall (with an arrangement inclined towards the interior of the syconium cavity) (Fig. 2A). In this phase, the internal bracts occupy the interior cavity of the syconium (Fig. 2A).

Phase A2. The morphological characterization is as described in the first phase, with an increase in the size of the syconium. Inside the syconium, the internal bracts are slightly lax, separated from each other, and their free ends extend towards the interior of the syconium. The stigmas of the pistillate flowers have a uniform distribution in the cavity of the receptacle (Fig. 4A), without developing papillae (Fig. 4B).

Phase B. The growth of the syconium continues, acquiring pale green macules; the basal bracts partially cover it (Fig. 1B). Inside the syconium, the pistillate flowers mature. The stigmas are receptive, forming a cohesive synstigma, with tight contact between the stigmatic branches through the entanglement of long papillae that compose the stigmatic surface (Fig. 4C-D). During this phase, the pollinator wasp enters the syconium (Fig. 1C) through the bracts of the ostiole, since the transition and internal bracts are less compact (Fig. 2B). It is common to find only one wasp, but up to ten were recorded. In some syconia, it can be inferred that a wasp entered because wings were found on the external bracts. Also, non-pollinating wasps were found ovipositing through the wall of the syconia (Fig. 1D).

Phase C1. The syconium continues to increase in size (Fig. 1E) while the fruits and wasps begin to develop. Dead wasps can be found among the bracts (Fig. 2C), and the ostiole is convex (Fig. 3C). Inside the syconium, the cavity is still well-defined, and no difference is detectable between the ovaries containing immature seeds and those containing wasps. The mean total A/O was $0.08 \pm s.d. 0.03$ (range 0.03-0.19). In the middle of the syconium near the ostiole, the mean A/O ratio was higher ($0.06 \pm s.d. 0.03$) than at the base of the syconium ($0.1 \pm s.d. 0.04$). The mean total number of pistillate flowers was $561.5 \pm s.d. 138.8$ and staminate flowers $43.7 \pm s.d. 17.3$.

Phase C2. The syconium continues to grow, and sometimes the external bracts turn reddish (Fig. 1F). The wasp larvae and fruits continue to develop, such that the syconium cavity nearly disappears (Fig. 1G). The transitional bracts are more compact (Fig. 2D), and the ostiole remains convex (Fig. 3D). The exoskeleton of the pollinator female wasp is compressed, dehydrated, and can be found in different degrees of decomposition. Frequently, it is also possible to observe nematodes or fungal hyphae feeding on the wasp.

Phase C3. The ostiole maintains the reddish color on the external bracts (Fig. 1H) and is nearly flattened (Fig. 3E). In the interior of the syconium, the development of the male flowers, fruits, and wasps is nearly complete. The bodies of the new wasps become visible through the ovary walls (Fig. 4E), which acquire a dark coloration, while those containing the fruits are yellowish.



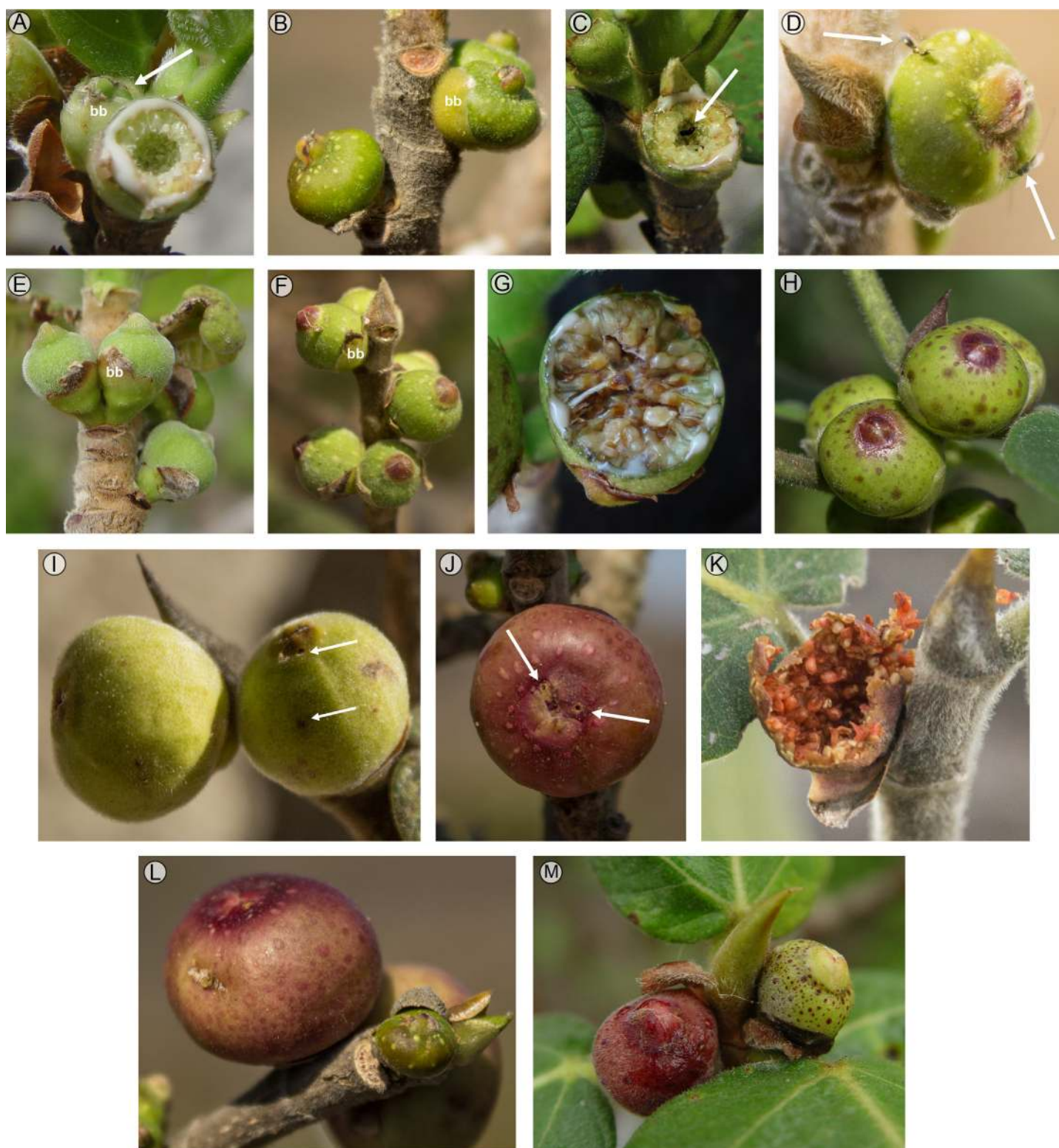


Figure 1: External characteristics of syconia development phases in *Ficus pringlei* S. Watson. A. top view syconium in Phase A1 (arrow, basal bract); B. Phase B; C. foundress female wasp in the syconium interior in Phase B; D. non-pollinating female wasps (arrows) on syconium in Phase B; E. Phase C1; F. Phase C2; G. syconium interior in Phase C2; H. Phase C3; I. syconia in Phase D with two exit tunnels (arrows) excavated by males; J. syconium in Phase E with two exit tunnels (arrows); K. syconium probably bitten by birds. L, M. syconia in different phases of development. bb=basal bracts.

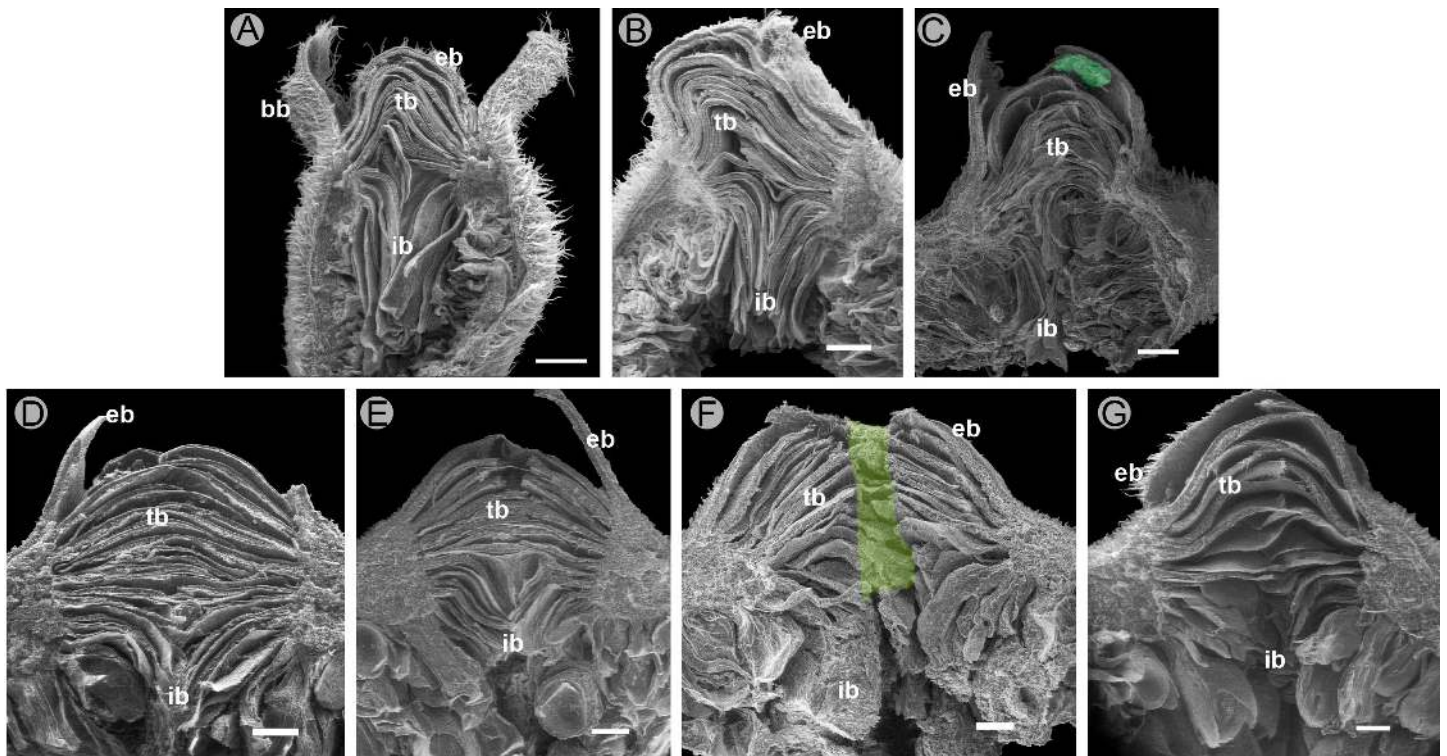


Figure 2: Longitudinal section of the syconium of *Ficus pringlei* S. Watson, in different stages of development. A. Phase A1; B. Phase B; C. Phase C1 with a pollinating wasp (green); D. Phase C2; E. Phase C3; F. Phase D with one exit tunnel excavated by males; G. Phase E. bb=basal bracts, eb=external bracts, tb=transitional bracts, ib=internal bracts. Scale bars: 500 µm.

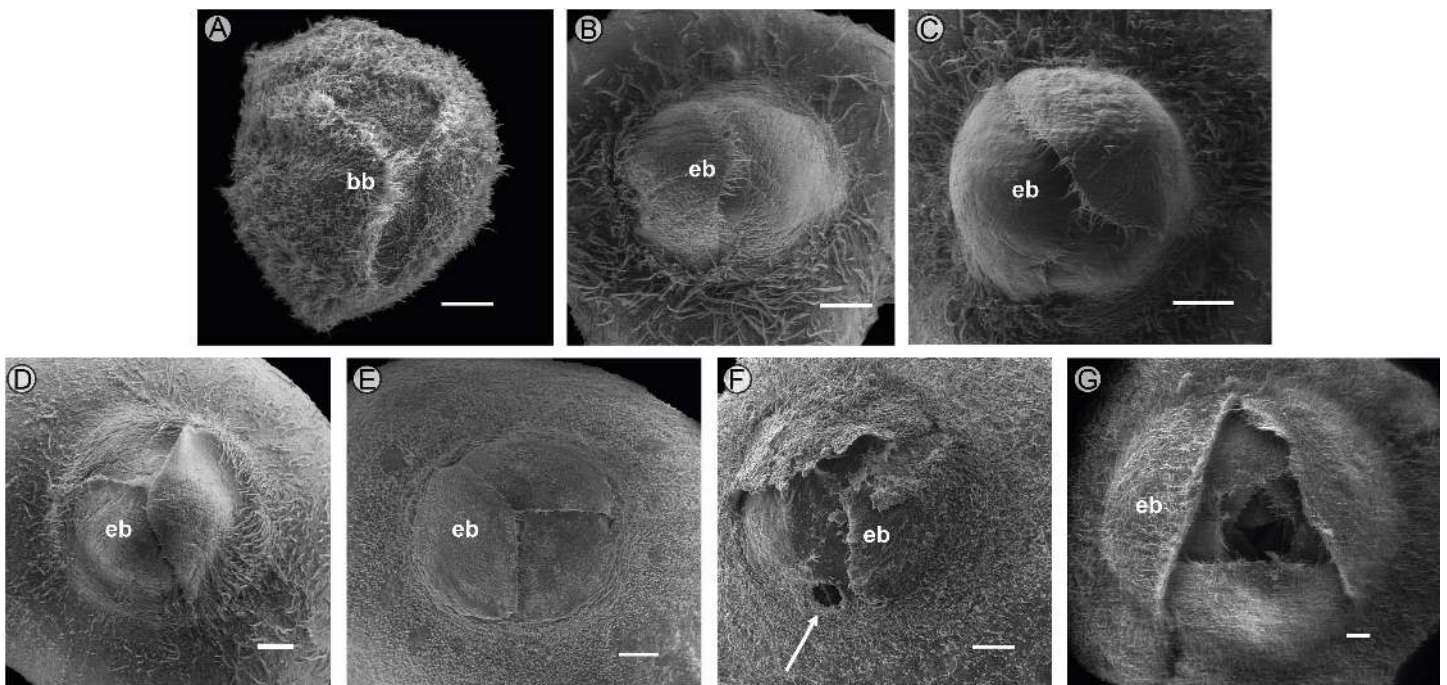


Figure 3: Superior view of the ostiole of *Ficus pringlei* S. Watson, in different stages of development. A. Phase A1; B. Phase B; C. Phase C1; D. Phase C2; E. Phase C3; F. Phase D with one exit tunnel excavated by males; G. Phase E. bb=basal bracts, eb=external bracts. Scale bars: 500 µm.

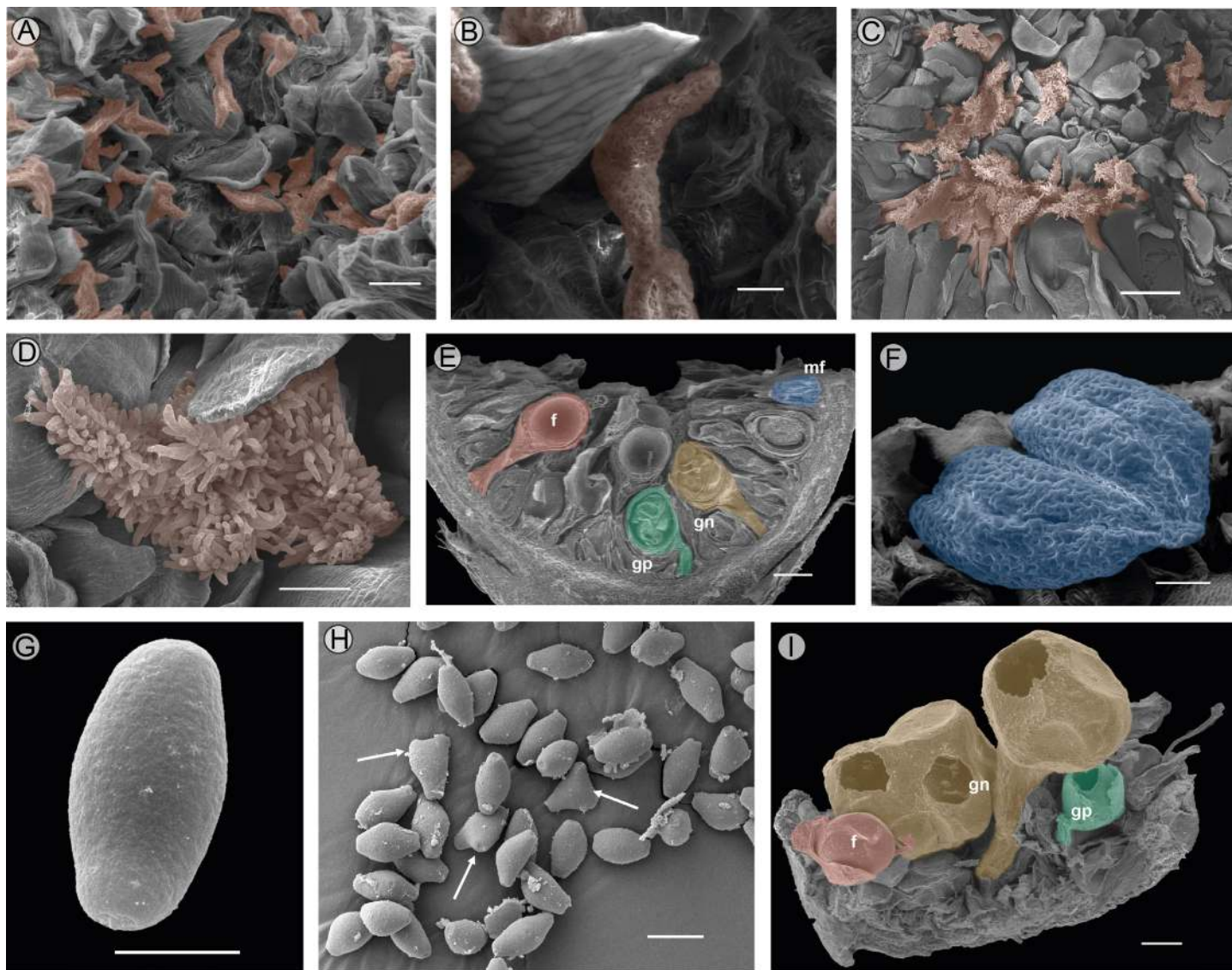


Figure 4: Internal morphology of different stages of development and pollen of *Ficus pringlei* S. Watson. A. stigmata in Phase A2; B. detail of stigmata in Phase A2; C. synstigma formed by the entanglement of styles and stigmatic papillae in Phase B; D. detail of stigmatic papillae; E. longitudinal section of the syconium in Phase C3; F. stamen with mature anther in Phase D; G. truncate obtuse rhombic pollen with psilate ornamentation; H. biporate and triporate pollen grains (arrows); I. section of syconium in Phase E. f=fruit, gn=galls of non-pollinating wasps, gp=galls of pollination wasp, mf=male flower. Scale bars: A=200 µm, B=50 µm, C=500 µm, D=100 µm, E=500 µm, F=100 µm, G=5 µm, H=10 µm, I=500 µm.

Phase D. The syconium turns yellowish and is slightly soft, and the external bracts turn brown (Fig. 1I). The male wasps of the genus *Pegoscapus* sp. are the first to emerge; they break the walls of the ovary with their mandibles and copulate with the females, facilitating their exit from the ovary wall. The male wasps excavate tunnels through the walls of the syconium (Fig. 1I-J) or the ostiole (Figs. 2F, 3F), which allows the fertilized and pollen-bearing female wasps to exit the syconium. Internally, the stamens are mature (Fig. 4F).

The pollen size is minute: mean polar axis 6.46 (range: 5.59–6.98 µm), mean equatorial axis 12.2 (11.23–13.52 µm), and the mean ratio of both axes 0.53 (0.42–0.61 µm). Based on the shape in equatorial view, pollen was mostly rhombic, with psilate ornamentation (Fig. 4G). Pollen grains are biporate or, more rarely, triporate (Fig. 4H).

Phase E. The syconia are reddish (Fig. 1J), glabrescent, with a slightly sweet taste, and the external and internal walls are very soft to the touch (Fig. 1K). The external and

transitional bracts are lax (Figs. 2G, 3G). Internally, the structures within the syconium cavity acquire an overall dark brown color. The staminate and pistillate flowers are senescent; hollow galls are observed without wasps inside them, and the seeds are ripe inside the fruits. The most prominent galls (>1 mm) correspond to non-pollinating wasps (Fig. 4I). The development of the syconia was asynchronous (i.e., we found different development phases of the syconium among different branches of the same tree; Fig. 1L, M).

Pollinating wasps of both sexes of *Pegoscapus* sp. were collected. The females are winged (Fig. 5A), with lamellae on the mandibles (Fig. 5B). They have pollen pockets, coxal combs on their fore coxae, and an elongated depression that was bordered by the comb (Fig. 5C, D). Males, in contrast, are wingless, blind, and do not have structures to transport pollen (Fig. 5E). In phase D, different non-pollinating female wasps were also observed. These non-pollinating insects are larger than pollinating wasps and have red eyes (Fig. 5F).

Ecological niche model

A total of 169 records were obtained with an elevation between 160 and 1806 m (Fig. 6, Appendix). The mean annual precipitation ranged from 661-1644 mm for these records, and the mean annual temperature was between 17.5-28.7 °C. In kuenm, 341 models were generated, and the best model selected was RM 5 and feature class quadratic and product. However, in biomod with 50 models, GBM and GLM performed best, followed by GAM, ANN, and MaxEnt. Four models generated with GBM (three models, ROC=0.814, 0.828, 0.803) and GLM (one model, ROC=0.805) were selected to build the ensemble model. The partial ROC value for the ensemble model was 0.921 ($p < 0.05$).

Nearly three quarters (74%) of the predicted suitable area to found *F. pringlei* was in the states of Jalisco and Michoacán. There was a gap in the center of the distribution, corresponding to the highlands west of the Trans-Mexican Volcanic Belt (TMVB) and Sierra Madre del Sur (SMS) (Fig. 6). The most important variable in the ENM was temperature seasonality (0.42), followed by annual mean temperature, annual precipitation, precipitation of the coldest quarter, precipitation of warmest quarter, annual mean

diurnal range, precipitation seasonality, isothermality, and precipitation of driest month (Fig. 7). However, only with temperature seasonality, a measure of temperature change over the year, and annual mean diurnal range, is possible to identify the climatic differences between the north and the south of the TMVB; both variables are higher in the former region (Fig. 7).

The total predicted area to locate *F. pringlei* corresponds to 21968 km²; however, this is drastically reduced when considering the land cover type. Agriculture and other types of vegetation cover that are not suitable to find this species cover 57% of the total predicted area. Of the remaining area (9651 km²; 43.9% of the total predicted area), primary (1594 km²) and secondary (3832 km²) TDF vegetation were the vegetation types with the largest area, followed by forests of oak (2426 km²) and oak-pine (1799 km²) (Fig. 6). When considering the suitable area accounting for vegetation types, only 1926 km² is contained within Natural Protected Areas. Most of this was north of the TMVB, with 74% recorded in the C.A.D.N.R. 043 Natural Resources Protection Area Forest Protection Zone in Nayarit and 7.8% in the La Primavera Forest Protection Zone and Wildlife Refuge. On the contrary, south of the TMVB, despite the existence of more Protected Natural Areas, only 18.2% (350.5 km²) of the total predicted suitable area was within Natural Protected Areas (Fig. 6).

Discussion

Development of syconia and pollination type

The most evident external morphological differences throughout the development phases of the syconium of *F. pringlei* are the gradual increase in size, changes in color, consistency, and shape of the ostiole (Figs. 1, 3). Transitional bracts are intertwined to form a helicoidal path towards the interior of the syconium cavity, like other representatives of the section *Americanae* (Miq.) Corner (Ramírez, 1974; Verkerke, 1989). Phase B, which is critical for pollination and lasts a few days, is mainly identified by the internal characteristics of the syconium and by the entry of the wasps. One of the most notorious internal characters was the presence of the receptive synstigma which, as in some species of the section *Americanae* (Teixeira et al., 2018, 2021), does not form a continuous surface. Rather,



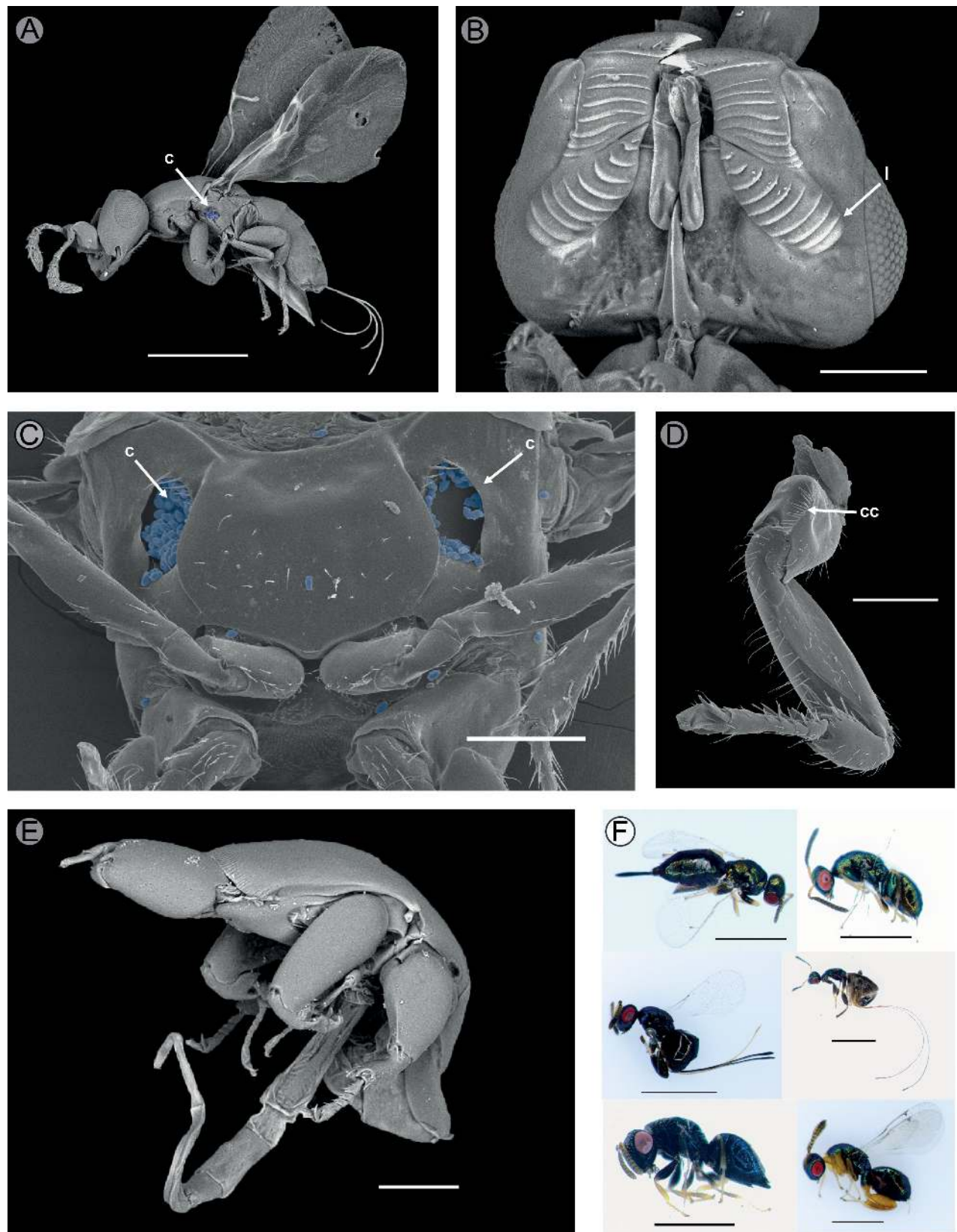


Figure 5: Pollinating (*Pegoscapus* sp.) and non-pollinating wasps of *Ficus pringlei* S. Watson. A. female wasp of *Pegoscapus* sp. (corbiculae, arrow); B. detail of the mandibles (arrow) of the female *Pegoscapus* sp.; C. corbiculae of *Pegoscapus* sp., pollen in blue; D. coxal combs on fore coxae and elongated depression bordered by the comb in *Pegoscapus* sp.; E. male wasp of *Pegoscapus* sp.; F. female non-pollinating wasps. c=corbiculae, cc=coxal combs, l=lamellas. Scale bars: A=500 μ m, B=100 μ m, C=100 μ m, D=100 μ m, E=200 μ m, F=1 mm.

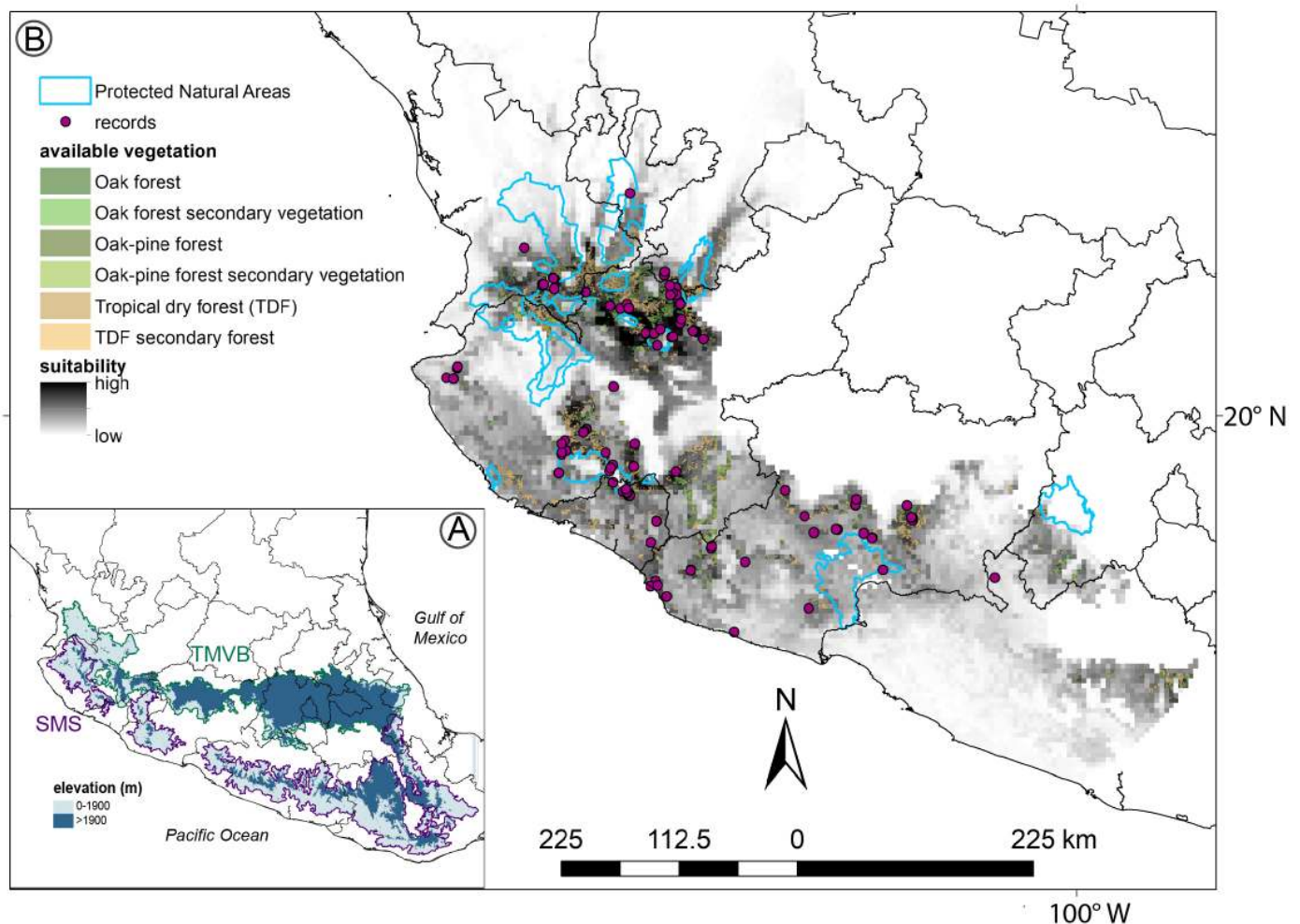


Figure 6: Potential geographical distribution of *Ficus pringlei* S. Watson, under current climate conditions. A. Trans-Mexican Volcanic Belt (TMVB) and Sierra Madre del Sur (SMS) biogeographical provinces; B. occurrence records, current potential geographical distribution, available vegetation, and Protected Natural Areas for *F. pringlei*.

the cohesion of the synstigma is due to the intertwining of the stigmatic branches and papillae of a few flowers. The synstigma may act to distribute pollen tubes to the flowers located near those that have been pollinated, thus modifying the fertilization pattern, and maximizing the male reproductive success of the plant when pollen deposition is limiting (Teixeira et al., 2018, 2021).

Although the pollinating wasps of *F. pringlei* have not been described, it belongs to the genus *Pegoscapus* Cameron, 1906, since unlike the other genus of wasps Agaonidae found in the Americas (*Tetrapus* Mayr, 1885), the mandibular appendices are lamellas and have external and coxal corbiculae (Fig. 5B-C) (Ramírez, 1970b). A single foundress wasp was found within the syconia, but some-

times as many as ten were recorded. An extraordinarily high number of female foundresses per syconium could be a consequence of the asynchrony in the reproductive events among the population of *Ficus* trees, resulting in a very high temporal variability in syconium availability. This variation often has consequences for the reproductive success of both the wasps and the plants. When more than one foundress wasp is found, females may have fewer offspring and females (Herre, 1989), which may imply an increase in interbreeding of the new generation of wasps, but a decrease in the potential for pollen dispersal per syconium. Therefore, pollinators may use the density of broken wings in the ostiole to decide which syconium to enter, preferring an empty one (Ramya et al., 2011).

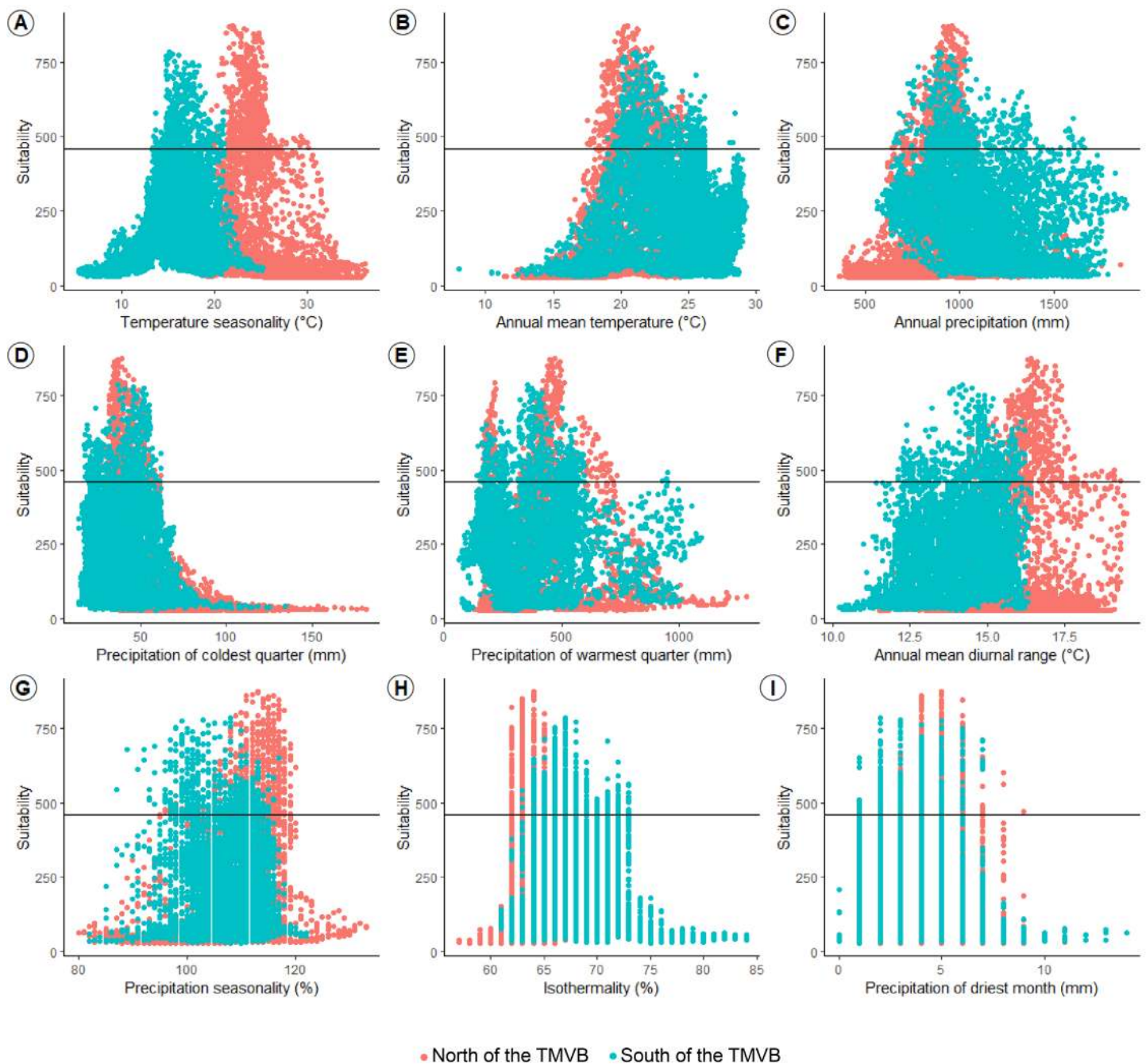


Figure 7: Habitat suitability and environmental variables in the ensemble model of *Ficus pringlei* S. Watson. The horizontal line indicates the value of the threshold to transform the model on presence and absence data. TMVB=Trans-Mexican Volcanic Belt.

Conversely, Phase D can be inferred by external characteristics of the syconium, such as slightly soft consistency to the touch, yellow coloration, and the presence of holes in the syconium wall or in the ostiole, through which the female wasps leave the syconium. Male tunneling activity near the ostiole may be driven by a higher proportion

of staminate flowers near the ostiole so that pollen can be loaded on females prior to their departure (Galil and Eisikowitch, 1968). From direct observations in fresh syconia, active pollination behavior was recorded in *F. pringlei*. These observations are supported by a low A/O ratio, the presence of synstigma and psilate pollen, as well as mor-

phenological traits to collect and store pollen efficiently in female wasps such as big sternal corbiculae, coxal corbiculae, and coxal combs. Pollen has been studied for few American species (e.g., Ibarra-Manríquez and Martínez-Hernández, 1997), but *F. pringlei* has pollen shape and ornamentation that is similar to other species that have active pollination (Wang et al., 2014).

A great diversity of non-pollinating wasps was found, some of which colonized the syconia during Phase B. Large galls generated by these wasps were also found. Gall size is usually characteristic of each wasp species and may structure the entire community of wasps occupying the syconium (Cardona et al., 2013; Compton et al., 2018) and even affect the fitness of the host *Ficus* tree (Zhang and Li, 2020) or the asynchrony of the development of the syconia (Krishnan and Borges, 2014). Therefore, it is essential to delve into the diversity, development, colonization, and temporal variation of these non-pollinating wasps.

Phase E was the most contrasting since the syconium turned reddish and was slightly sweet. The contrast between the syconium color and the foliage suggests that it may be dispersed by birds (Lomáscolo et al., 2008) over long distances (Shanahan et al., 2001). In some New World species, in which the seeds are primarily bird-dispersed, the production and maturation of syconia are less synchronous than in bat-dispersed syconia (Herre, 1996). Indeed, although determining the phenological strategy was not the objective of this work, the field observations throughout the study show that asynchrony in the crown of an individual is possible, since syconia in different phases of development can be recorded on branches (Fig. 1A, L-M). This strategy may allow the persistence of pollinator populations and increase opportunities to provide and receive pollen from other trees in small populations and highly seasonal environments (Ramírez, 1970a; Janzen, 1979; Gates and Nason, 2012; Smith and Bronstein, 1996).

Ecological niche model

In the search for presence data, *F. pringlei* is frequently sympatric with *F. cotinifolia* Kunth and they are morphologically similar in the absence of figs. This could lead to erroneous determinations of both species in the herbarium specimens and in the field. However, as Ibarra-Manríquez

et al. (2012) point out, *F. pringlei* can be distinguished because the leaves are always pubescent on the abaxial side, the tertiary venation is very conspicuous, and the syconium has a convex ostiole in the early stages of development.

Ficus pringlei is found mainly in the TDF, a vegetation type that has suffered extensive damage due to its widespread conversion to farmland, accompanied by uncontrolled cattle grazing and fires (Miles et al., 2006). The situation is critical in Mexico, where 73% of the original TDF has been lost and protected areas within this forest type are scarce (Trejo and Dirzo, 2000). In addition to land-use changes, climate change can reduce areas of this vegetation type, as is mentioned by Prieto-Torres et al. (2016) for the Balsas River Basin. Specifically, in the potential distribution area of *F. pringlei*, 145 km² of primary and secondary TDF vegetation was transformed into unsuitable habitats for this species, such as agricultural land or grassland, between 2016-2018 (INEGI, 2016; 2018). Therefore, it is important to establish strategies for conserving this endemic species, which is not currently recognized in any risk category of The International Union for Conservation of Nature of Threatened Species Red List (IUCN, 2021) or NOM-059-SEMARNAT-2010 (SEMARNAT, 2010), and only 20% of its potential suitable areas under current conditions is contained within Natural Protected Areas (Fig. 6).

Temperature seasonality was the main environmental factor that explained the distribution of *F. pringlei*. This climatic variable and others that were significant (e.g., annual precipitation) have been typically associated with *Ficus* species since they are mostly distributed in tropical forests, and they rarely exceed 2500 m elevation due to their low tolerance of low temperatures (Ramírez, 1969; Janzen, 1979; Ibarra-Manríquez et al., 2012). There was more significant variation in annual mean temperature seasonality north of the TMVB (Fig. 7), which could reflect a greater seasonal variation and affect plant traits, pollinator and parasitic fig wasp reproduction, as well as seed production (Peng et al., 2010; Krishnan et al., 2014; Zhang et al., 2019). For example, in the monoecious species *F. racemosa* L., although levels of within-tree reproductive asynchrony were similar across seasons, asynchrony had variable effects on pollinator and parasite reproduction, which could be linked to seasonal variation in syconium size (Krishnan et al., 2014).



However, future studies are required to corroborate the validity of these associations with seasonality in *F. pringlei*.

Finally, as has been observed in other groups of plants (e.g., Anguiano-Constante et al., 2021; López-Barra et al., 2021), it is possible that the TMVB, in addition to promoting a disjunct distribution in the species, acts as a geographical barrier to gene flow. In that case, it is essential to evaluate this possible isolate for *F. pringlei*, since it could help guide conservation efforts (Coates et al., 2018).

Conclusions

This study showed that the area suitable to locate *F. pringlei* is only distributed in western Mexico, mainly in TDF and, to a lesser extent, oak and oak-pine forest. Their syconium developmental phases were consistent with those described previously for other monoecious species in the genus. Likewise, through direct observations and different characters (e.g., synstigma, type of pollen, anther to ovule ratio, and the existence of coxal combs and corbiculae in the wasps), it was determined that the pollination behavior performed by wasps is active. Furthermore, it leaves open a series of questions that will be interesting to answer in the future. These include quantifying the degree of asynchrony of the syconia at the individual or population level in localities with different seasonal variations and its effect on plant traits (e.g., seed production and gene flow), describing pollinator and non-pollinating fig wasps and their reproduction, and identifying frugivorous fauna that depends on syconia availability in Phase E and its impact on seed dispersal. Additionally, it will be important to evaluate the TMVB's role as a possible barrier shaping the genetic structure of *F. pringlei* and therefore, guide the conservation units to the species.

Author contributions

APRC and GIM conceived and designed the study. APRC, APCR, GCT, and GIM contributed to acquiring and interpreting data. APRC and APCR developed ecological niche modeling analyses. APRC wrote the original draft. GIM wrote, reviewed, and edited the final document. All authors have read and agreed with the published version of the manuscript.

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Appendix: Presence data of *Ficus pringlei* S. Watson in Mexico and value of nine bioclimatic variables. Bio1=annual mean temperature (°C), Bio2=annual mean diurnal range (°C), Bio3=isothermality (%), Bio4=temperature seasonality (°C), Bio12=annual precipitation (mm), Bio14=precipitation of driest month (mm), Bio15=precipitation seasonality (%), Bio18=precipitation of warmest quarter (mm), Bio19=precipitation of the coldest quarter (mm). NA= No voucher since the individual was found without syconia. * New records.

State	Latitude	Longitude	Altitude	Vouchers (Herbarium)	Bio1	Bio2	Bio3	Bio4	Bio12	Bio14	Bio15	Bio18	Bio19
Colima	18.933611	-103.6475	329	<i>G. Ibarra 5709</i> (MEXU)	25.2	14.3	70	15.5	994	3	111	354	51
Colima	19.107958	-103.5979	515	<i>G. Ibarra 7159</i> (MEXU)*	23.9	14.1	69	14.9	996	2	110	364	47
Colima	19.108611	-103.5981	690	<i>G. Ibarra 5837</i> (IEB)	25.2	14.3	70	15.5	994	3	111	354	51
Colima	19.110767	-103.5992	543	NA*	21.1	13.9	68	14.8	952	3	108	370	41
Colima	19.110811	-103.5992	544	NA*	21.1	13.9	68	14.8	952	3	108	370	41
Colima	19.323033	-103.8308	691	<i>G. Ibarra 7162</i> (MEXU)*	21.1	13.9	68	14.8	952	3	108	370	41
Colima	19.324727	-103.8396	760	<i>G. Ibarra 7163</i> (MEXU)*	21.1	13.9	68	14.8	952	3	108	370	41
Colima	19.329008	-103.8311	886	<i>N. González 51</i> (IEB)	21.1	13.9	68	14.8	952	3	108	370	41
Colima	19.329206	-103.8344	878	<i>G. Ibarra 7164</i> (MEXU)*	21.1	13.9	68	14.8	952	3	108	370	41
Colima	19.332917	-103.8313	900	<i>M. Santana 2934</i> (IBUG)	21.1	13.9	68	14.8	952	3	108	370	41
Colima	19.342897	-103.8356	1078	<i>G. Ibarra 7165</i> (MEXU)*	25.6	13.2	71	14.8	867	2	108	501	24
Colima	19.343841	-103.8360	1079	NA*	25.2	13.7	71	13.6	873	2	110	360	38
Colima	19.346638	-103.8412	1102	<i>G. Ibarra 7241</i> (MEXU)*	25.2	13.7	71	13.6	873	2	110	360	38
Colima	19.346944	-103.8492	1180	<i>T. Cochrane 11757</i> (XAL)	21.2	13.8	67	14.8	1025	3	108	395	46
Colima	19.347004	-103.8394	976	NA*	25.2	13.7	71	13.6	873	2	110	360	38
Colima	19.348640	-103.8487	1200	<i>G. Ibarra 7166</i> (MEXU)*	25.2	13.7	71	13.6	873	2	110	360	38
Colima	19.351339	-103.8503	1189	<i>N. González 60</i> (IEB)	21.1	13.9	68	14.8	952	3	108	370	41
Colima	19.354444	-103.8514	1204	<i>R. Cuevas 5845</i> (IEB)	20.1	13.8	67	14.9	1052	4	104	416	51
Colima	19.360278	-103.8861	1420	<i>R. Cuevas 4041</i> (IBUG)	21.1	13.9	68	14.8	952	3	108	370	41
Colima	19.364167	-103.8600	1400	<i>G. Ibarra 5792</i> (IEB)	20.1	13.8	67	14.9	1052	4	104	416	51
Colima	19.364750	-103.8582	1404	<i>N. González 56</i> (IEB)	21.1	13.9	68	14.8	952	3	108	370	41
Colima	19.378889	-103.8589	1508	<i>G. Ibarra 6161</i> (MEXU)	25.2	14.3	70	15.5	994	3	111	354	51
Colima	19.436944	-103.9722	1600	<i>M. Santana 5215</i> (MEXU)	20.3	13.9	67	15.1	959	3	105	378	45
Colima	19.436944	-103.9722	1728	<i>M. Santana 5215</i> (MEXU)	23.9	14.1	69	14.9	996	2	110	364	47
Guerrero	18.636383	-100.7016	531	<i>F. González 6119</i> (MEXU)	28.4	15.1	62	20.8	946	1	116	201	16
Jalisco	19.517972	-104.4402	527	<i>F. Guzmán 185</i> (ZEA)	23.2	16.9	63	25.3	882	6	117	433	41
Jalisco	19.528363	-103.4331	1039	<i>L. Villareal 12726</i> (IBUG)	20.1	16.5	63	24.6	917	3	112	214	32
Jalisco	19.530258	-104.0024	1575	<i>G. Nieves 542</i> (IBUG)	20.4	16.4	64	22.8	997	6	110	472	46
Jalisco	19.560223	-103.9914	1214	<i>R. Cuevas 3751</i> (ZEA)	19.9	16.5	62	24.8	925	5	110	214	36
Jalisco	19.572638	-103.7969	1248	<i>M. Santana 7044</i> (ZEA)	21.6	17.3	63	25.1	916	5	117	196	33
Jalisco	19.583572	-103.9754	822	<i>A. Rodríguez 2054</i> (MEXU)	19.9	16.5	62	24.8	925	5	110	214	36
Jalisco	19.674722	-104.4144	1046	<i>C. Gómez 9</i> (IBUG)	23.2	17.1	63	24.6	939	5	115	203	36





Appendix. Continuation

State	Latitude	Longitude	Altitude	Vouchers (Herbarium)	Bio1	Bio2	Bio3	Bio4	Bio12	Bio14	Bio15	Bio18	Bio19
Jalisco	19.687778	-104.0347	1144	<i>F. Santana 6007</i> (IBUG)	18.6	16	62	23.5	976	5	114	479	35
Jalisco	19.692222	-104.4122	1267	<i>F. Santana 6916</i> (IBUG)	20.4	16.4	64	22.8	997	6	110	472	46
Jalisco	19.703722	-104.3720	1125	<i>G. Ibarra 7120</i> (MEXU)*	19.3	16.1	63	23.1	971	5	114	473	38
Jalisco	19.764722	-104.4142	1004	<i>U. Ramírez 7</i> (IBUG)	19.4	16.3	62	24.5	957	5	113	459	38
Jalisco	19.765305	-103.7871	1286	<i>E. Lott 424</i> (MO)	23.2	17.9	63	25.6	878	5	116	195	31
Jalisco	19.790556	-104.3856	1157	<i>R. Hernández 18</i> (IBUG)	19.3	16.1	63	23.1	971	5	114	473	38
Jalisco	19.858333	-104.2250	1033	<i>M. Santana 6747</i> (IBUG)	21.9	16.8	63	23.7	973	4	115	213	37
Jalisco	19.871906	-104.2177	1211	<i>G. Ibarra 7248</i> (MEXU)*	22.5	14.9	67	17.7	884	6	100	370	52
Jalisco	19.885325	-104.2008	1229	<i>G. Ibarra 7249</i> (MEXU)*	20.9	14.1	67	15.3	1207	4	106	488	62
Jalisco	19.885436	-104.2006	1229	NA*	21.7	15.6	62	22.8	803	8	95	382	51
Jalisco	19.887103	-104.1978	1243	<i>G. Ibarra 7250</i> (MEXU)*	21.7	15.6	62	22.8	803	8	95	382	51
Jalisco	20.240271	-103.9675	1417	NA*	23.1	15	66	17.9	815	4	99	338	49
Jalisco	20.240274	-103.9676	1413	<i>G. Ibarra 7247</i> (MEXU)*	22.3	14.6	67	16.9	963	5	102	389	55
Jalisco	20.307374	-105.3391	644	<i>G. Ibarra 7167</i> (MEXU)*	21.6	17.3	63	25.1	916	5	117	196	33
Jalisco	20.307389	-105.3406	652	<i>T. Cochrane 11941</i> (F)	21.5	17.4	62	28.8	715	6	112	322	41
Jalisco	20.314718	-105.4020	560	<i>G. Ibarra 7168</i> (MEXU)*	21.6	17.3	63	25.1	916	5	117	196	33
Jalisco	20.398217	-105.3085	685	NA*	21	17.2	63	24.9	929	5	116	445	35
Jalisco	20.406356	-105.3045	644	<i>G. Ibarra 7171</i> (MEXU)*	22.1	17.5	63	25.3	903	5	117	196	32
Jalisco	20.406438	-105.3043	641	<i>G. Ibarra 7180</i> (MEXU)*	20.9	16.8	63	24.2	918	4	113	216	33
Jalisco	20.409456	-105.3049	618	NA*	21.6	17.3	63	25.1	916	5	117	196	33
Jalisco	20.409862	-105.3050	615	NA*	23	13.6	69	19.4	1644	3	115	946	45
Jalisco	20.590868	-103.5925	1772	<i>L. Villareal 10072</i> (IBUG)	21.9	16.8	63	23.7	973	4	115	213	37
Jalisco	20.643352	-103.1967	1485	<i>R. Acevedo 1541</i> (XAL)	19	16	62	24.3	932	5	112	454	36
Jalisco	20.657830	-103.4735	1806	<i>L. Hernández 834</i> (IBUG)	21.9	16.8	63	23.7	973	4	115	213	37
Jalisco	20.662286	-103.4579	1786	<i>G. González 68</i> (MEXU)	21.9	16.8	63	23.7	973	4	115	213	37
Jalisco	20.687573	-103.6920	1400	<i>K. Manzano 6</i> (IBUG)	19.9	16.5	62	24.8	925	5	110	214	36
Jalisco	20.689918	-103.6232	1447	NA*	17.5	15.6	62	23.7	934	6	111	462	34
Jalisco	20.690276	-103.6227	1464	NA*	23	13.6	69	19.4	1644	3	115	946	45
Jalisco	20.691850	-103.6199	1457	NA*	22.8	13.7	69	19.8	1613	4	115	937	45
Jalisco	20.692013	-103.6198	1550	<i>O. Reyna 560</i> (IBUG)	19.9	16.5	62	24.8	925	5	110	214	36
Jalisco	20.692030	-103.6197	1457	NA*	23.1	13.4	70	19.4	1607	3	116	918	45
Jalisco	20.692294	-103.6196	1460	NA*	22.8	13.7	69	19.8	1613	4	115	937	45
Jalisco	20.692476	-103.6192	1522	NA*	22.8	13.7	69	19.8	1613	4	115	937	45



Appendix. Continuation

State	Latitude	Longitude	Altitude	Vouchers (Herbarium)	Bio1	Bio2	Bio3	Bio4	Bio12	Bio14	Bio15	Bio18	Bio19
Jalisco	20.692552	-103.6193	1457	<i>G. Ibarra 7245</i> (MEXU)*	22.8	13.7	69	19.8	1613	4	115	937	45
Jalisco	20.692647	-103.6192	1456	<i>G. Ibarra 7246</i> (MEXU)*	22.8	13.7	69	19.8	1613	4	115	937	45
Jalisco	20.695514	-103.6944	1367	<i>L. Villareal 9911</i> (IBUG)	21.9	16.8	63	23.7	973	4	115	213	37
Jalisco	20.701592	-103.2559	1136	NA*	21.9	16.8	63	23.7	973	4	115	213	37
Jalisco	20.703143	-103.2566	1122	<i>G. Ibarra 7072</i> (MEXU)*	21.9	16.8	63	23.7	973	4	115	213	37
Jalisco	20.703825	-103.2570	1117	NA*	21.9	16.8	63	23.7	973	4	115	213	37
Jalisco	20.706272	-103.2695	1099	NA*	21.9	16.8	63	23.7	973	4	115	213	37
Jalisco	20.707063	-103.2707	1079	NA*	21.9	16.8	63	23.7	973	4	115	213	37
Jalisco	20.707828	-103.2715	1072	NA*	21.2	16.6	64	22.9	944	4	116	212	36
Jalisco	20.707894	-103.2718	1071	<i>G. Ibarra 7071</i> (MEXU)*	21.9	16.8	63	23.7	973	4	115	213	37
Jalisco	20.710182	-103.2862	1500	<i>H. Hernández 78</i> (IBUG)	19.9	16.5	62	24.8	925	5	110	214	36
Jalisco	20.713348	-103.2775	1074	<i>G. Ibarra 7242</i> (MEXU)*	24.2	15.3	67	19	760	3	97	315	50
Jalisco	20.725278	-103.5642	1603	<i>J. Calzada 13533</i> (NY)	19.9	16.5	62	24.8	925	5	110	214	36
Jalisco	20.776316	-103.4006	1556	NA*	19.3	16.1	63	23.1	971	5	114	473	38
Jalisco	20.808611	-103.3875	1448	<i>A. Frías 347</i> (IBUG)	19.9	16.5	62	24.8	925	5	110	214	36
Jalisco	20.900570	-103.8384	1271	NA*	22.1	17.5	63	25.3	903	5	117	196	32
Jalisco	20.901333	-103.8370	1256	NA*	22.1	17.5	63	25.3	903	5	117	196	32
Jalisco	20.901413	-103.9082	1338	<i>G. Ibarra 7177</i> (MEXU)*	21	17.2	63	24.9	929	5	116	445	35
Jalisco	20.902248	-103.8368	1265	NA*	22.1	17.5	63	25.3	903	5	117	196	32
Jalisco	20.902406	-103.8369	1268	NA*	21	17.2	63	24.9	929	5	116	445	35
Jalisco	20.903333	-103.8369	1200	<i>G. Ibarra 5380</i> (MEXU)	20.9	17.3	63	25.4	893	4	118	434	34
Jalisco	20.906867	-103.8434	1265	<i>G. Cornejo 5138</i> (MEXU)*	21.6	17.3	63	25.1	916	5	117	196	33
Jalisco	20.909075	-103.8405	1262	NA*	19.3	16.1	63	23.1	971	5	114	473	38
Jalisco	20.909080	-103.8412	1259	<i>G. Ibarra 7122</i> (MEXU)*	19.3	16.1	63	23.1	971	5	114	473	38
Jalisco	20.909175	-103.8410	1254	NA*	19.3	16.1	63	23.1	971	5	114	473	38
Jalisco	20.909321	-103.8401	1246	NA*	19.3	16.1	63	23.1	971	5	114	473	38
Jalisco	20.909976	-103.8409	1231	NA*	19.3	16.1	63	23.1	971	5	114	473	38
Jalisco	20.922480	-103.9953	1405	NA*	21	17.2	63	24.9	929	5	116	445	35
Jalisco	20.922499	-103.9956	1396	<i>G. Ibarra 7178</i> (MEXU)*	21	17.2	63	24.9	929	5	116	445	35
Jalisco	20.936424	-103.3966	1266	<i>G. Ibarra 7231</i> (MEXU)*	22.5	14.9	67	17.7	884	6	100	370	52
Jalisco	20.936444	-103.3958	1266	<i>G. Ibarra 7229</i> (MEXU)*	24.4	14.3	68	16.1	1574	2	113	559	57
Jalisco	20.937624	-103.3964	1256	NA*	21.6	15.2	66	18.3	962	3	101	398	48
Jalisco	20.937630	-103.3963	1256	NA*	18.9	13.7	66	14.7	1049	5	100	420	56

Appendix. Continuation

State	Latitude	Longitude	Altitude	Vouchers (Herbarium)	Bio1	Bio2	Bio3	Bio4	Bio12	Bio14	Bio15	Bio18	Bio19
Jalisco	20.937795	-103.3944	1250	NA*	21.9	14.6	67	16.4	759	3	102	316	39
Jalisco	20.938847	-103.8522	1135	<i>L. Villareal 6092</i> (IBUG)	20.9	16.8	63	24.2	918	4	113	216	33
Jalisco	20.961802	-103.4089	1337	NA*	19.9	16.2	64	22	893	4	116	433	35
Jalisco	21.016667	-103.4833	1247	<i>P. Carrillo 1842</i> (NY)	18	15.9	62	24.6	911	6	111	445	35
Jalisco	21.017151	-103.4299	1231	NA*	20.7	14.5	66	15.9	864	6	96	367	54
Jalisco	21.026257	-103.4570	1216	NA*	22.5	14.5	67	16.4	981	4	104	398	54
Jalisco	21.026841	-103.4500	1259	NA*	20.7	14.5	66	15.9	864	6	96	367	54
Jalisco	21.028184	-103.4531	1270	NA*	20.7	14.5	66	15.9	864	6	96	367	54
Jalisco	21.030819	-103.4607	1234	<i>G. Ibarra 7240</i> (MEXU)*	20.1	14.1	66	15.7	938	5	98	382	53
Jalisco	21.032359	-103.4610	1245	NA*	22.3	14.6	67	16.9	963	5	102	389	55
Jalisco	21.032431	-103.4610	1246	<i>G. Ibarra 7232</i> (MEXU)*	20.5	14.8	66	17.2	724	4	89	321	49
Jalisco	21.033618	-103.4611	1246	NA*	20.9	14.1	67	15.3	1207	4	106	488	62
Jalisco	21.037829	-104.2045	1403	<i>J. Rzedowski 14270</i> (MEXU)	19.6	16.5	62	24.3	957	5	114	459	37
Jalisco	21.071399	-103.4339	884	NA*	19.3	16.1	63	23.1	971	5	114	473	38
Jalisco	21.071438	-103.4336	904	NA*	19.9	16.2	64	22	893	4	116	433	35
Jalisco	21.091460	-103.4867	1650	<i>P. Carrillo 1253</i> (IBUG)	23.2	17.9	63	25.6	878	5	116	195	31
Jalisco	21.868783	-103.8279	1660	<i>A. Flores 1846</i> (XAL)	22.6	17.6	63	25	902	5	116	200	32
Michoacán	18.698414	-103.3047	1390	NA*	26.2	15.8	69	14.8	858	1	113	161	19
Michoacán	18.701373	-103.3063	1412	NA*	28.5	15.2	65	18.3	678	3	109	141	23
Michoacán	18.70140	-101.6610	552	NA*	25.4	12.4	72	14.9	981	1	109	518	26
Michoacán	18.701412	-101.6605	552	NA*	25.8	12.4	72	15.7	1048	2	112	551	22
Michoacán	18.701458	-101.6601	552	NA*	22	14.3	71	12.7	1033	3	104	399	34
Michoacán	18.701548	-101.6608	552	NA*	23.5	16	68	16.6	1088	3	113	230	28
Michoacán	18.701608	-101.6605	552	NA*	26.1	12.5	72	15.9	1084	2	113	580	21
Michoacán	18.893513	-103.1275	1215	NA*	20	16.2	68	17	1146	4	111	240	32
Michoacán	18.905065	-103.1258	1253	NA*	21	14.4	66	15.4	1230	4	110	252	30
Michoacán	18.971535	-101.7535	684	NA*	26.9	16	68	17.2	861	1	112	171	18
Michoacán	18.973418	-101.7495	735	<i>G. Ibarra 7176</i> (MEXU)*	23.5	16	68	16.6	1088	3	113	230	28
Michoacán	19.008585	-101.8227	838	NA*	23.5	16	68	16.6	1088	3	113	230	28
Michoacán	19.041450	-102.0484	416	NA*	26.9	16	68	17.2	861	1	112	171	18
Michoacán	19.116658	-101.4056	1029	NA*	19.7	12.9	64	14.8	1323	4	104	260	36
Michoacán	19.135327	-101.4020	1098	NA*	24.6	14.8	66	15.9	944	1	117	178	19
Michoacán	19.139412	-101.4041	1052	<i>G. Ibarra 7137</i> (MEXU)*	21	13	64	16.1	1309	5	102	536	43





Appendix. Continuation

State	Latitude	Longitude	Altitude	Vouchers (Herbarium)	Bio1	Bio2	Bio3	Bio4	Bio12	Bio14	Bio15	Bio18	Bio19
Michoacán	19.141862	-101.4143	1132	NA*	21.3	12.4	65	15.2	1328	4	100	514	48
Michoacán	19.243748	-101.8925	739	<i>G. Ibarra 7146</i> (MEXU)*	26.2	12.3	73	13.3	1084	1	113	596	31
Michoacán	19.243762	-101.8931	746	NA*	28	15.8	66	18.2	757	2	107	158	24
Michoacán	19.244160	-101.8939	747	<i>I. Ek 415 *</i>	21	13	64	16.1	1309	5	102	536	43
Michoacán	19.245014	-101.8927	738	NA*	26	12.3	73	15.3	1044	2	110	566	26
Michoacán	19.248795	-101.8939	768	NA*	28.7	15.3	67	16.6	661	2	105	132	30
Michoacán	19.293950	-101.8842	986	<i>G. Ibarra 7175</i> (MEXU)*	26.9	16	68	17.2	861	1	112	171	18
Michoacán	18.178756	-102.9316	937	<i>N. González 18</i> (MEXU)	26.9	16	68	17.2	861	1	112	171	18
Michoacán	18.378799	-102.2964	943	<i>J. Soto 1711</i> (MEXU)	24.9	16	68	16.5	1004	3	112	215	23
Michoacán	18.478611	-103.5131	623	<i>B. Guerrero 879</i> (MEXU)	26.9	16	68	17.2	861	1	112	171	18
Michoacán	18.565829	-103.6492	160	<i>G. Ibarra 5968</i> (MEXU)	21	13	64	16.1	1309	5	102	536	43
Michoacán	18.569722	-103.5906	553	<i>B. Guerrero 224</i> (MEXU)	21	13	64	16.1	1309	5	102	536	43
Michoacán	18.610556	-103.6053	695	<i>B. Guerrero 869</i> (INIREB)	23.7	15.8	70	15	1043	3	111	211	30
Michoacán	18.698597	-103.3043	1389	<i>N. González 8</i> (MEXU)	28.7	15.3	67	16.6	661	2	105	132	30
Michoacán	18.701389	-101.6606	613	<i>G. Ibarra 2013</i> (MEXU)	25.5	15.7	69	14.7	872	1	113	167	20
Michoacán	18.771192	-102.8399	1355	<i>J. Soto 9234</i> (MEXU)	28.7	15.3	67	16.6	661	2	105	132	30
Michoacán	18.888501	-103.1327	1195	<i>J. Rzedowski 16670</i> (ENCB)	28.7	15.3	67	16.6	661	2	105	132	30
Michoacán	18.905000	-103.1258	1233	<i>G. Ibarra 2008</i> (MEXU)	28.2	15.6	66	18	708	2	106	150	25
Michoacán	18.969167	-101.7539	650	<i>G. Ibarra 5962</i> (MEXU)	21.3	12.4	65	15.2	1328	4	100	514	48
Michoacán	19.017036	-102.2502	303	<i>R. Torres 1538</i> (MEXU)	21.3	12.4	65	15.2	1328	4	100	514	48
Michoacán	19.042889	-102.0615	401	<i>M. Mendez 2009</i> (MEXU)	26.2	15.8	69	14.8	858	1	113	161	19
Michoacán	19.149444	-102.3292	1011	<i>WM. Leavenworth 1626</i> (MO)	28.7	15.3	67	16.6	661	2	105	132	30
Michoacán	19.243611	-101.4533	1750	<i>D. Ramírez s. n.</i> (MEXU)	24.5	15.6	69	14.3	924	2	112	179	23
Michoacán	19.369587	-102.4967	1209	<i>J. Soto 2457</i> (MEXU)	28.7	15.3	67	16.6	661	2	105	132	30
Nayarit	21.068167	-104.4768	1038	<i>O. Téllez 12709</i> (MEXU)	19.5	15.4	64	22.6	913	4	112	411	53
Nayarit	21.092283	-104.5746	904	<i>V. Rudd 3017</i> (MEXU)	19.5	15.4	64	22.6	913	4	112	411	53
Nayarit	21.100000	-104.5781	970	<i>M. Harker 723</i> (IBUG)	20.9	14.9	65	22.6	1096	3	116	738	62
Nayarit	21.100000	-104.5667	979	<i>O. Téllez 12834</i> (MEXU)	19.5	15.4	64	22.6	913	4	112	411	53
Nayarit	21.144962	-104.4759	1299	<i>G. Ibarra 7127</i> (MEXU)*	22	15.9	64	23.9	910	4	115	420	49
Nayarit	21.150868	-104.4836	1355	NA*	22	15.9	64	23.9	910	4	115	420	49
Nayarit	21.154460	-104.4873	1394	<i>G. Ibarra 7126</i> (MEXU)*	22	15.9	64	23.9	910	4	115	420	49

Appendix. Continuation

State	Latitude	Longitude	Altitude	Vouchers (Herbarium)	Bio1	Bio2	Bio3	Bio4	Bio12	Bio14	Bio15	Bio18	Bio19
Nayarit	21.154523	-104.4871	1393	NA*	22.7	16.4	64	25.5	872	5	115	412	45
Nayarit	21.409381	-104.7329	1212	<i>X. Madrigal 2309</i> (INIF)	19.5	15.4	64	22.6	913	4	112	411	53
Zacatecas	21.178349	-103.5332	1282	<i>G. Ibarra 7119</i> (MEXU)*	20.5	17.2	63	25.6	889	4	118	431	37
Zacatecas	21.178590	-103.5340	1259	NA*	20.5	17.2	63	25.6	889	4	118	431	37
Zacatecas	21.179600	-103.5308	1345	NA*	20.5	17.2	63	25.6	889	4	118	431	37
Zacatecas	21.179723	-103.5308	1343	NA*	20.5	17.2	63	25.6	889	4	118	431	37
Zacatecas	21.204827	-103.5292	1592	<i>J. Robles 20</i> (MEXU)	20.5	17.2	63	25.6	889	4	118	431	37

