UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO

POSGRADO EN CIENCIAS BIOLÓGICAS

INSTITUTO DE INVESTIGACIONES EN ECOSISTEMAS Y SUSTENTABILIDAD
ECOLOGÍA

LA INTERACCIÓN MUTUALISTA DE *Ficus pringlei* (MORACEAE) Y SUS AVISPAS POLINIZADORAS DEL GÉNERO *Pegoscapus* (AGAONIDAE): EVIDENCIAS GENÉTICAS Y ECOLÓGICAS SOBRE SU ESPECIFICIDAD

TESIS

PARA OPTAR POR EL GRADO DE:
DOCTORA EN CIENCIAS

PRESENTA:
ÁNGELA PATRICIA ROJAS CORTÉS

TUTOR PRINCIPAL DE TESIS: DR. GUILLERMO IBARRA MANRÍQUEZ
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COORDINACIÓN GENERAL DE ESTUDIOS DE POSGRADO
COORDINACIÓN DEL POSGRADO EN CIENCIAS BIOLÓGICAS
ENTIDAD: IIES - MORELIA
OFICIO: CGEP/CPCB/IIES-M/0918/2023
ASUNTO: Oficio de Jurado

M. en C. Ivonne Ramírez Wence
Directora General de Administración Escolar, UNAM
Presente

Me permito informar a usted que en la reunión ordinaria del Comité Académico del Posgrado en Ciencias Biológicas, celebrada el día **21 de agosto de 2023** se aprobó el siguiente jurado para el examen de grado de **DOCTORA EN CIENCIAS** de la estudiante **ROJAS CORTÉZ ANGELA PATRICIA** con número de cuenta **516491167** con la tesis **"LA INTERACCIÓN MUTUALISTA *DE FICUS PRINGLEI* (MORACEAE} Y SUS AVISPAS POLINIZADORAS DEL GÉNERO PEGOSCAPUS (AGAONIDAE): EVIDENCIAS GENÉTICAS Y ECOLÓGICAS SOBRE SU ESPECIFICIDAD",** realizada bajo la dirección del **DR. GUILLERMO IBARRA MANRIQUEZ** quedando integrado de la siguiente manera:

Presidente:	DR. LUIS ENRIQUE EGUIARTE FRUNS
Vocal:	DRA. NATALIA IVALÚ CACHO GONZÁLEZ
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Vocal:	DRA. CAROLINA GRANADOS MENDOZA
Vocal:	DR. EDUARDO RUÍZ SÁNCHEZ

Sin otro particular, me es grato enviarle un cordial saludo.

ATENTAMENTE
"POR MI RAZA HABLARÁ EL ESPÍRITU"
Ciudad Universitaria, Cd. Mx., a 21 de noviembre de 2023

COORDINADOR DEL PROGRAMA

DR. ADOLFO GERARDO NAVARRO SIGÜENZA

c. c. p. Expediente del alumno

AGNS/LTC/EARR/ltc

Agradecimientos

Agradezco al Posgrado en Ciencias Biológicas de la Universidad Nacional Autónoma de México por brindarme la oportunidad de permitirme ser una de sus estudiantes en el programa de doctorado, además por las facilidades logísticas, infraestructura y los recursos humanos que me permitieron desarrollar mi proyecto de investigación. Así mismo quiero agradecer el apoyo de la Dirección General del Personal Académico (DGAPA)- Programa de apoyo de investigación e Innovación Tecnológica (PAPIIT IN211620).

Expreso también mi gratitud al Consejo Nacional de Humanidades, Ciencias y Tecnologías (CONAHCYT) por el apoyo financiero durante el desarrollo de mis estudios al otorgarme la beca No. Registro 696852.

E igualmente agradezco a mi comité tutor, ya que gracias a ellos con sus recomendaciones mi trabajo fue enriquecido. A mi tutor el Dr. Guillermo Ibarra Manríquez por la orientación y confianza depositada en este tiempo, así mismo al Dr. Antonio González Rodríguez por sus atinados comentarios acerca de manuscrito y críticas constructivas al proyecto, y al Dr. Alejandro Zaldivar Riverón por sus aportaciones y consideraciones claves para el desarrollo de esta tesis.

Agradecimientos a título personal

A mi querida madre, quiero agradecerte de todo corazón por tu amor incondicional, comprensión y apoyo constante en mi vida.

A Lau, tu presencia ha sido un regalo invaluable en mi vida. Gracias por estar a mi lado, por brindarme tanto amor y por ser mi refugio, mi lugar seguro.

A Gathu, mi compañera en tantos procesos y mi calentagato en los momentos de frío. Gracias por desvelarte a mi lado.

A Edison, por tu apoyo incondicional. Gracias por estar allí cuando te necesité, por tu disposición para escucharme, animarme a seguir adelante y por ser esa persona en la siempre puedo contar.

A Memo, por tu apoyo incondicional que ha sido fundamental en la realización de esta tesis. Tu confianza en mí y tu dedicación a mi crecimiento académico han sido motores inspiradores. Gracias Memo, por brindarme no solo tu orientación profesional, sino también tu cariño que ha hecho de este viaje una experiencia enriquecedora. Gracias por recibirme en tu vida y compartirme ese amorcito por las plantas.

A Susana, gracias por alentarme a seguir adelante, por compartir tu pasión por los bichos, por hacer que la distancia se sintiera menos abrumadora y por ayudarme a mantenerme funcional en momentos difíciles. Tu compañía hizo que me sintiera escuchada y acompañada, como si estuviera en casa a pesar de la distancia.

A Jaime, mi gurú genómico, te agradezco enormemente por compartirme tu profundo conocimiento y por alentarme a continuar, incluso cuando los obstáculos parecían complicados. Tu guía ha sido esencial y ha iluminado mi camino en esta travesía genómica. Sin duda, mi camino no hubiera sido el mismo sin ti.

A la M. en C. Guadalupe Cornejo, Lupita, te agradezco enormemente por tu valiosa participación y apoyo en el proceso de colecta. También por tu cálida acogida que hizo que mi experiencia fuera aún más especial en cada salida y que me sintiera en familia.

A Ricardo Gaytán, por tus charlas reflexivas, tu constante ánimo y compañía. Gracias por acompañarme en mi travesía de la 'molecular'.

A Paulina Vázquez, mi profundo agradecimiento por acompañarme en los primeros pasos de este proyecto. Tu compañía fue invaluable, y compartir este viaje contigo hizo que el mar de inquietudes se sintiera menos abrumador.

A Jovana Jasso, por compartirme un poco de tu valioso conocimiento y por animarme a seguir indagando en el fascinante mundo de las avispas.

A Angie e Ingrid Lara, por su notable constancia y persistencia en el desafiante proceso de extracción de ADN. Su dedicación incansable de ambas fue esencial para esta investigación. Gracias por todo el apoyo prestado.

A Francisco Mora, por tu invaluable contribución en el ámbito estadístico. Tu experiencia y asesoramiento fueron importantes para la correcta interpretación de los datos. Gracias también por alentarme a cerrar procesos.

A Omar Chassin, mi profundo agradecimiento por ayudarme con las cuestiones de cómputo. Sin tu ayuda, este proceso habría sido considerablemente más complejo.

A Iván Ek, por su invaluable apoyo en el campo, por acompañarnos en todas travesías. Tu colaboración fue fundamental para el éxito de este proyecto.

A Toñito y Alejandro, quienes formaron parte de mi comité tutorial. Su invaluable ayuda y colaboración fueron esenciales para el éxito de mi tesis. A ambos, mi sincero agradecimiento por contribuir significativamente a este proyecto académico.

A Lencho, Ramón Cuevas, Arturo Solís y Pablo Carrillo, mi profundo agradecimiento por su invaluable ayuda en campo. Sin sus manos expertas, no hubiéramos podido obtener las muestras fundamentales para este proyecto.

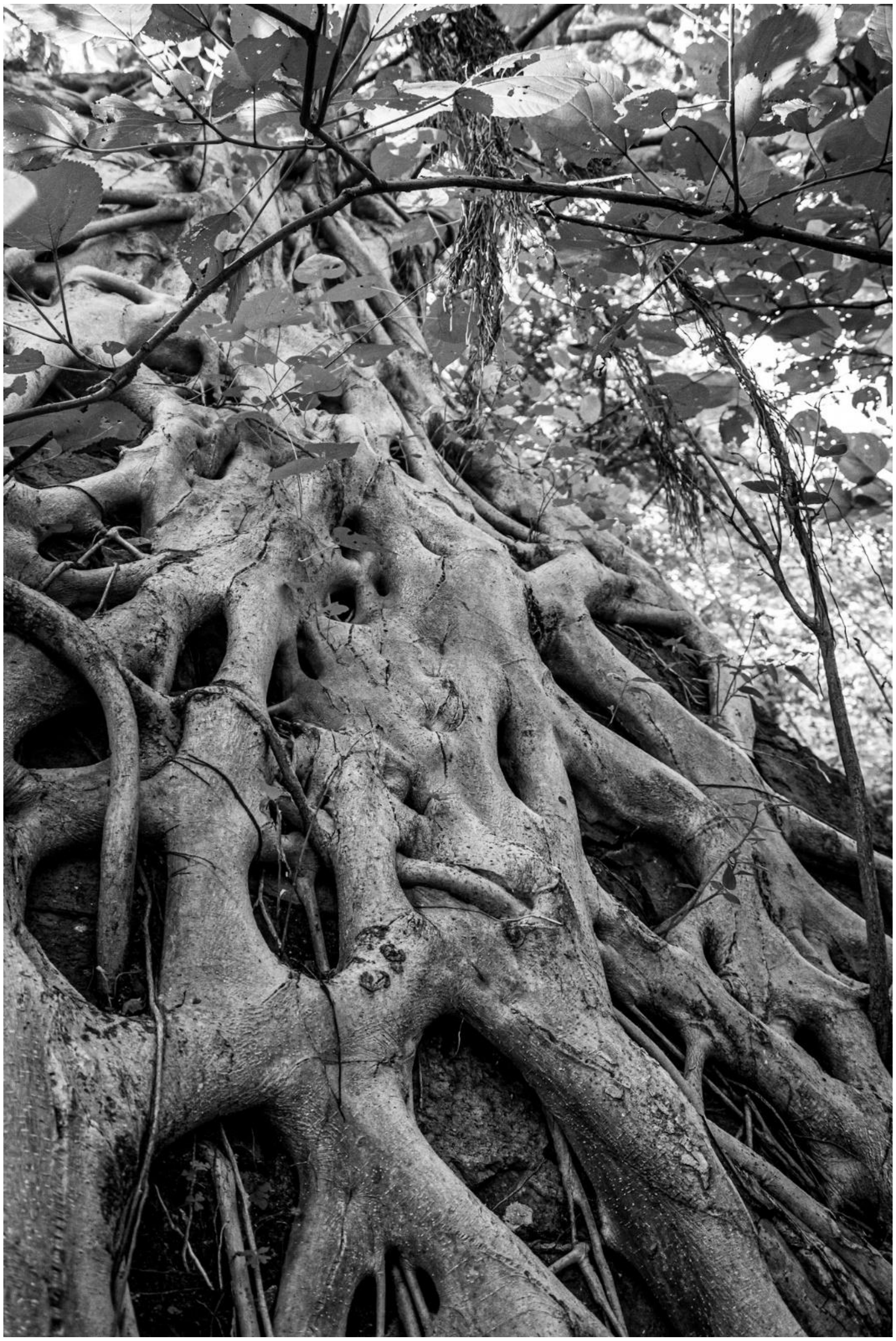
Agradezco al Dr. William Ramírez, por compartir su conocimiento y amor por este sistema tan complejo y lleno de sorpresas.

Agradezco al Dr. Orlando Hernández Cristóbal, por su colaboración en la toma de fotomicrografías en el microscopio electrónico de barrido de la Escuela Nacional de Estudios Superiores, Campus Morelia, UNAM.

Agradezco a los doctores Antonio González, Carolina Granados, Eduardo Ruíz, Luis Eguiarte e Ivalú Cacho, por su dedicación y esfuerzo en revisar y evaluar mi tesis. Sus valiosos comentarios y sugerencias contribuyeron significativamente a mejorar la calidad este trabajo.

Finalmente, quiero agradecer a cada persona que, de manera directa o indirecta, ha contribuido a mi crecimiento personal y académico en estos años. Su presencia ha dejado una huella significativa en mi camino. ¡Gracias totales!

Dedicada a Lau, mi madre y a la vida



"Are all botanists crazy? I began to think that anyone who would bother with such a genus as Eugenia must be a bit off; to say nothing of those who like to play with Ficus."

(from a letter of Merrill to Lam, 23 August 1949).

In Berg CC and Corner EJH. 2005. Moraceae: Ficeae. Flora Maleaisana 17: 1-70.

Resumen

Los mutualismos obligados de polinización, clave en la diversificación de grupos que los presentan, se observan en *Ficus* (Moraceae, Rosales), un género diverso, con más de 880 especies, polinizado exclusivamente por avispas de la familia Agaonidae. *Ficus* exhibe diferentes atributos relacionados con su reproducción. Los *Ficus* ginodioicos, generalmente son árboles pequeños, con altas densidades locales, bajos intervalos de dispersión de polen y niveles significativos altos de estructura genética. Por el contrario, las especies monoicas *Ficus*, predominan los árboles hemiepífitos y, se ha sugerido que presentan bajas densidades, dispersión de polen alta y poca estructura genética. La influencia en la variación genética y aislamiento poblacional en este mutualismo se atribuye a los rasgos de historia de vida, heterogeneidad ambiental y barreras geográficas. Sin embargo, no existen estudios poblacionales de polinizadores en el Neotrópico. En esta tesis analicé la interacción mutualista de *F. pringlei* y sus avispas polinizadoras (*Pegoscapus* sp.) en el oeste de México, en la Zona de Transición Mexicana, siendo el primer estudio a nivel poblacional y genómico de una especie de *Ficus* en América, explorando los procesos de diferenciación genética de ambos interactuantes. En el Capítulo 1 detallo el desarrollo del sícono y la polinización de *F. pringlei*, analizando su área de distribución y las variables climáticas que influyen en esta distribución. Este análisis sirvió como un marco de referencia para ubicar las poblaciones de esta especie. Las etapas de desarrollo de *F. pringlei* se asemejan a las de otras especies monoicas y el proceso de polinización es activo. *Ficus pringlei* se encuentra exclusivamente en el occidente de México, principalmente en bosques tropicales secos, con la temperatura estacional como factor clave que regula su distribución. Se observó una variación significativa en la estacionalidad de la temperatura en las poblaciones localizadas hacia el norte y sur de la Faja Volcánica Transmexicana (FVTM). En el Capítulo 2, presento el primer estudio poblacional y genómico de *Ficus* en América, utilizando 5,311 SNPs derivados de genomas completos a baja cobertura. Este análisis cuantifica la diversidad genética y explora la influencia de la FVTM y la heterogeneidad ambiental en su estructura genética, evidenciando una moderada diferenciación para ciertos grupos genéticos (F_{ST}= 0.208), explicada por la estacionalidad climática. La baja diferenciación entre poblaciones entre el norte y el sur de la FVTM sugiere que esta cadena montañosa no es una barrera al flujo genético. En el Capítulo 3, analicé la diversidad y estructura genética de las avispas *Pegoscapus* sp., polinizadoras de *F. pringlei*, utilizando el gen mitocondrial COI. Las avispas revelan alta diversidad (Hd=0.978) y una estructura moderada (F_{ST}= 0.257), con dos grupos genéticos separados por la FVTM y la región del Balsas, este último grupo con una alta diversidad genética. Aunque *F. pringlei* presenta estructura genética moderada, su potencial de dispersión de polen a larga distancia se ve limitado por las condiciones climáticas. La influencia de la FVTM en la estructura genética de las avispas sugiere la necesidad de análisis adicionales con diferentes marcadores, así como estudios morfológicos y moleculares para abordar la posible presencia de múltiples especies de avispas polinizadoras en *F. pringlei*. Investigaciones futuras deben explorar la asincronía en el desarrollo de los síconos bajo distintos patrones de temperatura, así como examinar el papel de secuencias heteroplásmicas o pseudogenes para una comprensión más completa de la variabilidad genética de las avispas.

Abstract

The obligatory mutualisms of pollination, key in the diversification of groups that exhibit them, is observed in *Ficus* (Moraceae, Rosales), a diverse genus with more than 880 species, exclusively pollinated by wasps of the Agaonidae family. *Ficus* exhibits different attributes related to its reproduction. Gynodioecious *Ficus* are generally small trees with high local densities, low pollen dispersal intervals, and significantly high levels of genetic structure. In contrast, monoecious *Ficus* species are predominantly hemiepiphytic trees, suggested to have low densities, high pollen dispersal, and low genetic structure. The influence on genetic variation and population isolation in this mutualism is attributed to life history traits, environmental heterogeneity, and geographical barriers. However, there are no population studies of pollinators in the Neotropics. In this thesis, I analyzed the mutualistic interaction of *F. pringlei* and its pollinating wasps (*Pegoscapus* sp.) in western Mexico, in the Mexican Transition Zone, being the first population and genomic study of a *Ficus* species in America, exploring the processes of genetic differentiation of both interactors. In Chapter 1, I detail the development of the syconium and the pollination of *F. pringlei*, analyzing its distribution area and the climatic variables that influence this distribution. This analysis served as a reference framework to locate the populations of this species. The developmental stages of *F. pringlei* resemble those of other monoecious species, and the pollination process is active. *Ficus pringlei* is exclusively found in western Mexico, mainly in tropical dry forests, with seasonal temperature as a key factor regulating its distribution. Significant variation in temperature seasonality was observed in populations located north and south of the Trans-Mexican Volcanic Belt (TMVB). In Chapter 2, I present the first population and genomic study of *Ficus* in America, using 5,311 SNPs derived from low-coverage whole genomes. This analysis quantifies genetic diversity and explores the influence of the TMVB and environmental heterogeneity on its genetic structure, revealing moderate differentiation for certain genetic groups (F_{ST}= 0.208), explained by climatic seasonality. Low differentiation between populations north and south of the TMVB suggests that this mountain range is not a barrier to genetic flow. In Chapter 3, I analyzed the diversity and genetic structure of the *Pegoscapus* sp. wasps, pollinators of *F. pringlei*, using the mitochondrial COI gene. The wasps show high diversity (Hd= 0.978) and moderate structure (F_{ST}= 0.257), with two genetic groups separated by the TMVB and the Balsas region, the latter group having high genetic diversity. Although *F. pringlei* shows moderate genetic structure, its potential for long-distance pollen dispersal is limited by climatic conditions. The influence of the TMVB on the genetic structure of wasps suggests the need for additional analyses with different markers, as well as morphological and molecular studies to address the possible presence of multiple species of pollinating wasps in *F. pringlei*. Future research should explore the asynchrony in the development of syconia under different temperature patterns and examine the role of heteroplasmic sequences or pseudogenes for a more complete understanding of the genetic variability of wasps.

Introducción general

Las interacciones ecológicas son un componente fundamental en la generación de la biodiversidad (Ehrlich & Raven, 1964; Suchan & Alvarez, 2015). Entre las angiospermas y los insectos, se considera que la polinización es el factor principal para explicar sus radiaciones (Dodd, Silvertown, & Chase, 1999; Bronstein, Alarcón, & Geber, 2006; Armbruster, 2014). Las angiospermas es el grupo de plantas terrestres más diverso, con aproximadamente 341,000 especies (WFO, 2023). Igualmente, dentro de los órdenes principales de insectos polinizadores, Coleoptera, Diptera, Himenoptera y Lepidoptera, existen al menos 340,000 especies (Ollerton, 2017). Aproximadamente, el 70 % de las angiospermas son polinizadas por insectos, el 17 % por otros animales, y solo una minoría por vectores abióticos, principalmente el viento (Ollerton, Winfree, & Tarrant, 2011). Sin embargo, para entender mejor la radiación de la diversidad en estos grandes grupos de plantas y animales, así como la complejidad de estas interacciones, es necesario profundizar en los procesos de especiación, diversificación y coevolución de estos mutualismos.

Una de las interacciones que ha despertado un amplio interés de investigación, por la estrecha relación de sus interactuantes, es el mutualismo obligado de polinización, donde los insectos ovipositan únicamente dentro de las estructuras reproductivas de las plantas al momento de polinizar las flores (Cook & Rasplus, 2003; Pellmyr, 2003; Kawakita & Kato, 2009). Solo se ha registrado este tipo de mutualismo en un pequeño número de géneros de plantas e insectos (Tabla 1), sin embargo, está presente en numerosas especies, que en algunos ecosistemas pueden llegar a ser ecológicamente dominantes (Hembry & Althoff, 2016). Entre los ejemplos mejor conocidos del mutualismo obligado de polinización está el género *Ficus* L. (familia Moraceae, orden Rosales) y sus avispas polinizadoras de la familia Agaonidae, superfamilia Chalcidoidea, y el de los géneros *Hesperoyucca* (Engelm.) Baker y *Yucca* L. (familia Asparagaceae, orden Asparagales) con sus polillas polinizadoras de los géneros *Parategeticula* Davis, 1967 y *Tegeticula* Zeller, 1873 (Ramírez, 1974; Janzen, 1979; Pellmyr, 2003; Herre, Jandér, & Machado, 2008). No obstante, en los géneros *Breynia* J.R. Forst. & G. Forst., *Glochidion* J.R. Forst. & G. Forst. y *Phyllanthus* L. (familia Phyllanthaceae, orden Malpighiales) también se presenta este tipo mutualismo con las polillas del género *Epicephala* Meyrick, 1880.

Tabla 1. Clasificación taxonómica de los organismos involucrados y principales características ecológicas de los mutualismos obligados de polinización.

	Phyllanthaceae-polillas	***Ficus*-Agaonidae**	***Yucca*-polillas**
Planta			
Familia	Phyllanthaceae	Moraceae	Asparagaceae
Géneros	*Breynia, Glochidion, Phyllanthus*	*Ficus*	*Hesperoyucca, Yucca*
Número de especies	Más de 500	Más de 750	Cerca de 40
Área de distribución	Pantropical	Pantropical	Norte y Centro América
Sistema sexual	Monoico	Monoico o funcionalmente dioico	Monoico
Floración	Continua o estacional	Continua o estacional	Estacional, de junio y julio
Especialización floral	Antesis nocturna, reducción de néctar, fusión de estilos y estambres	Una inflorescencia en forma de urna o sícono	Antesis nocturna, reducción de néctar
Polinizador			
Orden	Lepidoptera	Hymenoptera	Lepidoptera
Familia	Gracillariidae	Agaonidae	Prodoxidae
Géneros	*Epicephala*	20 géneros	*Parategeticula* y *Tegeticula*
Número de especies	Más de 500	Más de 750	Cerca de 20
Especies asociadas por huésped	1-4	1-9	1-6
Estructuras para recolectar polen	Sensilios en la probóscide	Corbículas	Tentáculos maxilares
Generaciones por año	Univoltina o multivoltina	Multivoltina	Univoltina

Modificado de (Kawakita & Kato, 2017).

Debido a que los organismos del mutualismo obligado de polinización dependen completamente unos de otros para reproducirse, y al hecho de que los polinizadores, a menudo, están especializados en una sola especie de planta, se ha propuesto que estos sistemas deberían promover la coespeciación e historias filogenéticas paralelas (Kiester, Researcher, & Schemske, 1984; Smith *et al.*, 2008). Sin embargo, en diferentes análisis cofilogenéticos, las filogenias congruentes parecen explicarse por otros procesos diferentes a la coespeciación (Althoff *et al.*, 2012). En *Yucca* y sus polinizadores se ha mostrado que existe una congruencia filogenética, debido a factores biogeográficos o por un seguimiento filogenético, por parte de las polillas, a los linajes de *Yucca* (Althoff *et al.*, 2012), mientras que en *Glochidion* y las especies de *Epicephala* se ha registrado una diversificación paralela, sin una congruencia perfecta entre las filogenias debido, probablemente, al cambio de huésped por parte de las polillas (Kawakita *et al.*, 2004). Es importante aclarar que no es posible descartar que el sistema haya evolucionado, principalmente, a través de procesos de diversificación coevolutiva y cierto nivel de seguimiento filogenético (Cruaud *et al.*, 2012). No obstante, se necesitan análisis adicionales que integren procesos microevolutivos con patrones macroevolutivos para comprender cómo la coevolución afecta la divergencia, la especiación y la extinción de los linajes coevolutivos (Althoff, Segraves, & Johnson, 2014).

Para entender integralmente el proceso coevolutivo y la especiación de los interactuantes en los mutualismos obligados de polinización, es necesario entender qué sucede con la variación genética de cada interactuante, en un contexto geográfico temporal y espacialmente dinámico. De esta forma, es posible distinguir interrupciones genéticas, producto de procesos históricos, o de eventos más recientes, los cuales podrían estar reduciendo el flujo genético y promoviendo aislamiento reproductivo (Avise, 2000). Así mismo, al integrar la variación genética de cada interactuante y cómo la selección natural actúa de forma diferencial, creando un mosaico de selección geográfica, sería posible determinar cómo la interacción coevolutiva varía geográficamente. Bajo el marco conceptual de la teoría del mosaico geográfico de coevolución propuesto por Thompson (1994), la selección puede ser recíproca en ciertos lugares, dando lugar a coevolución (puntos calientes), mientras que en otros lugares, la selección puede ser unidireccional o no tener interacción (puntos fríos). Este proceso puede estar influenciado por los cambios en la distribución de los alelos debido a mutaciones, flujo de genes, deriva génica u otros procesos (Gomulkiewicz *et al.*, 2007).

Al estar las especies interactuantes de los mutualismos obligados de polinización estrechamente relacionadas, se espera que respondan de forma similar a los factores extrínsecos (Alvarez *et al.*, 2010). No obstante, esta expectativa puede verse afectada por diversos factores, como la diferencia de las historias de vida de los hospederos y los polinizadores, la cual puede derivar en discrepancias en tiempos generacionales, en tamaños de población o capacidades de dispersión y con ello, en desajustes en la variación genética (Alvarez *et al.*, 2010; Kobmoo *et al.*, 2010). Otro factor muy importante es el número de especies involucradas, es decir, si es una interacción uno a uno o hay más especies en ella (Alvarez *et al.*, 2010). Por tal motivo, es posible que cada mutualismo presente patrones particulares de variación genética.

El género *Ficus* y sus avispas polinizadoras

Dentro de las angiospermas, *Ficus* es uno de los géneros más diversos en especies y formas de crecimiento. En el mundo se han descrito más de 880 especies, tanto en regiones tropicales como subtropicales, creciendo como árboles, arbustos, epífitas, hemiepífitas o lianas (Beck, 2019; POWO, 2023). El género se considera como un componente fundamental de los diferentes tipos de vegetación en los que se distribuye, ya que más de 1,200 especies de aves y mamíferos frugívoros consumen sus frutos durante todo el año, especialmente en épocas en que la disponibilidad de frutos disminuye a nivel comunitario (Shanahan *et al.*, 2001; Ibarra-Manríquez *et al.*, 2012; Beck, 2019).

Ficus presenta una inflorescencia peculiar en forma de urna, llamada sícono o higo, en cuyo interior se encuentran las flores pistiladas y/o estaminadas (Berg, 1989; Verkerke, 1989; Ibarra-Manríquez *et al.*, 2012; Piedra-Malagón *et al.*, 2019; Delgado-Perez *et al.*, 2020). El único acceso al interior del sícono es a través del ostíolo que es una pequeña abertura que puede funcionar como filtro selectivo para el ingreso de los polinizadores

(Delgado-Pérez *et al.*, 2022; Castro-Cárdenas *et al.*, 2022). Esta inflorescencia garantiza que la polinización de sus flores esté únicamente mediada por las avispas de la familia Agaonidae (Ramírez, 1974; Janzen, 1979). En *Ficus* hay dos sistemas reproductivos, monoico y dioico, con un número similar de especies (Berg, 1989). En las especies monoicas, tanto las avispas como las semillas se encuentran en el mismo sícono, mientras en las dioicas, los síconos de árboles funcionalmente femeninos dan lugar semillas, y los síconos masculinos al polen y a las avispas (Berg, 1989; Verkerke, 1989).

La familia Agaonidae incluye cientos de especies que están asociadas estrechamente con los síconos, pero específicamente las avispas polinizadoras tienen dietas especializadas en los óvulos agallados y varias características morfológicas que reflejan su asociación con las especies de *Ficus* (Weiblen, 2002). Las avispas hembra tienen alas excepcionalmente largas, que pueden estar asociadas con su dispersión pasiva por el viento. La cabeza es aplanada, con mandíbulas que presentan apéndices, que le ayudan a moverse a través del ostiolo y sus antenas presentan segmentos distales, con numerosos sensilios para localizar a su huésped (Kjellberg *et al.*, 2005). Además, las avispas con polinización activa presentan corbículas y peines coxales para transportar el polen, mientras que, por el contrario, las avispas con un comportamiento pasivo no presentan ninguna estructura especializada (Ramírez, 1969; Kjellberg *et al.*, 2005). Por otro lado, los machos no tienen alas, sus ojos están reducidos, tienen antenas simples y sus patas están modificadas para excavar entre las flores para buscar las flores tipo agalla, en cuyo ovario se encuentran encerradas las hembras (Kjellberg *et al.*, 2005). Estas características morfológicas están asociadas con los comportamientos que despliegan durante la polinización.

Los ciclos de vida de ambos interactuantes están en sincronía (Weiblen, 2002). En las especies monoicas, el proceso de polinización empieza con la atracción de las avispas hembra, portadoras de polen, a las flores receptivas, mediante la producción de compuestos orgánicos volátiles (Grison-Pigé *et al.* 2002, Proffit *et al.* 2020). Posteriormente, un número reducido de las avispas ingresan al interior del sícono, a través del ostiolo, para polinizar las flores pistiladas y ovipositar en un conjunto de ellas;

después de esto, las hembras mueren. Después de varias semanas, se da el desarrollo y maduración sexual de la nueva generación de avispas, donde los machos se aparean con las hembras. Posteriormente, las avispas hembra, en las especies de polinización activa, recogen el polen de las anteras para transportarlo en las corbículas. Luego, los machos cavan un túnel a través de las paredes del sícono o el ostíolo, permitiendo que las hembras fertilizadas y cargadas de polen salgan, en busca de otros síconos con flores pistiladas maduras, de manera que el ciclo de reproducción se repite. Por su parte, en el sícono se siguen madurando las semillas, para su posterior dispersión (Figura 1; Galil & Eisikowitch, 1968; Berg & Corner, 2005; Piedra-Malagón *et al.*, 2019).

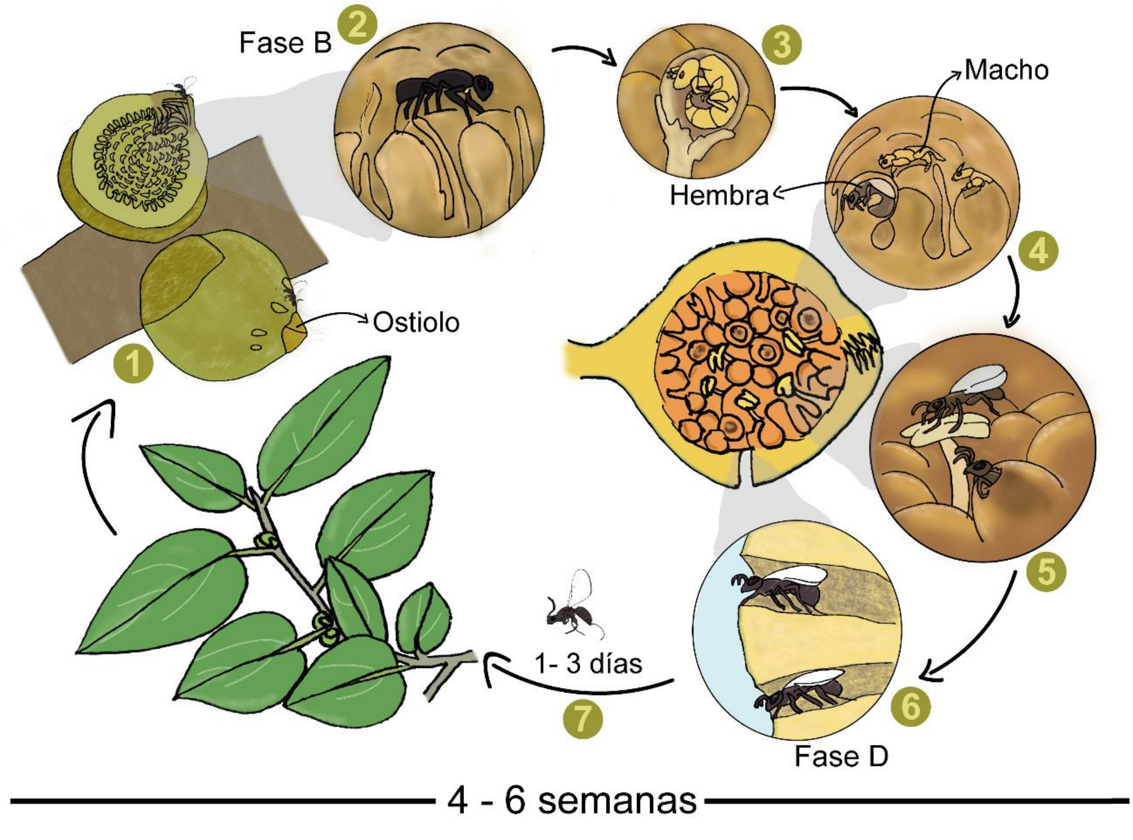


Figura 1. Ciclo de vida de *Ficus* y sus avispas polinizadoras en una especie monoica, el cual puede durar entre cuatro y seis semanas. 1) Mediante la producción de compuestos volátiles se da la atracción de las avispas hembra portadoras de polen (fase A del desarrollo del sícono). 2) Las avispas hembra, cargadas de polen, ingresan al sícono a través del ostíolo durante la fase B. En este proceso, un número limitado de avispas realiza

la polinización de las flores pistiladas y ovipositan en un conjunto de ellas. 3) Las flores pistiladas permiten el desarrollo de las larvas de avispa (flores agalladas) o semillas (fase C del sícono). 4) Después de varias semanas, se da el desarrollo y maduración sexual de la nueva generación de avispas, los machos emergen primero y fertilizan a las hembras (fase C del sícono). 5) Las hembras salen de sus agallas y recolectan polen de las flores masculinas. 6) Las hembras salen del sícono a través de túneles hechos por los machos. 7) Las hembras salen en búsqueda de otros síconos, este proceso puede durar entre uno y tres días (los puntos 5-7 se engloban dentro de la fase D del sícono), mientras las semillas siguen madurando para su posterior dispersión (fase E del sícono).

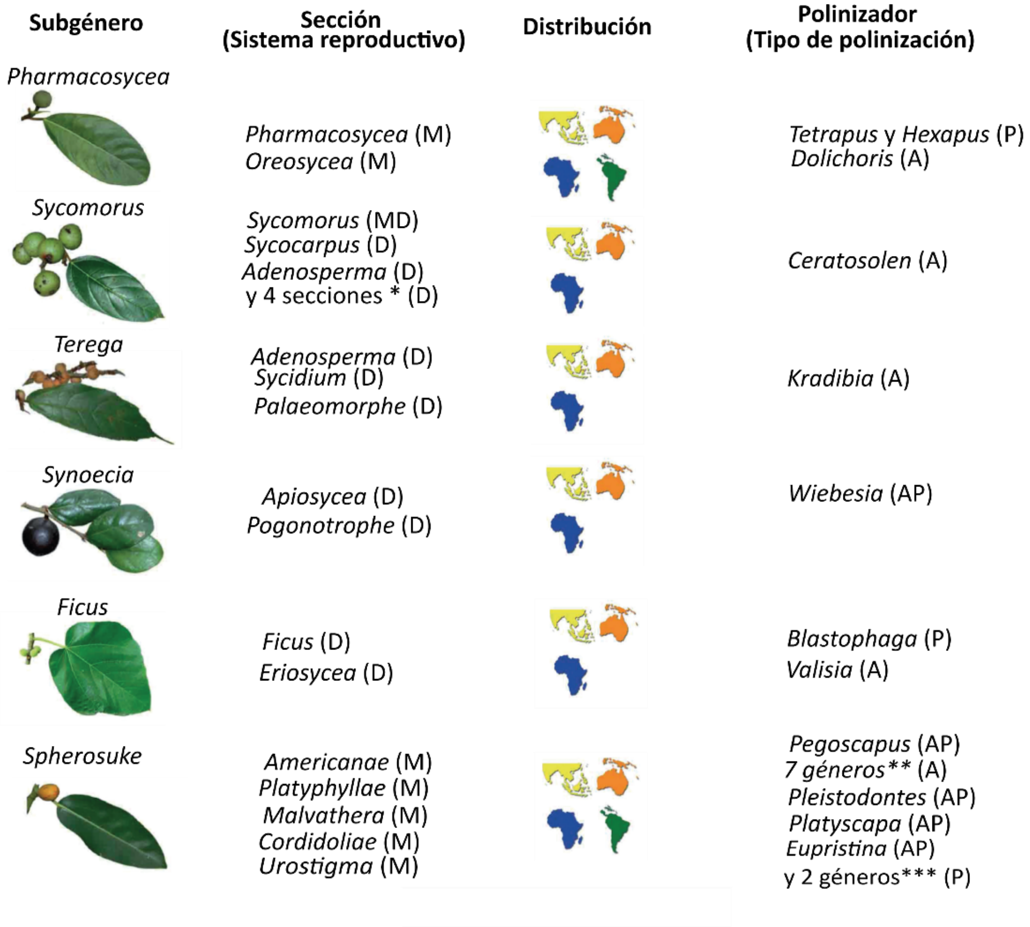
Esta estrecha relación entre *Ficus* y sus polinizadoras surgió hace aproximadamente 75 Ma (Cruaud *et al.*, 2012). Inicialmente, dada la observación de que los subgéneros y secciones de *Ficus* suelen ser polinizados por distintos géneros de avispas (Berg 1989, Cruaud *et al.* 2012; Figura 2), se sugirió que la especificidad entre las avispas polinizadoras y sus hospederos era la regla y el principal mecanismo de diversificación (Ramírez, 1970). Sin embargo, se han registrado cambios de hospedero o varias especies de avispas asociadas a una especie de *Ficus* o viceversa (Jousselin *et al.*, 2008; Cruaud *et al.*, 2012). Además, estudios recientes han detectado una incongruencia significativa entre todas las filogenias basadas en el núcleo, cloroplasto y mitocondria de las especies de *Ficus* con las filogenias de sus avispas polinizadoras, obtenidas con marcadores mitocondriales (e. g., Herre *et al.*, 1996). Estos hallazgos sugieren que se han producido cambios de huésped e hibridaciones a lo largo de la historia evolutiva de *Ficus*, incluso entre clados distantemente relacionados (Bruun Lund *et al.*, 2017; Wang *et al.*, 2021a).

La capacidad de hibridación de los *Ficus* es bien conocida, incluso entre taxa distantes y con distintos sistemas de polinización (Ramírez, 1994; Machado *et al.*, 2005; Jackson *et al.*, 2008; Wei *et al.*, 2014; Farache *et al.*, 2017; Wilde *et al.*, 2020). Además, estudios citogenéticos han determinado que si bien la mayoría de las especies y tejidos son diploides (2n=26), la poliploidía no es un fenómeno raro en este género (Condit, 1964; Falistocco, 2016).

Sin embargo, la historia filogenética de los *Ficus* sugiere que, aunque la hibridación es un proceso importante en la evolución de estas plantas, no se presenta una introgresión continua, sino la introgresión ha sucedido en pulsos dentro de linajes filogenéticamente estables (Gardner *et al.*, 2023). Por lo tanto, la alta diversidad de los *Ficus* puede ser producto del equilibrio entre la especialización del polinizador y la introgresión genética facilitada por la especificidad incompleta del polinizador (Gardner *et al.*, 2023).

Por otro lado, en las avispas polinizadoras la hibridación y la introgresión parecen jugar un papel insignificante en su historia evolutiva, ya que no se ha encontrado evidencias de introgresión; incluso entre especies con potencial de aparearse y reproducirse dentro del mismo sícono (Satler *et al.*, 2022). Sin embargo, se han registrado eventos raros de hibridación entre especies estrechamente relacionadas, pero los individuos F1 no se reproducen y se generan machos diploides, que suelen ser estériles (Molbo *et al.*, 2004). Por lo tanto, parece que la hibridación puede provocar un fallo en el sistema de determinación de sexo en estos insectos, ya que los machos normales se desarrollan a partir de huevos no fecundados y son haploides (n=5 o 6 según la especie; Liu *et al.* 2011, Sutton *et al.* 2017, Gokhman *et al.* 2019). Por ende, las barreras para una hibridación exitosa en las avispas son más restrictiva que en los huéspedes (Satler *et al.*, 2022).

Uno de los factores más importantes que promueven la especificidad en el sistema *Ficus*-Agaonidae es la síntesis de compuestos orgánicos volátiles (Hossaert-McKey *et al.*, 2010; Soler *et al.*, 2011; Proffit *et al.*, 2020). Estos compuestos desempeñan un papel central en la atracción de los polinizadores, ya que estos viven poco tiempo (1-3 días) y es fundamental para las avispas encontrar rápidamente higos receptivos para poder ovipositar (Weiblen, 2002; Jevanandam, Goh, & Corlett, 2013). Previamente, se sugería que las flores femeninas eran el lugar de síntesis de estos compuestos, sin embargo, con datos histológicos y del transcriptoma de las brácteas ostiolares, se observó que el ostiolo es el lugar de síntesis, y que las diferencias entre especies están determinadas principalmente por la variación de la expresión génica, más que por la diferenciación genética (Souza *et al.*, 2015; Yu *et al.*, 2015; Hu *et al.*, 2020).



Subgénero	Sección (Sistema reproductivo)	Distribución	Polinizador (Tipo de polinización)
Pharmacosycea	*Pharmacosycea* (M) *Oreosycea* (M)		*Tetrapus* y *Hexapus* (P) *Dolichoris* (A)
Sycomorus	*Sycomorus* (MD) *Sycocarpus* (D) *Adenosperma* (D) y 4 secciones * (D)		*Ceratosolen* (A)
Terega	*Adenosperma* (D) *Sycidium* (D) *Palaeomorphe* (D)		*Kradibia* (A)
Synoecia	*Apiosycea* (D) *Pogonotrophe* (D)		*Wiebesia* (AP)
Ficus	*Ficus* (D) *Eriosycea* (D)		*Blastophaga* (P) *Valisia* (A)
Spherosuke	*Americanae* (M) *Platyphyllae* (M) *Malvathera* (M) *Cordidoliae* (M) *Urostigma* (M)		*Pegoscapus* (AP) *7 géneros*** (A) *Pleistodontes* (AP) *Platyscapa* (AP) *Eupristina* (AP) y 2 géneros*** (P)

Figura 2. Clasificación y distribución de *Ficus*. El sistema reproductivo se indica como monoico (M) o dioico (D) y el tipo de polinización como pasiva (P) o activa (A). Nombres y rangos taxonómicos de *Ficus* según Berg & Corner (2005), actualizados por Pederneiras *et al.* (2015).**Bosscheria, Dammaropsis, Hemicardia, Papuayce.* ***Agaon*, *Alfonsiella*, *Allotriozoon*, *Courtella*, *Elisabethiella*, *Nigeriella* y *Paragaon*. ****Deilagaon* y *Waterstoniella*. Modificado de Cruaud *et al.* (2012).

Si bien el tamaño del genoma de las avispas polinizadoras es típico de los insectos (277-387 Mb), se ha observado una reducción de las familias de genes asociados a la capacidad quimiosensorial en comparación con otras avispas parasitas semigeneralistas de la misma superfamilia Chalcidoidea (Xiao *et al.*, 2013; Zhang *et al.*, 2020; Wang *et al.*, 2021b; Chen *et al.*, 2022). No obstante, los receptores olfatorios en las avispas asociadas a los *Ficus* pueden dividirse en más grupos. Los genes que codifican para estos receptores olfatorios tienen alta especificidad y tasa de evolución, lo que implica que su sistema sensorial olfativo es más complejo y que el reconocimiento olfativo es más amplio (Chen *et al.*, 2022). Estos receptores, además de identificar las sutiles diferencias de los compuestos orgánicos volátiles liberados por las diferentes especies de *Ficus*, también podrían facilitar el complejo intercambio de información entre las avispas (Chen *et al.*, 2022).

Otras familias de genes que se encuentran reducidas en el genoma de los Agaonidae son las asociadas a la respuesta desintoxicante e inmune innata (Chen *et al.*, 2022). Esta reducción puede deberse a que las avispas pasan casi toda su vida dentro de un huésped, ambiente en gran medida benigno, y, por tanto, existe poca necesidad de desintoxicación o protección ambiental (Xiao *et al.*, 2021). En el genoma mitocondrial de los Agaonidae también se han identificado genes asociados a la vía de fosforilación oxidativa (OXPHOS), es decir, el principal sistema de consumo de oxígeno y producción de trifosfato de adenosina (ATP). Estos genes han evolucionado significativamente más rápido en avispas polinizadoras, que en las no polinizadoras. Además, se han detectado señales de selección positiva en cinco genes mitocondriales en el linaje de los Agaonidae, lo que indica que los genes OXPHOS desempeñan un papel importante en la adaptación de los polinizadores (Zhou *et al.*, 2020).

Los anteriores ajustes genómicos no son los únicos fenómenos notables en la evolución del mutualismo *Ficus*-Agaonidae. Se ha observado que la regulación genética, mediante los factores de transcripción (FT), pueden impulsar divergencias en el desarrollo morfológico y la actividad fisiológica asociadas al dimorfismo sexual en las avispas (Sun *et al.*, 2015). Los datos transcriptómicos indican que los FT regulados positivamente en las hembras muestran un enriquecimiento significativo en el desarrollo de las alas, los ojos y las antenas en todas las etapas, desde la larva hasta el adulto. Mientras tanto, los FT regulados positivamente en machos adultos exhiben un enriquecimiento en el desarrollo de los genitales y el ritmo circadiano, que corresponden con el apareamiento y la protandría (Sun *et al.*, 2015). Además, datos transcriptómicos han revelado diferencias en los patrones de interacción con bacterias del género *Wolbachia*, según el sexo de las avispas, siendo mayor el número de genes regulados para la expresión genética de esta bacteria en los machos (Hou *et al.*, 2021).

En las especies de *Ficus*, a pesar de tener un genoma relativamente pequeño, entre 332 y 436 Mb, se han publicado pocos estudios sobre sus genomas (i.e., anotación, expresión génica y transcriptómica en especies silvestres) (pero ver He *et al.*, 2013; Usai *et al.*, 2020; Zhang *et al.*, 2020; Chakraborty *et al.*, 2022; Ashalatha, Arunkumar, & Gowda, 2023). La mayor parte de los estudios se han realizado en la higuera común o *F*. *carica* L., una especie común en todo el mundo y se han enfocado en características que favorecen la producción y mejoramiento de los síconos, como la partenocarpia, floración, desarrollo reproductivo, resistencia a hongos, color externo del higo, así como en su maduración y ablandamiento (Ercisli *et al.*, 2012; Rosianski *et al.*, 2016; Chai *et al.*, 2017; Cui *et al.*, 2019; Shirasawa *et al.*, 2020; Ikegami & Shirasawa, 2022).

En contraste, en las especies silvestres se ha explorado el gen Responsive-to-Antagonist1 (RAN1), responsable de la determinación del sexo en *F*. *carica*. Sin embargo, en *F*. *hispida* este gen no controla la determinación del sexo, pero probablemente participa en el desarrollo de las semillas (Zhang *et al.*, 2020). Además, se ha examinado la expresión genética de los metabolismos C3, C4 y CAM, indicando que algunos *Ficus* pueden alternar entre estos tres ciclos según de la biosíntesis de carbohidratos y aminoácidos, del metabolismo y las condiciones ambientales (Ashalatha *et al.*, 2023).

Variación y diferenciación genética

La dinámica de la genética poblacional en la interacción *Ficus*-Agaonidae ha sido ampliamente estudiada debido a su papel en la persistencia y adaptación mutua. Factores como los rasgos de historia de vida, la heterogeneidad ambiental y las barreras geográficas han jugado un papel importante en la variación genética y el aislamiento local de las poblaciones de estos organismos interactuantes (Souto-Vilarós *et al.*, 2019). Se ha subrayado, además, que cada interactuante se relaciona de forma diferencial con ciertos factores. Por ejemplo, las especies de *Ficus* que habitan en localidades poco heterogéneas ambientalmente pueden no presentar eventos significativos de diversificación. En contraste, en las avispas la adaptación, más que la dispersión, parece ser el factor limitante de su distribución geográfica y la coespeciación con su hospedero (Souto-Vilarós *et al.*, 2019).

En las eudicotiledóneas hay una correlación positiva entre la distancia al ecuador y la diversidad genética, mientras que la diferenciación sigue un patrón contrario (Gamba & Muchhala, 2020; De Kort *et al.*, 2021). Entre los factores que pueden restringir el flujo genético de estas plantas y reducir su diversidad genética en el trópico, se encuentran la orogenia heterogénea y la asincronía climática (Ricklefs, 1977; Martin & MCKay, 2004; Stein, Gerstner, & Kreft, 2014; Esquerré *et al.*, 2019; Gamba & Muchhala, 2020; De Kort *et al.*, 2021). Sin embargo, varias plantas tropicales, incluido el género *Ficus*, presentan niveles moderados o incluso altos de diversidad genética (Eguiarte, Pérez-Nasser, & Piñero, 1992; Nazareno & Carvalho, 2009; Heer *et al.*, 2015; López-Barrera *et al.*, 2021; Teixeira & Nazareno, 2021).

El tipo de polinización también juega un papel importante en la diferenciación poblacional (Gamba & Muchhala, 2020). En especies polinizadas por insectos pequeños, la diferenciación genética es mayor que en especies polinizadas por viento, insectos grandes o vertebrados. Este patrón probablemente se debe al flujo genético reducido que ejercen estos insectos, dada su menor capacidad de vuelo en comparación con abejorros y vertebrados, que pueden viajar a largas distancias, incluso a través de hábitats fragmentados (Dick *et al.*, 2008; Brunet, Larson-Rabin, & Stewart, 2012; Skogen *et al.*, 2019; Gamba & Muchhala, 2020). Sin embargo, es importante señalar que las especies de *Ficus*, polinizadas por avispas pequeños de hasta 2 mm, presenta una de las mayores distancias de fecundación en angiospermas (e. g., hasta 88 km en promedio), ya que estos insectos se dispersan pasivamente con el viento (Ahmed *et al.*, 2009). Este fenómeno desafía las expectativas convencionales, subrayando la complejidad de las adaptaciones evolutivas en este sistema mutualista.

Aunque las eudicotiledóneas tropicales muestran ciertos patrones en su dinámica genética, las especies de *Ficus* presentan características únicas que merecen una atención especial. Se ha sugerido que los sistemas reproductivos, relacionados con diversas características ecológicas, tienen una influencia en la variación genética en *Ficus*. Sus especies dioicas, presentes únicamente en el Paleotrópico, generalmente son árboles pequeños, con altas densidades locales, bajos rangos de dispersión de polen y niveles significativos de estructura genética (Nazareno, Alzate-Marin, & Pereira, 2013). Por el contrario, en las especies monoicas predominan los árboles hemiepífitos, aunque también se registran especies rupícolas o que germinan en el suelo (Ibarra-Manríquez *et al.*, 2012; Rojas-Cortés *et al.*, 2022), generalmente alcanzan el dosel de la vegetación, presentan densidades bajas, una dispersión de polen alta y bajos niveles de estructura genética (Ahmed *et al.*, 2009; Yang *et al.*, 2015). Sin embargo, hay pocos estudios en especies monoicas y su limitado alcance puede afectar la detección precisa de diferencias genéticas entre poblaciones (Tablas 2 y 3). Para comprender completamente la estructura genética de las especies de *Ficus*, es esencial expandir y diversificar los estudios existentes, asegurando un muestreo exhaustivo que abarque toda su distribución geográfica.

Los estudios genéticos poblacionales en la interacción *Ficus*-Agaonidae se han centrado principalmente en marcadores de ADN nuclear (Tablas 2 y 4). En el caso de los huéspedes, ofrecen una visión más completa del flujo genético, al reflejar tanto el movimiento del polen como el de las semillas (McCauley, 1994). Se destacan marcadores codominantes y dominantes, donde los primeros, microsatélites y polimorfismos de un solo nucleótido (SSR y SNP, respectivamente), identifican todos los alelos de un locus y permiten distinguir heterocigotos de homocigotos, mientras que los segundos, como polimorfismo en la longitud de fragmentos amplificados (AFLP), identifican un solo la presencia del alelo dominante. Los SSR, por su alta variabilidad y abundancia, son los marcadores más frecuentemente utilizados. Además, se han usado marcadores bioquímicos como las aloenzimas, que también son codominantes y que detectan variaciones en secuencias de aminoácidos (Wendel & Weeden, 1989). También se han explorado regiones de cloroplasto, mitocondria (que se heredan por vía materna) y de núcleo para discernir diferencias genéticas en las poblaciones (Tablas 4 y 5). A pesar del interés en la coevolución de los higos y las avispas, se destaca la falta de estudios genómicos y la limitada utilización de SNP, a pesar de su utilidad en organismos no modelo y en su capacidad de brindar información valiosa sobre los procesos ecológicos, genéticos y coevolutivos (Ekblom & Galindo, 2011; Davis, Houston, & Nason, 2015).

Los estudios de diversidad genética y diferenciación en *Ficus* revelan una carencia de estudios poblacionales con SNP en especies neotropicales, así como una escasez general para las especies monoicas (Tabla 2). Esto resalta la necesidad de realizar investigaciones más exhaustivas en estos grupos, contribuyendo así a un mejor entendimiento de la variabilidad genética de *Ficus*. Los microsatélites, marcadores predominantes en estos estudios, generalmente revelan una diversidad moderada, con valores de Heterocigosidad esperada (He) entre 0.3 y 0.82. Esta variabilidad es consistente, tanto en especies dioicas, como en monoicas. Los valores de He suelen ser superiores a la Heterocigosidad observada (Ho), sugiriendo un posible déficit de heterocigotos, que podría ser atribuible a fenómenos como la endogamia o un flujo genético limitado.

Las estimaciones de la estadística de diferenciación genética (F_{ST}) en *Ficus* con SSR (0.001-0.630) han mostrado diferenciaciones notables en especies dioicas y monoicas. Por ejemplo, *F. tikoua*, una especie dioica, exhibe una diferenciación alta entre dos poblaciones separadas por 470 km, destacando la influencia en este patrón de la topografía heterogénea (Chen *et al.*, 2011). Similarmente, *F. insipida*, una especie monoica, muestra diferenciación importante a través de la Cordillera de los Andes (Heer *et al.*, 2015). Estos ejemplos subrayan la importancia de factores geográficos en la diferenciación genética de estas especies. Por el contrario, los valores con SNP (0.001-0.395) muestran menores valores de F_{ST}.

En cuanto a los valores del coeficiente de endogamia (F_{IS}) con SSR, oscilan entre -0.210 y 0.451. En términos generales, las especies dioicas tienen valores de F_{IS} positivos, mientras que en las especies monoicas predominan valores negativos (Tabla 2). Esto sugiere que las poblaciones monoicas podrían ser más extensas y homogéneas en comparación con las dioicas. Además, en las especies dioicas, es probable que haya un mayor apareamiento entre individuos relacionados, lo que contribuye a la estructuración genética observada.

Los datos de secuenciación se han utilizado mayormente para cloroplasto en especies monoicas indican una mayor diversidad y diferenciación en cloroplasto que en la región nuclear ITS (Tabla 3). Aunque el tamaño del cloroplasto se mantiene relativamente constante entre especies (~160.000 pb) y tienen una estructura cuatripartita típica, investigaciones más detalladas sobre genomas completos de cloroplasto han revelado la presencia de regiones genéticas e intergénicas hipervariables (Huang *et al.*, 2022; Xia *et al.*, 2022; Zhang *et al.*, 2022). El análisis de estas regiones hipervariables podría ofrecer la oportunidad de obtener información detallada sobre patrones de migración, adaptación local y eventos evolutivos específicos, especialmente en relación con la dispersión de semillas en las poblaciones de *Ficus*.

Los estudios en avispas Agaonidae revelan la coexistencia de varias especies en un solo hospedero (Tabla 4). Al igual que en *Ficus*, hay una falta de investigaciones poblacionales con SNP en especies neotropicales. Los SSR revelan una diversidad genética moderada, con valores de He entre 0.160 y 0.812. Esta variabilidad es mayor en especies que habitan hospederos dioicos (0.300-0.812), que la registrada en los monoicos (0.160-0.778). En consonancia con la tendencia observada en *Ficus*, los valores de He son superiores a Ho con SSR.

La estimación de FST con SSR (0 y 0.581) en las avispas Agaonidae generalmente revela diferenciaciones altas. Al considerar los marcadores SNP, se observa una mayor variabilidad y valores muy altos de FST (0.004-0.933), destacando la sensibilidad del marcador elegido en la evaluación de la diferenciación genética. Los valores de FIS con SSR varían entre 0.091 y 0.877, lo que puede indicar cierta estructuración genética en las poblaciones de avispas.

Tabla 2. Atributos genéticos en especies de *Ficus* mediante marcadores moleculares. Ho: Heterocigosidad observada; He: Heterocigosidad esperada; F_{ST}: Diferenciación genética entre poblaciones; F_{IS}= Coeficiente de endogamia. Abreviaturas: D (dioica), M (monoica), N (Neotrópico), P (Paleotrópico).

Especie	**Marcador (loci)**	**Ho**	**He**	**F_{ST}**	**F_{IS}**	**Referencias**
F. arfakensis ^{DP}	SSR (10)	0.380	0.660	0.360	-	(Segar *et al.*, 2017)
	SNP (4718)	-	-	0.011-0.155	-	(Souto-Vilarós *et al.*, 2019)
F. carica ^{DP}	SSR (6)	0.700	0.820	0.056-0.063	0.128	(Saddoud *et al.*, 2007)
	SSR (6)	-	-	0.164	-	(Baraket *et al.*, 2011)
F. coronata ^{DP}	SNP (7492)	0.291	0.257	0.013-0.395	-0.062	(Wilde *et al.*, 2021)
F. cyrtophylla ^{DP}	SSR (7)	-	0.660	-	-	(Zhou & Chen, 2010)
F. exasperata ^{DP}	SSR (7)	0.473	0.770	-	0.390	(Dev *et al.*, 2011)
F. hahliana ^{DP}	SSR (10)	0.460	0.550	0.030-0.330	-	(Segar *et al.*, 2017)
F. hirta ^{DP}	SSR (9)	0.698	0.710	0.021-0.181	-0.066	(Yu *et al.*, 2019)
	SSR (9)	0.525-0.731	0.613-0.755	0.011-0.047	-0.196-0.345	(Lu *et al.*, 2022)
F. hispida ^{DP}	SSR (7)	0.520	0.639	-	0.230	(Dev *et al.*, 2011)
F. itoana ^{DP}	SNP (8988)	-	-	0.003-0.340	-	(Souto-Vilarós *et al.*, 2019)
F. pumila ^{DP}	SSR (6)	0.340	0.430	0.021-0.206	0.205	(Chen *et al.*, 2008)
	SSR (10)	0.380	0.530	0.060	0.287	(Wang *et al.*, 2009)
	SSR (8)	0.573	0.554	0.094		(Liu *et al.*, 2013)
	SSR (8)	0.660	0.630	0.123	0.035	(Liu *et al.*, 2015)
F. sarmentosa ^{DP}	SSR (9)	0.527	0.619	0.055	0.108	(Wang *et al.*, 2018)
F. septica ^{DP}	SSR (13)	0.355	0.401	0.016-0.242		(Rodriguez *et al.*, 2017)
F. tikoua ^{DP}	SSR (6)	0.278-0.388	0.364-0.563	0.042-0.627	0.102-0.451	(Chen *et al.*, 2011)

F. trichocerasa $^{\mathrm{DP}}$	SNP (11066)	-	-	0.001-0.273	-	(Souto-Vilarós *et al.*, 2019)
F. wassa $^{\mathrm{DP}}$	SNP (17312)	-	-	0.001-0.081	-	(Souto-Vilarós *et al.*, 2019)
F. arpazusa $^{\mathrm{MN}}$	Aloenzimas (10)	0.482	0.409	-	-0.178	(Nazareno & Carvalho, 2009)
F. bonijesulapensis $^{\mathrm{MN}}$	SSR (9)	-	0.300	-	-	(Duarte, Carvalho, & Vieira, 2015)
F. citrifolia $^{\mathrm{MN}}$	SSR (12)	0.559	0.664	0.024	-0.210	(Nazareno *et al.*, 2013)
	SSR (11)	-	0.790	-	0.054	(Heer *et al.*, 2015)
F. eximia $^{\mathrm{MN}}$	SSR (12)	0.608	0.677	0.014	-0.209	(Nazareno *et al.*, 2013)
F. insipida $^{\mathrm{MN}}$	SSR (9)	-	0.580	0.030-0.552	-0.021	(Heer *et al.*, 2015)
	AFLP (130)	-	-	0.125	-	(Poelchau & Hamrick, 2012)
F. obtusifolia $^{\mathrm{MN}}$	SSR (7)	-	0.690	-	0.147	(Heer *et al.*, 2015)
F. yoponensis $^{\mathrm{MN}}$	SSR (8)	-	0.640	-	-0.056	(Heer *et al.*, 2015)
F. bizane $^{\mathrm{MP}}$	SSR (8)	-	-	0.120	-	(Deng *et al.*, 2020a,b)
F. craterostoma $^{\mathrm{MP}}$	SSR (12)	-	-	0.050	-	(Deng *et al.*, 2020a,b)
F. racemosa $^{\mathrm{MP}}$	SSR (9)	0.285	0.352	0.001-0.630	0.227	(Bain *et al.*, 2016)
F. sur $^{\mathrm{MP}}$	SSR (10)	-	-	0.040	-	(Deng *et al.*, 2020a,b)

Tabla 3. Atributos genéticos en especies de *Ficus* mediante secuencias genéticas. SEC: tipo de secuencias de ADN; Hd: diversidad haplotípica; π: diversidad nucleotídica; F_{ST}: Diferenciación genética entre poblaciones; G_{ST}: medida de diferenciación genética ajustada. Abreviaturas: cp (cloroplasto), D (dioica), M (monoica), N (Neotrópico), P (Paleotrópico).

Especies	**SEC**	**Hd**	**π**	**F_{ST}**	**Referencia**
Ficus hirta ^{DP}	cp	0.229	-	0.729	(Yu *et al.*, 2010)
F. bonijesulapensis ^{MN}	cp	0.848	0.001	0.837*	(Vieira *et al.*, 2015)
F. insipida ^{MN}	cp	0.650-0.700	0.031-0.037	0.720*	(Honorio Coronado *et al.*, 2014)
	cp	0.614	0.002	0.648	(Costa *et al.*, 2017)
	ITS	0.192	0.0005	0.030	(Costa *et al.*, 2017)
F. aghatodifolia ^{MP}	cp	0.589	0.003	0.899	(Costa *et al.*, 2017)
	ITS	0.101	0.0002	-0.170	(Costa *et al.*, 2017)
F. altissima ^{MP}	cp	0.245	0.001	0.958	(Huang, Darwell, & Peng, 2021)

* G_{ST}

En los estudios que abordan regiones mitocondriales o de núcleo en avispas Agaonidae, se observa una menor proporción en las que viven en especies monoicas (Tabla 5). La variación genética de la mitocondria es mayor en comparación con la región del ITS. Los valores de diversidad haplotípica (Hd) en la mitocondria oscilan entre 0.535 y 0.975, mientras que para ITS el valor es de 0.428. Esta discrepancia se refleja también en los valores de diversidad nucleotídica (π), donde la mitocondria muestra un rango de 0.003-0.010, mientras que ITS registra un valor de 0.001. Estos patrones sugieren una mayor complejidad genética en la mitocondria.

No obstante, recientemente han aumentado los estudios que debaten la fiabilidad de los marcadores mitocondriales en avispas Agaonidae. Dos fenómenos relevantes en este debate son la heteroplasmia y los NUMT (segmentos de ADN mitocondrial nuclear) (Song *et al.*, 2008; Chen *et al.*, 2014; Ricardo, Françoso, & Arias, 2020). La presencia de estas secuencias se ha interpretado, a menudo, como la presencia de especies crípticas, especialmente en ausencia de conocimientos taxonómicos y ha contribuido a sobreestimar el número de especies y a inducir a error en las inferencias sobre estructura genética y diferenciación (Cruaud *et al.*, 2017; Ricardo *et al.*, 2020).

La interacción *Ficus*-Agaonidae se caracteriza por una dinámica compleja, influenciada por varios factores. Aunque la investigación en especies neotropicales aun presenta diversas lagunas en su conocimiento, la topografía y otros elementos geográficos emergen como factores importantes en la evolución de los hospederos (Chen *et al.*, 2011; Heer *et al.*, 2015; Bain *et al.*, 2016; Deng *et al.*, 2020c). Los sistemas reproductivos, con especies dioicas mostrando mayor apareamiento entre individuos relacionados y las monoicas exhibiendo extensiones y homogeneidad genética, indican adaptaciones diferenciales a las presiones ambientales. La mayor diferenciación genética en las avispas respecto a los hospederos sugiere que, el apareamiento selectivo y la sensibilidad a las condiciones climáticas, podrían impulsar una tasa acelerada de especiación en los polinizadores, desacoplando los procesos de especiación, evidenciado en la coexistencia de varias avispas en un solo hospedero (Compton *et al.*, 2009; Darwell, Al-Beidh, & Cook, 2014; Bain *et al.*, 2016; Rodriguez *et al.*, 2017; Yu *et al.*, 2019; Satler *et al.*, 2019).

En resumen, se sugiere que las barreras geográficas y las adaptaciones influyen en las divergencias poblacionales, generando un desacople temporal en la relación uno a uno. No obstante, esto último podría ser transitorio, ya que se ha sugerido que las avispas tienen el potencial de imponer nuevas barreras reproductivas, restaurando la relación uno a uno (Souto-Vilarós *et al.*, 2019).

Tabla 4. Atributos genéticos en especies de Agaonidae mediante marcadores moleculares codominantes. Ho: Heterocigosidad observada; He: Heterocigosidad esperada; F_{ST}: Diferenciación genética entre poblaciones; F_{IS}= Coeficiente de endogamia. Abreviaturas: D (dioica), M (monoica), N (Neotrópico), P (Paleotrópico).

Especies	**Hospedero**	**Marcador (loci)**	**Ho**	**He**	**F_{ST}**	**F_{IS}**	**Referencias**
Blastophaga nipponica, B. taiwanenesis, B. tannoensis, B. yeni	*Ficus erecta* ^{DP}, *F. formosana* ^{DP}, *F. tannoensis* ^{DP}, *F. vaccinioides* ^{DP}	SSR (13)	-	-	0.0092-0.561	-	(Wachi *et al.*, 2016)
		SNP (1000)	-	-	0.075-0.933	-	(Wachi *et al.*, 2016)
Ceratosolen armipes, C. kaironkensis	*F. itoana* ^{DP}	SNP (26083)	-	-	0.004-0.872	-	(Souto-Vilarós *et al.*, 2019)
Ceratosolen solitarius	*F. arfakensis* ^{DP}	SNP (39033)	-	-	0.013-0.667	-	(Souto-Vilarós *et al.*, 2019)
Ceratosolen bisulcatus jucundus	*F. septica* ^{DP}	SSR (7)	0.210	0.300	0.560	-	(Lin *et al.*, 2011)
Ceratosolen sp. 1		SSR (7)	0.360	0.450	0.560	-	(Lin *et al.*, 2011)
		SSR (15)	-	-	-0.001-0.018	0.236	(Lin, Yeung, & Li, 2008)
Ceratosolen sp.	*F. tikoua* ^{DP}	SSR (20)	0.232	0.812	0.232	0.714	(Deng *et al.*, 2020c)
Ceratosolen "TRI"	*F. trichocerasa* ^{DP}	SNP (8303)	-	-	0.004-0.811	-	(Souto-Vilarós *et al.*, 2019)
Kradibia wassae	*F. wassa* ^{DP}	SNP (16304)	-	-	0.095-0.893	-	(Souto-Vilarós *et al.*, 2019)
Valisia javana	*F. hirta* ^{DP}	SSR (9)	0.405	0.580	0.121	0.316	(Tian *et al.*, 2015)
Valisia sp. 1		SSR (9)	0.427	0.558	0.026-0.329	0.275	(Yu *et al.*, 2019)
Valisia sp. 2		SSR (9)	0.342	0.442	0.019-0.086	0.272	
Valisia sp. 3		SSR (9)	0.421	0.512	0.212-0.424	0.169	

Valisia sp. 4		SSR (9)	0.381	0.517	0.180-0.440	0.338	
Valisia sp. 5		SSR (9)	0.349	0.402	0.194-0.581	0.091	
Valisia sp. 6		SSR (9)	0.244	0.395	0.168-0.581	0.383	
Valisia sp. 7		SSR (9)	0.359	0.442	0.034-0.485	0.147	
Valisia sp. 8		SSR (9)	0.521	0.607	0.210-0.348	0.093	
Valisia sp. 9		SSR (9)	0.317	0.399	0.228-0.465	0.161	
Wiebesia pumilae	*F. pumila* ^{DP}	SSR (10)	0.670	0.720	0.059	-	(Liu *et al.*, 2015)
Wiebesia sp. 1		SSR (10)	0.648	0.733	-	0.119	(Liu *et al.*, 2013)
		SSR (9)	0.589	0.681	0.030	0.137	(Tong *et al.*, 2021)
Pegoscapus hoffmeyeri sp. A	*F. obtusifolia* ^{MN}	SSR (13)	0.430	-	0.008	0.848	(Molbo *et al.*, 2004)
Pegoscapus hoffmeyeri sp. B		SSR (13)	0.245	-	0.016	0.877	
Pegoscapus gemellus sp. A		SSR (13)	0.310	-	-0.048	0.404	
Pegoscapus gemellus sp. B		SSR (13)	0.666	-	-0.002	0.169	
Ceratosolen fusciceps	*F. racemosa* ^{MP}	SSR (7)	0.636	0.778	0.001-0.039	0.190	(Kobmoo *et al.*, 2010)
Ceratosolen sp.		SSR (7)	0.110	0.160	0.414-0.497	0.300	
Pleistodontes imperialis	*F. rubiginosa* ^{MP}	SSR (8)	0.110-0.470	0.400-0.760	0.280-0.540	-	(Sutton *et al.*, 2017)

Tabla 5. Atributos genéticos en especies de Agaonidae mediante secuencias genéticas. SEC: tipo de secuencia genética de ADN; Hd: diversidad haplotípica; π: diversidad nucleotídica; F_{ST}: diferenciación genética entre poblaciones. Abreviaturas: mt (mitocondria), D (dioica), M (monoica), N (Neotrópico), P (Paleotrópico).

Especies	**Hospedero**	**SEC**	**Hd**	**π**	**F_{ST}**	**Referencia**
Blastophaga nipponica, B. taiwanenesis, B. tannoensis, B. yeni	*F. erecta* ^{DP}, *F, formosana* ^{DP}, *F. tannoensis* ^{DP}, *F. vaccinioides* ^{DP}	mt	-	-	0.011-0.944	(Wachi *et al.*, 2016)
Ceratosolen bisulcatus jucundus	*F. septica* ^{DP}	mt	0.935	0.003	-	(Lin *et al.*, 2011)
Ceratosolen sp.		mt	0.967	0.003	-	(Lin *et al.*, 2011)
		mt	0.975	0.003	-	Lin et al., 2008
Ceratosolen sp.	*F. tikoua* ^{DP}	mt	0.798	0.007	0.425	(Deng *et al.*, 2020c)
		ITS	0.428	0.001		
Valisia javana	*F. hirta* ^{DP}	mt	0.921	0.004	0.262	(Tian *et al.*, 2015)
Valisia sp. 1			0.933	0.000	0.446	(Yu *et al.*, 2019)
Valisia sp. 2			0.972	0.000	0.172	
Valisia sp. 3			0.923	0.004	-	
Valisia sp. 4			0.881	0.004	-	
Valisia sp. 5			0.309	0.000	-	
Valisia sp. 6			1	0.001	0.126	
Valisia sp. 7			0.944	0.001	0.824	
Valisia sp. 8			0.909	0.008	-	
Valisia sp. 9			0.714	0.004	-	
Wiebesia sp.	*F. pumila* ^{DP}	mt	0.779	0.003	0.425	(Chen *et al.*, 2012)
		ITS	0.428	0.001	-	
Eupristina verticillata	*F. microcarpa* ^{MP}	mt	0.535-0.900	0.001-0.004	-	(Sun *et al.*, 2011)
Pleistodontes imperialis	*F. rubiginosa* ^{MP}	mt	-	0.007-0.010	-	(Sutton *et al.*, 2017)

La interacción de *Ficus* y sus avispas polinizadoras en el Neotrópico

La región del Neotrópico abarca desde México hasta el sur de Sudamérica y es una de las regiones más biodiversas de la Tierra (Antonelli & Sanmartin, 2011). En el Neotrópico hay varios biomas, comunidades y hábitats (Antonelli *et al.*, 2018a; Liu *et al.*, 2023). La diversificación neotropical ha sido resultado de una compleja interacción de factores bióticos y abióticos (Antonelli & Sanmartin, 2011). Entre los procesos abióticos se encuentran los cambios hidrológicos y topográficos provocados por el levantamiento de las montañas y por los cambios climáticos (Hoorn *et al.*, 2010; Antonelli & Sanmartin, 2011; Mastretta-Yanes *et al.*, 2015). En los procesos bióticos están las interacciones y la evolución de rasgos específicos de cada organismo (Fine, Mesones, & Coley, 2004; Antonelli *et al.*, 2018a).

En el Neotrópico se encuentran dos subgéneros monoicos de *Ficus*: *Spherosuke* Raf. y *Pharmacosycea* (Miq.) Miq (Figura 2). Dentro de estos subgéneros, hay secciones específicas que han colonizado América por diferentes rutas, en diferentes periodos y presentan diferencias en cuanto a su polinización y características morfológicas (Berg & Corner, 2005; Cruaud *et al.*, 2012; Pederneiras, Mansano, & Romaniuc-Neto, 2017; Pederneiras *et al.*, 2018). La sección *Pharmacosycea* (Miq.) Griseb. (ca. 31 spp.), del subgénero *Pharmacosycea* colonizó América desde Europa a través de Norteamérica, durante el Eoceno temprano (ca. 42 Ma; Pederneiras *et al.*, 2018). Los árboles de esta sección tienen un proceso de germinación en el suelo y exhiben un hábito exclusivamente terrestre. Aunque la mayoría de los miembros de esta sección tienen una polinización por avispas del género *Tetrapus* Mayr; se ha sugerido la existencia de otro género, *Hexapus* Ramírez (Berg & Corner, 2005; Ibarra-Manríquez *et al.*, 2012; Ramírez, 2016).

Por otro lado, la sección *Americanae* (Miq.) Corner (ca. 120 spp.), del subgénero *Spherosuke* Raf., tuvo su origen en África y colonizó Sudamérica en el Eoceno tardío (ca. 39 Ma; Pederneiras *et al.*, 2018). Los árboles de esta sección pueden germinar en condiciones rupícolas o adoptar hábitos epífitos, hemiepífitos o estranguladores, estos últimos, a medida en la que se crecen, matan el árbol en el germinaron. Su polinización es llevada a cabo por avispas del género *Pegoscapus* Cameron (Berg & Corner, 2005; Ibarra-Manríquez *et al.*, 2012).

La investigación sobre las avispas polinizadoras de *Ficus* en la región Neotropical es limitada en cuanto estudios poblacionales. Un estudio abordó la endogamia en dos pares de avispas cripticas que coexisten en *F*. *obtusifolia*, sugiriendo que las especies con un menor número fundadoras tienen niveles más altos de endogamia. Además, examinaron la diferenciación genética entre avispas muestreadas en diferentes árboles y momentos, sin encontrar evidencia de desequilibrio genotípico (Molbo *et al.*, 2004). En cuanto a los hospederos, la investigación en genética poblacional es igualmente limitada, mayormente utilizando marcadores de cloroplasto y nucleares. Estos estudios han revelado una baja diferenciación nuclear entre las poblaciones de hospederos (Poelchau & Hamrick, 2013; Honorio Coronado *et al.*, 2014, 2019; Costa *et al.*, 2017).

Los estudios poblacionales se han realizado principalmente en *F*. *insipida*, una especie de la sección *Pharmacosycea*, que se encuentra desde México hasta la selva de la Amazonia occidental (Poelchau & Hamrick, 2013; Honorio Coronado *et al.*, 2014; Costa *et al.*, 2017). Sus patrones filogeográficos pueden ser explicados por barreras ecoclimáticas, como la cordillera de los Andes y las zonas secas del norte de Sudamérica, pero también por fluctuaciones climáticas del Cuaternario (Poelchau & Hamrick, 2013; Honorio Coronado *et al.*, 2014, 2019; Costa *et al.*, 2017). En *F*. *bonijesulapensis*, una especie de la sección *Americanae*, endémica de los afloramientos rocosos de los bosques secos de Brasil, la estructura y diversidad genética están también relacionadas con fluctuaciones climáticas del Cuaternario (Vieira *et al.*, 2015). Heer et al. (2015) encontraron que las especies de ambas secciones de *Ficus* pueden presentar baja estructura genética, debido a la dispersión a larga distancia del polen, aunque las semillas tienen una dispersión localizada y hay establecimiento agrupado de plántulas.

La Zona de Transición Mexicana (ZTM) es un territorio de gran complejidad geológica, geográfica y climática, que ha desempeñado un papel fundamental en la generación de diferenciación genética en diversos grupos de especies y en la megadiversidad de la región neotropical (Halffter & Morrone, 2017; Anguiano-Constante *et al.*, 2021). Esta región se caracteriza por ser el punto de encuentro entre las regiones Neártica y Neotropical (Halffter & Morrone, 2017) y se ha propuesto que los sistemas montañosos, como la Faja Volcánica Transmexicana (FVTM) en la ZTM, desempeñan un papel crucial en la diversificación biótica de México, teniendo impactos diferenciados en diversos taxones (Mastretta-Yanes *et al.*, 2015). También se ha sugerido que las tierras bajas de la FVTM han funcionado como una barrera física, al introducir una discontinuidad geográfica y generar diferenciación genética, causando patrones discordantes entre especies con baja y alta capacidad de dispersión (Arbeláez-Cortés, Roldán-Piña, & Navarro-Sigüenza, 2014; López-Barrera et al., 2021; Schramm, Valdez-Mondragón, & Prendini, 2021; Zarza, Reynoso, & Emerson, 2008). Sin embargo, el patrón de diversidad y estructura genética en las especies de *Ficus* y sus polinizadores de esta región no es hasta conocido en este momento.

En México, de las 25 especies de *Ficus*, tres son endémicas (Ibarra-Manríquez *et al.*, 2012; Hernández-Esquivel *et al.*, 2020). Una de estas especies es *Ficus pringlei* S. Watson (Moraceae, subgén. *Spherosuke,* secc. *Americanae*), la cual se encuentra, mayormente, en bosques tropicales estacionalmente secos del oeste de México, particularmente en los estados de Colima, Guerrero, Jalisco, Michoacán, Nayarit y Zacatecas (Serrato *et al.*, 2004; Ibarra-Manríquez *et al.*, 2012; Rojas-Cortés *et al.*, 2022). Esta especie de *Ficus* es polinizada por avispas no descritas taxonómicamente del género *Pegoscapus* (Agaonidae). No obstante, otras especies de *Ficus* muestran una distribución más amplia, especialmente en los bosques tropicales perennifolios y bosques húmedos de montaña (Serrato, Ibarra-Manríquez, & Oyama, 2004; Ibarra-Manríquez *et al.*, 2012).

La interacción de *F. pringlei-Pegoscapus* sp. aún no ha sido estudiada, pero se trata de un caso interesante para entender a las interacciones mutualistas. *Ficus pringlei*, a pesar de su restringida distribución, encuentra su hábitat principal en el bosque tropical estacionalmente seco del oeste de México. Este entorno específico, con una compleja historia ambiental y biogeográfica, podría albergar barreras geográficas y ecológicas significativas. Por lo tanto, explorar el papel de estas barreras para comprender mejor la dinámica de este mutualismo es esencial. Además, establecer el grado de especificidad y congruencia en la variación y diferenciación genética de sus interactuantes aportaría información relevante para comprender cómo se diversifican y cuáles son los procesos que contribuyen al mantenimiento y diferenciación de los linajes involucrados en el mutualismo.

El objetivo principal en esta tesis fue entender, desde una perspectiva microevolutiva, la interacción mutualista entre *F. pringlei* y sus avispas polinizadoras. De esta manera, analicé la diversidad y diferenciación genética a nivel de población de ambas especies. La tesis está estructurada en tres partes. El Capítulo 1 describo aspectos de la historia natural de *F. pringlei*, con énfasis en su biología reproductiva, describiendo las etapas de desarrollo del sícono y su polinización. Además, examinó su distribución potencial, su variación ambiental y las áreas con ambientes que podrían ser adecuados para su distribución para, posteriormente, elegir las localidades para logar un muestreo representativo para estudiar la genética poblacional de la especie. En el Capítulo 2 estudié los patrones de diversidad genética y los factores que han dado forma a la estructura genética de *F. pringlei*, a lo largo de su área de distribución, con el uso de SNPs nucleares, debido a su alta abundancia, facilidad de genotipado, cobertura relativamente densa en el genoma y al muestreo eficiente de las variaciones genéticas. Esta elección se fundamentó en las limitaciones de los marcadores tradicionales, como secuencias o microsatélites para el cloroplasto o el núcleo, que no ofrecen la misma combinación de ventajas en términos de eficacia y eficiencia en la obtención de información genética detallada. En el Capítulo 3 presento la diversidad y estructura genética de la avispa *Pegoscapus* sp., polinizadora de *F. pringlei*, a partir de secuencias de COI, que resultaron útiles por su alta variabilidad y uso extenso en otros estudios de avispas Agaonidae. Finalmente, incluyo una discusión general, que integra los resultados de los distintos capítulos y las conclusiones derivadas de los mismos.

En el material suplementario incluyo el cuento infantil *El secreto mejor guardado del universo*, que se presentó a la convocatoria del libro digital de cuentos infantiles de Sociedad Científica Mexicana de Ecología, con la intención de transmitir la importancia de la relación mutualista entre las especies de *Ficus* y sus avispas polinizadoras.

CAPÍTULO 1. Reproductive biology and potential distribution of *Ficus pringlei* (Moraceae), a microendemic New World species

Publicado en Acta Botanica Mexicana 129: e2048. DOI: https://doi.org/10.21829/abm129.2022.2048

Localidades donde se encuentra *Ficus pringlei*. Arriba: paisaje fragmentado en Tequila, Jalisco (marzo de 2019). Abajo: Un paisaje con mayor cobertura vegetal en Cuatro Caminos, Michoacán (agosto de 2019).

Acta Botanica Mexicana

Research article

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

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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.

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Received: March 16, 2022.
Reviewed: May 20, 2022.
Accepted by Marie-Stéphanie Samain: June 3, 2022.
Published Online first: June 27, 2022.
Published: Acta Botanica Mexicana 129(2022).

To cite as: Rojas-Cortés, A.P., A.P. Cuervo-Robayo, G. Cornejo-Tenorio and G. Ibarra-Manríquez. 2022. Reproductive biology and potential distribution of *Ficus pringlei* (Moraceae), a microendemic New World species. Acta Botanica Mexicana 129: e2048. DOI: https://doi.org/10.21829/abm129.2022.2048

e-ISSN: 2448-7589

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; Jousselin 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 (Jousselin 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 (Jousselin 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 (Jousselin 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 (Truiller 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±*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±*s.d.* 0.03) than at the base of the syconium (0.1±*s.d.* 0.04). The mean total number of pistillate flowers was 561.5±*s.d.* 138.8 and staminate flowers 43.7±*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.

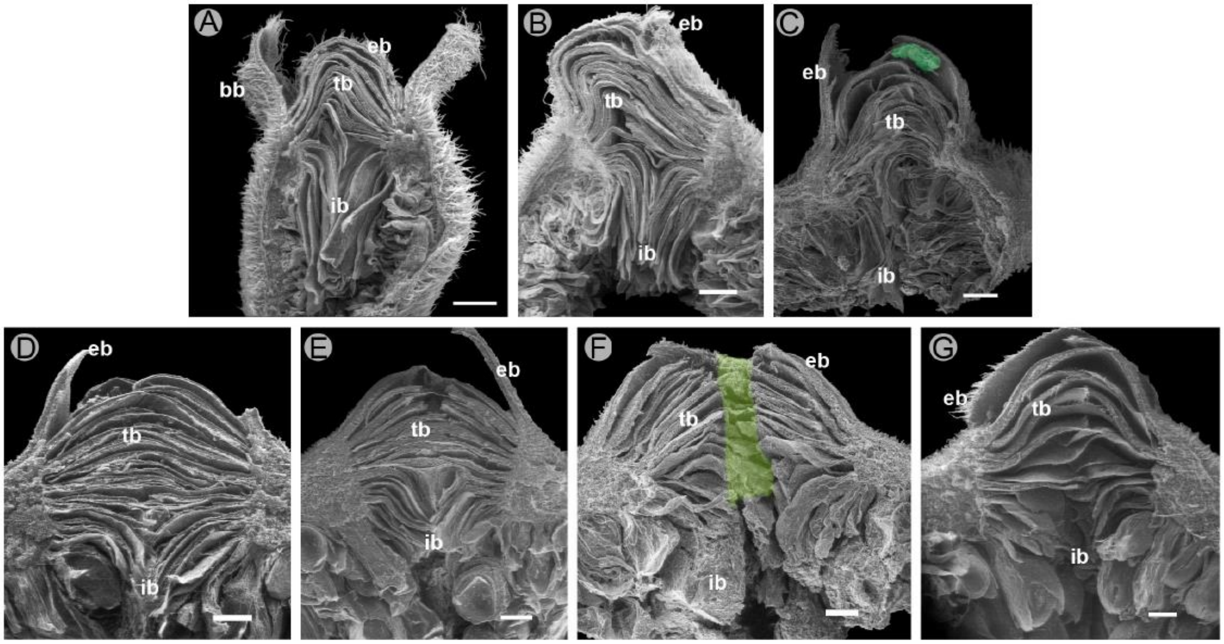

Figure 2: Longitudinal section of the syconium of *Ficus pringlei* S. Watson, in different stages of development. A. Phase A1; B. Phase B; C. Phase C1 with a pollinating wasp (green); D. Phase C2; E. Phase C3; F. Phase D with one exit tunnel excavated by males; G. Phase E. bb=basal bracts, eb=external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

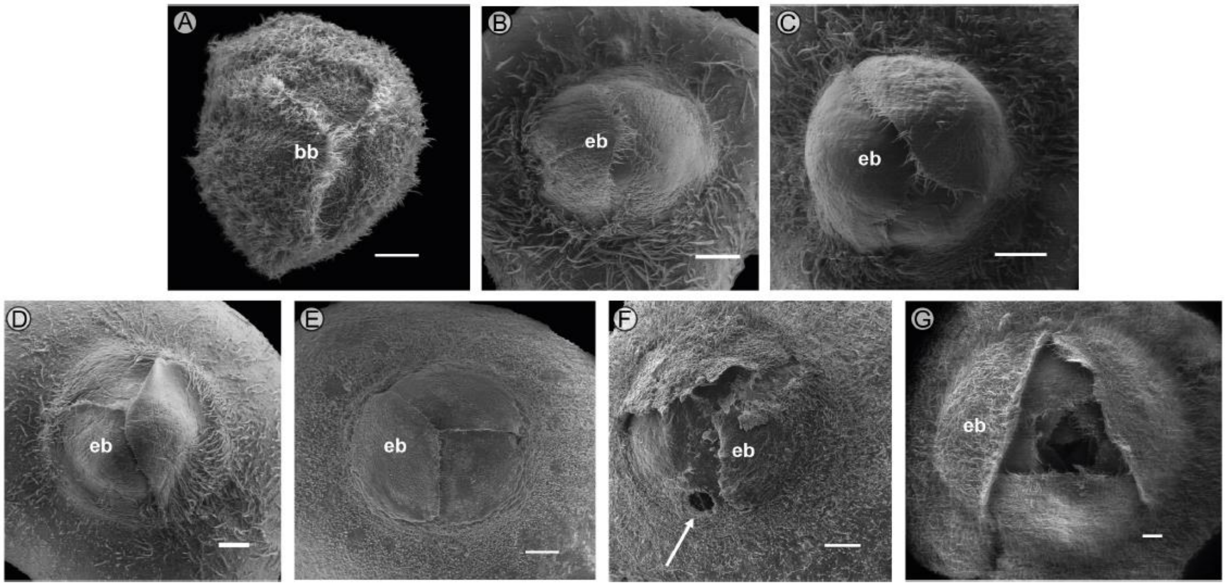

Figure 3: Superior view of the ostiole of *Ficus pringlei* S. Watson, in different stages of development. A. Phase A1; B. Phase B; C. Phase C1; D. Phase C2; E. Phase C3; F. Phase D with one exit tunnel excavated by males; G. Phase E. bb=basal bracts, eb=external bracts. Scale bars: 500 µm.

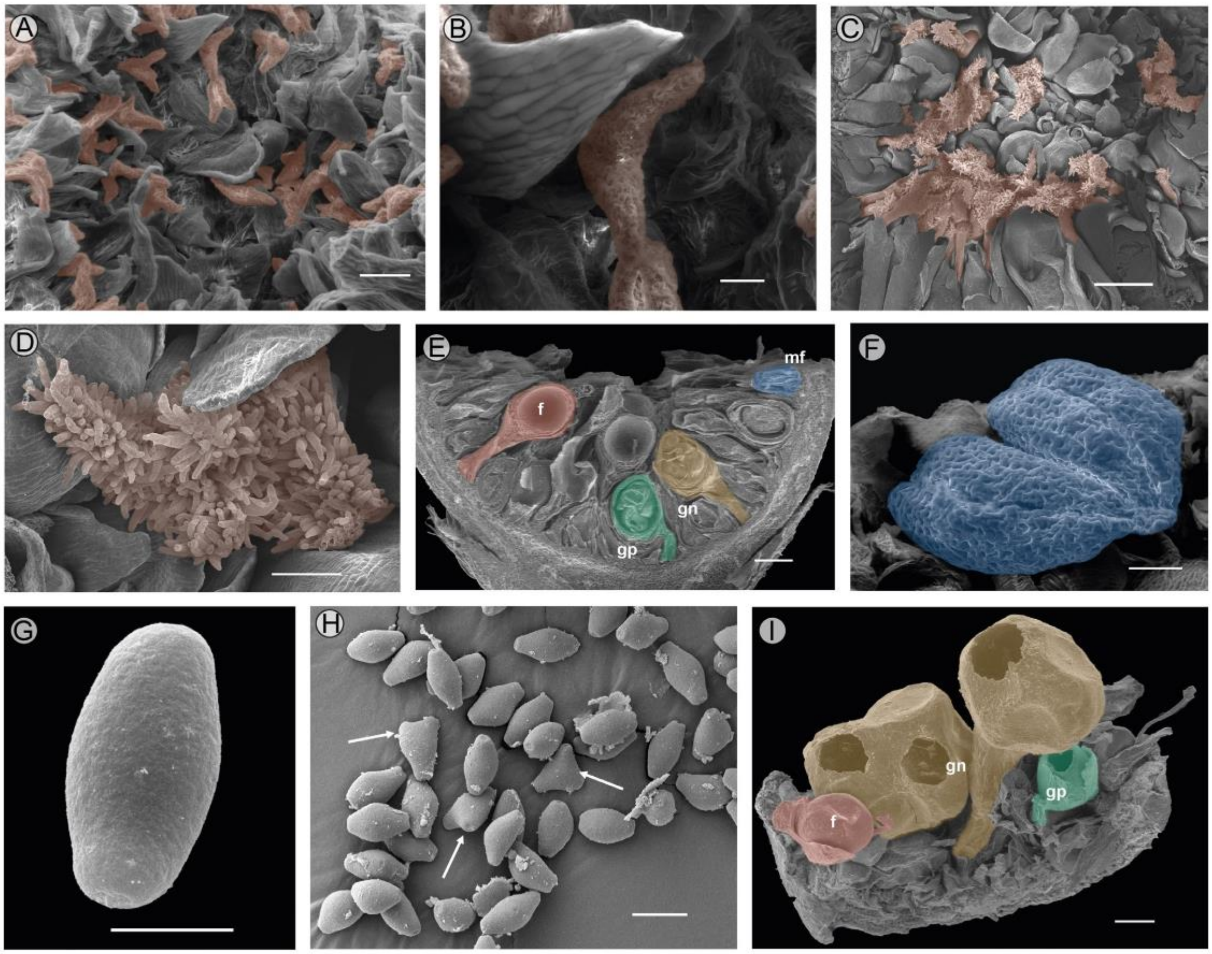

Figure 4: Internal morphology of different stages of development and pollen of *Ficus pringlei* S. Watson. A. stigmata in Phase A2; B. detail of stigmata in Phase A2; C. synstigma formed by the entanglement of styles and stigmatic papillae in Phase B; D. detail of stigmatic papillae; E. longitudinal 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 km^2; 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 km^2; 43.9% of the total predicted area), primary (1594 km^2) and secondary (3832 km^2) TDF vegetation were the vegetation types with the largest area, followed by forests of oak (2426 km^2) and oak-pine (1799 km^2) (Fig. 6). When considering the suitable area accounting for vegetation types, only 1926 km^2 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 km^2) 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,

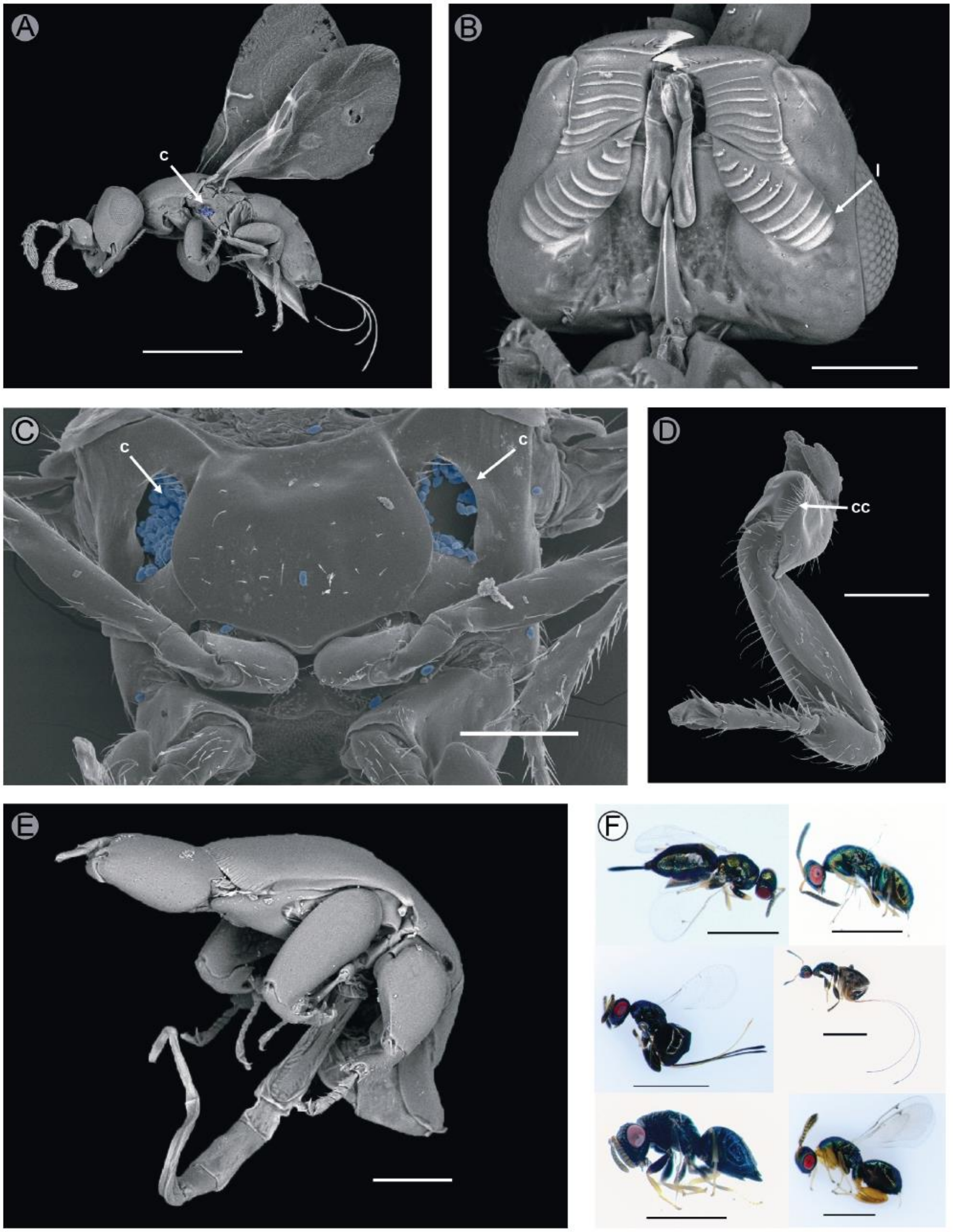

Figure 5: Pollinating (*Pegoscapus* sp.) and non-pollinating wasps of *Ficus pringlei* S. Watson. A. female wasp of *Pegoscapus* sp. (corbiculae, arrow); B. detail of the mandibles (arrow) of the female *Pegoscapus* sp.; C. corbiculae of *Pegoscapus* sp., pollen in blue; D. coxal combs on fore coxae 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 µm, B=100 µm, C=100 µm, D=100 µm, E=200 µm, F=1 mm.

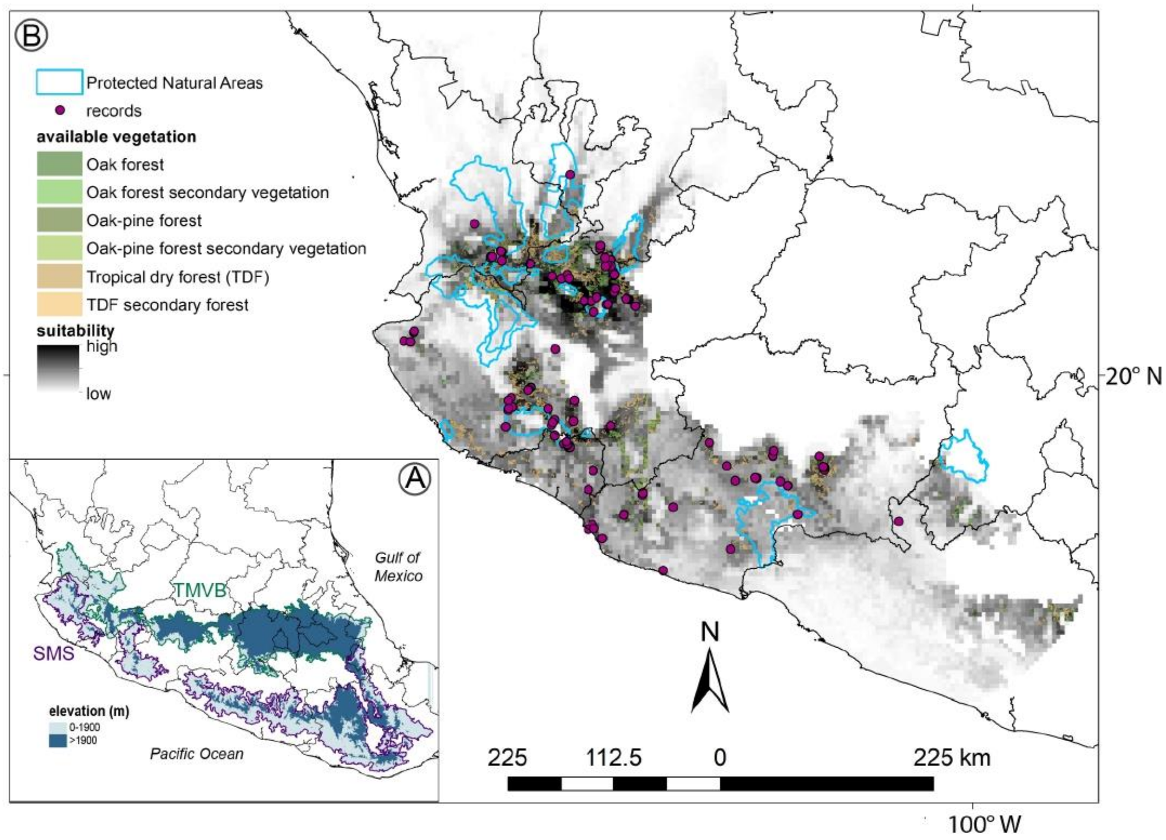

Figure 6: Potential geographical distribution of *Ficus pringlei* S. Watson, under current climate conditions. A. Trans-Mexican Volcanic Belt (TMVB) and Sierra Madre del Sur (SMS) biogeographical provinces; B. occurrence records, current potential geographical distribution, available vegetation, and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

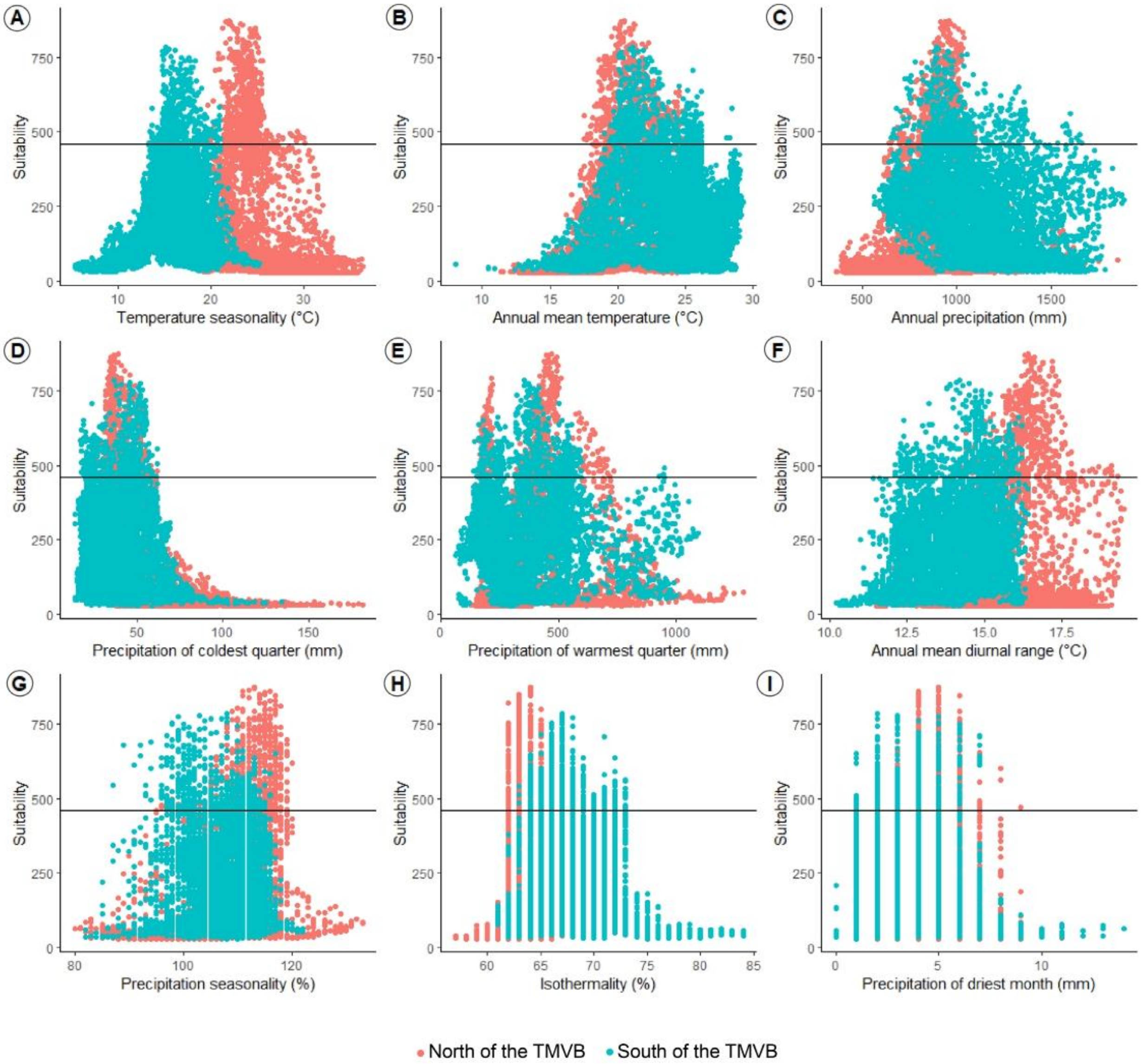

Figure 7: Habitat suitability and environmental variables in the ensemble model of *Ficus pringlei* S. Watson. The horizontal line indicates the value of the threshold to transform the model on presence and absence data. TMVB=Trans-Mexican Volcanic Belt.

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 morphological 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 km^2 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 for 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-Barrera 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.

Funding

This work was supported by the Dirección General del Personal Académico (DGAPA)- Programa de apoyo de investigación e Innovación Tecnológica (PAPIIT IN211620) of the Universidad Nacional Autónoma de México (UNAM).

Acknowledgements

We thank Iván Leonardo Ek Rodríguez for field assistance; to the microscopy laboratory of Escuela Nacional de Estudios Superiores (ENES), unidad Morelia, for the assistance with the photographs of the scanning electron microscope. This paper is a partial fulfillment of the requirements for APRC to obtain the doctoral degree from the Posgrado en Ciencias Biológicas (UNAM), and she acknowledges the scholarship provided by the Consejo Nacional de Ciencia y Tecnología (CVU 696852).

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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	*G. Ibarra 5709* (MEXU)	25.2	14.3	70	15.5	994	3	111	354	51
Colima	19.107958	-103.5979	515	*G. Ibarra 7159* (MEXU)*	23.9	14.1	69	14.9	996	2	110	364	47
Colima	19.108611	-103.5981	690	*G. Ibarra 5837* (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	*G. Ibarra 7162* (MEXU)*	21.1	13.9	68	14.8	952	3	108	370	41
Colima	19.324727	-103.8396	760	*G. Ibarra 7163* (MEXU)*	21.1	13.9	68	14.8	952	3	108	370	41
Colima	19.329008	-103.8311	886	*N. González 51* (IEB)	21.1	13.9	68	14.8	952	3	108	370	41
Colima	19.329206	-103.8344	878	*G. Ibarra 7164* (MEXU)*	21.1	13.9	68	14.8	952	3	108	370	41
Colima	19.332917	-103.8313	900	*M. Santana 2934* (IBUG)	21.1	13.9	68	14.8	952	3	108	370	41
Colima	19.342897	-103.8356	1078	*G. Ibarra 7165* (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	*G. Ibarra 7241* (MEXU)*	25.2	13.7	71	13.6	873	2	110	360	38
Colima	19.346944	-103.8492	1180	*T. Cochrane 11757* (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	*G. Ibarra 7166* (MEXU)*	25.2	13.7	71	13.6	873	2	110	360	38
Colima	19.351339	-103.8503	1189	*N. González 60* (IEB)	21.1	13.9	68	14.8	952	3	108	370	41
Colima	19.354444	-103.8514	1204	*R. Cuevas 5845* (IEB)	20.1	13.8	67	14.9	1052	4	104	416	51
Colima	19.360278	-103.8861	1420	*R. Cuevas 4041* (IBUG)	21.1	13.9	68	14.8	952	3	108	370	41
Colima	19.364167	-103.8600	1400	*G. Ibarra 5792* (IEB)	20.1	13.8	67	14.9	1052	4	104	416	51
Colima	19.364750	-103.8582	1404	*N. González 56* (IEB)	21.1	13.9	68	14.8	952	3	108	370	41
Colima	19.378889	-103.8589	1508	*G. Ibarra 6161* (MEXU)	25.2	14.3	70	15.5	994	3	111	354	51
Colima	19.436944	-103.9722	1600	*M. Santana 5215* (MEXU)	20.3	13.9	67	15.1	959	3	105	378	45
Colima	19.436944	-103.9722	1728	*M. Santana 5215* (MEXU)	23.9	14.1	69	14.9	996	2	110	364	47
Guerrero	18.636383	-100.7016	531	*F. González 6119* (MEXU)	28.4	15.1	62	20.8	946	1	116	201	16
Jalisco	19.517972	-104.4402	527	*F. Guzmán 185* (ZEA)	23.2	16.9	63	25.3	882	6	117	433	41
Jalisco	19.528363	-103.4331	1039	*L. Villareal 12726* (IBUG)	20.1	16.5	63	24.6	917	3	112	214	32
Jalisco	19.530258	-104.0024	1575	*G. Nieves 542* (IBUG)	20.4	16.4	64	22.8	997	6	110	472	46
Jalisco	19.560223	-103.9914	1214	*R. Cuevas 3751* (ZEA)	19.9	16.5	62	24.8	925	5	110	214	36
Jalisco	19.572638	-103.7969	1248	*M. Santana 7044* (ZEA)	21.6	17.3	63	25.1	916	5	117	196	33
Jalisco	19.583572	-103.9754	822	*A. Rodríguez 2054* (MEXU)	19.9	16.5	62	24.8	925	5	110	214	36
Jalisco	19.674722	-104.4144	1046	*C. Gómez 9* (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	*F. Santana 6007* (IBUG)	18.6	16	62	23.5	976	5	114	479	35
Jalisco	19.692222	-104.4122	1267	*F. Santana 6916* (IBUG)	20.4	16.4	64	22.8	997	6	110	472	46
Jalisco	19.703722	-104.3720	1125	*G. Ibarra 7120* (MEXU)*	19.3	16.1	63	23.1	971	5	114	473	38
Jalisco	19.764722	-104.4142	1004	*U. Ramírez 7* (IBUG)	19.4	16.3	62	24.5	957	5	113	459	38
Jalisco	19.765305	-103.7871	1286	*E. Lott 424* (MO)	23.2	17.9	63	25.6	878	5	116	195	31
Jalisco	19.790556	-104.3856	1157	*R. Hernández 18* (IBUG)	19.3	16.1	63	23.1	971	5	114	473	38
Jalisco	19.858333	-104.2250	1033	*M. Santana 6747* (IBUG)	21.9	16.8	63	23.7	973	4	115	213	37
Jalisco	19.871906	-104.2177	1211	*G. Ibarra 7248* (MEXU)*	22.5	14.9	67	17.7	884	6	100	370	52
Jalisco	19.885325	-104.2008	1229	*G. Ibarra 7249* (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	*G. Ibarra 7250* (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	*G. Ibarra 7247* (MEXU)*	22.3	14.6	67	16.9	963	5	102	389	55
Jalisco	20.307374	-105.3391	644	*G. Ibarra 7167* (MEXU)*	21.6	17.3	63	25.1	916	5	117	196	33
Jalisco	20.307389	-105.3406	652	*T. Cochrane 11941* (F)	21.5	17.4	62	28.8	715	6	112	322	41
Jalisco	20.314718	-105.4020	560	*G. Ibarra 7168* (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	*G. Ibarra 7171* (MEXU)*	22.1	17.5	63	25.3	903	5	117	196	32
Jalisco	20.406438	-105.3043	641	*G. Ibarra 7180* (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	*L. Villareal 10072* (IBUG)	21.9	16.8	63	23.7	973	4	115	213	37
Jalisco	20.643352	-103.1967	1485	*R. Acevedo 1541* (XAL)	19	16	62	24.3	932	5	112	454	36
Jalisco	20.657830	-103.4735	1806	*L. Hernández 834* (IBUG)	21.9	16.8	63	23.7	973	4	115	213	37
Jalisco	20.662286	-103.4579	1786	*G. González 68* (MEXU)	21.9	16.8	63	23.7	973	4	115	213	37
Jalisco	20.687573	-103.6920	1400	*K. Manzano 6* (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	*O. Reyna 560* (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	*G. Ibarra 7245* (MEXU)*	22.8	13.7	69	19.8	1613	4	115	937	45
Jalisco	20.692647	-103.6192	1456	*G. Ibarra 7246* (MEXU)*	22.8	13.7	69	19.8	1613	4	115	937	45
Jalisco	20.695514	-103.6944	1367	*L. Villareal 9911* (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	*G. Ibarra 7072* (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	*G. Ibarra 7071* (MEXU)*	21.9	16.8	63	23.7	973	4	115	213	37
Jalisco	20.710182	-103.2862	1500	*H. Hernández 78* (IBUG)	19.9	16.5	62	24.8	925	5	110	214	36
Jalisco	20.713348	-103.2775	1074	*G. Ibarra 7242* (MEXU)*	24.2	15.3	67	19	760	3	97	315	50
Jalisco	20.725278	-103.5642	1603	*J. Calzada 13533* (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	*A. Frías 347* (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	*G. Ibarra 7177* (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	*G. Ibarra 5380* (MEXU)	20.9	17.3	63	25.4	893	4	118	434	34
Jalisco	20.906867	-103.8434	1265	*G. Cornejo 5138* (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	*G. Ibarra 7122* (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	*G. Ibarra 7178* (MEXU)*	21	17.2	63	24.9	929	5	116	445	35
Jalisco	20.936424	-103.3966	1266	*G. Ibarra 7231* (MEXU)*	22.5	14.9	67	17.7	884	6	100	370	52
Jalisco	20.936444	-103.3958	1266	*G. Ibarra 7229* (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	*L. Villareal 6092* (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	*P. Carrillo 1842* (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	*G. Ibarra 7240* (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	*G. Ibarra 7232* (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	*J. Rzedowski 14270* (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	*P. Carrillo 1253* (IBUG)	23.2	17.9	63	25.6	878	5	116	195	31
Jalisco	21.868783	-103.8279	1660	*A. Flores 1846* (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	*G. Ibarra 7176* (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	*G. Ibarra 7137* (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	*G. Ibarra 7146* (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. Ek 415* *	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	*G. Ibarra 7175* (MEXU)*	26.9	16	68	17.2	861	1	112	171	18
Michoacán	18.178756	-102.9316	937	*N. González 18* (MEXU)	26.9	16	68	17.2	861	1	112	171	18
Michoacán	18.378799	-102.2964	943	*J. Soto 1711* (MEXU)	24.9	16	68	16.5	1004	3	112	215	23
Michoacán	18.478611	-103.5131	623	*B. Guerrero 879* (MEXU)	26.9	16	68	17.2	861	1	112	171	18
Michoacán	18.565829	-103.6492	160	*G. Ibarra 5968* (MEXU)	21	13	64	16.1	1309	5	102	536	43
Michoacán	18.569722	-103.5906	553	*B. Guerrero 224* (MEXU)	21	13	64	16.1	1309	5	102	536	43
Michoacán	18.610556	-103.6053	695	*B. Guerrero 869* (INIREB)	23.7	15.8	70	15	1043	3	111	211	30
Michoacán	18.698597	-103.3043	1389	*N. González 8* (MEXU)	28.7	15.3	67	16.6	661	2	105	132	30
Michoacán	18.701389	-101.6606	613	*G. Ibarra 2013* (MEXU)	25.5	15.7	69	14.7	872	1	113	167	20
Michoacán	18.771192	-102.8399	1355	*J. Soto 9234* (MEXU)	28.7	15.3	67	16.6	661	2	105	132	30
Michoacán	18.888501	-103.1327	1195	*J. Rzedowski 16670* (ENCB)	28.7	15.3	67	16.6	661	2	105	132	30
Michoacán	18.905000	-103.1258	1233	*G. Ibarra 2008* (MEXU)	28.2	15.6	66	18	708	2	106	150	25
Michoacán	18.969167	-101.7539	650	*G. Ibarra 5962* (MEXU)	21.3	12.4	65	15.2	1328	4	100	514	48
Michoacán	19.017036	-102.2502	303	*R. Torres 1538* (MEXU)	21.3	12.4	65	15.2	1328	4	100	514	48
Michoacán	19.042889	-102.0615	401	*M. Mendez 2009* (MEXU)	26.2	15.8	69	14.8	858	1	113	161	19
Michoacán	19.149444	-102.3292	1011	*WM. Leavenworth 1626* (MO)	28.7	15.3	67	16.6	661	2	105	132	30
Michoacán	19.243611	-101.4533	1750	*D. Ramírez* s. n. (MEXU)	24.5	15.6	69	14.3	924	2	112	179	23
Michoacán	19.369587	-102.4967	1209	*J. Soto 2457* (MEXU)	28.7	15.3	67	16.6	661	2	105	132	30
Nayarit	21.068167	-104.4768	1038	*O. Téllez 12709* (MEXU)	19.5	15.4	64	22.6	913	4	112	411	53
Nayarit	21.092283	-104.5746	904	*V. Rudd 3017* (MEXU)	19.5	15.4	64	22.6	913	4	112	411	53
Nayarit	21.100000	-104.5781	970	*M. Harker 723* (IBUG)	20.9	14.9	65	22.6	1096	3	116	738	62
Nayarit	21.100000	-104.5667	979	*O. Téllez 12834* (MEXU)	19.5	15.4	64	22.6	913	4	112	411	53
Nayarit	21.144962	-104.4759	1299	*G. Ibarra 7127* (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	*G. Ibarra 7126* (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	*X. Madrigal 2309* (INIF)	19.5	15.4	64	22.6	913	4	112	411	53
Zacatecas	21.178349	-103.5332	1282	*G. Ibarra 7119* (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	*J. Robles 20* (MEXU)	20.5	17.2	63	25.6	889	4	118	431	37

CAPÍTULO 2. Genomic diversity and structure of a Neotropical microendemic fig tree

Manuscrito

Individuos de *Ficus pringlei*. Arriba: Zacatecas (agosto de 2021). Abajo: Huaxtla, Jalisco (marzo de 2020).

Genomic diversity and structure of a Neotropical microendemic fig tree

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Abstract

Genetic diversity is a key component of evolution and unraveling factors that promote genetic differentiation in space and time is a central question in evolutionary biology. One of the most diverse and ecologically important tree genera in tropical forests worldwide is *Ficus* (Moraceae). It has been suggested that, given the great dispersal capacity of pollinating fig wasps (Chalcidoidea; Agaonidae), the spatial genetic structure, particularly in monoecious fig species, should be weak. However, no studies have addressed the factors that determine the genetic structure of *Ficus* species in regions of high geological, geographic, and climatic complexity, such as the Mexican Transition Zone. Using nuclear single nucleotide polymorphisms (5,311 SNPs) derived from low-coverage whole genomes and 17 populations, we analyzed the population genomics of *Ficus pringlei* to characterize neutral and adaptive genetic variation and structure and its association with geographic barriers such as the Trans-Mexican Volcanic Belt and environmental heterogeneity. From a genomic data set of 71 individuals, high genetic diversity and three genetic clusters were recorded, despite the putative long distances of pollen dispersal. The results suggest that gene flow is limited by climatic conditions, particularly temperature seasonality and annual precipitation. Also, the low differentiation between populations between the north and south of the Trans-Mexican Volcanic Belt (F_{ST}= 0.021) suggests that this mountain range may not be a strong barrier to gene flow for this species. This study provides information on the possible mechanisms underlying the genetic variation of endemic species of the tropical dry forest of western Mexico, such as *F. pringlei*.

Keywords: gene flow, population structure, *Ficus*, tropical dry forest, Mexican Transition Zone

INTRODUCTION

Genetic diversity is one of the essential components of biological diversity and unraveling the factors that contribute to its origin and maintenance is a central question in evolutionary biology (Amos, 1998). Levels and geographic distribution of genetic diversity can be shaped by factors such as spatial isolation, climatic heterogeneity, and geographic barriers. However, characteristics of the life history of the species can also lead to population divergence (Hamrick, Godt, & Sherman-Broyles, 1992; Gelmi-Candusso, Heymann, & Heer, 2017). It has been suggested that widely distributed, wind-pollinated woody species have greater diversity and less differentiation between populations than endemic, animal-pollinated species with other life forms (Hamrick *et al.*, 1992; Dick *et al.*, 2007). Therefore, the study of genetic diversity and the factors that shape it allow a better understanding of the complex evolutionary histories of species.

Ficus L. (Moraceae) is a genus with high species diversity and great variation in ecological characteristics that can shape genetic diversity patterns (Nazareno *et al.*, 2013; Heer *et al.*, 2015; Beech *et al.*, 2017). Each of the approximately 800 *Ficus* species has an obligate pollination system carried out exclusively by wasps of the Agaonidae family, which can only reproduce in the pistillate flowers of the fig inflorescence or syconium (Ramírez, 1970; Janzen, 1979; POWO, 2023). Although these wasps are generally highly mobile dispersers that rely on passive dispersal by wind, their mobility, and therefore the gene flow of their hosts, can be affected by factors such as the sexual system of plants (Nason, Herre, & Hamrick, 1998; Ahmed *et al.*, 2009; Nazareno *et al.*, 2013). It has been suggested that wasps from monoecious hosts have a greater dispersal capacity than wasps in dioecious species, which is reflected in the weak genetic structure of monoecious *Ficus* (Nazareno *et al.*, 2013; Yang *et al.*, 2015; Bain *et al.*, 2016). Considering the extensive gene flow observed in monoecious species, there might be limited differentiation and adaptation to local environments, and little to no evidence of isolation by distance between populations (Bain *et al.*, 2016). However, studies on neotropical trees reveal a strong genetic differentiation in species with broader distributions (Lowe *et al.*, 2018). This suggests that geographic barriers or Quaternary climatic fluctuations may play a crucial role in facilitating genetic differentiation in Neotropical species (Honorio Coronado *et al.*, 2014; Vieira *et al.*, 2015; Heer *et al.*, 2015).

The Neotropics encompass many biomes with a particular history of landscapes and biotic evolution (Hughes, Pennington, & Antonelli, 2013). The Mexican Transition Zone (MTZ), where the Nearctic and Neotropical regions converge (Halffter & Morrone, 2017; Anguiano-Constante *et al.*, 2021), is characterized by great geological, geographic, and climatic complexity, and has contributed to the genetic variation of species and megadiversity. In this area, it has been suggested that recent mountainous complexes, such as the Trans-Mexican Volcanic Belt (TMVB), are main drivers of diversification in Mexico and have affected taxa in different ways (Mastretta-Yanes *et al.*, 2015). For example, in the lowlands, the TMBV has functioned as a barrier by introducing a genetic discontinuity in several species with different dispersal capabilities (Zarza, Reynoso, & Emerson, 2008; Arbeláez-Cortés, Roldán-Piña, & Navarro-Sigüenza, 2014; López-Barrera *et al.*, 2021; Schramm, Valdez-Mondragón, & Prendini, 2021). However, the pattern of genetic diversity and structure in the *Ficus* species of this region is unknown.

Within the MTZ is *Ficus pringlei* S. Watson (subg. *Spherosuke* Raf.*,* sect. Americanae (Miq.), a monoecious endemic species (Ibarra-Manríquez *et al.*, 2012). This species primarily occurs in the dry tropical forests of western Mexico, characterized by high biological diversity and deforestation rates (Ceballos & Garcia, 1995; Ceballos *et al.*, 2010; Rojas-Cortés *et al.*, 2022). The distribution of this species is fragmented by the highlands to the west of the TMVB and the northeast of the Sierra Madre del Sur (SMS) (Rojas-Cortés *et al.*, 2022). In addition to these geographical features that can act as barriers, in the northern areas of the TMVB, a higher degree of seasonality of temperature has been observed (Rojas-Cortés *et al.*, 2022), which could contribute to the differentiation and heterogeneity within the distribution of the species. Nonetheless, the genetic diversity of this species and how mountain systems and environmental heterogeneity can shape it is yet to be determined.

This is the first study to investigate the population genomics of a Neotropical *Ficus* species using single nucleotide polymorphisms (SNPs). The aims of this study were twofold: a) to characterize neutral and adaptive genetic variation and structure of *F. pringlei* and b) evaluate the impact of environmental and geographic factors on the patterns of genetic variation. We hypothesized that geographic isolation, enforced by physical barriers such as TMVB and SMS, would diminish gene flow, leading to the differentiation between populations located north and south of these barriers, despite the high pollen dispersal capacity proposed for monoecious species. Considering the variation in climatic conditions within the distribution range of the species and potential limitations on the dispersal of pollinators or seed dispersers, we also anticipate a correlation between environmental variation and genetic structure patterns. Similarly, the restriction of genetic exchange due to geographic barriers can amplify the effects of genetic drift and local adaptation, resulting in a more pronounced genetic structure among populations compared to monoecious species with higher gene flow.

METHODS

Study system

Ficus pringlei distributes in Colima, Guerrero, Jalisco, Michoacán, Nayarit, and Zacatecas states, in the Pacific coast and Central Mexico (Rojas-Cortés *et al.*, 2022). Unlike most species of the subgenus *Spherosuke*, which have a hemiepiphytic or strangler habit, *F. pringlei* is rupiculous, growing on rocky outcrops or cliffs, reaching up to 12 m in height (Ibarra-Manríquez *et al.*, 2012). The figs develop in the axil of the leaves and, when ripe, are red, ca. 1.5 cm in diameter and suggesting mainly birds dispersal (Lomáscolo, Speranza, & Kimball, 2008; Rojas-Cortés *et al.*, 2022). As in other *Ficus* species, the development of figs in *F. pringlei* is coupled with the wasps life cycle (Rojas-Cortés *et al.*, 2022). In phase B or female, wasps of the genus *Pegoscapus* Cameron 1906 oviposit and pollinate flowers with receptive stigmas. In phase D or male, the new generation of female wasps leaves the syconium laden with pollen. The development of figs within the crown of the trees of this species can be asynchronous, so there are in a given tree syconia in different stages of development (Rojas-Cortés *et al.*, 2022) or synchronous.

Sampling, DNA extraction, and sequencing

Through the distribution of *F. pringlei*, the young leaves of 71 individuals were sampled from 17 localities between 2018 and 2020 (Table 1, Figure 1A). In each plant, between 3-4 young leaves were dried in silica gel and voucher specimen was collected and deposited in the National Herbarium of Mexico (MEXU) and the Herbarium of the Missouri Botanical Garden (MO).

Genomic DNA was isolated from 50 mg of dry leaf tissue using a standard protocol (Doyle & Doyle, 1990). Prior to the DNA extraction process, a prewash step was implemented using a washing buffer containing Tris-HCl, EDTA, NaCl, 2-mercaptoethanol, and polyvinylpyrrolidone, aiming to eliminate secondary compounds (Li *et al.*, 2007). DNA concentrations were quantified with a Qubit (Thermo Fisher Scientific, Massachusetts, USA) and the quality of the extraction with NanoDrop 2000 (Thermo Fisher Scientific, Massachusetts, USA). DNA was diluted with TE buffer to obtain approximately 10 ng/ µl. The extractions were sent to SNPsaurus LLC (Eugene, OR) for library preparation and low-coverage whole-genome sequencing.

A complete genome library was prepared with 5 ng of genomic DNA, fragmented with the Nextera DNA Flex reagent (Illumina, Inc), which also ligates the adapter sequences to the ends of the fragments. Whole genome sequencing was made to an average of 8 X read depth on a NovaSeq 6000 S4 lane (Illumina, Inc., San Diego, California, USA), with 150 bp reads from paired ends.

Bioinformatics and genotyping

First, reads from each sample were trimmed using the BBDuk of BBTools (Bushnell, 2020). Trimming removed the Nextera adapter sequences, and quality-trimmed the end of the reads as needed. Next, the reads were extended using tadpole (BBTools) with default parameters, and the extended reads were used as input for the *novo* assembly of the sample with more raw reads (29,272,027) in SPAdes (Prjibelski *et al.*, 2020) with a k=104.

For each individual, the reads from the organelles were removed using NOVOPlasty (Dierckxsens, Mardulyn, & Smits, 2016).

Alignments in SAM format were sorted, indexed, and compressed in bam format using SAMTOOLS version 1.9 (Danecek *et al.*, 2021) and nest processed with Picardtools (Broad Institute, 2019). The Picard processing validated read pairing, removed duplicate reads, and added read groups for analysis in the Genome Analysis Toolkit (GATK, Poplin et al., 2018). The call of genotypes was performed in GATK Unified Genotyper v.4.2 using as assembly reference the contigs with 10,000 base pairs or more and the best practices workflow by individuals VCFs using 'HaplotypeCaller', specifying–ERC GVCF, and then calling population variants using 'GenotypeGVCFs'(Van der Auwera *et al.*, 2013). We then used GATK 'VariantFiltration' to further hard filter based on GATK's best practices recommendation (QD < 2.5, QUAL < 40.0, SOR > 2.5, FS > 50.0, MQ < 50.0, MQRankSum < -10.5, ReadPosRankSum < -4.0), and used GATK 'SelectVariants' to include only variants that met all filtering thresholds. Sets of variants were further filtered using vcftools v.0.1.15 (Danecek *et al.*, 2011) to remove SNPs with low frequency alleles in the population (MAF < 0.05), with more than two alleles at a single position, or in linkage disequilibrium (window size 100, step size 100 and R^2 threshold 0.25). Also, we filtered out SNPs outside Hardy-Weinberg Equilibrium (*P*-value 0.001), with mean depth values lower than 10, with a proportion greater than 0.75 of missing data, and a final step was taken to reduce paralogous sections of single SNPs by applying a threshold of 250 base pairs (thin 250).

Diversity and population structure analyses

The observed (Ho) and expected (He) heterozygosity and coefficient of inbreeding (F_{IS}) by localities were estimated with the dartR package (Gruber *et al.*, 2018). Pairwise relatedness were calculated using vcftools for all individuals, where a value negative indicates an inverse or negative genetic relationship, a value close to 0 indicates no significant or apparent genetic relationship, and a value close to 0.5 indicates high genetic similarity (Manichaikul *et al.*, 2010). Population structure was assessed using principal component analysis (PCA) on all individuals using the adegenet package (Jombart, 2008). The individual ancestry coefficients were estimated based on sparse non-negative matrix factorization algorithms (sNMF) through the package LEA (Frichot & François, 2015). The sNMF is comparable to other widely used programs, such as Admixture and Structure but is computationally faster and robust to departures from traditional population genetic model assumptions, such as Hardy–Weinberg equilibrium (Frichot & François, 2015). Ancestry coefficients were estimated for 1–15 ancestral populations (K) using 200 replicates for each K. The cross-entropy criterion was then used to determine the best K based on the prediction of masked genotypes.

Overall F_{ST} was calculated in the hierfstat package (Goudet, 2004). Pairwise genetic distance among localities and genetic distance between gene clusters suggested by PCA were estimated by Weir and Cockerham's F_{ST} (Weir & Cockerham, 1984) using StAMPP (Pembleton, Cogan, & Forster, 2013). All analyses were performed in R v 4.2.2 (R-Core-Team, 2022). The spatial method Fast and Flexible Estimation Effective Migration Surfaces (FEEMS, Marcus et al., 2021) was implemented to detect patterns of genetic diversity across a landscape that deviates from a null expectation of isolation by distance (IBD). This method was applied as an exploratory tool to find regions of the landscape that may act as biogeographic barriers in this system (*e.g.,* The TMVB or/and SMS).

Environmental data

Nineteen environmental variables were initially used to investigate the relationship between genetic variation and environmental gradients. These variables were obtained from high-resolution monthly climate surfaces in geographical coordinates (Datum WGS-84) with a spatial resolution of 3 arc seconds (~ 90 m) for the 1910 -2009 period (Cuervo-Robayo *et al.*, 2014). A correlation test with a threshold of 0.85 was used to mitigate the problem of collinearity among the variables. This implies that if a strong correlation was found between two variables, only the one with the highest representativeness of climatic conditions throughout the year and influence on the seasonal environment of *F. pringlei* was retained.

Identification of variation under selection

To enhance the robustness of outlier detection in this study, three methods to detect SNPs under selection were used: Redundancy Analysis (RDA, Capblancq & Forester, 2021; Forester, Lasky, Wagner, & Urban, 2018), the latent factor mixed model (LFMM, Caye, Jumentier, Lepeule, & François, 2019) and PCAdapt (Luu, Bazin, & Blum, 2017). RDA has been used as a Genome-Environment Association (GEA) method with high detection power and a low false positive rate in identifying adaptation signatures (Forester *et al.*, 2018; Capblancq & Forester, 2021). Simple RDA was performed, treating 5,311 SNPs as response variables and six environmental variables as explanatory variables. Data were imputed based on sNMF-estimated ancestry coefficients, using the imputation function of the LEA package to reduce the potential effects of missing sites. Outliers were identified on each of the first three ordination axes as SNPs with a locus score of ±3 SD from the mean score for that axis RDA, as Forester *et al*. (2018) suggested.

Besides RDA, LFMM, a univariate GEA method, was performed using the R package lfmm (Caye *et al.*, 2019). The number of latent factors was chosen following sNMF-estimated ancestry coefficients ($K = 4$). As a complement to GEA methods, PCAdapt analysis was applied to identify SNPs putatively under selection pressure because they deviate from the typical distribution of the test statistic Z (Luu *et al.*, 2017). Two principal components (K = 2) were used for estimating the test statistics. The list of putative adaptive SNPs was obtained for both methods under an expected false discovery rate of $\alpha = 0.05$ using the R package qvalue (Storey *et al.*, 2022).

A VennDiagram package (Chen & Boutros, 2011) was used to identify all putative adaptive SNPs identified and shared among the three methods of identification of outlier SNPs. Two datasets were built after identifying putative SNPs under selection: neutral and putatively adaptive. The first dataset included all SNPs (5,289 SNPs), except SNPs identified by at least two methods of identifying SNPs under selection and the second dataset included 22 SNPs.

Partitioning genomic variation

Redundancy analysis (RDA) was conducted on the neutral and putative adaptative SNPs datasets separately to estimate how environmental variables and geographic distance influence genomic variation. A matrix of genotypes by individual and two additional matrices of environmental variables and spatial autocorrelation were included. To determine how much of the genetic variation in *F. pringlei* is explained by each set of variables or by the combination of them, the RDA was partitioned by running three models: a full model with environmental and spatial autocorrelation variables; a partial model in which spatial autocorrelation explains genetic data conditioned on environmental noncollinear variables, and a partial model in which environmental noncollinear variables explain genetic data conditioned on spatial autocorrelation.

For all the RDA models, the significance of the partitioning was tested using 999 permutations with the R function *anova.cca*. In addition, to test the importance of each environmental and spatial variable in the SNP variation, another permutation test was performed, where the parameter "by" was set to "margin" in the anova.cca function. This test determines the marginal effects of each variable by eliminating the confounding effect of the other variables.

Distance-based Moran's eigenvector maps (dbMEMs) were used in RDA to model the effect of spatial autocorrelation on SNP variation. dbMEMs represent a spectral decomposition of the spatial relationships among the sampling sites (Dray *et al.*, 2012). The first eigenvectors usually describe broad spatial structures, encompassing the spatial variation in the whole sampled area, while the last eigenvectors describe fine spatial structures, which may capture variation at the scale of sampling sites (Dray *et al.*, 2012). The truncation threshold was set to the length of the longest edge of the minimum spanning tree to compute the MEMs, and only positives were retained using the adespatial package (Dray *et al.*, 2022). After that, to further avoid high collinearity, variables were selected based on variance inflation factors (VIFs) and excluded variables with a VIF ≥ 10 (Borcard, Gillet, & Legendre, 2011).

RESULTS

Genomic diversity and structure analysis

For 71 individuals, 1,171,156,667 reads, and 287 GB of raw data were generated, with an average of 15,615,422.23 ± 5,633,235.98 reads per individual. After filtering, 5,311 SNPs were retained. The overall Ho, He, and F_{IS} values were 0.263, 0.234, and -0.124, respectively. The observed heterozygosity ranged from 0.203 in locality 16 to 0.300 in locality 7, while the average gene diversity (He) fluctuated from 0.136 in locality 16 to 0.228 in locality 6. In all localities, Ho was higher than He, indicating an excess of heterozygotes (Table 1). Relatedness with values greater than 0.26 were found between all individuals from locality 17 (mean 0.27), meaning that these individuals could be parent-offspring or full sib. In contrast, 66 % of the plants north of the TMVB (relatedness values range -0.35 and 0.21, mean 0.06) and 50 % of the plants south of the TMVB (relatedness values range -0.38 and 0.24, mean 0.04) showed at least one value higher than 0.11 with another individual, meaning that could be second-degree relatives. In addition, seven plants from the north and south of the TMVB (1.1, 4.5, 5.1, 11.4, 12.4, 12.4, 12.4, 14.2, 14.3, 14.6, 16.1) had relatedness values of less than zero, meaning that they are not closely related to other individuals (Figure 2).

The first two component axes in the PCA explained 6.7% of the variation depicting three clusters (Figure 1C). The first cluster includes to localities North of the TMVB (1-9). Another cluster was formed by sites South of the TMVB (localities 10-16). The third cluster was included plants from locality 17 (Churumuco). The sNMF analysis, based on the optimal value *K*=4 of the cross-entropy criterion (Figure 3), identified the same three genetic clusters of PCA, in addition to another one cluster including plants from all localities (Figure 1 A-B).

The overall F_{ST} value between localities was low (0.042). For most pairs of localities, F_{ST} was significant ($P < 0.05$), with values between -0.006 to 0.208 (Figure 4). Locality 17 (Churumuco) was less related to other localities (paired F_{ST} ranging from 0.098 to 0.208). The paired F_{ST} between the genetic groups suggested by PCA (i.e., North, South, and Churumuco) was significant ($p < 0.05$), with values between 0.021 and 0.098. The highest genetic differentiation was found between the locality 17 with the North (F_{ST}=0.098) and the South (F_{ST}=0.08), while between the North and the South, there was the least differentiation (F_{ST}=0.021). FEEMS analysis suggested uneven gene flow across the landscape (Figure 1D). Areas with low rates of gene flow corresponded to the uplands to the west of TMVB and SMS. Low rates of gene flow were also recorded near locality 12 (Colima) and to the south of the distribution at locality 17 (Churumuco).

Partitioning genomic variation

From the full set of SNPs (5,311), 253 (4.76%) outlier SNPs were detected using the three detection methods. RDA was the least conservative (154 SNPs), PCAdapt was intermediate (106 SNPs), and LFMM was the most conservative (16 SNPs). For these variables, according to the RDA, 75 SNPs were correlated to bio4 (temperature seasonality), 21 SNPs to bio12 (annual precipitation) and bio18 (precipitation of warmest quarter), 16 SNPs to bio19 (precipitation of coldest quarter), 14 SNPs to bio 17 (precipitation of driest quarter), and 9 SNPs to bio1 (annual mean temperature). According to LFMM analysis bio18 was the variable with the most correlated SNPs (9), followed by bio1 (4 SNPs), and bio19 (3 SNPs). The three methods detected only one candidate SNP in common, and only 22 candidate SNPs were shared across two methods (Figure 5).

Of the seven spatial variables, MEM1, which describes broad spatial structures, was the only one highly correlated with temperature seasonality (r=-0.93, Figure 6). A total of 11 explanatory variables were retained for the RDA using the neutral and putative adaptative SNPs, five spatial variables (MEM2, MEM4, MEM5, MEM6, MEM7), and six environmental variables (bio1, bio4, bio12, bio17, bio18, and bio19). The RDA results explained a small but significant portion of the genetic variation of the 5,289 neutral SNPs (adj. $R^2 = 0.061$, $p < 0.001$; Table 2). Partial RDA was performed to unlock the contribution between spatial autocorrelation and environment variables. RDA showed that environmental variables explained 12.3 % (adj. $R^2 = 0.045$, $p < 0.001$; Table 2) of SNP variation, while spatial autocorrelation captured by dbMEMs explained 8.4 % (adj. $R^2 = 0.019$, $p < 0.001$; Table 2). For the RDA, using SNPs identified as putatively adaptative explained a moderate but significant portion of the genetic variation (adj. $R^2 = 0.393$, $p < 0.001$; Table 2). Partitioning of total variance analysis indicated that the environment explained 33.7 % (adj. $R^2 = 0.339$, $p < 0.001$; Table 2) of outlier SNP variation, while spatial autocorrelation explained 13.4 % (adj. $R^2 = 0.099$, $p < 0.001$; Table 2).

After confirming the association between genomic variation and environmental and spatial variables, the effects of individual variables were investigated. The simple RDA models use the putatively adaptative and neutral datasets with separate adjustments for each variable. For neutral data, permutation tests showed that ten variables were significantly associated with SNP variation ($p < 0.05$; Table 3). For putatively adaptive data, permutation tests showed that nine variables were significantly associated with SNP variation ($p < 0.05$; Table 3). The most important environmental variable in both data sets was bio4, while bio17 explained the smallest portion of genetic variation (Table 3). On the other hand, the spatial variable that best explained the genetic variation in both data sets was MEM2. Simple RDA biplots (Fig. 2) with putatively adaptative and neutral data sets showed a population structure consistent with the three genetic clusters identified by PCA.

In both data sets, the first RDA axis corresponded to genetic differentiation in North, South, and Churumuco (Figure 7). However, in the putatively adaptative data set, the differentiation between the three groups was less clear (Figure 7). RDA biplots also provided more information on the relative importance of environmental gradients. For example, individuals north of the TMVB were associated with greater temperature seasonality and lower mean annual temperature. In contrast, individuals from the south and Churumuco were associated with higher mean annual temperature and lower temperature seasonality (Figure 7). On the second RDA axis, individuals from Churumuco were negatively associated with precipitation related variables. In contrast, individuals from the south, although being less clustered than Churumuco, were positively associated with these variables (Figure 7).

DISCUSSION

Despite their low population density, several tropical plants present moderate or even high levels of genetic diversity and, in many cases, an excess of heterozygotes (Eguiarte *et al.*, 1992; Nazareno & Carvalho, 2009; Heer *et al.*, 2015; Teixeira & Nazareno, 2021). In the case of *F. pringlei*, the moderate levels of He and negative values of F_{IS} for all locations may be related to its perennial life form, cross-pollination, pollen dispersal over long distances, and random mating of its individuals facilitated by the often reproductive asynchrony of its individuals (Rojas-Cortés *et al.*, 2022), as has been suggested for other *Ficus* species (Nazareno & Carvalho, 2009; Nazareno *et al.*, 2013; Heer *et al.*, 2015).

A perennial habit can allow greater production of seeds and pollen in multiple reproductive seasons, which would increase the genetic variability of the population (Hamrick & Godt, 1996). Cross-pollination and the dispersal of pollen over long distances also could allow a greater exchange of alleles between individuals and populations (Hamrick *et al.*, 1992; Hamrick & Godt, 1996), as observed in individuals with lower relatedness values (Figure 2). On the other hand, variability in the times of flowering and seed maturation can increase the probability of mating with individuals with different alleles, facilitate random mating, and increase the heterozygosity of the population (Gates & Nason, 2012). However, within the *Ficus* genus, an excess of heterozygotes is not a prevalent pattern, as an excess of homozygotes has been observed primarily in paleotropical monoecious and dioecious species, which may arise to reduced pollen dispersal, localized seed dispersal, and subsequent mating among closely related individuals(Wang *et al.*, 2009; Yu *et al.*, 2010).

Genomic variation

Limited local differentiation in *Ficus* species, the complex topography with the presence of geographic barriers, and heterogeneity in environmental conditions are factors that have promoted genetic structuring in some species (Harrison, 2005; Honorio Coronado *et al.*, 2014; Heer *et al.*, 2015; Rodriguez *et al.*, 2017; Souto-Vilarós *et al.*, 2019; Deng *et al.*, 2020c). Environmental heterogeneity was a better predictor of genomic variation, than spatial autocorrelation, implying that that its differentiation is mainly driven by isolation by environment. However, spatial autocorrelation was also associated with a considerable proportion of genomic variation, suggesting that also neutral processes may also influence the structure of genetic diversity in *F. pringlei*.

Specifically, the RDA analysis indicated that environmental gradients explained a substantial portion of SNP genomic variation and that temperature seasonality and annual precipitation were the most important drivers of genomic variation. These variables are climatic factors strongly associated with the tropical and subtropical distribution geographic range of species of *Ficus* because of their low tolerance to low temperatures (Janzen, 1979; Ibarra-Manríquez *et al.*, 2012; Rojas-Cortés *et al.*, 2022). However, temperature seasonality was highly correlated with MEM1, suggesting that this environment variable has a broad spatial arrangement.

Wasp dispersal may be an important factor for explaining the correlation between environmental heterogeneity and genomic variation, because despite being highly mobile dispersers, they live for a short time (1-2 days) outside the syconium and are particularly vulnerable to abiotic stress(Nason *et al.*, 1998; Ahmed *et al.*, 2009; Jevanandam *et al.*, 2013; Segar *et al.*, 2017; Souto-Vilarós *et al.*, 2019; Gigante *et al.*, 2021; Xu, Wang, & Yu, 2021; van Kolfschoten *et al.*, 2022). For example, to the south of TMVB, locality 17 is moderately differentiated, there are high relatedness values between individuals, limited genetic connectivity with the other localities. This locality presents higher temperatures and lower precipitation than the nearest locality (locality 16, located 30 km apart), and these environmental characteristics may limit the longevity of the wasps and, therefore, their dispersal capacity (Gigante *et al.*, 2021; Xu *et al.*, 2021).

Patterns of FEEMS gene flow could be attributable to geography, particularly of uplands to the west of TMVB and SMS, which had been previously identified by inferring gene flow for several species found on both sides of the TMVB (Gándara & Sosa, 2014; Ruiz-Sanchez & Ornelas, 2014; Anguiano-Constante *et al.*, 2021; López-Barrera *et al.*, 2021). However, there is low differentiation between individuals north and south of the TMVB in *F. pringlei* that may be generated by a limited in gene flow, which can be possible throughout of the lower elevation zones located to the east of the TMVB, as has been suggested for other dry habitat species (Contreras-Negrete *et al.*, 2021). Additionally, areas north of the TMVB have higher seasonality in temperature and precipitation that can affect the dynamics between the reproductive phenology of the plant and the reproduction of the wasps (Peng, Compton, & Yang, 2010). In these seasonal environments, plants can be asynchronous in their syconia production, producing syconia in different stages within the same individual , as reported for *F. pringlei* (Rojas-Cortés *et al.*, 2022). The presence of syconia in different stages within the same tree allows the persistence of their wasps, causing asynchronous populations to reproduce out of phase of other populations whose syconia production is synchronous, which can favor isolation and subsequent differentiation (Smith & Bronstein, 1996; Martin *et al.*, 2009).

Another mechanism that could magnify the genetic structure of fig populations is the localized seed dispersal that in some species may be more restricted than pollen movement (Yu & Nason, 2013; Honorio Coronado *et al.*, 2014; Souto-Vilarós *et al.*, 2019). The main seed dispersers of *F. pringlei* are still unknown, but Rojas-Cortés *et al*. (2022) suggested that birds may be dispersal vectors, since the mature syconia are red. The size and behaviors frugivores affect seed-mediated gene flow. Small birds usually produce short-distance dispersal, while larger birds can produce long-distance dispersal (Jordano *et al.*, 2007). Seed-dispersing vertebrates are also likely to have dispersal linked to temporal climate variability; in more stable environments, species are more likely to be sedentary (Sheard *et al.*, 2020). In the future, to better understand the drivers of the genetic structure of *F. pringlei*, our results should be combined with information on the dispersers and their contribution to gene flow.

Genetic divergence and the role of adaptation

Local adaptation plays a crucial role in the genetic structuring of the mutualistic relationship between *Ficus* and fig wasps and is potentially responsible for species-level divergence and the process of co-speciation (Tian *et al.*, 2015; Rodriguez *et al.*, 2017; Souto-Vilarós *et al.*, 2019). Restricted gene flow by climatic conditions and by non-random mating in wasps would facilitate their isolation and genetic differentiation, which, in turn, would limit gene flow of their hosts and eventually lead to parapatric speciation in both interactants of the mutualism (Orsini *et al.*, 2013; Tian *et al.*, 2015; Souto-Vilarós *et al.*, 2019). This local adaptation and speciation process probably occurs initially in wasps since they have shorter generation times, which would explain why the one-to-one relationship between the host and pollinating wasps frequently breaks (Darwell *et al.*, 2014; Rodriguez *et al.*, 2017; Yu *et al.*, 2019; Souto-Vilarós *et al.*, 2019). This process has been mainly observed in dioecious species, probably due to its grouped establishment and lower pollen dispersal in comparison to monoecious figs (Nazareno *et al.*, 2013). However, the local adaptation process may occur in monoecious species in regions with highly heterogeneous environments, such as where *F. pringlei* is found.

CONCLUSIONS

This study provides genomic information on the diversity and the mechanisms underlying the genomic variation of *F. pringlei*. Although a weak or negligible genetic structure is often assumed for the *Ficus* species, in *F. pringlei* we detected three distinct genetic groups, suggest limited gene flow affected by climatic conditions, particularly temperature seasonality, and annual precipitation. The populations of *F. pringlei* north and south of the TMVB and the SMS have limited gene flow, leading to differentiation. The low differentiation among populations North and South of TMVB suggests that this mountain range may not be a strong barrier to gene flow for *F. pringlei*.

However, further studies are needed on the reproductive phenology of this species and the role of seed dispersers in gene flow to improve our understanding of this complex interaction. Likewise, to comprehend more integrally the diversification of this species and the process of cospeciation, it would be valuable to conduct studies on the genetic variation of wasps, among other studies.

AUTHOR CONTRIBUTIONS

Ángela P. Rojas-Cortés: Conceptualization (lead); data curation (lead); formal analysis (lead); funding acquisition (supporting); investigation (lead); methodology (equal); project administration (equal); visualization (lead); writing – original draft (lead); writing – review and editing (equal). **Jaime Gasca-Pineda**: Conceptualization (supporting); data curation (supporting), formal analysis (supporting); investigation (supporting); methodology (equal); writing – review and editing (equal). **Antonio González-Rodríguez**: Conceptualization (supporting); Resources (equal); writing – review and editing (equal). **Guillermo Ibarra-Manríquez**: Conceptualization (supporting); funding acquisition (lead); investigation (equal); project administration (equal); Resources (equal); writing – review and editing (equal).

ACKNOWLEDGEMENTS

The first author thanks Posgrado en Ciencia Biologicas UNAM and Consejo Nacional de Humanidades, Ciencias y Tecnologías for the support of the graduate studies and PhD scholarship she received (No. 696852). We thank the people who helped in field work, particularly Iván Leonardo Ek Rodríguez, Ma. Guadalupe Cornejo Tenorio, and Lorenzo Sánchez Sánchez. Besides, we thank Libny Ingrid Lara De La Cruz for support in laboratory activities.

FUNDING INFORMATION

This work was supported by the Dirección General del Personal Académico (DGAPA)-Programa de apoyo de investigación e Innovación Tecnológica (PAPIIT IN211620) of the Universidad Nacional Autónoma de México (UNAM).

CONFLICT INTEREST

All authors claim no conflict of interest.

DATA AVAILABILITY STATEMENT

Genotypes and descriptions of individual and environmental data are archived on DRYAD https://doi.org/10.5061/dryad.z8w9ghxjg.

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Table 1. Sampling site information and genetic estimates at the locality level based on 5,311 SNPs. N: number of individuals; He (SD): Expected heterozygosity (standard deviations); Ho (SD): observed heterozygosity (standard deviations); F_{IS}= Inbreeding coefficient.

Localities	**Longitude**	**Latitude**	**State**	**N**	**He (SD)**	**Ho (SD)**	**F_{IS}**
North lineage				35			
1. Zacatecas	-103.5322	21.1791	Zacatecas	4	0.191 (0.186)	0.237 (0.270)	-0.059
2. San Cristóbal	-103.4583	21.0313	Jalisco	5	0.218 (0.173)	0.265 (0.270)	-0.084
3. Huaxtla	-103.3959	20.9374	Jalisco	5	0.223 (0.172)	0.281 (0.256)	-0.122
4. Guadalajara	-103.3432	20.8383	Jalisco	6	0.210 (0.166)	0.238 (0.224)	-0.024
5. La Primavera	-103.6193	20.6925	Jalisco	4	0.191 (0.185)	0.239 (0.267)	-0.070
6. Tequila	-103.8515	20.9050	Jalisco	6	0.228 (0.167)	0.283 (0.246)	-0.128
7. Magdalena	-103.9955	20.9225	Jalisco	2	0.197 (0.212)	0.300 (0.362)	-0.123
8. Nayarit	-104.4834	21.1513	Nayarit	3	0.210 (0.195)	0.293 (0.315)	-0.145
South lineage				32			
9. El Tuito	-105.3298	20.3814	Jalisco	4	0.207 (0.185)	0.269 (0.279)	-0.125
10. Colotepec	-103.9676	20.2403	Jalisco	2	0.164 (0.213)	0.272 (0.382)	-0.217
11. Autlán	-104.2520	19.8332	Jalisco	4	0.198 (0.182)	0.244 (0.265)	-0.062
12. Colima	-103.7578	19.2604	Colima	6	0.218 (0.167)	0.259 (0.233)	-0.081
13. Coalcomán	-103.3465	18.7177	Michoacán	4	0.217 (0.182)	0.280 (0.274)	-0.117
14. Cuatro Caminos	-101.9175	19.2187	Michoacán	6	0.206 (0.170)	0.233 (0.225)	-0.021
15. Chipícuaro	-101.4025	19.1363	Michoacán	4	0.220 (0.184)	0.291 (0.283)	-0.143
16. La Huacana	-101.7515	18.9725	Michoacán	2	0.136 (0.203)	0.203 (0.342)	-0.027
17. Churumuco	-101.6605	18.7015	Michoacán	4	0.181 (0.203)	0.277 (0.347)	-0.328

Table 2. Redundancy analyses with neutral and putative adaptive datasets to partition among-population genetic variation in *Ficus pringlei* into the environment (env), spatial autocorrelation (space), and their combined effects, as measured by adjusted R^2. F: Dependent matrix of allele frequencies per individual. **p <0.001

	Neutral (5289)			**Putative adaptive (22)**		
	Inertia (variance)	**Proportion of total variance**	**Adjusted R^2 (p-value)**	**Inertia (variance)**	**Proportion of total variance**	**Adjusted R^2 (p-value)**
Full model: F~env+space	236.2	0.208	0.061**	4.457	0.488	0.393**
Pure enviroment: F~env|space	139.05	0.123	0.045**	3.346	0.337	0.339**
Pure space: F~space|env	95.28	0.084	0.019**	1.227	0.134	0.099**
Total unexplained	896.78	0.792		4.669	0.512	
Total inertia	1133	1		9.126	1	

Table 3. List of selected explanatory variables contributing to the genomic variation. Values in bold are significant.

	Neutral (5289)			**Putative adaptive (22)**		
	Variance	***F***	***p*-value**	**Variance**	***F***	***p*-value**
bio1: annual mean temperature	18.48	1.22	**0.010**	0.27	3.45	**0.003**
bio4: temperature seasonality	26.44	1.74	**0.001**	0.82	10.4	**0.001**
bio12: annual precipitation	24.72	1.63	**0.001**	0.58	7.37	**0.001**
bio17: precipitation of driest quarter	18.88	1.24	**0.003**	0.32	4.01	**0.001**
bio18: precipitation of warmest quarter	22.92	1.51	**0.001**	0.58	7.39	**0.001**
bio19: precipitation of coldest quarter	17.55	1.15	**0.021**	0.13	1.60	0.105
MEM2	23.33	1.53	**0.001**	0.58	7.33	**0.001**
MEM4	18.91	1.24	**0.006**	0.29	3.72	**0.001**
MEM5	18.12	1.19	**0.013**	0.23	2.94	**0.005**
MEM6	17.77	1.17	**0.019**	0.17	2.18	**0.031**
MEM7	16.51	1.09	0.079	0.09	1.19	0.275

Figure legends

Figure 1. Geographical population structure and migration surface for *Ficus pringlei*. A) Geographic distribution of 17 sampling localities. For more detailed information about sampling locations, see Table 1. Circles represent the assignment proportion to the four genetic clusters identified in sNMF. B) Individual ancestry coefficient for 71 individuals obtained with sNMF, K=4, and based on 5,311 SNPs; each vertical line depicts one individual. C) Structure of *F. pringlei* using principal component analysis (PCA), with each point colored according to its respective locality. Each dot indicates one individual and is colored according to sampling localities. D) Estimated effective migration surface plots. Highlighted blue regions have higher-than-expected migration rates, and orange-shaded regions have lower-than-expected migration rates. Points represent sampled locations.

Figure 2. The overview diagram of genomic relatedness between the 71 sequenced individuals.

Figure 3. Cross-entropy and geographical population structure with alternative levels of K=2-10 estimated with sNMF. The lowest value represents the most likely number of genomic clusters in the Cross-entropy. Note that the result for K=4 can be found in Figure 1A.

Figure 4. Genetic distances among sampling localities. The numbers in each cell indicate non-significant FST Pairwise values (P-values > 0.05).

Figure 5. Numbers of significant outlier SNPs detected by three different methods for genome scans.

Figure 6 Pearson correlation matrix of spatial distribution and environmental variables. Variables in bold were used in the final RDA models.

Figure 7. Distance-based redundancy analysis biplot reveals the influence of spatial autocorrelation and environmental variables on genomic variation in *F. pringlei*. A) Biplot of simple RDA with the neutral dataset. B) Biplot of simple RDA with putative adaptive dataset. Arrows represent environmental variables that drive the observed population structure. Moran's eigenvector map (MEM) vectors represent geographical isolation at

different spatial scales. Dots represent individuals. bio1: annual mean temperature; bio4: temperature seasonality; bio12: annual precipitation; bio17: precipitation of driest quarter; bio18: precipitation of warmest quarter; bio19: precipitation of coldest quarter. Variables in bold are significant.

Figure 1

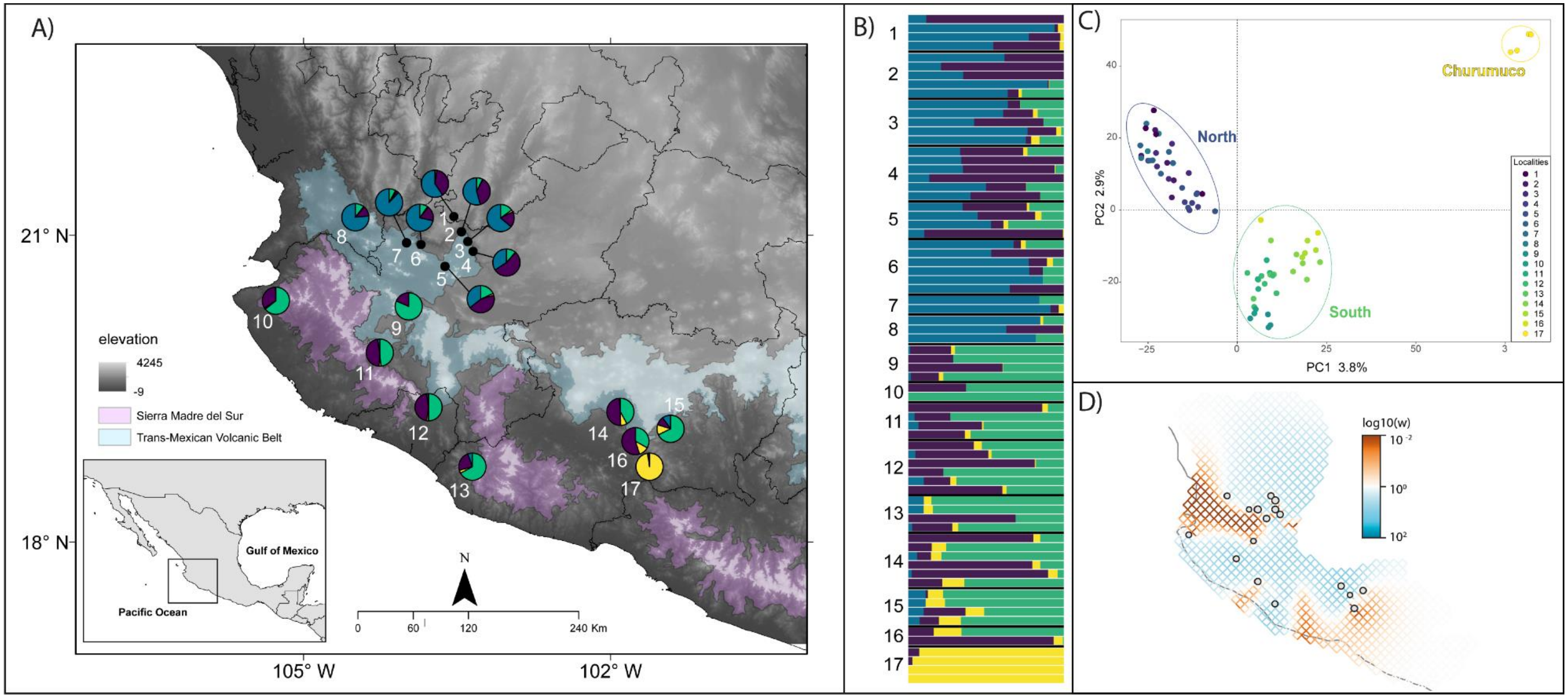

A)
21° N
18° N
105° W
102° W
elevation
4245
-9
Sierra Madre del Sur
Trans-Mexican Volcanic Belt
Gulf of Mexico
Pacific Ocean
N
0
60
120
240 Km
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
B)
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
C)
Churumuco
North
South
40
20
0
-20
-25
0
25
50
3
PC1 3.8%
PC2 2.9%
Localities
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
D)
log10(w)
10^{-2}
10^{0}
10^{2}

Figure 2

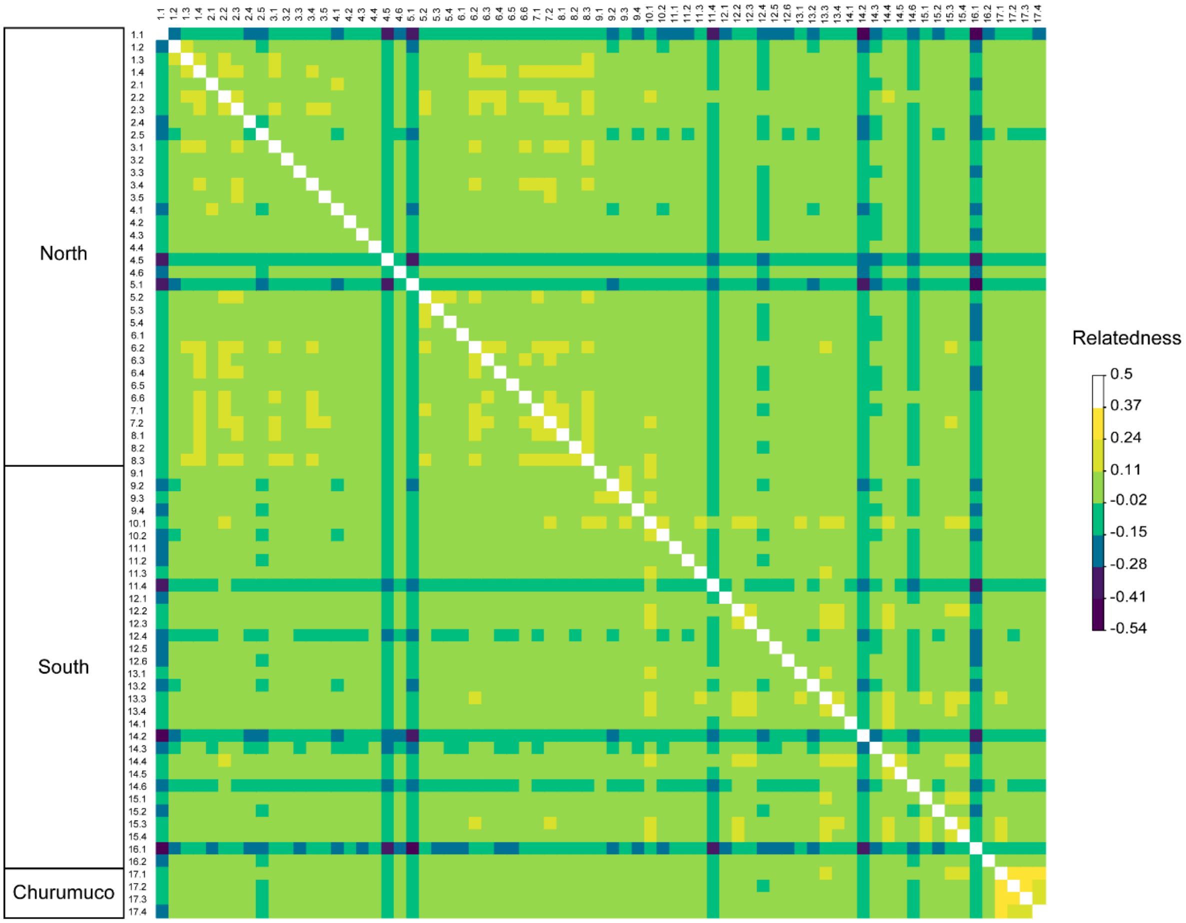

North
South
Churumuco
1.1
1.2
1.3
1.4
2.1
2.2
2.3
2.4
2.5
3.1
3.2
3.3
3.4
3.5
4.1
4.2
4.3
4.4
4.5
4.6
5.1
5.2
5.3
5.4
6.1
6.2
6.3
6.4
6.5
6.6
7.1
7.2
8.1
8.2
8.3
9.1
9.2
9.3
9.4
10.1
10.2
11.1
11.2
11.3
11.4
12.1
12.2
12.3
12.4
12.5
12.6
13.1
13.2
13.3
13.4
14.1
14.2
14.3
14.4
14.5
14.6
15.1
15.2
15.3
15.4
16.1
16.2
17.1
17.2
17.3
17.4
Relatedness
0.5
0.37
0.24
0.11
-0.02
-0.15
-0.28
-0.41
-0.54

Figure 3

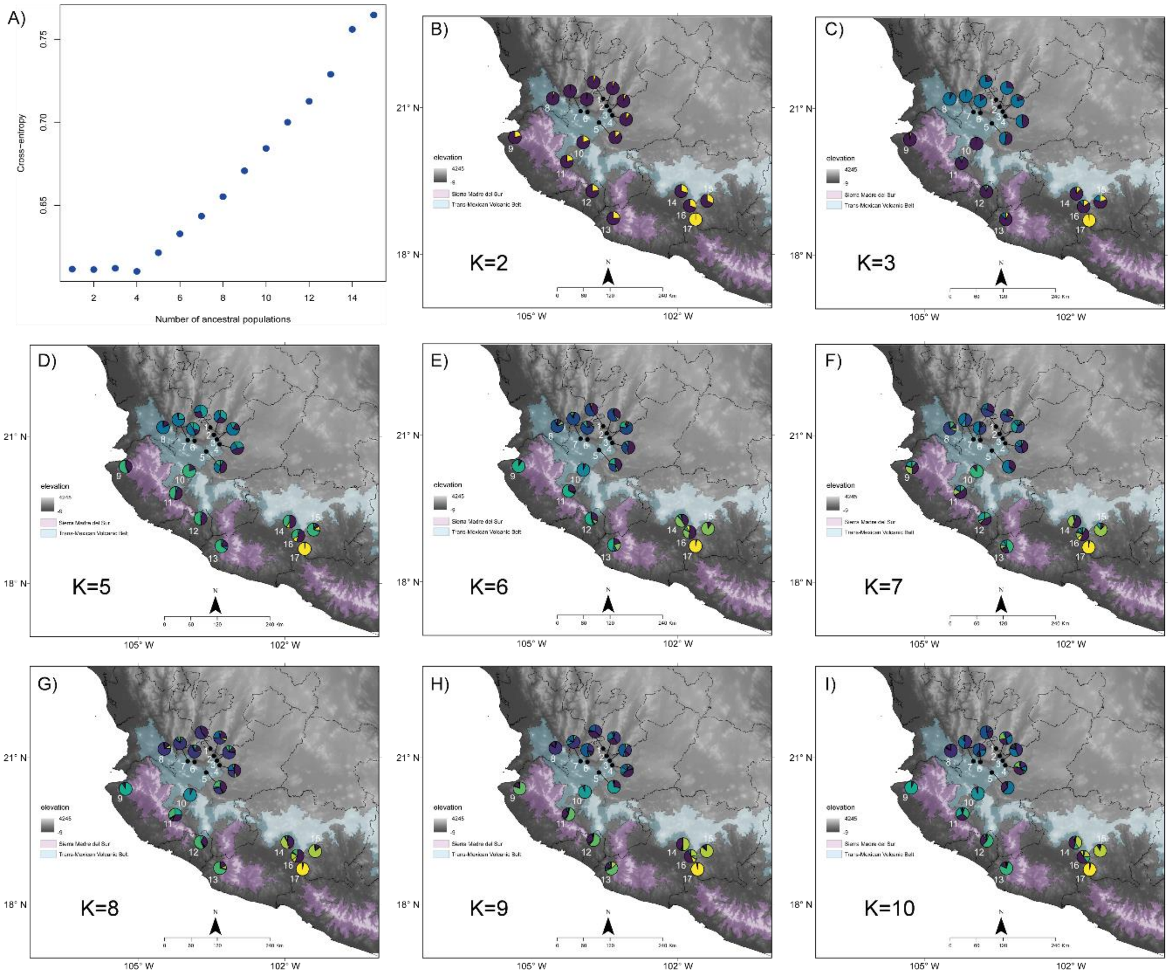

A)
Cross-entropy
0.65
0.70
0.75
2
4
6
8
10
12
14
Number of ancestral populations
B)
C)
D)
E)
F)
G)
H)
I)
K=2
K=3
K=5
K=6
K=7
K=8
K=9
K=10
21° N
18° N
105° W
102° W
elevation
4245
-6
Sierra Madre del Sur
Trans-Mexican Volcanic Belt
N
0 60 120 240 Km
5
8
9
10
11
12
13
14
15
16
17

Figure 4

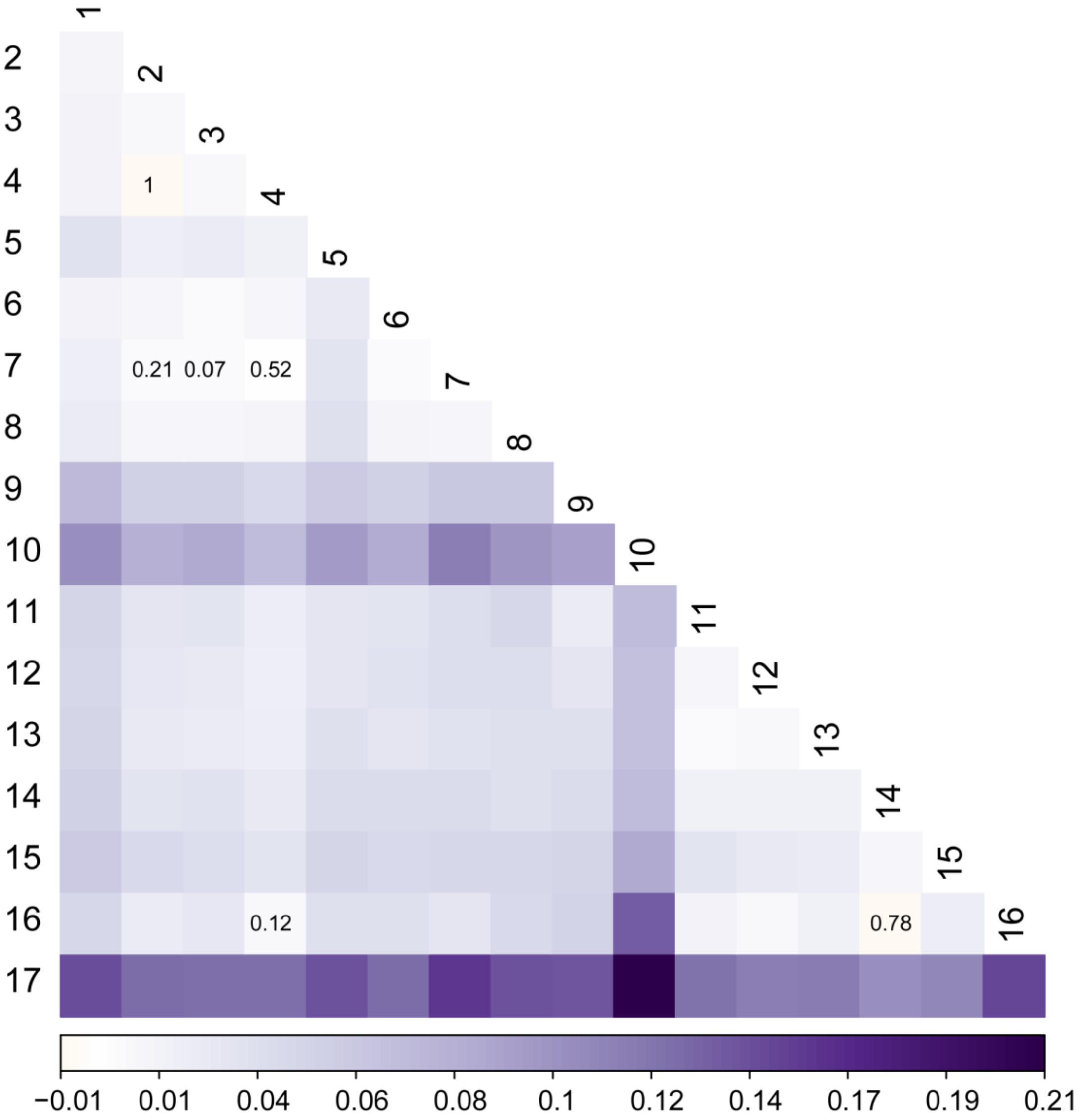

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
1
0.21
0.07
0.52
0.12
0.78
−0.01
0.01
0.04
0.06
0.08
0.1
0.12
0.14
0.17
0.19
0.21

Figure 5

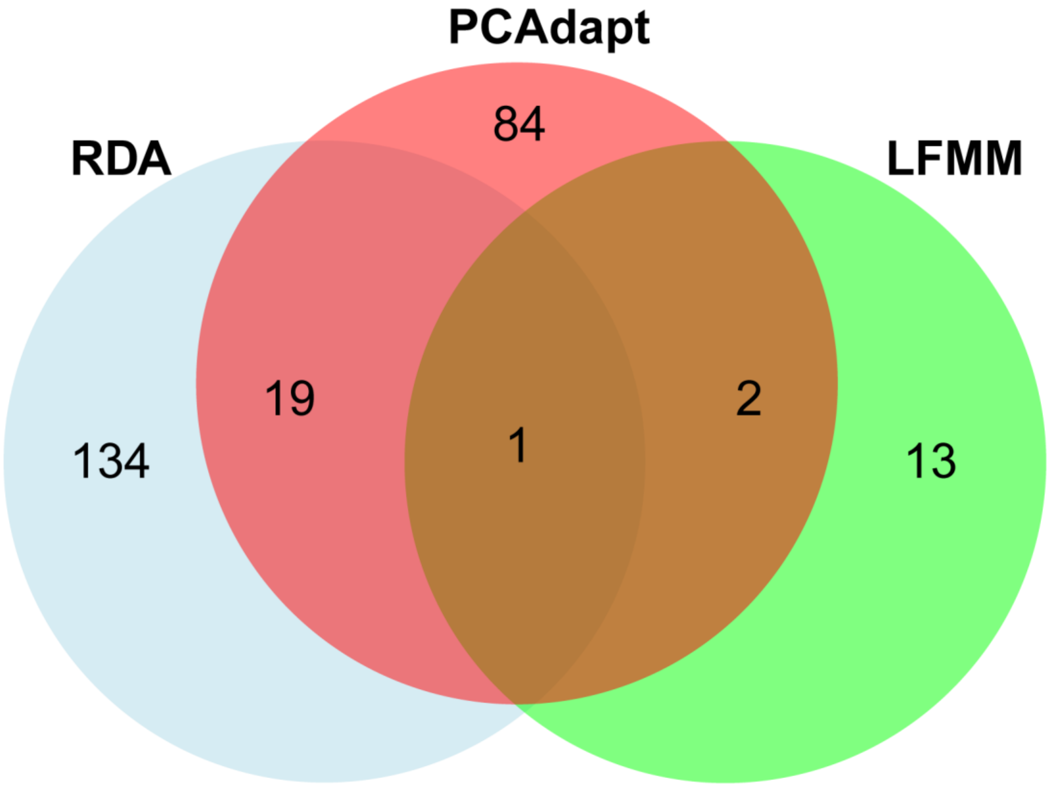


Figure 6

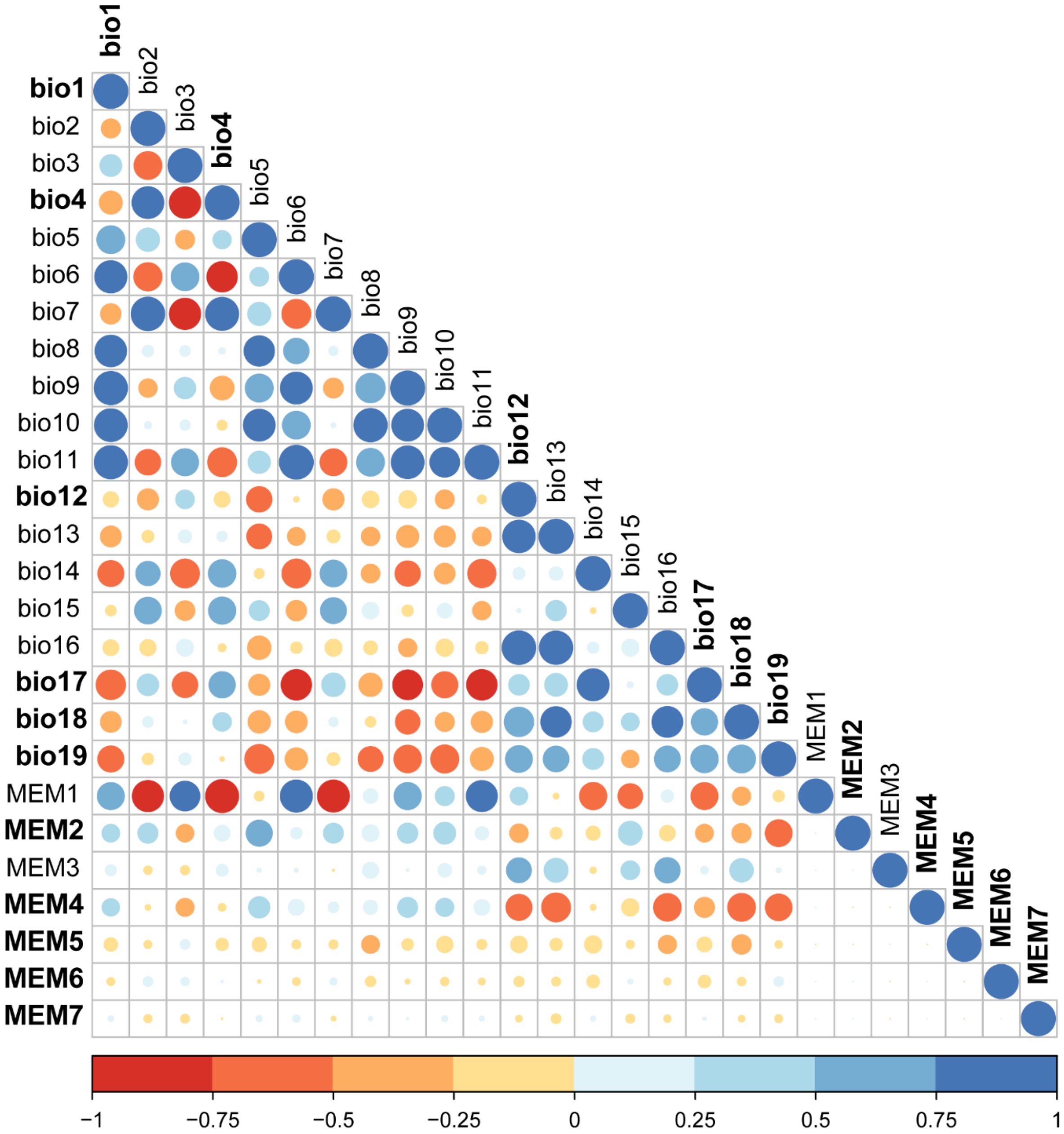
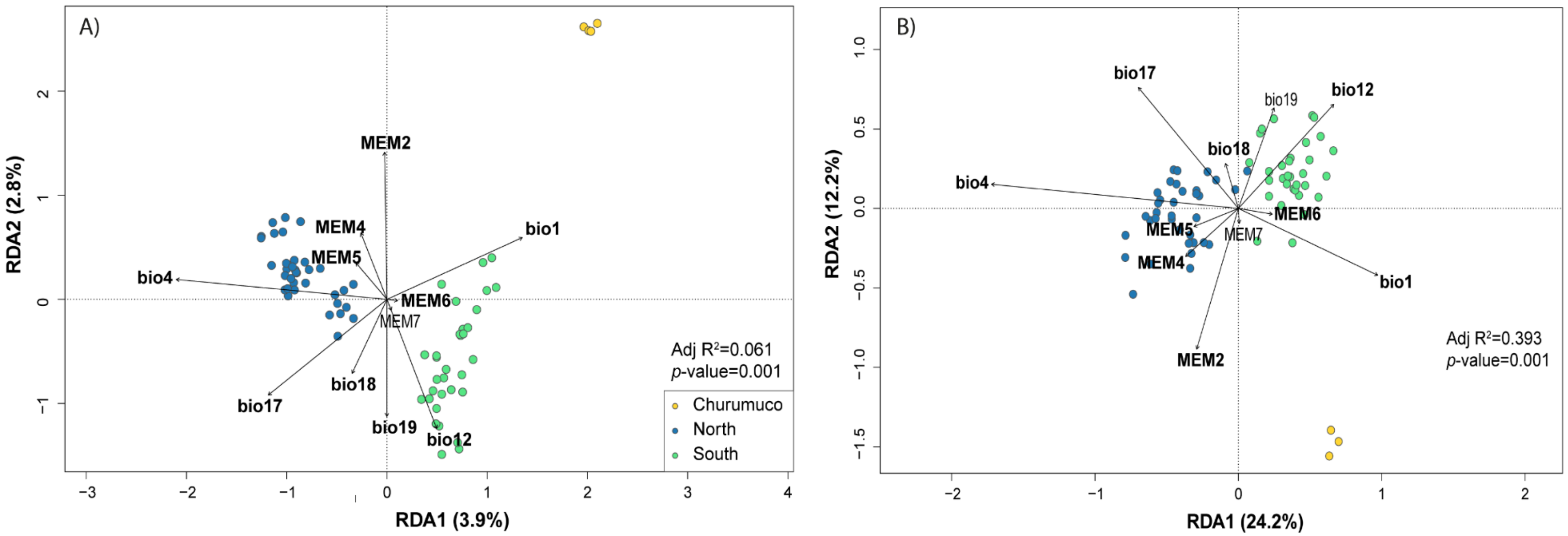

bio1
bio2
bio3
bio4
bio5
bio6
bio7
bio8
bio9
bio10
bio11
bio12
bio13
bio14
bio15
bio16
bio17
bio18
bio19
MEM1
MEM2
MEM3
MEM4
MEM5
MEM6
MEM7
−1
−0.75
−0.5
−0.25
0
0.25
0.5
0.75
1

Figure 7


A)
RDA1 (3.9%)
RDA2 (2.8%)
MEM2
MEM4
MEM5
MEM6
MEM7
bio1
bio4
bio12
bio17
bio18
bio19
Adj R²=0.061
p-value=0.001
Churumuco
North
South
−3
−2
−1
0
1
2
3
4
−1
0
1
2
B)
RDA1 (24.2%)
RDA2 (12.2%)
bio17
bio19
bio12
bio18
bio4
MEM6
MEM5
MEM7
MEM4
bio1
MEM2
Adj R²=0.393
p-value=0.001
−2
−1
0
1
2
−1.5
−1.0
−0.5
0.0
0.5
1.0

CAPÍTULO 3. Genetic structure of pollinating wasps *Pegoscapus* sp. of *Ficus pringlei* S. Watson in the Mexican Transition Zone

Manuscrito

Paisaje en la localidad de Chipicuaro, Michoacán. Arriba: Temporada de lluvias, en julio de 2019. Abajo: Temporada de secas, en abril de 2021.

Genetic structure of pollinating wasps *Pegoscapus* sp. of *Ficus pringlei* S. Watson in the Mexican Transition Zone

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Abstract

The study of the interaction between fig trees (*Ficus*, Moraceae) and their pollinating wasp species (Agaonidae) has mainly focused on ecological and evolutionary aspects due to their high diversity, unusual reproductive biology, and their tightly linked life histories. The population genetics of fig-pollinating wasp species, however, has been barely explored. Here, we characterized the genetic diversity and structure of an undescribed pollinating wasp species of *F. pringlei*, a Neotropical fig tree that is endemic to the central part of the Mexican Pacific lowlands. We sequenced a fragment of the cytochrome oxidase I (COI) mitochondrial DNA gene for a total of 148 wasps that were collected throughout their host´s distribution range. We found high levels of genetic diversity (Hd= 0.978) and moderate genetic differentiation among populations (F_{ST}= 0.257). Our findings indicate that the highlands located west of the Transmexican Volcanic Belt (TMVB) and the morphotectonic province of the Sierra Madre del Sur may play a crucial role as natural barriers, promoting genetic differentiation between populations of pollinating fig wasps. However, we also observed the presence of some shared haplotypes between the northern and southern TMVB populations, suggesting the possibility of occasional genetic exchanges through corridors or habitats that facilitate connectivity between these areas. We also found well-differentiated clades that could represent distinct evolutionary lineages (*i.e.*, species). This study highlights the necessity of conducting further studies for a better understanding of the population dynamics and structure of mutualistic pollinator wasp species.

Keywords: Agaonidae wasps, *Americanae* section, neotropical *Ficus*, *Pegoscapus*, population genetics, *Spherosuke* subgenus.

INTRODUCTION

Brood pollination mutualisms are specialized interactions in which insects exclusively oviposit within the reproductive structures of plants, thus both having benefit from this obligate association (Hembry & Althoff, 2016). Despite being uncommon, this obligate mutualism occurs in various tropical taxa with an important ecological role (Hembry & Althoff, 2016). One of the most comprehensively studied cases of this interaction occurs between *Ficus* L. (Moraceae) species and their pollinating wasps of the family Agaonidae.

The plant genus *Ficus* (Moraceae) is a diverse group that currently contains more than 800 described species that are mainly distributed in tropical and subtropical regions around the world (POWO, 2023). This genus is characterized by having a closed inflorescence called syconium, which contains either or both pistillate and staminate flowers and whose only access to the interior is through the ostiole (Berg, 1990). Fig trees are exclusively pollinated by wasp species of the family Agaonidae, which are commonly known as fig wasps (Ramírez, 1970). Syconia with receptive flowers attract pollen-bearing female wasps by producing volatile organic compounds (Grison-Pigé *et al.*, 2002; Proffit *et al.*, 2020); however, only a limited number of females enter the syconium, pollinate the female flowers, and oviposit in some of them (Herre *et al.*, 2008; Piedra-Malagón *et al.*, 2019). Once the larvae develop, males mate with females when they are still in the syconium and subsequently dig a tunnel through the walls of the syconium or ostiole, allowing the fertilized and pollen-laden females to emerge and disperse in search of other syconia with receptive pistillate flowers (Galil & Eisikowitch, 1968; Piedra-Malagón *et al.*, 2019; Delgado-Perez *et al.*, 2020).

Recent molecular techniques have revealed the existence of a number of *Ficus* species with multiple pollinators living either in allopatry, parapatry or sympatry (Lin *et al.*, 2008; Rodriguez *et al.*, 2017; Yu *et al.*, 2019; Deng *et al.*, 2020c). Nevertheless, little is known about the genetic structure of *Ficus* species and whether and how it is correlated with the geographic distribution and genetic diversity of their pollinating wasps. It has been observed that some life history traits of the pollinating-wasp species, such as their dispersal ability and reproductive system, may modify their genetic structure and that of their host plants (Lin *et al.*, 2008; Nazareno *et al.*, 2013; Bain *et al.*, 2016). For instance, paternity analyses have revealed that some fig wasps transport pollen between monoecious *Ficus* species more than 100 km away (Ahmed *et al.*, 2009), whereas in dioecious species, the wasps gene flow is considerably more localized (Chen *et al.*, 2011). In contrast, Zavodna *et al*. (2005) reported that in some dioecious *Ficus* species there is also considerably long-distance pollen dispersal (> 40 km).

Other factors that could shape the genetic variation and lead to speciation of pollinator wasps are geographical barriers, climatic heterogeneity, and local adaptation (Chen *et al.*, 2012; Rodriguez *et al.*, 2017; Yu *et al.*, 2019; Souto-Vilarós *et al.*, 2019; Deng *et al.*, 2020c). Ecological niche and potential distribution models coupled with genetic population analyses of pollinators of widely distributed *Ficus* species have revealed that topography is a relevant factor of genetic divergence and speciation of pollinators, due to geographic isolation in sites that functioned as shelters (i.e., refugia) during unfavorable climatic periods (Deng *et al.*, 2020c). Topography is now considered part of the explanation for the exceptional biodiversity of the Neotropics (Rull, 2011; Antonelli *et al.*, 2018b).

The Mexican Transition Zone (MTZ) is a region of considerable geomorphological and climatic complexity, and it has contributed to the current genetic variation and diversity of species in Mexico (Halffter & Morrone, 2017). In this region, mountain ranges such as the Trans-Mexican Volcanic Belt (TMVB) and Sierra Madre del Sur (SMS) have been proposed as drivers of diversification , affecting taxa in different ways (Mastretta-Yanes *et al.*, 2015). In the lowlands, these mountain complexes have served as barriers by introducing genetic discontinuities in several animal species that are poorly dispersed and some that are highly vagile (Zarza *et al.*, 2008; Arbeláez-Cortés *et al.*, 2014; Schramm *et al.*, 2021). However, the diversity patterns and genetic structure of wasp pollinator species of *Ficus* in this region has been scarcely understood.

In this study, we use mitochondrial DNA sequence data to characterize the levels of diversity and genetic structure of the undescribed pollinating wasps of *F. pringlei* that is endemic to the central part of the Mexican Pacific lowlands (Ibarra-Manríquez *et al.*, 2012;

Rojas-Cortés *et al.*, 2022). Our results indicate that the complex geological and paleoclimatic history of western Mexico led to a marked geographic genetic structure between populations of pollinating wasps located to the north and south of the TMVB and SMS mountain ranges, as reported in previous studies of taxa with similar geographic distribution (*e.g*. Arbeláez-Cortés *et al.*, 2014; López-Barrera *et al.*, 2021; Schramm *et al.*, 2021; Zarza *et al.*, 2008).

MATERIALS AND METHODS

Host species

Ficus pringlei is found in scattered populations in the central part of the Mexican Pacific lowlands in the states of Colima, Guerrero, Jalisco, Michoacán, Nayarit, and Zacatecas (Serrato *et al.*, 2004; Ibarra-Manríquez *et al.*, 2012). Unlike most species of the subgenus *Spherosuke*, which have a hemiepiphytic or strangler habit (Berg & Corner, 2005), this fig species is rupicolous growing on rocky outcrops or cliffs (Figure 1A). The development of syconia in the species is asynchronous (Figure 1B), and it has a diverse associated community of wasps, including its pollinator *Pegoscapus* sp. (Figure 1C) and several non-pollinating chalcidoid species, all still undescribed (Rojas-Cortés *et al.*, 2022).

Sampling

We collected specimens belonging to the undescribed species of *Pegoscapus* in 16 localities from 2018 to 2020 (Figure 2A, Table 1). In each locality, we collected syconia from one to seven host trees and kept them in plastic bags to allow wasp's emergence. Wasps were mainly collected from mature syconia, but some founder wasps were occasionally obtained from receptive or female phase syconia. The pollinating fig wasps that emerged were preserved in absolute ethanol and stored at 4 °C until DNA extraction. We analyzed two to eleven female fig wasps from each population, choosing one to four specimens per fig.

DNA extraction and amplification

We used two DNA extraction and sequencing strategies to recover the cytochrome oxidase subunit I (COI) marker. The first strategy was conducted for 101 *Pegoscapus* specimens. Genomic DNA was extracted from the whole body of individual female wasps with the Invitrogen PureLink Genomic DNA kit (Invitrogen, Carlsbad, CA, USA), following the manufacturer's protocol, with the modification of applying half of the volume of the reagents to obtain higher DNA concentrations. We used the primers COI1-1-Ple and COI-2M (Su *et al.*, 2008) to amplify an 855 bp fragment of the cytochrome oxidase I mitochondrial DNA gene. The thermal cycling program consisted of an initial denaturation step at 94 °C for 4 min, then 35 cycles of 94 °C for 30 s, 50 °C for 1 min and 72 °C for 1 min, followed by a final extension step at 72 °C for 10 min. PCR products were purified and sent for sequencing to Macrogen (Rockville, MD, USA).

The second strategy was performed for 47 specimens. Genomic DNA was extracted from the abdomen of female wasps using the Sunnucks and Hales protocol (Sunnucks & Hales, 1994). REPLI-g multiple displacement amplification (MDA; Qiagen, Valencia, CA, USA) was used to increase the amount of DNA, which allows complete genomes to be amplified without using PCR (Qiagen, 2020). Genomic DNA concentrations were quantified using a Qubit (Thermo Fisher Scientific, Massachusetts, USA) and the quality by spectrophotometry (NanoDrop 2000 Thermo Fisher Scientific, Massachusetts, USA). Genomic DNA was diluted with TE buffer to obtain approximately 10 ng/ µl. The extractions were sent to SNPsaurus LLC (Eugene, OR) for library prep and sequencing. We used 5 ng of genomic DNA for library preparation and then they further fragmented with the Nextera DNA Flex reagent (Illumina, Inc), which also ligates the adapter sequences to the ends of the DNA fragments. Whole genome sequencing was performed to an average of 8X read depth on a NovaSeq 6000 S4 lane (Illumina, Inc., San Diego, California, USA), generating 150 bp pair end reads.

Raw reads were trimmed and filtered with Geneious 10.2.6 (Kearse *et al.*, 2012) and harvested to assemble the above COI fragment. Cleaned reads were used to perform by-reference assemblies in Geneious 10.2.6 (Kearse *et al.*, 2012), using as seed an available COI sequence (868 bp) of *Pegoscapus kraussi* Grandi, 1952 (AB308345.1). Assemblies were annotated in the MITOS 2 webserver (Bernt *et al.*, 2013), utilizing the invertebrate mitochondrial genetic code.

COI sequences obtained from both sequencing strategies showed positions with ambiguous bases, with most of them with synonymous substitutions and lacking stop codons, indicating potential heteroplasmy. All ambiguous sites were scored with IUPAC ambiguity codes and the mitochondrial haplotypes, for each samples were separated using the software DnaSP v5 (Librado & Rozas, 2009). The dataset of inferred haplotypes is hereafter called a phased dataset. All sequences were aligned with MEGA X software (Kumar *et al.*, 2018).

Genetic diversity and structure

The number of haplotypes (*h*), number of polymorphic sites (*S*), haplotype diversity (*Hd*), and nucleotide diversity (π) were calculated for the entire phased dataset and each population with the software DnaSP v5 (Librado & Rozas, 2009). A haplotype network was constructed using the Neighbor-Net algorithm with SPLITSTREE 4.14.2 (Huson & Bryant, 2006). The presence of phylogeographic structure ($N_{ST}> G_{ST}$) was evaluated from 100,000 permutations with PERMUT 1.2.1 (Pons & Petit, 1996). Genetic differentiation was estimated with molecular analyses of variance (AMOVA) in Arlequin v.3.5 (Excoffier & Lischer, 2010) with 50,000 permutations.

The genetic structure was investigated through the spatial analysis of molecular variance (SAMOVA) with SAMOVA 1.0 (Dupanloup, Schneider, & Excoffier, 2002). This method is based on a simulated annealing procedure aimed at identifying groups of populations that are geographically homogeneous and maximally differentiated in terms of the among-group component (F_{CT}) of the overall genetic variance. The program was run for 10,000 iterations from each of 10 random initial conditions and tested all the grouping options, with the predefined number of groups (K) ranging from 2 to 16. We also performed Tajima's D (Tajima, 1989) and Fu's Fs (Fu, 1997) tests for the complete phased dataset and each population using Arlequin with 10,000 permutations.

Phylogenetic analysis

The phylogenetic relationships among haplotypes were estimated using PhyML 3.0 (Guindon *et al.*, 2010) implemented in http://www.atgc-montpellier.fr/phyml/. The GTR+G evolutionary model for the entire dataset was selected using the SMS software (Lefort, Longueville, & Gascuel, 2017), based on the Akaike Information Criterion (AIC). Branch support was obtained using the transfer bootstrap analysis (Lemoine *et al.*, 2018) with 100 replicates. The resulting tree was edited with the program Figtree V 1.8.4 (Rambaut, 2018). Genetic distances were calculated with the Kimura-2-Parameter (K2P) model using MEGAX. This analysis included sequences of 31 other individuals of *Pegoscapus* sampled from other *Ficus* species. Specimens of *Platyscapa fischeri* Wiebes, 1977 were included as the outgroup, since this genus is closely related to *Pegoscapus* (Cruaud *et al.*, 2012). A list with the examined specimens, their localities, and their GenBank accession numbers is provided in Table S1.

RESULTS

Diversity and genetic structure

The COI sequences that were generated for the 148 P*egoscapus* specimens included 119 haplotypes and 66 polymorphic sites. Sixty of these haplotypes were restricted to single population. The total haplotype (*Hd*) and nucleotide diversity (π) were 0.978 and 0.006, respectively. The data obtained for each population are given in Table 1. The H3-H38 haplotypes (L1; Figure 2) were only recorded in northern populations of the TMVB, whereas most of the haplotypes of the L2 group are present in the south of this morphotectonic province, though some haplotypes are on both sides (Figure 2). The most frequent haplotype (>28 sequences) was H85, which only was recorded in populations south of the TMVB. Localities 2 and 16 had the highest number of haplotypes, with 18 and

17 haplotypes, respectively. Population 5, on the other hand, had only three haplotypes. Haplotypes from H98 to H104, found exclusively south of the TMVB, showed greater differentiation than others (L3; Figure 2). Specimens with different haplotypes within the same tree or even the same syconium were obtained. For instance, two specimens were sampled from the same syconium in population 8, which had four phased haplotypes (H9, H22, H96, and H105).

The analysis in PERMUT indicated significant and strong phylogeographic structure in the populations (N_{ST} = 0.441; G_{ST} = 0.025, p <0.05). According to the AMOVA, the highest percentage of variation (84.3 %) was explained by differences within populations, whereas only 25.7 % was found among populations (Table 2). The prevalence of shared haplotypes within populations resulted in moderate genetic differentiation (F_{ST}=0.257). The SAMOVA analysis suggested two groups, with a value of F_{CT} = 0.310 (Figure 2): northern cluster (localities 1-8) and southern cluster (9-16).

Finally, Tajima's D test was not significant (p > 0.05) in any locality, although negative it was for 10 localities. Similarly, the total values for groups obteined (north and south of the TMVB) were negative but non-significant (Table 1).

Phylogenetic analyses

The topology obtained in the phylogenetic analysis (Figure 3) included all *Pegoscapus* haplotypes collected from their host *F*. *pringlei*, in a single, strongly supported clade, though it also had haplotypes of specimens collected in *F*. *cotinifolia* Kunth, a species in which pollinators have been previously described as *P*. *kraussi*. This clade had three subclades: Subclade A, encompassing 94 % of the haplotypes, distributed in both the northern and southern regions of the TMVB; and subclades B and C, which include haplotypes only found in the southern part of the TMVB, specifically in Michoacán state. Corrected pairwise differences between subclades ranged from 2 to 2.3 %, while differences within each subclade varied between 0.1 % and 0.2 % (Table S2).

DISCUSSION

Genetic diversity and structure

The pollinating wasps of *F. pringlei* exhibited high levels of genetic variation (*Hd* = 0.978), despite the restricted distribution of their host. These levels of variation might be due to the high evolutionary rates of the mtDNA, coupled with short generation times and a large population size in the examined species (Weiblen, 2002; Xiao *et al.*, 2012). Additionally, the wasps collected within a same *F. pringlei* tree or even within the same syconium significantly varied in their haplotypes. The presence of several founder wasps (up to 12 female wasps) could contribute to this genetic variation, since they could come from different locations, which consequently decreases genetic homogeneity. Several other studies in pollinator wasps showing high variation in the COI marker (Lin *et al.*, 2008; Chen *et al.*, 2012; Yu *et al.*, 2019; Deng *et al.*, 2020c).

Localities 13-16 have the highest genetic diversity, as they contain the most divergent haplotypes (Figure 2). These localities at the southern end of the distribution of *F*. *pringlei* belonging to the tropical dry forest of the Balsas region, has been considered as a center of diversification and endemism, that thrives in drier and more seasonal conditions than some regions closer to the Pacific coast (Espinosa-Organista *et al.*, 2008; Ceballos *et al.*, 2010). This environmental variation may favor the genetic differentiation and limited gene flow of the examined wasps, as they are very sensitive to high temperatures and low relative humidity, which may limit the longevity of wasps and their dispersal capacity (Janzen, 1979; Gigante *et al.*, 2021; Xu *et al.*, 2021).

The complex physiography of the TMVB, coupled with Pleistocene climatic oscillations, have been instrumental in shaping the genetic diversity and population dynamics of numerous animal species found in the seasonally dry tropical lowland forests of western Mexico (Zaldívar Riverón, León Regagnon, & Nieto Montes De Oca, 2004; Devitt, 2006; Zarza *et al.*, 2008; Pringle *et al.*, 2012; Schramm *et al.*, 2021). Specifically, the rugged terrain and diverse microclimates within the highlands SMS and west of the TMVB morphotectonic province, may act as natural barriers, promoting genetic differentiation among populations of *F. pringlei* pollinating wasps. In particular, the SAMOVA analysis revealed that mountainous regions effectively divide localities into two genetic clusters (north and south), indicating restricted gene flow between these areas. However, the presence of shared haplotypes between the northern and southern TMVB populations suggests that there are also occasional genetic exchanges, through corridors or habitats to allow connectivity between these areas. Thus, a dynamic relationship exists between isolation and genetic connectivity, accentuating the complexity of genetic processes in this region. Understanding this interplay would provide valuable insights into how diverse environments shape the evolutionary dynamics of fig wasp's species in the MTZ.

One to one rule?

Three subclades were recorded within the clade that recovered all *Pegoscapus* haplotypes collected in *F. pringlei*, but the average genetic distance among three clades was 2.3 %. This among-clade genetic distance is smaller than those recorded between other *Pegoscapus* species (Molbo *et al.*, 2003), as well as in species of other agaonid genera, whose distances are > 4 % (WANG *et al.*, 2005; Chen *et al.*, 2012; Yu *et al.*, 2019; Deng *et al.*, 2020c).

On the other hand, pollinator wasps collected from *F. cotinifolia* in clade C were previously described as *P. kraussi*. To define whether one or more wasp species are in *F. pringlei* and in the described clade, it will be necessary to deepen the morphological characterization of these wasps and to perform systematic studies with pollinators of other sympatric host species, particularly those found in *F. cotinifolia*.

Shared pollinators between hots have been reported in the *Ficus* of sect. *Americanae* (Molbo *et al.*, 2003; Machado *et al.*, 2005; Cook & Segar, 2010; Satler *et al.*, 2019). One factor that could promote this is the recent origin of this section, which may have prevented the evolution of specific barriers and unique wasp lineages; in this case, events of hybridization in these species can be occur, so that genetically well-defined lineages of wasps could interact with less genetically defined *Ficus* groups (Machado *et al.*, 2005; Satler *et al.*, 2019). Another factor that may promote pollinator sharing is host switch, which could occur despite the selective pressures derived from the appropriate recognition of the host and the compatibility that promote specificity, both in *Ficus* and its pollinating wasps (Satler *et al.*, 2019).

Identification heteroplasmic sequences or nuclear mitochondrial pseudogenes (NUMTs) in agaonid wasps is increasingly common (Chen *et al.*, 2014; Cruaud *et al.*, 2017; Li *et al.*, 2021). The presence of these sequences has often been interpreted as the presence of cryptic species, especially in the absence of taxonomic knowledge, and has contributed to overestimate the number of species and to mislead inferences about their genetic structure and differentiation (Cruaud *et al.*, 2017; Ricardo *et al.*, 2020). Further studies, including additional nuclear markers will be critical to understand the genetic structure, speciation, and differentiation of this insect group.

CONCLUSIONS

This study provided evidence on the mitochondrial genetic diversity and the possible mechanisms underlying the evolution of a pollinating wasp species associated to *F. pringlei*. High genetic diversity and moderate genetic differentiation have been recorded among wasp populations of other species, although a weak or negligible genetic structure is often assumed in the wasps of monoecious hosts.

Our results show that the highlands west of TMVB and SMS significantly influenced the genetic structure of these pollinating wasp species. The number of pollinator species associated to *F. pringlei* should be further assessed based on a detailed morphological examination, as well as on further molecular studies employing nuclear, markers. Additionally, taxonomic studies are warranted to enhance our understanding of the broader taxonomic relationships within this ecological interaction.

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Table 1. Genetic variation and localities of specimens assigned to *Pegoscapus* sp. that were collected in *Ficus pringlei*. N: number of wasps; *h*= number of haplotypes; *S*= polymorphic sites; *Hd*= haplotype diversity; *π*=nucleotide diversity; D_T=Tajima's D.

Localities	Longitude	Latitude	State	Trees (syconia)	N	Sequences	*h*	*S*	*Hd*	*π*	D_T
NORTH				26(49)	64	128	52	32	0.962	0.004	-1.344
1. Zacatecas	-103.5322	21.1791	Zacatecas	3(8)	8	16	9	11	0.892	0.003	-0.387
2. San Cristóbal	-103.4583	21.0313	Jalisco	7(11)	13	26	18	17	0.967	0.004	-0.638
3. Huaxtla	-103.3959	20.9374	Jalisco	3(5)	5	10	9	12	0.978	0.005	0.499
4. Guadalajara	-103.3432	20.8383	Jalisco	3(3)	6	12	6	10	0.863	0.004	0.460
5. La Primavera	-103.6193	20.6925	Jalisco	2(2)	2	4	3	3	0.833	0.002	0.168
6. Tequila	-103.8515	20.9050	Jalisco	4(8)	11	22	11	14	0.922	0.004	-0.569
7. Magdalena	-103.9955	20.9225	Jalisco	2(2)	5	10	5	7	0.822	0.003	-0.165
8. Nayarit	-104.4834	21.1513	Nayarit	2(10)	14	28	16	22	0.944	0.005	-0.931
SOUTH				27(61)	84	168	71	54	0.958	0.005	-1.160
9. El Tuito	-105.3298	20.3814	Jalisco	2(7)	10	20	10	11	0.911	0.004	0.385
10. Autlán	-104.2520	19.8332	Jalisco	4(8)	11	22	11	15	0.883	0.004	-0.241
11. Colima	-103.7578	19.2604	Colima	4(8)	12	24	11	10	0.913	0.003	-0.095
12. Coalcomán	-103.3465	18.7177	Michoacán	4(8)	15	30	11	10	0.876	0.003	0.323
13. Cuatro Caminos	-101.9175	19.2187	Michoacán	4(9)	13	26	15	21	0.932	0.005	-0.917
14. Chipícuaro	-101.4025	19.1363	Michoacán	4(10)	10	20	12	26	0.958	0.007	-0.776
15. La Huacana	-101.7515	18.9725	Michoacán	1(3)	4	8	4	16	0.750	0.007	-0.202
16. Churumuco	-101.6605	18.7015	Michoacán	4(8)	9	18	17	22	0.993	0.008	0.415
TOTAL				53(110)	148	296	119	66	0.978	0.006	-1.671

Table 2. Results obtained from the analysis of molecular variance (AMOVA) of the examined specimens of *Pegoscapus* sp. d.f.: degrees of freedom**$p < 0.001$.

	df	Sum of squares	Estimated variance	Percent	Fixation Indices
No groups defined					
Among populations	15	216.815	0.682	25.69	
Within populations	280	552.503	1.973	74.31	F_{ST}=0.257**
Total	295	769.318	2.655		

Figure legends

Figure 1. *Ficus pringlei* and its wasps of *Pegoscapus* sp. **A**) Rupicolous habit of *F. pringlei* from Tequila, Jalisco. **B**) Syconia in different development phases. **C**) Female wasp of *Pegoscapus* sp.

Figure 2. **A**) Population locations (names as in Table 1) and distribution of the three groups of haplotypes derived from the phylogenetic analysis performed. **B**) Neighbor-net of mitochondrial COI haplotypes. Red line corresponds to the barrier indicated by SAMOVA, which separates the localities into two groups: North (localities 1-8) and South (localities 9-16). Pie charts represent frequencies of haplotype groups found at each sampling location. The graph size is proportional to sample size.

Figure 3. Phylogenetic inference of haplotype relationships obtained with maximum likelihood. H-numbers (H1-H119) are haplotypes of wasps *Pegoscapus* sp. collected in *Ficus pringlei*. Black squares represent posterior probabilities ≥ 0.80; gray squares represent posterior probabilities < 0.80.

Figure 1

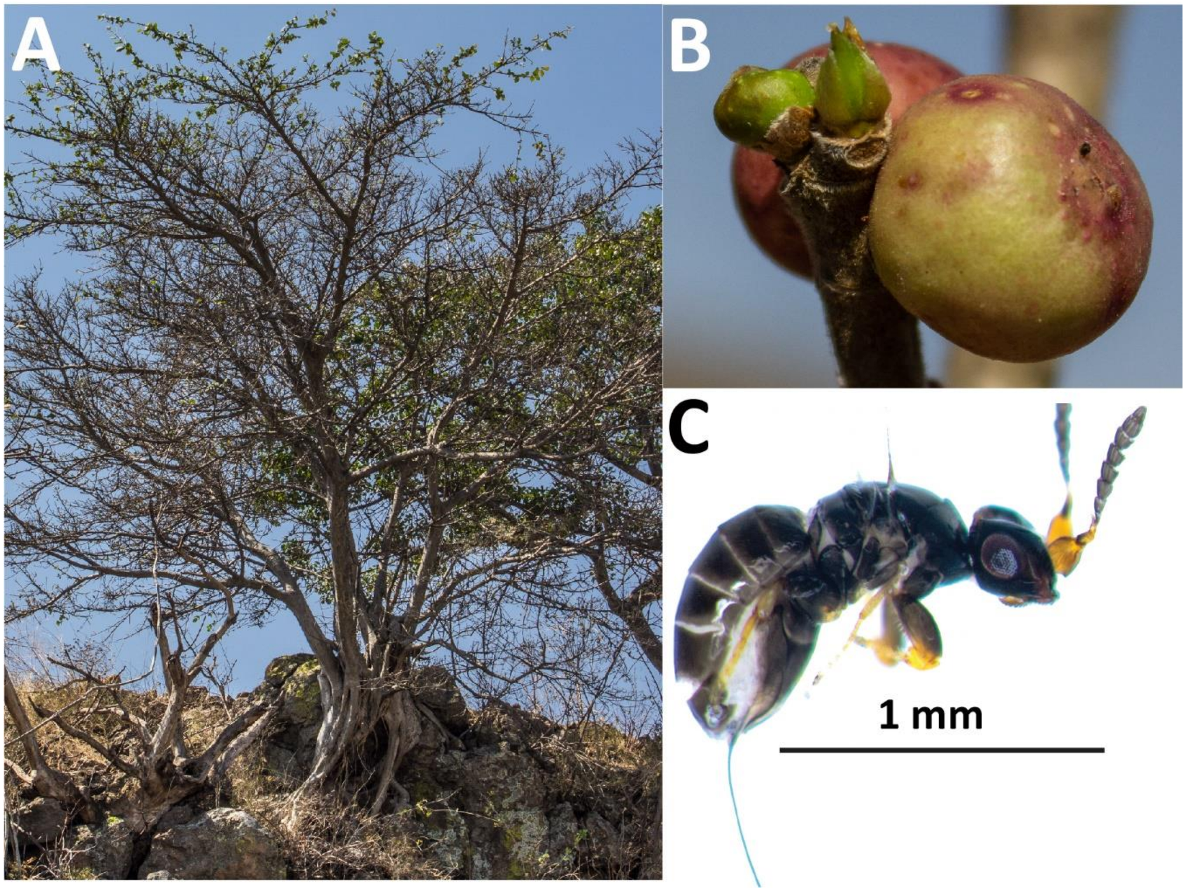
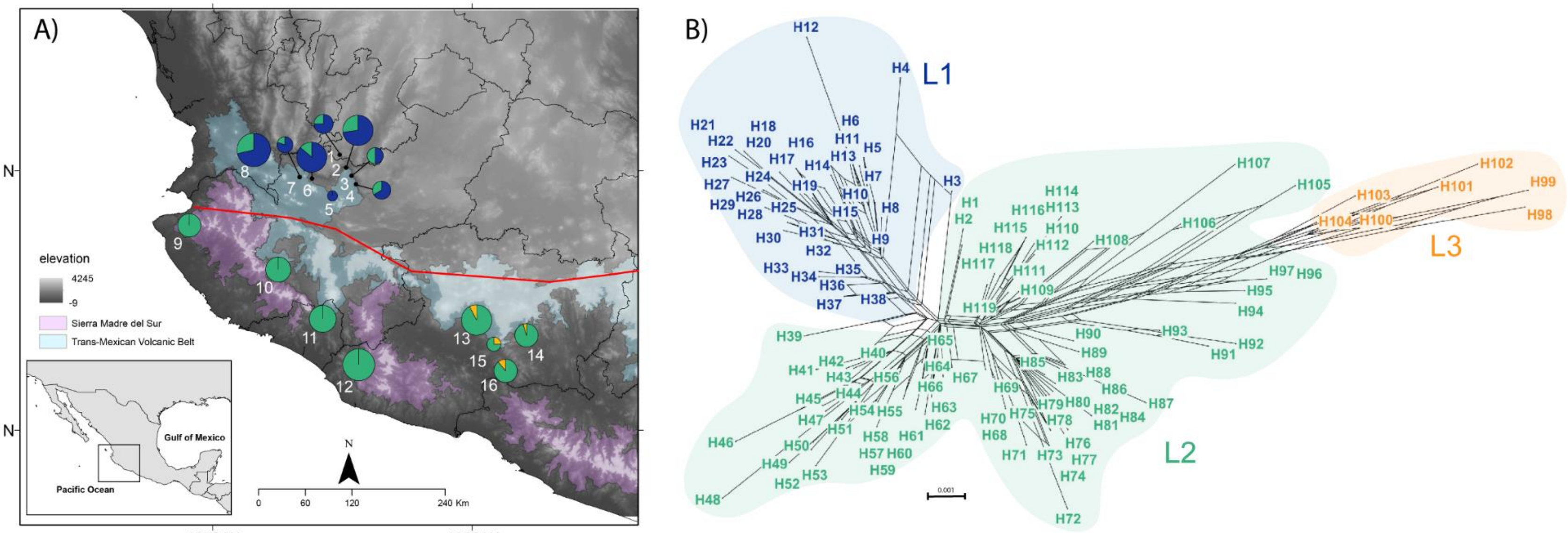


Figure 2



A)
21° N
18° N
105° W
102° W
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
elevation
4245
-9
Sierra Madre del Sur
Trans-Mexican Volcanic Belt
Gulf of Mexico
Pacific Ocean
N
0
60
120
240 Km
B)
L1
L2
L3
H12
H4
H21
H18
H22
H20
H16
H6
H11
H5
H23
H17
H13
H14
H7
H24
H27
H26
H19
H10
H3
H29
H28
H25
H15
H8
H30
H31
H9
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H33
H34
H35
H36
H37
H38
H1
H2
H117
H118
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H54
H53
H52
H51
H50
H49
H48
H47
H46
H45
H44
H43
H42
H41
H40
H39
0.001

Figure 3

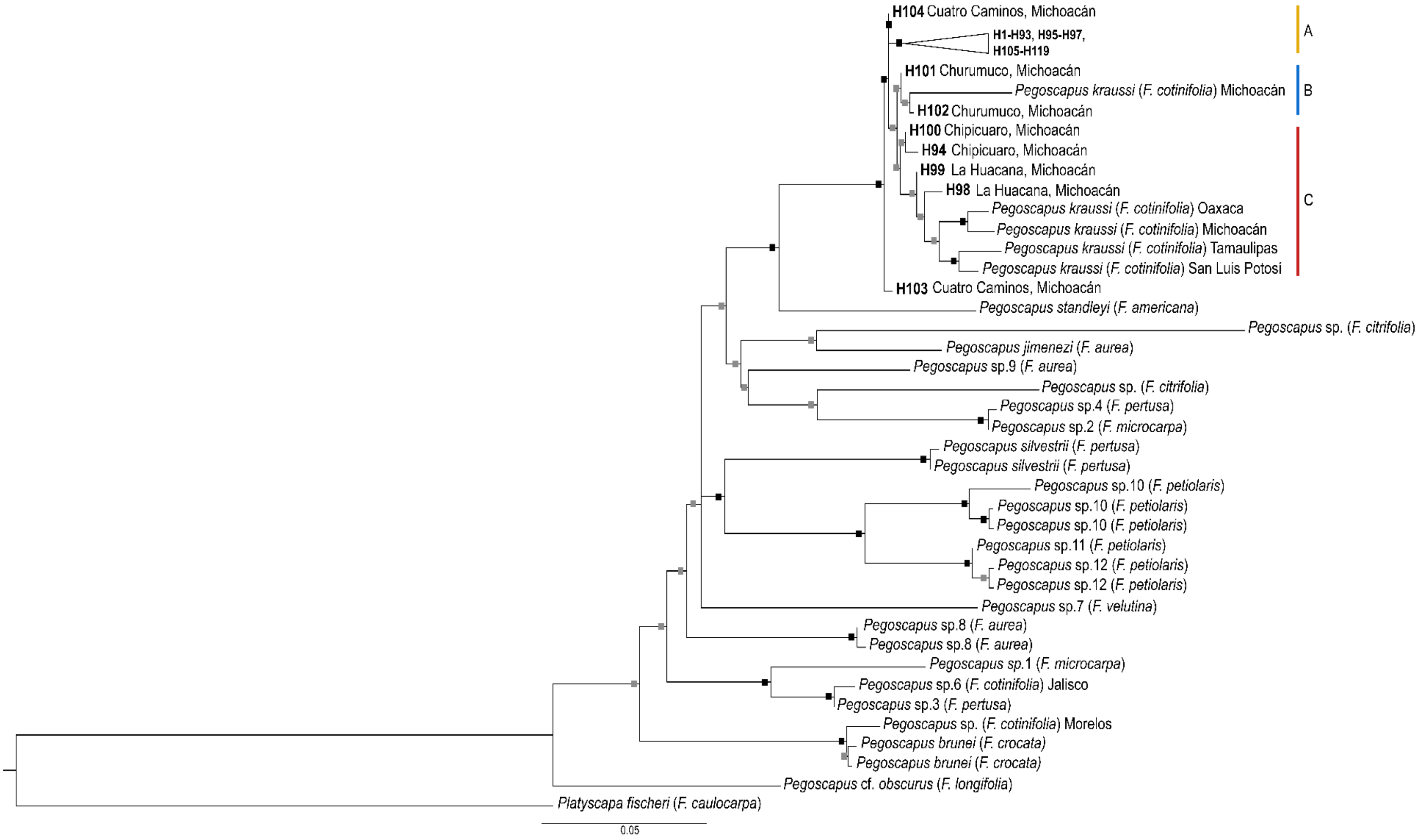
H104 Cuatro Caminos, Michoacán
H1-H93, H95-H97,
H105-H119
A
H101 Churumuco, Michoacán
Pegoscapus kraussi (*F. cotinifolia*) Michoacán
B
H102 Churumuco, Michoacán
H100 Chipicuaro, Michoacán
H94 Chipicuaro, Michoacán
H99 La Huacana, Michoacán
H98 La Huacana, Michoacán
Pegoscapus kraussi (*F. cotinifolia*) Oaxaca
C
Pegoscapus kraussi (*F. cotinifolia*) Michoacán
Pegoscapus kraussi (*F. cotinifolia*) Tamaulipas
Pegoscapus kraussi (*F. cotinifolia*) San Luis Potosí
H103 Cuatro Caminos, Michoacán
Pegoscapus standleyi (*F. americana*)
Pegoscapus sp. (*F. citrifolia*)
Pegoscapus jimenezi (*F. aurea*)
Pegoscapus sp.9 (*F. aurea*)
Pegoscapus sp. (*F. citrifolia*)
Pegoscapus sp.4 (*F. pertusa*)
Pegoscapus sp.2 (*F. microcarpa*)
Pegoscapus silvestrii (*F. pertusa*)
Pegoscapus silvestrii (*F. pertusa*)
Pegoscapus sp.10 (*F. petiolaris*)
Pegoscapus sp.10 (*F. petiolaris*)
Pegoscapus sp.10 (*F. petiolaris*)
Pegoscapus sp.11 (*F. petiolaris*)
Pegoscapus sp.12 (*F. petiolaris*)
Pegoscapus sp.12 (*F. petiolaris*)
Pegoscapus sp.7 (*F. velutina*)
Pegoscapus sp.8 (*F. aurea*)
Pegoscapus sp.8 (*F. aurea*)
Pegoscapus sp.1 (*F. microcarpa*)
Pegoscapus sp.6 (*F. cotinifolia*) Jalisco
Pegoscapus sp.3 (*F. pertusa*)
Pegoscapus sp. (*F. cotinifolia*) Morelos
Pegoscapus brunei (*F. crocata*)
Pegoscapus brunei (*F. crocata*)
Pegoscapus cf. *obscurus* (*F. longifolia*)
Platyscapa fischeri (*F. caulocarpa*)
0.05

Table S1. GenBank accession numbers and details of sequences used in the phylogenetic analysis. * Mexico localities.

Host *Ficus*	**Wasps deposited as**	**Locality**	**Accession numbers GenBank**
F. americana	*Pegoscapus standleyi*	Tamul, San Luis Potosí*	AB308342
F. aurea	*Pegoscapus jimenezi*	Temazcal, Oaxaca*	AB308348
	Pegoscapus sp.8	Los Tuxtlas, Veracruz*	AB308349
			AB308350
	Pegoscapus sp.9	Alta Cima, Tamaulipas*	AB308351
F. cotinifolia	*Pegoscapus Kraussi*	Gómez Farias, Tamaulipas*	AB308343
		Tamul, San Luis Potosí*	AB308344
		San Pedro Huamelula, Oaxaca*	AB308345
	Pegoscapus sp.6	Chamela, Jalisco*	AB308346
F. crocata	*Pegoscapus bruneri*	Valle de Tehuacán-Cuicatlan, Oaxaca*	AB308352
		San Mateo de Mar, Oaxaca*	AB308353
F. microcarpa	*Pegoscapus* sp.1	Cuatro Caminos, Oaxaca*	AB308336
	Pegoscapus sp. 2	Arroyo Azul, Oaxaca*	AB308337
F. pertusa	*Pegoscapus* sp.3	Tehuantepec, Oaxaca*	AB308338
	Pegoscapus sp. 4	Los Tuxtlas, Veracruz*	AB308339
	Pegoscapus silvestrii	Los Tuxtlas, Veracruz*	AB308340
			AB308341
F. petiolaris	*Pegoscapus* sp.10	San Pedro Huamelula, Oaxaca*	AB308354
		Las Estacas, Morelos*	AB308355
			AB308356
	Pegoscapus sp.11	San Carlos, Sonora*	AB308357
	Pegoscapus sp.12	Las Palmas, Baja California Sur*	AB308358
			AB308359
F. velutina	*Pegoscapus* sp.7	La Cañada, Cuernavaca, Morelos*	AB308347
F. caulocarpa	*Platyscapa fischeri*	Isobe, Ishigaki-shi, Okinawa, Japan	AB488709
F. cotinifolia	*Pegoscapus* sp.	Axochiapan, Morelos*	LC647646
	Pegoscapus Kraussi	Lázaro Cárdenas, Michoacán*	LC647647
		Santiago Tangamandapio, Michoacán*	LC647648
F. longifolia	*P. cf. obscurus*	Brazil	JN103286
F. trigona	*Pegoscapus* sp.	Brazil	JN103329
F. citrifolia	*Pegoscapus* sp.	Brazil	MG214355

Table S2. The average K2P distances between clusters genetics of *Pegoscapus* collected in *F. pringlei.* The values above the diagonal correspond to standard deviation.

	A	**B**	**C**
A		0.0041	0.0052
B	0.0165		0.0045
C	0.0299	0.0203	

Discusión general y conclusiones

Ahondando en el conocimiento de una higuera endémica del Neotrópico

Previo al presente trabajo, el conocimiento sobre *F. pringlei* se limitaba a descripciones taxonómicas de la especie, especialmente lo referente a sus caracteres diagnósticos y una aproximación general de su distribución en México (Serrato *et al.*, 2004; Ibarra-Manríquez *et al.*, 2012). Por ejemplo, con respecto a las colectas de herbario depositadas en distintos herbarios de México y del mundo, se encontraron frecuentemente determinaciones erróneas, particularmente de los ejemplares que carecen de síconos, ya que las hojas de *F. pringlei* pueden ser confundidas con las de *F. cotinifolia* (Ibarra-Manríquez *et al.*, 2012).

Después de una revisión del material depositado en el Herbario Nacional (MEXU, Universidad Nacional Autónoma de México), concluí que la distribución de *F. pringlei* abarca desde Nayarit hasta Guerrero (Figura 6, Capítulo 1). Esta especie es endémica al occidente de México y se encuentra principalmente en bosques tropicales estacionalmente secos y, en menor medida, en bosques de *Quercus* y *Quercus-Pinus*. La distribución de la especie está separada por las tierras altas, al oeste de la Faja Volcánica Transmexicana (FVTM) y Sierra Madre del Sur (SMS), y está altamente relacionada con la estacionalidad de la temperatura, cuya variación no es homogénea en toda el área de su distribución. Al norte de la FVTM se registra una mayor estacionalidad de esta variable climática (Capítulo 1), la cual podría afectar la sincronía en la producción de síconos, la reproducción y supervivencia de las avispas que la polinizan, así como la producción de semillas, como ha sido destacado para otras especies de *Ficus* (Peng *et al.*, 2010; Krishnan & Borges, 2014; Zhang *et al.*, 2019).

Las fases de desarrollo del sícono de *F. pringlei* son consistentes con las descritas previamente para otras especies monoicas de *Ficus* (Ramírez, 1974; Verkerke, 1989; Piedra-Malagón *et al.*, 2019; Delgado-Perez *et al.*, 2020). Las diferencias morfológicas externas más evidentes a lo largo del desarrollo del sícono fueron el aumento gradual de tamaño, la forma del ostiolo, así como cambios de color y de consistencia (Figuras 1 y 3, Capítulo 1). En la fase B, que es crítica para la polinización y dura solo unos pocos días, se observó la presencia de un sinestigma, es decir, el entrelazamiento de las ramas estigmáticas y las papilas de algunas flores, lo que sugiere la presencia de un comportamiento activo en la polinización. Este comportamiento fue respaldado por una baja relación de anteras-óvulos, la presencia de polen psilato (liso), así como rasgos morfológicos en las avispas hembra para recolectar y almacenar el polen de forma eficiente, como corbículas esternales grandes, corbículas y peines coxales (Figuras 4 y 5, Capítulo 1).

Aunque determinar la estrategia fenológica no era el objetivo de este trabajo, las observaciones de campo mostraron que la asincronía en la copa de los individuos es posible, ya que frecuentemente se registraron síconos en diferentes fases de desarrollo (Figura 1L-M, Capítulo 1). Esta estrategia puede permitir la persistencia de poblaciones de polinizadores y aumentar las oportunidades de proporcionar y recibir polen de otros árboles, lo que ha sido asociado a poblaciones pequeñas y ambientes altamente estacionales (Ramírez, 1970; Janzen, 1979; Smith & Bronstein, 1996; Gates & Nason, 2012).

Las avispas polinizadoras de *F. pringlei* aún no han sido descritas, pero pertenecen sin duda al género *Pegoscapus* Cameron, 1906, ya que a diferencia del otro género de avispas Agaonidae encontrado en América (i.e., *Tetrapus* Mayr, 1885), los apéndices mandibulares son laminares y tienen corbículas en las coxas anteriores y en el mesoesternón (Figura 5B-C, Capítulo 1). Frecuentemente se registró una sola avispa fundadora dentro del sícono, pero, en ocasiones, se contabilizaron hasta diez individuos. El alto número de hembras fundadoras por sícono podría ser consecuencia de la asincronía en los eventos reproductivos de la población. Esta asincronía resulta en una variabilidad temporal en la disponibilidad de síconos, los cuales pueden estar en diferentes etapas de desarrollo, en un mismo árbol. Esta variabilidad puede generar escasez de síconos en una misma etapa, lo que a su vez aumenta las posibilidades de encontrar más avispas cuando se encuentra un sícono receptivo.

Diversidad y estructura genética de *F. pringlei* y sus avispas polinizadoras

En este trabajo se desarrolló el primer estudio, a nivel poblacional y genómico, de una especie de *Ficus* en América. Se analizaron 17 localidades cubriendo el área de distribución de *F. pringlei* y se obtuvieron 5,311 SNPs. Se registraron niveles generales moderados de He (0.234) y valores negativos de F_{IS} (-0.124) –esto es, un exceso de heterocigotos- para todas las localidades (Capítulo 2). Estos valores pueden estar relacionados con varios aspectos del ciclo de vida de esta especie. Por ejemplo, el crecimiento perenne, la polinización cruzada, y la posibilidad de que ocurra una dispersión del polen a grandes distancias. Además, se destaca el papel del apareamiento aleatorio, facilitado por la asincronía reproductiva, donde la heterogeneidad en las épocas de floración y maduración de las semillas puede incrementar la probabilidad de reproducirse con individuos con alelos diferentes, facilitar el apareamiento aleatorio y aumentar la heterocigosidad de la población (Gates & Nason, 2012).

A pesar de que *F. pringlei* presenta una estructura genética general baja (F_{ST}= 0.042), se observa una diferenciación genética moderada en ciertas poblaciones (F_{ST}= -0.006 a 0.208). Se registraron tres grupos genéticos: uno hacia el norte de la FVTM, otro hacia el sur, y el tercero en la localidad de Churumuco (Figura 1, Capítulo 2). La heterogeneidad ambiental fue un mejor predictor de la variación genómica que la autocorrelación espacial. Esto sugiere que el aislamiento ambiental puede ser el principal factor que contribuye su variación genómica. Específicamente, se observó que la estacionalidad de la temperatura y la precipitación anual tuvieron la mayor influencia en la variación genómica de *F. pringlei* (Figura 2, Capítulo 2). Estas variables ambientales pueden afectar la dinámica reproductiva, tanto de la planta, como de las avispas (Peng *et al.*, 2010). Por ejemplo, la localidad Churumuco tiene temperaturas más altas y humedad relativa más baja que la localidad más cercana. Estas condiciones pueden limitar la longevidad de las avispas, su capacidad de dispersión y, en consecuencia, la genética de poblaciones de sus hospederos (Nason *et al.*, 1998; Ahmed *et al.*, 2009; Jevanandam *et al.*, 2013; Segar *et al.*, 2017; Souto-Vilarós *et al.*, 2019; Gigante *et al.*, 2021; Xu *et al.*, 2021; van Kolfschoten *et al.*, 2022).

La autocorrelación espacial también se asoció con una proporción de la variación genómica, lo que sugiere que los procesos neutros tienen un efecto detectable en la estructura de la diversidad genética de esta especie. Las tierras altas al oeste de la FVTM y SMS también desempeñan un papel en la configuración de la diversidad genética de *F. pringlei*. Sin embargo, la baja diferenciación entre individuos al norte y al sur de la FVTM puede ser generada por barreras incompletas al flujo génico, facilitado por las zonas que presentan elevaciones más bajas, como acontece hacia el oriente de la FVTM. Esta hipótesis se ha sugerido para *Neltuma* (=*Prosopis*) *laevigata* (Humb. & Bonpl. ex Willd.) Britton & Rose, el mezquite característico de los hábitats secos de México (Contreras-Negrete *et al.*, 2021).

Las avispas polinizadoras de *F. pringlei* se analizaron a lo largo de toda la distribución geográfica del *Ficus* (16 localidades). Se registraron altos niveles de variación genética (Hd= 0.978). lo que podría deberse a las altas tasas evolutivas del ADNmt y, posiblemente, a un gran tamaño de sus poblaciones y a sus cortos tiempos de generación (Weiblen, 2002; Xiao *et al.*, 2012). Se encontraron diferentes haplotipos en avispas recolectadas dentro del mismo individuo o incluso dentro del mismo sícono, lo que indica la presencia de varias avispas fundadoras lo que podría contribuir a esta variación genética, sobre todo si las avispas proceden de diferentes áreas. Sin embargo, esto podría generar menos descendencia y un cambio de proporción de sexos en la descendencia (Herre, 1989), lo que afectaría su genética poblacional.

En las localidades del extremo sur de la distribución de *F. pringlei*, en el bosque seco tropical de la región del Balsas, se registraron los índices de diversidad genética más altos y los haplotipos más divergentes para las avispas polinizadoras de *F. pringlei*. En estas localidades (Capítulo 2), las condiciones son más secas y estacionales que en algunas regiones más cercanas a la costa del Pacífico (Espinosa-Organista *et al.*, 2008; Ceballos *et al.*, 2010). Esta variación ambiental puede favorecer la diferenciación y la adaptación local de las avispas, ya que estos insectos son muy sensibles a las altas temperaturas y a la baja humedad relativa, lo que puede limitar su tiempo de vida y capacidad de dispersión (Gigante et al., 2021; Janzen, 1979; Xu et al., 2021).

Para las avispas no se intentó analizar una posible asociación de la variación genética con la heterogeneidad ambiental del área de distribución de *F. pringlei*, ya que sólo se estudió un gen mitocondrial COI. Sin embargo, se registró una estructura genética moderada (F_{ST}= 0.257), moldeada por las tierras altas al oeste de la FVTM y SMS, que podrían actuar como una barrera al flujo genético. Se detectaron tres grupos genéticos: uno al norte de la FVTM, otro al sur de la FVTM y uno último en la región del Balsas, en Michoacán (Figura 2, Capítulo 3). Este resultado concuerda con la relación que se ha asignada a la orogenia del FVTM como un factor que ha impulsado la divergencia de varias especies animales que se encuentran en los bosques tropicales estacionalmente secos de las tierras bajas del occidente de México (Zaldívar Riverón *et al.*, 2004; Devitt, 2006; Zarza *et al.*, 2008; Pringle *et al.*, 2012; Schramm *et al.*, 2021).

El papel de la adaptación local en el proceso de diversificación

La adaptación local desempeña un papel fundamental en el proceso de diversificación en la interacción mutualista entre las especies de *Ficus* y sus avispas polinizadoras (Tian *et al.*, 2015; Souto-Vilarós *et al.*, 2019). La presencia de heterogeneidad ambiental y la divergencia genética son factores clave en la generación de nuevas especies y en el proceso de coespeciación de la interacción *Ficus*-Agaonidae (Tian *et al.*, 2015; Rodriguez *et al.*, 2017; Souto-Vilarós *et al.*, 2019). En entornos climáticos contrastantes, el apareamiento selectivo de las avispas y su respuesta al estrés abiótico podría limitar el flujo genético, facilitando su aislamiento y diferenciación genética. Esto, a su vez, podría restringir el flujo genético de los hospederos y conducir eventualmente a una especiación parapátrica en ambos interactuantes (Orsini *et al.*, 2013; Tian *et al.*, 2015; Souto-Vilarós *et al.*, 2019).

La adaptación local en esta interacción puede implicar cambios en rasgos importantes para la polinización y reproducción, como la morfología floral, la sincronización de la floración, la composición química de los compuestos orgánicos volátiles emitidos por los higos y la respuesta al estrés abiótico (Soler *et al.*, 2011;

Rodriguez *et al.*, 2017; Deng *et al.*, 2020c). Este proceso, que podría reflejar un mosaico geográfico de coevolución, se observaría primero en las avispas, debido a sus ciclos generacionales más cortos (Thompson, 2005). A diferencia de la propuesta de este último autor, tras la adaptación local de ciertas poblaciones (puntos calientes), no se restablece el contacto con otras poblaciones sin adaptación local (puntos fríos), llevando a una coevolución generalizada. Por el contrario, se observa un fenómeno de aislamiento y especiación (Figura 2), reforzando así la complejidad de la dinámica evolutiva en esta interacción (Darwell *et al.* 2014, Rodriguez *et al.* 2017, Souto-Vilarós *et al.* 2019, Yu *et al.* 2019).

Estudios recientes que emplearon marcadores genómicos, han revelado que la especiación ecológica es un fenómeno común (McCulloch *et al.*, 2021; McCulloch & Waters, 2023). Este proceso puede dar lugar a radiaciones adaptativas y al origen de nuevas especies, aprovechando las oportunidades ecológicas (Yoder *et al.*, 2010). Sin embargo, se necesitan más investigaciones usando datos genómicos y métodos de análisis adecuados para evaluar el papel específico de la adaptación local en la generación de nuevas especies en países con alta heterogeneidad ambiental, como México. Un enfoque más detallado en estos contextos permitirá en el futuro comprender la contribución de la adaptación local al proceso de diversificación biológica.

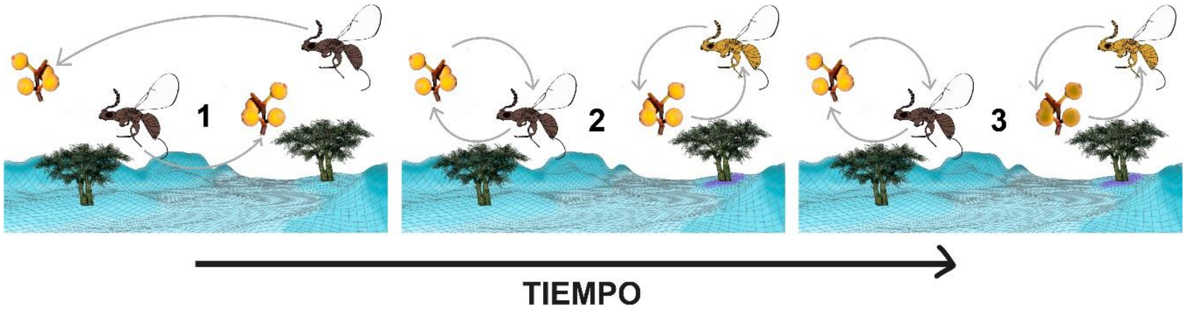

Figura 2. Proceso de coespeciación y adaptación local: 1) Inicialmente hay homogeneidad ambiental, donde las avispas y *Ficus* mantienen una conectividad genética. 2) Por diferentes procesos, se produce heterogeneidad ambiental y las avispas experimentan adaptación local, lo que influye en el flujo genético de los hospederos. 3) Con el paso del

tiempo, se observa una restauración a nivel local de la relación uno a uno entre las avispas y sus hospederos. Esto se debe al continuo aislamiento reproductivo ejercido por las avispas en los hospederos, lo que eventualmente podría desembocar en a especiación parapátrica en ambos interactuantes.

Conservación

Ficus pringlei se encuentra principalmente en bosques tropicales estacionalmente secos, un tipo de vegetación en situación crítica en México, donde se ha perdido más del 73 % del bosque original, y cuya representación en el sistema de áreas protegidas es escasa (Trejo y Dirzo, 2002). Además, la cuenca del Balsas es un centro de diversificación y endemismo (Flores-Tolentino *et al.*, 2021), pero los cambios en el uso del suelo y el cambio climático puede reducir las áreas de este tipo de vegetación. Estos impactos en la región del Balsas podrían afectar directamente la diversidad genética del mutualismo *F. pringlei* y sus polinizadores, ya que en esta área se presenta de forma exclusiva uno de los grupos genéticos del hospedero y la mayor diversidad genética en las avispas.

En el área de distribución potencial de *F. pringlei*, entre 2016 y 2018, se han transformado 145 km^2 de vegetación primaria y secundaria de BTS a hábitats no aptos para esta especie, como terrenos agrícolas o pastizales (Capítulo 1). Por lo tanto, es urgente establecer estrategias de conservación para esta especie de *Ficus* endémica, que actualmente no se encuentra reconocida en ninguna categoría de riesgo de la Lista Roja de Especies Amenazadas de *The International Union for Conservation for Nature of Threatened Species* (IUCN), ni en la NOM-059-SEMARNAT-2010, y sólo el 20 % de sus áreas potencialmente aptas en las condiciones actuales se encuentra dentro de Áreas Naturales Protegidas (Figura 6, Capítulo 1).

Perspectivas

El presente trabajo deja una serie de inquietudes relevantes que se pueden abordar en el futuro para mejorar nuestro entendimiento del mutualismo *Ficus*-Agaonidae. En el caso de las plantas, por ejemplo, sería interesante cuantificar detalladamente el grado de asincronía en la producción de síconos a nivel individual y poblacional en una localidad o entre localidades con, por ejemplo, diferentes variaciones de temperatura. Este estudio permitiría comprender mejor cómo las condiciones climáticas influyen en la sincronización de la producción de frutos y su impacto en la producción de semillas y en el flujo genético de la especie (Smith & Bronstein, 1996; Martin *et al.*, 2009; Gates & Nason, 2012).

Identificar y estudiar la diversidad de animales frugívoros que se alimentan de los síconos en la fase E permitiría comprender mejor las interacciones entre *F. pringlei* y otros organismos en su entorno. Además, investigar qué especies de animales son más eficientes en la dispersión de las semillas y si existe una correlación entre la presencia y actividad de ciertos animales frugívoros y la distribución espacial de las semillas, proporcionará una mejor comprensión del impacto de estos animales en el proceso de dispersión de las semillas (Heer *et al.*, 2015). Estas investigaciones podrían involucrar no sólo el monitoreo de la presencia y actividad de animales frugívoros, sino la recolección y análisis de semillas dispersadas y el mapeo de su distribución espacial.

En los aspectos genómicos, es necesario en el futuro realizar la secuenciación, ensamblado y anotación de genomas completos con alta cobertura y calidad, así como la generación de transcriptomas que permitan analizar los genes que se expresan, tanto en diversas especies de *Ficus* neotropicales, como en sus avispas polinizadoras. Estas tareas permitirán obtener una base de datos genómica más amplia y representativa, facilitando la identificación de patrones genéticos comunes y específicos de diferentes grupos de especies, así como revelar genes clave y regiones genómicas asociadas con la adaptación al mutualismo y la coevolución entre *Ficus* y sus polinizadores.

Asimismo, la comparación de los genomas de diferentes especies de *Ficus*, provenientes de distintas regiones geográficas, ayudará a entender cómo factores ambientales y geográficos han influido en la evolución y diversificación de las interacciones mutualistas en este grupo de plantas (Xu *et al.*, 2021). De esta manera, se podrían identificar variaciones genéticas específicas asociadas a adaptaciones locales y determinar si existen patrones genómicos que reflejen la historia evolutiva de las relaciones mutualistas en *Ficus*.

Evaluar la estrechez y generalidad de la especialización en la polinización de F. pringlei requiere una comprensión más profunda de la taxonomía de las avispas polinizadoras asociadas. Hasta la fecha, la especie de avispa presente en F. pringlei no ha sido descrita(Rojas-Cortés *et al.*, 2022). Esta información taxonómica permitirá establecer comparaciones con otras especies de avispas polinizadoras de *Ficus* en diferentes regiones geográficas, lo que contribuirá a una mejor comprensión de la diversidad y la coevolución en este grupo de organismos.

Por otro lado, es crucial profundizar en la caracterización morfológica de las avispas que interactúan con *F. cotinifolia* y *F. pringlei*, considerando que estas especies son frecuentemente simpátricas, presentan similitudes morfológicas (Ibarra-Manríquez *et al.*, 2012) y comparten polinizadores estrechamente relacionados. Se requiere llevar a cabo estudios sistemáticos y poblacionales de ambas especies de *Ficus* y sus avispas, para comprender mejor la naturaleza de su interacción e impacto en su evolución.

La caracterización morfológica detallada de las avispas de estas especies de *Ficus* permitiría identificar posibles diferencias o similitudes en sus rasgos morfológicos, lo que brindará información importante sobre la especificidad de la relación entre las avispas y cada especie de *Ficus*. Asimismo, los estudios sistemáticos y poblacionales permitirían evaluar la existencia de posibles barreras reproductivas entre poblaciones y analizar la influencia de factores como la distancia geográfica y la conectividad genética.

De manera similar, es importante investigar las secuencias heteroplásmicas o pseudogenes mitocondriales nucleares en las avispas, ya que podrían revelar información valiosa sobre la dinámica evolutiva y los procesos genéticos que ocurren entre las avispas y sus hospederos (Ricardo *et al.*, 2020; Li *et al.*, 2021). Las secuencias heteroplásmicas, término que se refiere a la presencia de múltiples secuencias de ADN mitocondrial dentro de un individuo, pueden revelar patrones de variación genética que son importantes para comprender la interacción *Ficus*-Agaonidae. Estas secuencias pueden surgir debido a eventos como la recombinación mitocondrial, la duplicación del genoma mitocondrial o la transferencia horizontal de genes, lo que puede tener implicaciones significativas en la adaptación y la diversificación de las especies (Minhas *et al.*, 2023).

Por otro lado, pseudogenes mitocondriales nucleares -copias no funcionales de genes mitocondriales que se encuentran en el núcleo de la célula- pueden surgir a través de procesos de transferencia génica horizontal o a través de eventos de duplicación y pseudogenización (Chen *et al.*, 2014; Françoso *et al.*, 2016). Su estudio podría revelar la historia evolutiva y los procesos de coevolución entre las avispas y los hospederos, proporcionando información sobre eventos de transferencia génica, cambios en la función de genes mitocondriales y su impacto en la adaptación y diversificación de ambas partes.

Asimismo, es esencial determinar el efecto del número de avispas fundadoras, evaluando su influencia en el número de avispas y semillas, y comprender sus consecuencias ecológicas y genéticas, es fundamental para comprender la dinámica y la evolución de esta interacción mutualista. Estudiar las consecuencias genéticas de diferentes números de fundadoras permitiría comprender la diferenciación genética y la capacidad de adaptación entre las poblaciones de avispas y de *Ficus* (Herre, 1989). Además, al explorar los efectos ecológicos de los diferentes números de fundadoras, sería posible examinar cómo estas variaciones influyen en la eficacia de la polinización y la reproducción de *Ficus*, así como en la adecuación de las avispas (Herre, 1989).

En un futuro, explorar la gran diversidad, desarrollo, colonización y variación temporal de las avispas no-polinizadoras ayudará a comprender mejor las interacciones complejas que ocurren en el mutualismo entre *Ficus* y sus polinizadores. Aunque estas avispas no tienen un papel directo en la polinización, su presencia puede tener un impacto significativo en la aptitud, tanto del hospedero como de las avispas polinizadoras. Investigar la diversidad de estas avispas no-polinizadoras también permitiría comprender mejor cómo influyen en la dinámica de la comunidad de avispas polinizadoras en el interior del sícono (Cardona, Kattan, & Chacón de Ulloa, 2013; Compton *et al.*, 2018). Estudiar los diferentes estadios de desarrollo y los momentos en los que estas avispas colonizan los higos dará una visión más completa de su ciclo de vida y su interacción con los hospederos, así como evaluar su impacto potencial en la aptitud del hospedero y de las avispas polinizadoras (Krishnan & Borges, 2014; Zhang & Li, 2020).

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Material suplementario: Cuento infantil.

El secreto mejor guardado del universo

El secreto mejor guardado del universo

Ángela P. Rojas-Cortés
Lau Rojas
Ilustradora Kaity Kazinsky


¡Que lindo árbol,
vamos a ver sus flores!
¡Adiós Noa!


¿Y sus flores?
¿Las puedes ver?


¡una flor vampiro!
Pis, soy Guillo, el hogar y alimento de muchos animales y te voy a contar un secreto... **¡El secreto mejor guardado del universo!**
Mis flores siempre están escondidas y nunca ven la luz del sol


¡Mira! Aquí están en este higo
Son muy pequeñitas y no son coloridas como las rosas o los girasoles, pero son importantes para que haya más árboles como yo


¡UN BICHO!
ishu! ishu!
¡CUIDADO!
Es Lupita, la avispa
y está trabajando


¿Trabajando?
Si, ella es la única que
puede entrar a ver mis flores
y polinizarlas


¡Hola Lupita!
¡¡Uff, esto es muy agotador!! Tengo que poner polen y huevos, polen y huevos en todas estas flores


¿Y por qué pones tus huevos ahí?
mmm... ¡¡Que rico, higos con chilito como me gustan!!
Porque no puedo proteger siempre a mis bebes y ellos necesitan un lugar seguro, calientito y con muuucha comida


¿Y después qué pasa?
Los bebes se transforman en adultos hembras y machos dentro del higo. Y únicamente las hembras tienen alas
¿Qué onda, vamos por un elotito?
ups, se me olvido que no puedes volar


¿Y los machos?
Que el viento te lleve lejos, te extrañaré
No te preocupes, nuestros bebes estarán bien
No tienen alas, pero tienen unas mandíbulas muy fuertes para abrir un huequito para que salgan las hembras a polinizar otros Guillos


¿Y entonces los machos se quedan encerrados?
Si, porque son un alimento importante para otros animales como murciélagos o aves que ayudan a dispersar las semillas de Guillo
¡Gracias Pepe por ayudar a dispersar a mis hijos!


¡¡Wow, que buen equipo hacen todos!!
Sin Guillo, no habría refugio y comida para muchos animales. Sin Lupita, las diminutas flores de Guillo no serías polinizadas. Sin Pepe, no se dispersarían las semillas de Guillo.
O sea, se necesitan unos a los otros para poder vivir.
¿Y crees que todos hacemos parte de un equipo como este, en el que todos somos importantes?