UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO

POSGRADO EN CIENCIAS BIOLÓGICAS

INSTITUTO DE ECOLOGÍA

BIOLOGÍA EVOLUTIVA

UNA APROXIMACIÓN FILOGENÉTICA, MORFOLÓGICA, ECOLÓGICA Y GENÉTICA PARA ENTENDER LA DINÁMICA EVOLUTIVA DE OYAMELES MEXICANOS (*Abies*, Pinaceae).

TESIS

QUE PARA OPTAR POR EL GRADO DE DOCTOR EN CIENCIAS

PRESENTA:

JORGE CRUZ NICOLÁS

TUTOR PRINCIPAL: DR. JUAN PABLO JARAMILLO CORREA

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POSGRADO
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COORDINACIÓN DEL POSGRADO EN CIENCIAS BIOLÓGICAS
ENTIDAD INSTITUTO DE ECOLOGÍA
OFICIO CPCB/398/2020
ASUNTO: Oficio de Jurado

M. en C. Ivonne Ramírez Wence
Directora General de Administración Escolar, UNAM
P r e s e n t e

Me permito informar a usted que en la reunión ordinaria del Subcomité de Ecología y Biología Evolutiva del Posgrado en Ciencias Biológicas, celebrada el día **23 de marzo de 2020**, aprobó el siguiente jurado para la presentación de examen para obtener el grado de **DOCTOR EN CIENCIAS,** del estudiante **CRUZ NICOLÁS JORGE**, con número de cuenta: **516021364** con la tesis titulada:**"UNA APROXIMACIÓN FILOGENÉTICA, MORFOLÓGICA, ECOLÓGICA Y GENÉTICA PARA ENTENDER LA DINÁMICA EVOLUTIVA DE OYAMELES MEXICANOS (*Abies*, Pinaceae)",** bajo la dirección del **Dr. JUAN PABLO JARAMILLO CORREA,** quedando integrado de la siguiente manera:

Presidente:	DR. JUAN FRANCISCO ORNELAS RODRÍGUEZ
Vocal:	DRA. ALICIA MASTRETTA YANES
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Suplente:	DRA. ALEJANDRA VÁZQUEZ LOBO YURÉN
Suplente:	DR. SANTIAGO RAMÍREZ BARAHONA

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

A T E N T A M E N T E
"POR MI RAZA HABLARÁ EL ESPÍRITU"
Cd. Universitaria, Cd. Mx., a 23 de junio de 2020

COORDINADOR DEL PROGRAMA

POSGRADO EN CIENCIAS BIOLOGICAS
COORDINACIÓN

DR. ADOLFO GERARDO NAVARRO SIGÜENZA

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Contents

RESUMEN

El género *Abies* (Mill.) es un componente característico de las zonas montañosas de México, como tal su distribución tiende a ser restringida y fragmentada exceptuando algunos casos en el centro del país. Al igual que en otras coníferas mexicanas, los ciclos glaciales del Pleistoceno han influido de manera determinante en la historia evolutiva de los oyameles mexicanos, marcando etapas de aislamiento, expansión, contacto secundario entre especies y poblaciones, que han influido de una forma determinante en los procesos de especiación y divergencia del género. En este sentido, los trabajos pioneros para el género *Abies* en México, han puntualizado que el clado donde se encuentran los oyameles mexicanos es totalmente distinto en relación con sus congéneres de Norteamérica, aunque las relaciones filogenéticas hasta ahora no son claras. El primer objetivo de este trabajo fue determinar las relaciones filogenéticas de los oyameles mexicanos. Una vez que se cubrió este objetivo, se postuló que en el proceso de especiación y divergencia las especies pueden o no adquirir ciertas propiedades contingentes como diferenciación de nicho climático, adaptaciones específicas, y una posible diferenciación morfológica o genética. Para este objetivo, se tomó como modelo de estudio un complejo de oyameles del centro de México (*Abies flinckii* y *A. religiosa*), para realizar un par de estudios a nivel poblacional, con la finalidad de comparar algunos parámetros genéticos, morfológicos y ecológicos, entre especies y entre poblaciones de la misma especie, con la finalidad de entender mejor la dinámica evolutiva de estas especies y poblaciones.

Mediante la reconstrucción filogenética se identificaron nueve linajes independientes que se agruparon de acuerdo con los principales sistemas montañosos de México. Se propone a la especiación alopátrida como el modelo más plausible para explicar el proceso de especiación en estos oyameles mexicanos, debido a que los linajes presentaron una fuerte afinidad geográfica con sistemas montañosos, los cuáles han funcionado como refugios para estas poblaciones. Sin

embargo, a medida que se avanzó de norte a sur se observó mayor reticulación entre los linajes del centro y entre los linajes del sur, lo que sugiere que procesos de introgresión, aislamiento, contacto secundario están ocurriendo o han tenido lugar en el pasado reciente. El primer estudio poblacional (Capítulo III) con dos especies del centro de México, una de ellas con distribución más restringida y fragmentada (*A. flinckii*) y la otra con una distribución relativamente más amplia, evidenció que los patrones clinales observados entre caracteres morfológicos y la geografía/ambiente en *A. flinckii* están influenciados por procesos estocásticos, mientras que, en *A. religiosa,* la selección natural prevalece en al menos un par de caracteres, principalmente en el grosor de la hoja. En el segundo estudio poblacional (Capítulo IV) analizamos la centralidad del nicho en el contexto de la teoría neutral (Kimura, 1968, 1983) y casi neutral de evolución molecular (Ohta, 1973). Se encontró que en *A. flinckii* hubo una correlación negativa entre la variación genética neutral y la centralidad del nicho, lo que indica mayor acumulación de diversidad genética a medida que nos acercamos al "optimo ambiental". En los demás casos no hubo relación alguna, a partir del análisis de patrones de diversidad genética se infirió que la historia demográfica y tamaños poblacionales ancestrales han influido de manera más determinante que la centralidad del nicho ecológico en la cantidad de variación genética neutral y la acumulación de mutaciones deletéreas en estas especies.

Finalmente se discute que en este complejo del centro de México distintas fuerzas evolutivas están operando en el proceso de divergencia y especiación. Es decir, que a nivel de género los procesos estocásticos han sido importantes en el proceso de divergencia. Y a nivel local en el centro de México, la variación morfológica observada en forma de clinas puede atribuirse a procesos estocásticos en *A. flinckii*, y en *A. religiosa* puede atribuirse a la selección direccional para el grosor de las hojas, mientras que el nicho ecológico climático fue similar en este par de especies. A partir de estos datos se discute que las diferencias genéticas, morfológicas y ecológicas no van a

la misma velocidad ni en la misma dirección, por lo que el proceso de especiación al que están sometidas estas especies y poblaciones es complejo, y en más de un caso se reflejará en discordancias taxonómicas. Se concluye con que las herramientas utilizadas en el presente estudio nos brindan una perspectiva más amplia de la dinámica evolutiva de estas especies y sus poblaciones.

ABSTRACT

The genus *Abies* (Mill.) is a characteristic component of the mountainous areas of Mexico, as such its distribution tends to be restricted and fragmented, except in some cases in the center of Mexico. Pleistocene glacial cycles have had a decisive influence on the evolutionary history of Mexican firs, promoting isolation, expansion periods and secondary contact between populations and species. These processes have had a decisive influence on the processes of speciation and divergence of the genus. Previous studies in the genus *Abies* in Mexico, have pointed out that the clade of the Mexican firs is different in relation to their counterparts in North America, although the phylogenetic relationships are not clear. The first goal of this study was to determine the phylogenetic relationships of the Mexican firs. Then, it was postulated that in the speciation and divergence process the species might have certain contingent properties such as climatic niche differentiation, specific adaptations, genetic differentiation and a possible morphological differentiation. For this goal, a complex of firs from central Mexico was chosen as a study model (*Abies flinckii* and *A. religiosa*), to carry out a couple of studies at the population level, in order to compare genetic, morphological and ecological parameters, between species and populations, in order to better understand the evolutionary dynamics of these species and populations.

Through the phylogenetic reconstruction, nine independent lineages were identified that were grouped according to the main mountain systems of Mexico. Allopatrid speciation is proposed as the most plausible model to explain the speciation process in these Mexican firs, because the lineages presented a strong geographic affinity, it is possible that isolated mountain systems have functioned as refuges for these natural populations. A pattern of isolation by distance with direction North to South was detected in Mexican firs. Furthermore, higher reticulation was observed between lineages overall in Central and South of Mexico. It suggests that processes of

introgression, isolation, secondary contact are occurring or have taken place in the recent past. The first population study (Chapter III) with two species from central Mexico showed that in *A. flinckii* (with more restricted and fragmented distribution) the morphological variation expressed in shape of clines is affected by stochastic processes, whereas in *A. religiosa*, (with a wider distribution), the natural selection prevails in at least a couple of characters. In the second population study (Chapter IV), we analyze the niche centrality in the context of the neutral and quasi-neutral theory proposed by Kimura (1968, 1983) and Ohta (1973), respectively. *A. flinckii* had a negative correlation between the neutral genetic variation and the centrality of the niche, indicating a greater accumulation of genetic diversity as we get closer to the "*optimal conditions*". In the other cases there was no relationship, from the analysis of patterns of genetic diversity it was inferred that demographic history and ancestral population sizes have influenced more decisively than the niche centrality in the amount of neutral genetic variation and accumulation of deleterious mutations.

Finally, it is argued that in this complex in central México, different evolutionary forces are operating in the process of divergence and speciation. In other words, at the gender level, stochastic processes have been important in the divergence process. Locally in central Mexico, the observed morphological variation in shape of clines can be attributed to stochastic processes in *A. flinckii*. In *A. religiosa*, it can be attributed to directional selection for leaf thickness and resin duct position, while the climatic niche was similar in this pair of species. From these data it is argued that genetic, morphological and ecological differences are not coupled. In this way, the speciation process in these species and populations are complex, and in more one case will be find taxonomic mismatches. It is concluded that the tools used in the present study give us a broader perspective of the evolutionary dynamics of these species and their populations.

CAPÍTULO I: INTRODUCCIÓN

Uno de los principales objetivos de la biología evolutiva es comprender cómo y por qué se diversifican los organismos vivos. Los patrones de diversidad de las especies han sido moldeados por procesos evolutivos a lo largo del tiempo, de tal suerte que el número de especies que observamos hoy es el resultado de los procesos de especiación y extinción del pasado (Magallón & Sanderson, 2001). La formación de nuevas especies es considerada un proceso temporal gradual durante el cual algunas poblaciones alcanzan diversos grados de diferenciación (Harrison, 1998), que después de un "suficiente" número de generaciones podemos reconocer como especies.

Si bien los estudios comparativos a través de filogenias son muy valiosos, estos dejan un cierto grado de incertidumbre sobre los procesos intraespecíficos o aquellos que ocurren durante los estados tempranos de los procesos de diferenciación. Idealmente estos deben ser complementados con estudios a nivel poblacional, donde se consideren patrones de diversidad genética, morfológica y ecológica para así llenar el vacío dejado por los estudios comparativos. A continuación, describimos en forma concreta los mecanismos de especiación que serán tomados como referencia en el desarrollo de esta tesis.

1.2. Mecanismos de especiación

La diversidad de formas biológicas que se asignan como entes diferentes es muy compleja y el grado de diferenciación entre estos entes biológicos es muy amplia, de ahí que existen muchos conceptos de especie (Wilkins, 2011); estos dependen en gran medida del grupo de organismos objeto de estudio y de los aspectos en los que se enfocan. En principio, el concepto biológico de especie de Mayr (1982), que se refiere a grupos de poblaciones que se reproducen entre sí y que

están reproductivamente aisladas de otros grupos, es un buen referente para la delimitación de especies. Sin embargo, no puede ser aplicado a todos los organismos vivos; por ejemplo, a los organismos asexuales. Otras limitaciones existen cuando el aislamiento reproductivo entre taxones es bajo o nulo; como en el caso de muchas especies de plantas con polinización cruzada. Cuando se desea abordar el problema desde la perspectiva morfológica, la separación de especies tampoco es tan obvia. Con frecuencia sucede que las diferencias pueden ser sutiles, y no es claro en qué punto las diferencias son suficientes para establecer un umbral de corte, sobre todo cuando la diferenciación es gradual. De hecho, si nuestro grupo de estudio es de reciente divergencia, y comparte características con taxones hermanos, se debe recurrir a otras alternativas para diferenciarlos (Eaton *et al.*, 2015).

De todos los conceptos de especie el *concepto evolutivo*, que define a las especies como *linajes independientes con sus propias tendencias evolutivas y destino histórico* (Simpson, 1961), destaca por cumplir con la característica de universalidad y contenido teórico. Sin embargo, este concepto no indica del todo bien cómo delimitarlas. Esto puede ser subsanado por conceptos operacionales (Zachos, 2016). A nuestra disposición tenemos más de 20 de estos conceptos, muchos de los cuales hacen referencia a una situación particular y están basados en similitudes de algún tipo (Wilkins, 2011; Zachos, 2016).

La similitud morfológica, genética, ecológica, o de comportamiento como parte de nuestro proceso cognitivo ha estado presente en la forma de clasificar a los organismos, aún antes de la edad media. Sin embargo, a diferencia de los primeros naturalistas, Charles Darwin, en *El Origen de las especies* (1859), tuvo el acierto de explicar la similitud entre organismos a partir de su genealogía o ascendencia común. Es decir, Darwin nota que los organismos descendientes de un antepasado común deberían ser más semejantes entre sí, que aquellos con los que no están

relacionados. De esta forma, la teoría evolutiva considera que la clasificación debe reflejar e ilustrar los mecanismos evolutivos de los seres vivos. En este sentido uno de los conceptos más utilizados en las últimas décadas ha sido el concepto filogenético de especie: "*el grupo más pequeño de poblaciones dentro del cual hay un patrón parental de ancestría y descendencia donde hay una combinación única de estados de carácter*" (Cracraft, 1997; Wheeler & Platnick, 2000); el uso extendido de este concepto se debe en parte a que brinda lineamientos para reconocer la presencia de linajes independientes y establecer relaciones filogenéticas entre ellos.

Una vez que estamos de acuerdo en reconocer la presencia de determinado número de linajes, el paso siguiente es indagar sobre los posibles mecanismos que llevan a esta independencia. Para este propósito se han propuesto distintos escenarios invocando principalmente la separación espacial o alopatría que, aunque puede no ser la responsable directa de la especiación, si se le considera un factor muy importante. A continuación, se describen brevemente algunos mecanismos de especiación (Figura 1; Morrone, 2000; Wiley & Lieberman, 2011).

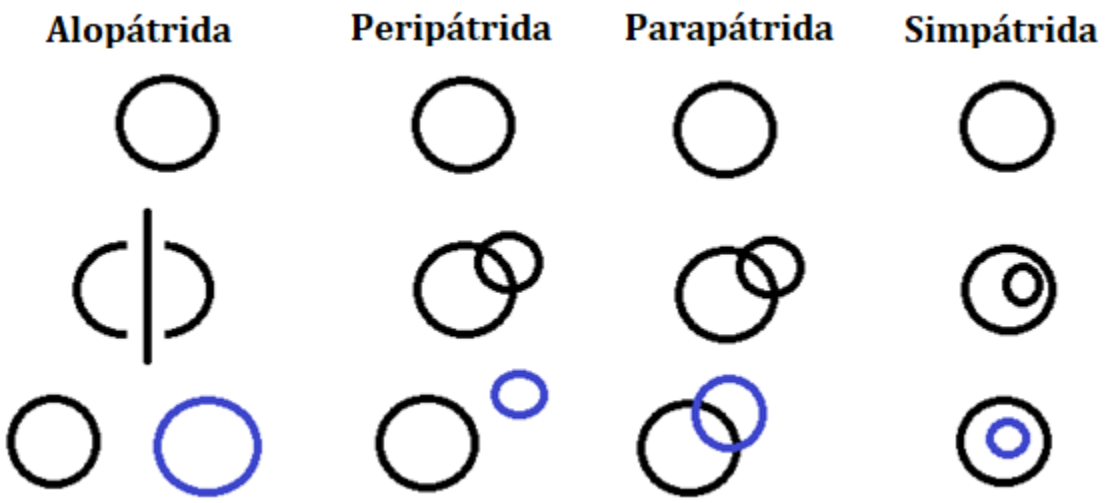


Figura 1. Modelos de especiación alopátrida, peripátrida, parapátrida y simpátrida. En la especiación alopátrida se supone la separación geográfica de dos poblaciones a partir de una población ancestral producto de una barrera. En la especiación peripátrida una nueva especie surge en la periferia de una población central de mayor tamaño. En la especiación parapátrida dos poblaciones de una especie ancestral se diferencian por procesos estocásticos y selección local a pesar de no existir una separación completa. En la especiación simpátrida se diferencian una o más especies nuevas a partir de una especie ancestral sin que exista una separación geográfica de las poblaciones.

Especiación alopátrida, también conocida como especiación geográfica o por vicarianza, supone una gran población en la que surge una barrera geográfica física o una discontinuidad climática (factor extrínseco) que la divide en dos, y a partir de las cuales se generan dos entidades nuevas y distintas entre sí. Este modo de especiación se ve beneficiado por la ausencia de entrecruzamiento o flujo génico entre las dos subpoblaciones. Por lo tanto, la independencia de linajes puede ocurrir por simple deriva génica. En este caso, las barreras reproductivas serían un subproducto de la diferenciación, y no el primer paso (Coyne & Orr, 2004). Este aislamiento reproductivo puede ser resultado entonces de procesos histórico-demográficos (Gao *et al.*, 2012), de ventajas adaptativas de diferentes caracteres ante presiones de selección diferenciadas, o de la

acumulación diferencial de mutaciones entre subpoblaciones, dependiendo de su tamaño efectivo (Palstra & Ruzzante, 2008; Charlesworth, 2009).

Debido al supuesto de no entrecruzamiento, hay un cierto consenso en que la especiación alopátrida es la más frecuente y que incluso debería proponerse como un modelo nulo (Coyne & Orr, 2004; Warren *et al.*, 2014). Es decir, que el aislamiento es un detonante para que ocurra el proceso de especiación. Por ejemplo, en especies continentales, los recurrentes ciclos glaciales del Pleistoceno se han hipotetizado reiteradamente como promotores de nuevas especies, dadas las discontinuidades ambientales y el aislamiento que generaron en especies de afinidad templada (Haffer, 1969; Avise, Walker, & Johns, 2010; April *et al.*, 2013).

Especiación peripátrida: en este modelo, una submuestra de la población se aísla en la periferia de la distribución de una especie, propiciando un cuello de botella. Luego, ocurre la diferenciación de la nueva población. Generalmente se cree que la deriva génica es el principal promotor de este tipo de especiación, aunque no puede descartarse la importancia de la selección natural y la consecuente adaptación local en hábitats extremos (Gavrilets & Hastings, 1996; Sobel *et al.*, 2010). Algunos consideran este tipo de especiación como una variante de la especiación alopátrida (Losos & Glor, 2003). Sin embargo, la especiación peripátrida generaría un patrón biogeográfico, donde las nuevas especies tienen una distribución marginal y un tamaño poblacional efectivo pequeño con respecto a las especies parentales (Arbelaéz-Cortés *et al.*, 2015; Castellanos-Morales *et al.*, 2016); lo cual es ciertamente distinto al modelo clásico de especiación alopátrida. La especiación peripátrida puede detectarse más facilmente en islas oceánicas o 'islas' en el cielo en zonas de alta montaña (McCormack, Huang, & Knowles, 2009), donde como su nombre lo indica se trata de pequeñas poblaciones aisladas por una barrera física en el caso de las islas oceánicas, o por una barrera ambiental en el caso de las islas en el cielo. Podemos mencionar

a los frailejones del género *Espeletia* ubicados en páramos de Sudámerica (Padilla-González, Diazgranados, & Da Costa, 2017) y a *Picea rubens* en Norteamérica (Perron *et al.*, 2000; Jaramillo-Correa & Bousquet, 2003), particularmente durante las dinámicas glaciales del Pleistoceno.

Especiación simpátrida: ocurre sin que exista segregación geográfica en una misma población; de esta manera, dos especies son simpátricas si sus áreas de distribución se traslapan o coinciden, con individuos físicamente capaces de encontrarse y reproducirse con una frecuencia moderada (Mallet *et al.*, 2009). Este modelo parte entonces de una población panmíctica, en donde la selección disruptiva modifica los patrones de cruzamiento y promueve la adaptación local (Bolnick & Fitzpatrick, 2007). Bajo este escenario, se espera selección en contra de los migrantes y una reducida viabilidad de los híbridos, seguida de mecanismos de aislamiento reproductivo y reforzamiento (Zachos, 2016). Esta selección puede actuar sobre caracteres y loci que contribuyan al aislamiento en un determinado hábitat, permitiendo la especiación aún en presencia de flujo génico. En este modelo, la inviabilidad de los híbridos es producto de la selección del hábitat y no de una separación geográfica (Coyne & Orr, 2004; Rieseberg & Willis, 2007; Nosil, Funk, & Ortiz-Barrientos, 2009; Nosil & Schluter, 2011). Aunque este patrón es mucho menos frecuente (Bolnick & Fitzpatrick, 2007), se ha documentado durante cambios de hospederos en insectos fitófagos (Feder & Filchak, 1999; Nosil, 2004), en ambientes aislados como islas oceánicas, y en gradientes de profundidad en lagos postglaciales (Bolnick & Fitzpatrick, 2007). También hay casos interesantes con *Coregonus cupleaformis* (Salmonidae), donde la divergencia adaptativa de caracteres morfológicos, de comportamiento, tasa de crecimiento y posible evolución de barreras reproductivas se ha asociado a una base genética, al generar híbridos inviables en zonas de simpatría (Rogers & Bernatchez, 2006, 2007).

Especiación parapátrida: en este modelo, dos poblaciones de una especie parental se diferencian, pero las especies resultantes pueden compartir una pequeña zona de contacto y aun así generar aislamiento reproductivo. Las poblaciones locales tienden a diferenciarse en respuesta a procesos estocásticos y selección local. La parapatría puede involucrar la adaptación de poblaciones a sus hábitats locales, formando clinas en la distribución espacial. El flujo génico entre poblaciones a lo largo de una clina se puede interrumpir por efecto de selección contra los híbridos o entre individuos adaptados a diferentes ambientes (Arbelaéz-Cortés *et al.*, 2015). Este modelo puede inferirse a partir de un patrón biogeográfico en el que las distribuciones de las especies hermanas colindan estrechamente con zonas híbridas entre ellas. Este patrón sin embargo también puede ocurrir luego de un contacto secundario entre linajes que divergieron en alopatría. Existen alternativas para distinguir entre estas dos posibilidades a través de un análisis en la forma de las clinas, evaluando patrones alternativos de introgresión actual e histórica, identificando marcadores asociados a zonas híbridas o verificando la existencia de aislamiento por distancia (Endler, 1977; Szymura & Barton, 1986; Fitzpatrick, 2013; Eaton *et al.*, 2015). También se pueden usar experimentos en campo bajo condiciones controladas y evaluar caracteres candidatos a selección específicos (Sakaguchi *et al.*, 2018). La especiación parapátrida desde el punto de vista geográfico representa un punto intermedio entre la especiación alopátrida y simpátrida (Mallet *et al.*, 2009). Algunos incluso proponen un tipo de especiación alo-parapátrida, en donde las poblaciones entran en contacto antes de que se haya producido una divergencia completa. En este caso el reforzamiento (mecanismo de aislamiento prézigotico producido para evitar la formación de híbridos con menor eficacia biológica) sería un factor determinante (Perfectti, 2002).

De las ideas hasta aquí expuestas, podemos vislumbrar que el abanico de posibilidades para analizar los cambios que se van dando durante la especiación es enorme; sin embargo, para propósitos específicos de este trabajo y con la finalidad de introducir al lector en lo subsecuente,

proponemos en el Cuadro 1 algunos escenarios posibles con respecto a los cambios esperados a nivel morfológico, genético y ecológico en los diferentes modos de especiación.

Cuadro 1. Cambios relevantes que se esperan durante diferentes procesos de especiación en caracteres ecológicos, genéticos y morfológicos.

Especiación	Nicho ecológico (climático)	Genético	Morfológico
Alopátrida	Se espera un predominio de nichos ecológicos similares (McCormack, Zellmer, & Knowles, 2010; Imada, Kawakita, & Kato, 2011).	Diferenciación gradual paso a paso en poblaciones relativamente continuas (Kimura & Weiss, 1964); la diferenciación puede estar en función del tamaño efectivo de las poblaciones (Palstra & Ruzzante, 2008; Charlesworth, 2009); poca o ninguna diferenciación si esta es reciente (Orr & Smith, 1998).	No es un prerrequisito encontrar diferencias morfológicas (Hoskin *et al.*, 2005). Si existen podrían atribuirse a procesos estocásticos (Cruz-Nicolás *et al.*, 2020).
Peripátrida	Se infiere por una reducción en el tamaño poblacional con una posterior expansión en el nicho ecológico, en un periodo de tiempo corto (Blair *et al.*, 2014; Castellanos-Morales *et al.*, 2016).	Podemos esperar poblaciones pequeñas y con variación reducida en relación con su especie parental (Gavrilets & Hastings, 1996). Acumulación diferencial de diversidad genética parcialmente deletérea.	No es un prerrequisito observar diferencias morfológicas. Si existen pueden ser un subproducto de la deriva génica y pueden expresarse por patrones geográficos entre localidades (Lande, 1991).
Parapátrida	Poco traslape de nichos ecológicos, derivado de diferentes preferencias ambientales (Graham *et al.*, 2004).	Contacto secundario después de divergencia en alopatría (Graham *et al.*, 2004). Es posible observar clinas en las frecuencias génicas dentro de las zonas híbridas. La mutación y la deriva podrían generar aislamiento reproductivo (Gavrilets, 2000).	Se espera que los individuos en ambientes similares presenten características morfológicas similares. No puede descartarse la variación geográfica (Radford, Cousens, & Michael, 2004; Roda *et al.*, 2013).

Cuadro 1. Cambios relevantes que se esperan durante diferentes procesos de especiación en caracteres ecológicos, genéticos y morfológicos. (continuación…).

Especiación	Nicho ecológico (climático)	Genético	Fenotípico
Simpátrida	Posible divergencia de nicho promoviendo especiación (McCormack *et al.*, 2010), incluso puede observarse a través de variables ambientales individuales (Savolainen *et al.*, 2006).	Selección disruptiva que promueve el mantenimiento de polimorfismos, mantenidos por selección contra migrantes e híbridos. Reforzamiento para promover aislamiento reproductivo (Bolnick & Fitzpatrick, 2007).	Se esperan diferencias solo si las características morfológicas son ecológicamente funcionales (Bolnick & Fitzpatrick, 2007).

1.3. La reconstrucción filogenética.

La clasificación cladista o filogenética (Hennig, 1966) considera que un taxón se agrupará con otro según su proximidad a su antepasado común y formando grupos monofiléticos. Bajo esta consideración, el mecanismo de especiación produciría la separación de una especie en dos o más entidades distintas (Mayr, 1982). El propósito de la reconstrucción filogenética es tratar de inferir las relaciones de parentesco o ancestría-descendencia a partir de un conjunto de datos representativos (Martínez-Castilla, 2007). Inicialmente, la filogenética tradicional utilizó datos morfológicos derivados de la medición y cuantificación de propiedades fenotípicas. Después, a partir de la secuenciación Sanger, las secuencias de ADN sirvieron de base para efectuar dichas clasificaciones (Hillis & Moritz, 1990). Uno de los problemas recurrentes que enfrentan este tipo de análisis es la discrepancia entre árboles de genes y árboles de especies; debido a que la historia evolutiva de genes individuales no siempre representa la divergencia de las especies. Estas diferencias se pueden dar por hibridación, paralogía, transferencia genética horizontal, selección

balanceadora o negativa y diferenciación incompleta de linajes (Rosenberg, 2003; Degnan & Rosenberg, 2006, 2009). Limitaciones adicionales se presentan en especies con divergencia reciente o con retención de polimorfismos ancestrales (Aguirre-Planter *et al.*, 2012). En estos casos, los métodos llamados de "nueva generación" (*Next Generation Sequencing; NGS*) han representado, si no la solución, por lo menos una mejora que brinda una mejor resolución. Estos utilizan un muestreo más exhaustivo del genoma, minimizando la discrepancia entre árboles de genes y de especies en clados con reciente divergencia (Hipp *et al.*, 2014; Hamon *et al.*, 2017). Estos métodos incluso han servido para corroborar las propuestas previas con base en caracteres morfológicos (Escudero *et al.*, 2014; Massatti, Reznicek, & Knowles, 2016).

Los árboles obtenidos a partir de la reconstrucción filogenética brindan un significado de la historia evolutiva de un grupo de taxones. Los métodos asumen que las similitudes son el resultado de ancestría compartida. Exceptuando la transferencia genética horizontal y el flujo génico para las cuales usamos redes, existen otros procesos como las fluctuaciones en los tamaños poblacionales, que pueden tener efectos profundos sobre la diversidad y similitud genética de las poblaciones y también pueden sesgar las relaciones filogenéticas (Kalinowski, 2009; Garamszegi & González-Voyer, 2014).

Otro de los aspectos del que dan un indicio las filogenias y que en las últimas décadas ha tenido una mención especial en la literatura es el de las radiaciones adaptativas. Adicionalmente a la filogenia, tienen que estudiarse adaptaciones específicas con métodos comparativos y/o experimentos controlados y su relación con oportunidades ecológicas en el proceso de especiación (Glor, 2010). También se ha propuesto que las radiaciones en periodos cortos de tiempo podrían darse por aislamiento sin especiación ecológica, lo que se ha llamado radiación no adaptativa (Rundell & Price, 2009; Czekanski-Moir & Rundell, 2019).

1.4. De los estudios comparativos a los estudios poblacionales.

La separación y divergencia de linajes puede ser conceptualizada en términos de procesos evolutivos: mutación, selección natural, flujo génico y deriva génica. Las propiedades sobre las que inciden son muy diversas por ejemplo, pueden ser cualitativas, cuantitativas, ventajas selectivas o fisiológicas (De Queiroz, 2005; Figura 2). Si bien es cierto que la especiación y la adaptación a través de selección natural son fundamentales en evolución, son inseparables para comprender los cambios en los organismos vivos. Desde luego, nuestra forma de percibir estos cambios estará sin duda influida por la escala temporal y espacial en que se aborde el problema. Puede considerarse una población a través de un intervalo de tiempo muy prolongado (millones de años), lo que podría llamarse un linaje. Pero también las poblaciones pueden ser consideradas como existentes en un instante en el tiempo; en este caso serían una sección transversal (o un segmento) de un linaje. En esta fase la población o conjunto de poblaciones (metapoblación), pudieron haber adquirido propiedades contingentes (por ejemplo, aislamiento reproductivo, caracteres diagnósticos y monofilia) con respecto a la población ancestral. Durante este proceso, la metapoblación puede pasar a traves de un estado polifilético, a un parafilético y finalmente a monofilia (De Queiroz, 2005). En coníferas, y otras especies con tiempos generacionales largos y bajas tasas de mutación, tenemos también evolución reticulada, producto de una reducida acumulación de nuevas mutaciones después de la divergencia, introgresión y retención de polimorfismos, por lo que dificilmente esperaríamos una representación dicotómica de la filogenia (Liepelt *et al.*, 2010; Bouillé, Senneville, & Bousquet, 2011; Aguirre-Planter *et al.*, 2012; Semerikova & Semerikov, 2014).

En el proceso de divergencia evolutiva hay adquisición de un número diferente de propiedades, no solo la acumulación de cambios genéticos. En este proceso podrían ocurrrir

cambios morfológicos y ecológicos, los cuales no ocurrirán de forma simultánea, por consiguiente el grado de divergencia genética, ecológica y morfológica será variable (De Queiroz, 2005; Phadnis & Orr, 2009; Nosil & Schluter, 2011). Determinar estas propiedades adquiridas, o su ausencia, nos situa en el plano de los estudios poblacionales. Dichos estudios nos permiten determinar divergencias a nivel especies y poblaciones (Figura 2).

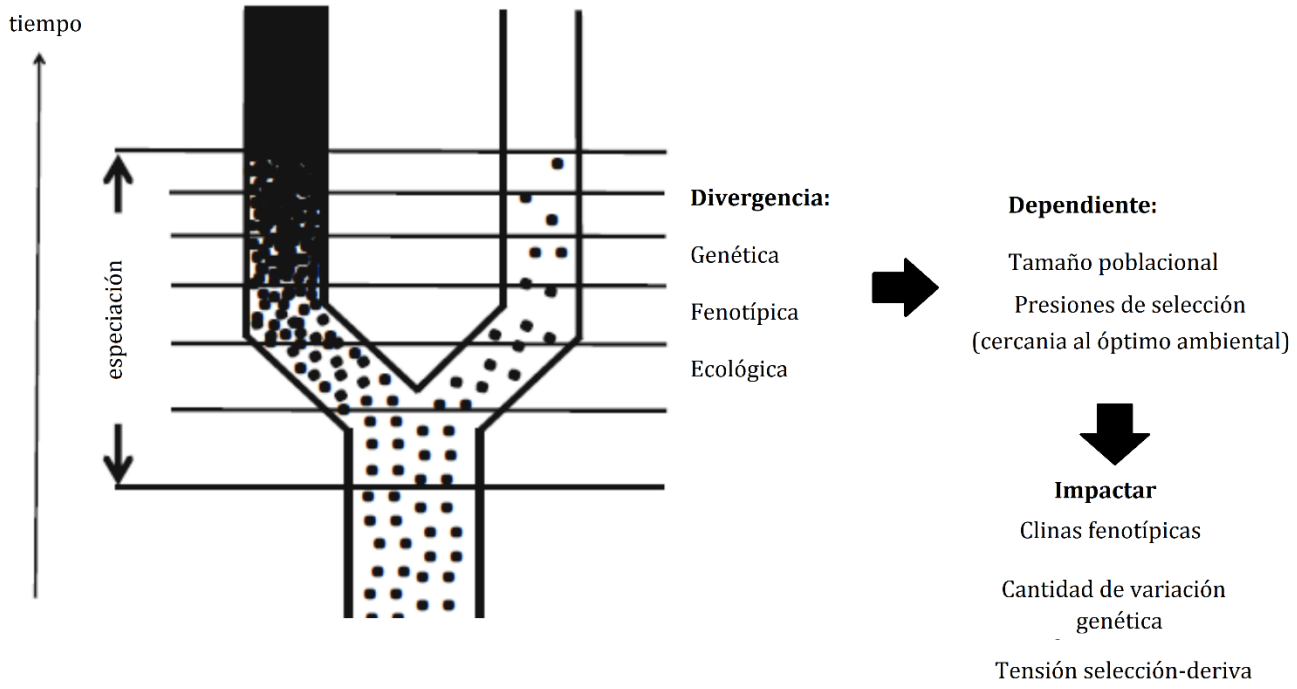


Figura 2. Representación de la divergencia de dos linajes a través del tiempo a partir de un solo linaje. La zona difusa representa distintos subprocesos que ocurren durante la divergencia que puede ser genética, fenotípica y ecológica (Modificada a partir de de Queiroz, (2005) y Zachos (2016). Se postula que la velocidad o el grado de divergencia estará influido por los tamaños poblacionales y las presiones de selección. A su vez esto puede verse reflejado o impactar en la cantidad de variación genética o en clinas fenotípicas.

1.5. La variación genética

La variación genética neutral es el resultado de un balance entre la mutación y la deriva génica (Kimura, 1983) y como tal, está sujeta a reglas estocásticas. La deriva génica, entendida como un cambio aleatorio de frecuencias alélicas en poblaciones de tamaños finitos, es una de las fuerzas evolutivas más importantes que causan cambios en las frecuencias de los alelos, y la eventual pérdida o fijación de estos (Crow & Kimura, 1970; Nielsen & Slatkin, 2013); este hecho podría poner en riesgo la existencia de las poblaciones. Por lo tanto, conocer la variación neutral es indispensable para inferir procesos histórico-demográficos y determinar la vulnerabilidad de las poblaciones.

Por otro lado, la variación adaptativa está sujeta a la interacción entre la selección natural y la deriva génica, y se espera que esta interacción impacte la adecuación de los organismos (Holderegger, Kamm, & Gugerli, 2006). Tanto la deriva génica como la selección natural modifican las frecuencias alélicas y genotípicas; sin embargo, la primera actúa simultáneamente sobre todos los loci del genoma, mientras que la segunda sólo lo hace sobre algunos loci específicos (Whitehead & Crawford, 2006; Steiner, Weber, & Hoekstra, 2007). La selección natural incrementa de una manera más rápida la frecuencia de genes favorables en determinada población, pero es necesario que exista una variación suficiente sobre los cuales la selección pueda actuar (Nei & Kumar, 2000).

La mutación como generadora de variación genética en el corto plazo es una fuerza débil, pero es determinante a largo plazo para la evolución molecular. Una forma de clasificar a las mutaciones puntuales está dada por los cambios que producen en el código genético. Aquellas que no causan un cambio de aminoácido son llamadas sustituciones *sinónimas*; contrariamente, las sustituciones *no sinónimas* originarán cambios de aminoácido. En general, los cambios sinónimos se asumen como neutrales (Nielsen & Slatkin, 2013); este tipo de sustituciones son las más

frecuentes y su acumulación está influenciada por el tamaño efectivo de la población (Nei & Kumar, 2000; Eyre-Walker & Keightley, 2007). Los cambios no sinónimos se asumen como (parcialmente) deletéreos y la selección negativa en estos sitios es más fuerte (Schmidt *et al.*, 2008; Nielsen & Slatkin, 2013). Por lo tanto, se espera que estas variantes sean eliminadas por la selección purificadora (Nei & Kumar, 2000).

Una forma de estimar la eficiencia en la eliminación de variantes deletéreas es a través del cociente entre los cambios sinónimos y no sinónimos (π_N/π_S), donde un valor cercano a cero indica una selección purificadora muy eficiente y valores cercanos o mayores a uno sugieren una selección muy poco eficiente o relajada (McDonald & Kreitman, 1991). Una alta eficiencia de este tipo de selección explica por qué muchas secuencias de proteínas no han cambiado o lo han hecho muy poco durante el proceso evolutivo de las poblaciones y especies (Andolfatto, 2005; Buschiazzo *et al.*, 2012). En relación a la eficacia de la selección, Ohta (1973) sugirió que poblaciones con grandes tamaños efectivos serían más eficientes en eliminar las variaciones deletéreas; se espera entonces que el tamaño efectivo de las poblaciones sea determinante en la acumulación de variación neutral y deletérea en las poblaciones a lo largo de las generaciones.

El tamaño efectivo, N_e, representa una población ideal en la cual todos los organismos tienen la misma probabilidad de dejar progenie; además de cumplir con ciertas premisas como la igualdad en la proporción de sexos, y el apareamiento al azar (Hamilton, 2009; Hedrick, 2011). En términos evolutivos, N_e indica la cantidad de individuos que están contribuyendo a la poza génica en una determinada población y, por lo tanto, nos habla de la capacidad de respuesta de determinadas poblaciones a ciertos cambios ambientales. Sewall Wright (1930) fue el primero en reconocer que una disminución de heterocigosis en función del tamaño poblacional a una proporción de $1/2N$ resulta una reducción casi lineal en la cantidad de variación genética. Simulaciones teóricas

sugieren que un solo periodo con tamaño efectivo poblacional pequeño (como un cuello de botella) puede producir pérdida de heterócigos. De forma natural, una población puede sufrir un cuello de botella cuando un pequeño grupo de emigrantes de una población parental funda una nueva población (Hartl & Clark, 1997). Si esta población crece, sus niveles de heterocigosis muy probablemente incrementarán; aunque los alelos raros de la población parental ya no existirán en esta nueva población. Si la población permanece con un tamaño pequeño, la variación genética seguramente disminuirá o se perderá por completo (Futuyma, 2005).

1.6. Variación morfológica y patrones clinales.

La variación fenotípica entre individuos es esencial para entender los procesos de adaptación, ya que la selección natural tiende a operar sobre esta variación (Shafer & Wolf, 2013; Karhunen *et al.*, 2014). El fenotipo está influenciado por un componente genético, un componente ambiental y la interacción de los mismos (Falconer, 1981). Muchos caracteres ecológicamente importantes exhiben herencia poligénica y requieren análisis genéticos cuantitativos (Podolsky, 2001). El número mínimo de genes para producir cambios notables en un carácter cuantitativo varía de unos cuantos genes a decenas de ellos (Lande, 1981; Anderson, Lee, & Mitchell-Olds, 2011). Por esta razón, se espera que la variación neutral y la adaptativa no se comporten igual, debido a que la selección natural actuará sobre loci específicos y los procesos estocásticos afectarán el genoma completo. En poblaciones con tamaños efectivos pequeños, es más probable que la deriva génica limite los procesos adaptativos debido a la mayor probabilidad de fijación de alelos deletéreos (Futuyma, 2005). Contrariamente, la selección natural será más eficiente en poblaciones con tamaños efectivos más grandes. La probabilidad de fijación de una nueva mutación tiene tres posibilidades: ser fuertemente deletérea [$2Ns << 1$, $\mu \sim 0$], cercana a la neutralidad [$-1 < 2Ns < 1$, μ

$\sim 1/2N$] o fuertemente ventajosa [$2Ns >> 1$, $\mu \sim 2s$]. El destino de una nueva mutación dependerá entonces del tamaño poblacional (N) y del coeficiente de selección (s). Por ejemplo, un alelo que es muy ventajoso en una población con tamaño grande, puede comportarse como uno neutral en una población pequeña (Nielsen & Slatkin, 2013). De esta forma, también se espera que la adaptación local impulse la divergencia fenotípica en poblaciones grandes, aunque esto puede ser contrarrestado por efectos del flujo génico, o de la plasticidad fenotípica (Savolainen, Pyhäjärvi, & Knürr, 2007). Investigar las causas detrás de los patrones de variación fenotípica y de diferenciación genética es un paso crucial para determinar qué papel juegan los procesos neutrales y selectivos detrás de la diferenciación fenotípica medible (Leinonen *et al.*, 2006, 2008).

Haciendo referencia a procesos locales, y específicamente a la variación fenotípica clinal producto de los gradientes geográficos o ambientales y mencionada en el Cuadro 1, estamos interesados en determinar si estos cambios se pueden atribuir a diferencias genéticas o ambientales. Con este propósito se pueden diseñar experimentos de jardín común, donde una vez eliminada la variación ambiental, las diferencias fenotípicas restantes pueden atribuirse a efectos genéticos (Allendorf & Luikart, 2007). En el caso de árboles y otros organismos con largos tiempos generacionales, es frecuente que este tipo de experimentos solo existan para unas pocas especies con importancia económica y en determinadas regiones (Yang, Yeh, & Yanchuk, 1996). En su ausencia, se suele recurrir a otras aproximaciones para discriminar si las diferencias fenotípicas observadas en poblaciones naturales son producto de procesos estocásticos o adaptativos. Una forma de hacerlo es comparando la diferenciación genética entre poblaciones con marcadores moleculares, F_{ST} (Wright, 1965), con una medida para evaluar diferenciación en caracteres cuantitativos, Q_{ST} (Spitze, 1993), o un *proxy* de este, P_{ST} (Storz, 2002). La idea es que en un paisaje heterogéneo, donde los individuos experimentan presiones selectivas diferentes, podemos suponer que las diferencias a nivel molecular y fenotípico están dadas por la deriva y la selección

local divergente (Kremer & Le Corre, 2012). Si $Q_{ST} > F_{ST}$ para un carácter determinado, habría evidencia de que el carácter ha experimentado más diversificación de la esperada de acuerdo con un modelo neutral, sugiriendo selección direccional. Si $Q_{ST} < F_{ST}$, habría evidencia de que los caracteres están bajo selección estabilizadora, dado que los mismos fenotipos son favorecidos en diferentes poblaciones. Finalmente, si $Q_{ST} = F_{ST}$, la cantidad de diferenciación en el carácter cuantitativo es similar a la esperada en un modelo neutro (Allendorf & Luikart, 2007; Whitlock, 2008).

1.7. El nicho ecológico y la diversidad genética.

Debido al impacto del tamaño efectivo en el curso evolutivo de las poblaciones, se han hecho intentos en las últimas décadas por relacionar algunos parámetros con los tamaños poblacionales; entre ellos mencionaré dos que se abordan en este trabajo: el *tamaño del área* de distribución (en términos de superficie) y la distancia al *centroide del nicho ecológico*. Por ejemplo, se espera que una mayor superficie de distribución se traduzca en mayor número de individuos y tamaño efectivo, y por lo tanto en mayor diversidad genética, y a la inversa (Frankham, 1996; Leimu & Fischer, 2008).

El segundo parámetro se ha popularizado más recientemente con el advenimiento de algoritmos para modelar el nicho ecológico. Una de las definiciones más populares de nicho ecológico, es aquella que considera un hipervolumen *n*-dimensional compuesto por un espacio abstracto de ambientes potenciales en los que un organismo puede vivir (Hutchinson, 1957). A partir de estas ideas, y para efectos prácticos, se ha propuesto la especificidad de nichos *grinelianos*, definidos por variables escenopoéticas. Es decir, las condiciones ambientales que no

se agotan por el uso de los individuos (ej: temperatura y precipitación), y que pueden definirse a escalas más gruesas (Soberón, 2007a). Debido a esta estructura interna, se espera que en el centroide del nicho ecológico se den condiciones óptimas para la sobrevivencia y reproducción de las especies, lo que traería como consecuencia una mayor abundancia de individuos y, por ende, mayor diversidad genética (Maguire, 1973; Martínez-Meyer *et al.*, 2013; Lira-Noriega & Manthey, 2014). Si bien es cierto que hay evidencia empírica sobre estas ideas, esta relación se puede ver ensombrecida total o parcialmente por causas como la centralidad histórica de las poblaciones, la ausencia de equilibrio demográfico-poblacional, ciertas características de las historias de vida y la capacidad de dispersión de las especies (Duncan *et al.*, 2015; Pironon *et al.*, 2015; Osorio Olvera, Falconi, & Soberón, 2016).

Hasta donde sabemos, este tipo de aproximaciones se han hecho con variación neutral es decir, aquella que no está relacionada con la adecuación de los individuos (Lira-Noriega & Manthey, 2014). También la centralidad del nicho ha permitido inferir señales de adaptación local en el genoma mediante asociaciones estadísticas, es decir, se ha observado una relación positiva entre la distancia al centroide del nicho y el número de *SNPs* (*Single Nucleotide Polymorphism*, por sus siglas en inglés) candidatos a selección (Aguirre-Liguori *et al.*, 2017). En el presente trabajo hacemos una extensión entre centralidad y la teoría casi neutral, es decir tratamos de relacionar la centralidad del nicho con la acumulación de mutaciones deletéreas en poblaciones naturales. Además, verificamos si existe un acoplamiento entre la divergencia genética y la diferenciación de nichos ecológicos. El entender estas relaciones (genético-ambiental), junto con la comparación de nichos ecológicos y variables ambientales específicas, nos ayudará a entender el papel de los procesos ecológicos en la divergencia de especies/poblaciones e inferir cómo podría verse afectada la biodiversidad con los cambios globales actuales.

1.8. El género Abies como modelo de estudio.

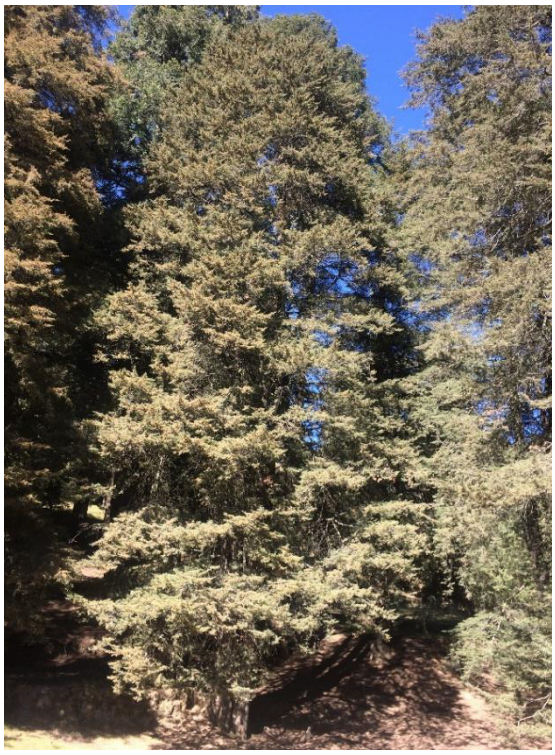
Los árboles del género *Abies* Mill. (Pinaceae), llamados comúnmente oyameles, abetos o pinabetes, son de afinidad templada y se encuentran distribuidos en Eurasia y Norteamérica. Su límite sur se encuentra en las zonas montañosas de Mesoamérica (Farjon, 2010). En nuestro país, este género posee aproximadamente ocho especies y alrededor de ocho variedades (Gernandt & Pérez-De La Rosa, 2014). Aunque abarca un área relativamente pequeña del territorio nacional de aproximadamente 149, 458 ha. (SEMARNAT, 2010a). *Abies* ocupa el cuarto lugar en producción maderable del país (SEMARNAT, 2016). Los bosques de *Abies* albergan una gran cantidad de especies de flora y fauna (incluida la mariposa monarca *Danaus plexippus*), además ayudan en la recarga y conservación de mantos acuíferos, la retención de suelo y la captura de carbono. Aunque a la fecha únicamente *A. flinckii, A. hickelii, A. guatemalensis, A. vejarii* y *A. concolor* se encuentran bajo alguna categoría de riesgo (SEMARNAT, 2010b), es conveniente notar que en la zona central de su distribución algunas poblaciones naturales de *A. religiosa* e incluso una localidad de *A. hidalgensis*, se encuentran bajo un elevado impacto antropogénico (principalmente cambio de uso de suelo, tala clandestina y lluvia ácida), lo que podría traducirse en cambio de categoría en un futuro cercano.

Los oyameles son árboles corpulentos, siempre verdes, resinosos, de copa simétrica y aguda, con hojas lineares y perennes; las estructuras fértiles están arregladas en conos polínicos simples los cuales se producen en la parte inferior de las ramillas y los conos ovulados compuestos, que se encuentran en las ramas más altas (Figura 3); estos son erguidos, sésiles, agrupados y bastante resinosos; de forma cilíndrica u oval (Martínez, 1948; Hernández, 1985). Los oyameles pueden empezar a producir estructuras reproductivas desde los 20 años; sin embargo, el pico

reproductivo se alcanza alrededor de los 50 (Avila-Bello, López-Mata, & Mandujano, 2015). Aunque son árboles monoicos, los conos polínicos y ovulados se encuentran separados espacialmente, favoreciendo la dispersión y polinización anemófila, con lo que se incrementa la tasa de polinización cruzada (Eckenwalder, 2009).

a)

c)



b)

d)

Figura 3. Imágenes de dos especies de oyamel del centro de México. Árbol adulto (a) y una población natural de *Abies flinckii* en el Nevado de Colima (b). Árbol adulto (c) y un cono ovulado de *Abies religiosa* (d) en el ejido Río Frío, Ixtapaluca, Edo. Méx. Las imágenes *a* y *b* fueron proporcionadas por G. I. Gilés-Pérez.

En México, los bosques de oyamel se desarrollan en las Sierras Madre Oriental, Occidental y del Sur, así como en la Faja Transversal Mexicana (Figura 4); generalmente entre los 2000 y 3600 msnm (Hernández, 1985). A nivel local, el área de mayor distribución del género se encuentra en este último macizo montañoso. En el resto su distribución se encuentran de forma más aislada, formando pequeñas poblaciones (Rzedowski, 2006).

En términos genéticos, su número cromosómico es 2n=24 (Mergen & Bubley, 1963). Aunque los tamaños de genoma en estas especies suelen ser gigantescos, y no se tiene el dato específico de especies mesoamericanas, en *Abies alba* se ha estimado en alrededor de 18 gigabases (Mosca *et al.*, 2019), lo que representa una longitud seis veces mayor a la del genoma humano (3.2 Gb). Las grandes dimensiones del genoma en este tipo de especies se han atribuido a un elevado número de secuencias repetitivas de ADN, que se han acumulado durante su historia evolutiva; estos elementos repetitivos parecen estar originados por transposones (Nystedt *et al.*, 2013).

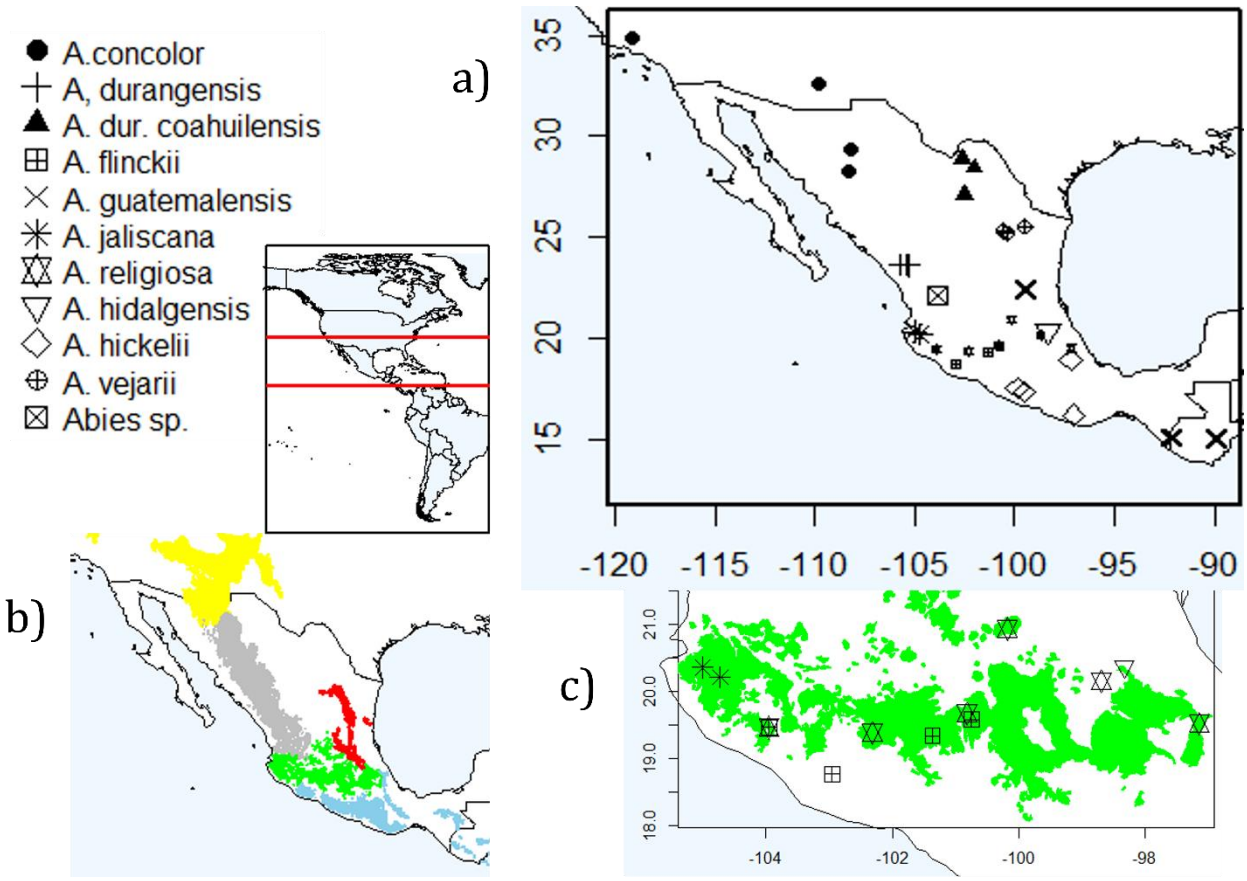


Figura 4. Localidades del género *Abies* muestreadas en el presente trabajo en los límites con E.U.A. y Guatemala (a). Principales sistemas montañosos de México en amarillo norte de México, en gris Sierra Madre Occidental, en rojo Sierra Madre Oriental, en verde Faja Volcánica Trans Mexicana y en azul Sierra Madre del Sur (b). Muestras de *Abies* en la Faja Volcánica Transmexicana (c).

El género hermano de *Abies* es *Keteleeria* y su divergencia se ha fechado hacia el final del Cretácico (92-102 millones de años); la diversificación del grupo corona de los *Abies* modernos se ha estimado en un intervalo que fluctúa entre los 13.5 y los 20.1 millones de años (Semerikova *et al.*, 2018). Aunque las clasificaciones filogenéticas a nivel local suelen ser controversiales debido a la evolución reticulada (Liepelt *et al.*, 2010; Semerikova & Semerikov, 2014), el número de especies en el mundo se encuentra alrededor de 40 (Eckenwalder, 2009). Específicamente para el caso de las especies mesoamericanas, hasta el momento se han descrito entre 6 a 10 dependiendo del autor (Cuadro 2).

Cuadro 2. Propuestas taxonómicas para el género *Abies* en México (Liu 1971; Farjon & Rushforth, 1989).

Liu	**Farjon y Rushforth**
Sect. Oyamel	*Sect. Oiamel*
A. religiosa, A. hickelii	*A. religiosa, A. vejarii, A. hickelii*
Sect. Grandis	*Sect. Grandis*
A. durangensis, A. guatemalensis, A. concolor	*A. concolor, A. durangensis, A. guatemalensis, A. flinckii*
Sect. *Vejarianae*	
A. vejarii	

Estas clasificaciones sin embargo no han considerado a *Abies hidalgensis* (Debreczy & Rácz, 1995) ni a *A. jaliscana* (Vázquez-García *et al.*, 2014). *A. mexicana* (Martínez, 1948) se considera actualmente como un sinónimo de *A. vejarii*. Además, los últimos estudios que han analizado las relaciones filogenéticas del género consideran a las secciones *Grandis* y *Oiamel*, donde se encuentran las especies Mesoamericanas, como un solo grupo (Aguirre-Planter *et al.*, 2012; Xiang *et al.*, 2018).

La historia del género es complicada, el registro fósil es muy escaso, pero hay evidencia de polen en el estado de Veracruz para el Plioceno medio, por lo que se sugiere que los oyameles ya estaban presentes en el territorio de lo que hoy es México hace unos cinco millones de años (Graham, 1999). Aunque desde el punto de vista evolutivo este tiempo es breve, las relaciones filogenéticas han mostrado que los oyameles Mesoamericanos consistentemente forman un clado monofilético dentro del género (Aguirre-Planter *et al.*, 2012). Además, de acuerdo con el Cuadro 2 podemos esperar ya algún grado de divergencia/especiación, puesto que desde el punto de vista

taxonómico existen diferencias fenotípicas medibles. Si se considera que los hábitats con menor altitud y características ambientales distintas han actuado como barreras para la dispersión de estos organismos que se distribuyen principalmente en zonas más altas (Lozano-García *et al.*, 1993; Caballero *et al.*, 2010; Ferrari *et al.*, 2012), podemos proponer a la especiación alopátrida como modelo de especiación más plausible, ya que bajo este modelo se requiere aislamiento y tiempo (Coyne & Orr, 2004). Estos elementos estarían operando sobre la mayor parte de la distribución del género *Abies* en México.

A esta hipótesis habría que agregar la gran heterogeneidad topográfica y microambiental donde se desarrollan estas especies (Rzedowski, 2006). Prueba de ello son las diferencias en patrones fenológicos y la asociación con algunas especies del bosque mesófilo de montaña, especialmente en algunas poblaciones del extremo oeste de la distribución en el centro de México (Mantilla-Blandón, 2006; Cuevas-Guzmán *et al.*, 2011; Vázquez-García *et al.*, 2014). Esto implica que, si bien es cierto que el aislamiento ha sido un impulso para la diferenciación, por lo menos a nivel genético, las condiciones locales también podrían tener su contribución particular al menos en ciertas regiones geográficas. De ahí que las zonas montañosas se hayan propuesto en reiteradas ocasiones como una fuente importante de endemismos (Gámez *et al.*, 2012; Mastretta-Yanes *et al.*, 2018). Lo anterior convierte a los organismos de zonas montañosas en laboratorios "naturales" para el estudio de la especiación. Específicamente en el centro de México, que es donde se encuentra gran parte de la distribución del género. Los estudios pioneros con marcadores nucleares y de cloroplasto sugirieron la presencia de dos especies divergentes (*A. flinckii* y *A. religiosa*; Aguirre Planter, Furnier, & Eguiarte, 2000; Jaramillo-Correa *et al.*, 2008). A estas dos especies la evidencia morfológica agrega una especie más (*A. jaliscana*; Vázquez-García *et al.*, 2014). Pero este no es el único caso; en el sur de México, para *A. hickelii*, se han propuesto un par de variedades, lo mismo para el norte de México, en *A. vejarii* (Martínez, 1948; Gernandt & Pérez-

De La Rosa, 2014). Estos antecedentes nos hablan de la complejidad de los procesos locales y que operan de manera simultánea a la diferenciación a escalas mayores. Es decir, no es suficiente con proponer un modelo de especiación, es probable que haya que proponer al menos dos de ellos. El presente trabajo intenta llenar estos vacíos, analizando diferencias morfológicas y genéticas en la zona de contacto de dos especies del centro de México.

A diferencia de las islas oceánicas, donde la teoría biogeográfica de islas sugiere que la diversidad de especies es producto de un balance entre colonización y extinción (Vellend, 2003), las *sky islands* se distinguen por su historia climática y dinámica de dispersión. La separación o interconexión entre "islas" dependerá de la proximidad existente entre montañas, las cuáles servirán como puntos de unión entre islas, de tal suerte que, podríamos observar poblaciones de oyamel con distribuciones relativamente continuas y otras con mayor fragmentación. Esto nos lleva a proponer que en determinadas regiones donde el aislamiento se ha prolongado por más tiempo, se espere mayor divergencia con respecto a las poblaciones donde ha existido mayor contacto. Los estudios genéticos del género *Abies* en México con distintos marcadores genéticos (isoenzimas, microsatélites nucleares y de cloroplasto) señalan que la deriva génica ha tenido un papel importante en la diferenciación de muchas poblaciones, sobre todo aquellas más aisladas. Así mismo, se ha inferido una limitada capacidad de dispersión del polen (Aguirre-Planter *et al.*, 2000; Jaramillo-Correa *et al.*, 2008). Estos hallazgos refuerzan la idea de que en las islas en el cielo, la combinación del efecto del aislamiento con el efecto de los gradientes altitudinales promueve la divergencia por deriva génica y selección natural (McCormack *et al.*, 2009).

El análisis de la historia demográfica de un par de especies del centro de México (*A. flinckii* y *A. religiosa*) a partir de secuencias codificantes (*Porin Mip1, 1-6 xilosiltransferasa, Fructosa 1,6 difosfato aldolasa, Heat shock, Lhca4, ArMybVI, ArMybIX* y *ArMybSTR*) sitúa su divergencia a finales

del Plioceno (Giles-Pérez, 2017), para estructurarse genéticamente durante el Pleistoceno a través de una colonización este-oeste en la parte central de México (Múgica-Gallart, 2013; Giles-Pérez, 2017). La estructura genética sugiere que al menos dentro de un cierto grupo de poblaciones el flujo génico es restringido por lo que pudieran existir diferencias genéticas y ecológicas. El explorar correlaciones entre procesos genéticos y ecológicos nos ayudará a entender los procesos de diferenciación entre especies. En este sentido, si los nichos de las especies son similares, como se espera en general para las especies de afinidad templada, incluso aquellas separadas geográficamente (Alba-Sánchez *et al.*, 2010; Peterson, 2011), se esperaría una especiación alopátrida para el género *Abies* en México.

A partir de las ideas esbozadas anteriormente, en esta tesis nos hemos propuesto abordar inicialmente una perspectiva macroevolutiva, para posteriormente analizar la diferenciación morfológica, genética y ecológica a nivel poblacional para el género *Abies* en México. El presente escrito se ha estructurado en tres partes. En el capítulo II (este capítulo se está estructurando de forma tal que en unos meses sea sometido a revisión para su publicación) hemos inferido la independencia de linajes del género *Abies* en Mesoamérica, a través de una filogenia elaborada con datos de Genotipado por secuenciación (*Genotyping-by-sequencing, GBS*). A partir de las relaciones filogenéticas, proponemos los posibles mecanismos de especiación/diferenciación. Con estas relaciones en mente, evaluamos en los capítulos siguientes algunos aspectos de divergencia a nivel genético, morfológico y ecológico (Cuadro 1 y Figura 2). Para ello tomamos como modelo de estudio un par de especies del centro de México, *Abies flinckii* y *Abies religiosa.* En el capítulo III, comparamos la diferenciación morfológica y genética. Inicialmente, determinamos si la divergencia fenotípica observada en forma de clinas obedece a un patrón de aislamiento por distancia o de aislamiento por adaptación a través de comparaciones P_{ST}-F_{ST}. Los resultados de este capítulo fueron publicados en el *Botanical Journal of the Linnean Society*. Posteriormente, en el

capítulo IV, analizamos estas mismas especies a la luz de las condiciones ambientales que han experimentado desde el Último Máximo Glacial y sus posibles consecuencias sobre la eficacia de la selección purificadora en los patrones de variación neutral. Para esto, estimamos el óptimo ambiental a partir del centroide del nicho ecológico (actual e histórico) y lo comparamos con la cantidad de diversidad genética. Este capítulo ya se sometió a revisión en una revista científica internacional. Finalmente, en el capítulo V se hace una discusión general a partir de los conceptos delineados en esta introducción y se presentan las conclusiones del presente trabajo.

CAPITULO II: Unravelling evolutionary processes driving the diversification of a temperate conifer lineage after its migration into the tropics

RESUMEN

Un hecho importante para entender procesos evolutivos en especies de divergencia reciente es la formulación de hipótesis filogenéticas. Específicamente, hablando de especies con largos ciclos generacionales, grandes tamaños efectivos, elevado flujo génico y retención de polimorfismos ancestrales, como las coníferas, establecer las relaciones filogenéticas suele ser complicado. Para este propósito se generaron datos de genotipado por secuenciación (GBS, por sus siglas en inglés) en 45 individuos de oyamel (*Abies*, Pinaceae) distribuidos desde el sur de Estados Unidos hasta Guatemala, con nueve especies (de acuerdo con su descripción morfológica) y 88, 464 polimorfismos de nucleótido único (*SNPs*, por sus siglas en inglés). Para inferir el árbol de especies se utilizaron Análisis de Cuartetos, con SVDquartets y de Máxima Verosimilitud, con RAxML. Con la finalidad de observar la reticulación entre especies y detectar patrones de aislamiento por distancia, se construyó una red a partir de las distancias genéticas entre individuos, se realizó un análisis de admixture y un análisis de componentes principales. Se encontraron nueve linajes independientes estructurados geográficamente, que corresponden a los siguientes taxa: *Abies concolor,* al sur de Estados Unidos y norte de México; en la Sierra Madre Occidental *A. durangensis*; en la Sierra Madre Oriental *A. durangensis* var. *coahuilensis* y *A. vejarii*; en la Faja Volcánica Transmexicana *A. religiosa*, *A. flinckii* y *A. jaliscana*; en la Sierra Madre del Sur *A. hickelii* y *A. guatemalensis*. Se encontró que *A. hidalgensis* y una muestra de *A. guatemalensis* del estado de San Luis Potosí no mostraron una relación clara, por lo que sugerimos realizar estudios más exhaustivos en esta región para clarificar sus relaciones. A medida que se avanzó de norte a sur se observó mayor reticulación y ancestría mezclada, con un patrón de aislamiento por distancia. Se propone que las zonas montañosas han funcionado como refugios para estas especies y poblaciones, permitiendo el proceso de especiación alopátrida. Finalmente, se propone un modelo de evolución basado en una radiación alopátrica no adaptativa.

Unravelling evolutionary processes driving the diversification of a temperate conifer lineage after its migration into the tropics

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ABSTRACT

An important issue for understanding important evolutionary processes at closely related species of recent divergence is to reconstruct phylogenetic relationships. This is complicated in species with long live cycles, weak reproductive isolation mechanisms and ancestral large effective population sizes, such as conifers. In this study we elucidate the phylogenetic relationships of endemic Mesoamerican firs (*Abies*, Pinaceae) which are distributed discontinuously in temperate and subtropical montane forest in Mesoamerica. We used Genotyping by Sequencing (GBS) in 45 individuals in nine species with 88, 464 SNPs from Southern United States to Guatemala and Maximum Likelihood and quartet analyses to infer phylogenetic relationships. We established phylogenetic relationships for Mesoamerican firs, the phylogeny showed a strong geographic structure from North to South. In this way, we proposed the allopatric processes including reticulation and an isolation by distance framework. In general, phylogenetic clades fit to geographic regions of Mesoamerica. We found nine lineages confined to principal mountain systems 1) *Abies concolor* to the South of the United States and North of México, 2) *A. durangensis* in the Sierra Madre Occidental, 3) *A. durangensis* var. *coahuilensis* and *A. vejarii* in the Sierra Madre Oriental, 4) *A. religiosa*, *A. flinckii* and *A. jaliscana* in the Trans Mexican Volcanic Belt, 5) *A. hickelii* and *A. guatemalensis* in the Sierra Madre del Sur. We proposed a model of evolution based on non-adaptive allopatric processes by isolation in multiple mountain refuges have played an important role to the speciation and endemism in this biodiverse region.

Keywords

Abies, genotyping-by-sequencing, Mexico, tropical mountains, non-adaptive radiation, allopatric speciation.

1. Introduction

Understanding how phylogenetic relationships correlate with the geographic and ecological distribution of species and unravelling the evolutionary forces underneath species divergence is one of the main challenges in evolutionary biology. Resolving phylogenetic relationships among long-lived or slowly evolving taxa remains a major task, as phylogenies are often blurred by inconsistencies derived from incomplete lineage sorting and reticulate evolution. Species with reticulated histories do not fit the expected bifurcating model in which sister taxa show similar divergence rates from an outgroup (Semerikova & Semerikov, 2014). Among the possible causes to explain the lack of bifurcation we might cite, retention of ancestral polymorphisms (Liepelt *et al.*, 2010; Aguirre-Planter *et al.*, 2012), a reduced accumulation of new mutations after divergence (Bouillé *et al.*, 2011), introgressive hybridization following secondary contact (Liepelt *et al.*, 2010) and/or local adaptation and diversification along a small number of environmental or geographic axes, which may be reflected in only few genes (Rundell & Price, 2009). Such processes tend to arise in dynamic regions characterized by geological or climatic instability, in which recurrent landscape changes allow for rapid population isolation and secondary contact (Czekanski-Moir & Rundell, 2019).

Trees evolving in the tropics embody an evolutionary dichotomy, which may engender several of the processes driving reticulate evolution. On one hand, they are out-crossing long-lived taxa, with a predominant position in the ecosystem, which favors evolutionary stasis and diminishes diversification rates (Crisp & Cook, 2011). On the other hand, the tropics harbor extremely species-rich habitats, where countless biotic interactions prompt disruptive selection and species divergence (Haine, Martin, & Cook, 2006; Levin, 2006). Such a dichotomy is particularly compelling for conifers that have migrated from temperate zones into tropical regions. Conifers are characterized by a slow accumulation of new mutations, a high retention of ancestral polymorphisms, and large interspecific genetic exchanges (Semerikova & Semerikov, 2014); all of them favored by large ancestral effective population sizes and vast genomes enriched with duplicated genes that are preserved by low recombination rates (Jaramillo-

Correa, Verdú, & González-Martínez, 2010). Nevertheless, except for the lowland forests in South America, central Africa and India, conifers have successfully colonized many tropical environments, sometimes forming biodiversity hotspots in places like México, Northern Central America and Southern China (Sundaram *et al.*, 2019).

Several evolutionary outcomes can be expected when a conifer lineage moves into a tropical environment. For instance, given the typically low accumulation rate of new mutations in conifers, such a lineage might experience little to no diversification, and simply reflect an isolation by distance pattern (Mimura & Aitken, 2007). Under such a framework, any potential morphological variation between populations could be the result of phenotypic plasticity or non-adaptive forces (Cruz-Nicolás *et al.*, 2020). On the other hand, such a lineage may undergo rapid diversification (i.e. species radiation), prompted by population isolation and fueled by multiple colonization or hybridization events (Kozak, Weisrock, & Larson, 2006).

The implication and timing of selective forces during a species radiation may condition whether the resulting taxa might have diversified, displaced or conserved their ecological niches (Czekanski-Moir & Rundell, 2019). For instance, if selection operates from the very beginning, and drives species radiation, the result would be a collection of closely related taxa inhabiting different environments, which may or not over overlap geographically (Losos *et al.*, 2006). Under such circumstances, a strong selection against hybrids should help preserve the species' identity (Van Der Sluijs *et al.*, 2008). On the other hand, the outcome of a non-adaptive radiation would be a variety of taxa occupying very similar environments with little to no geographic overlap, and limited reproductive isolation (Rundell & Price, 2009; Czekanski-Moir & Rundell, 2019). In some particular cases, divergent selection might act upon the resulting taxa of such a radiation, which would generate character or niche displacement in one or more species, and produce a tension hybrid zone in the regions of secondary contact (Rundell & Price, 2009; Czekanski-Moir & Rundell, 2019).

The mountain forests of Mesoamerica provide an ideal setting for surveying conifer evolution in the tropics and testing how it accommodates to the predictions above. Mesoamerican montane forests are distributed along a highly fragmented 'sky-island' system with mostly homogeneous climatic conditions, where niche conservation seems the rule (Martínez-Méndez *et al.*, 2016). Such a system was formed during various geologically active periods that spanned the last 60 Ma (Ferrari *et al.*, 2012). More recently, the climatic oscillations of the Pleistocene further provided the ideal framework for conifer populations expansion (during the cold periods) and contraction (in the interglacials), which respectively prompted secondary contact and population isolation (Mastretta-Yanes *et al.*, 2015).

Firs (*Abies* Miller) are a predominant component of Mesoamerican mountain forests, particularly at elevations between 2,500 and 4,000 m a.s.l. (Martínez, 1948; Hernández, 1985; Rzedowski, 2006). According to the palynological record and past phylogenetic studies (Graham, 1976, 1999; Aguirre-Planter *et al.*, 2012; Xiang *et al.*, 2015; Semerikova *et al.*, 2018), firs colonized Mesoamerica from western North America during the Pliocene (5 Ma), and then underwent what it seems a rapid diversification. Depending on the authority, between six and ten fir species can be now recognized in these forest (Martínez, 1948; Farjon & Rushforth, 1989; Debreczy & Rácz, 1995; Eckenwalder, 2009; Vázquez-García *et al.*, 2014). Such a diversification may fit several of the previously discussed outcomes. Indeed, all phylogenetic attempts to-date have failed to disentangle species relationships (or to recover species at all), likely because of reticulated evolution or little to no-divergence (Aguirre-Planter *et al.*, 2012).

Here, we used a large SNP dataset derived from genotyping-by-sequencing to investigate the phylogenetic relationships of Mexican firs and test the above-discussed evolutionary outcomes. More specifically, we asked: (*i*) do phylogenetic clades (if present) fit better the taxonomic descriptions of firs or the geographic regions of Mesoamerica; (*ii*) can we detect more than one fir expansion wave into Mesoamerica; (iii) is there any evidence for a species radiation. The potential answers to these questions are discussed in the light of the conservation/taxonomic status of Mesoamerican firs, and from an evolutionary point of view.

2. Materials and Methods

2.1. Sampling, DNA extraction and next-generation sequencing

We initially sampled foliage from 45 individuals previously collected in 33 natural fir populations (1-2 indivs/pop) along a latitudinal gradient between the southwestern USA and Guatemala (Fig. 1).

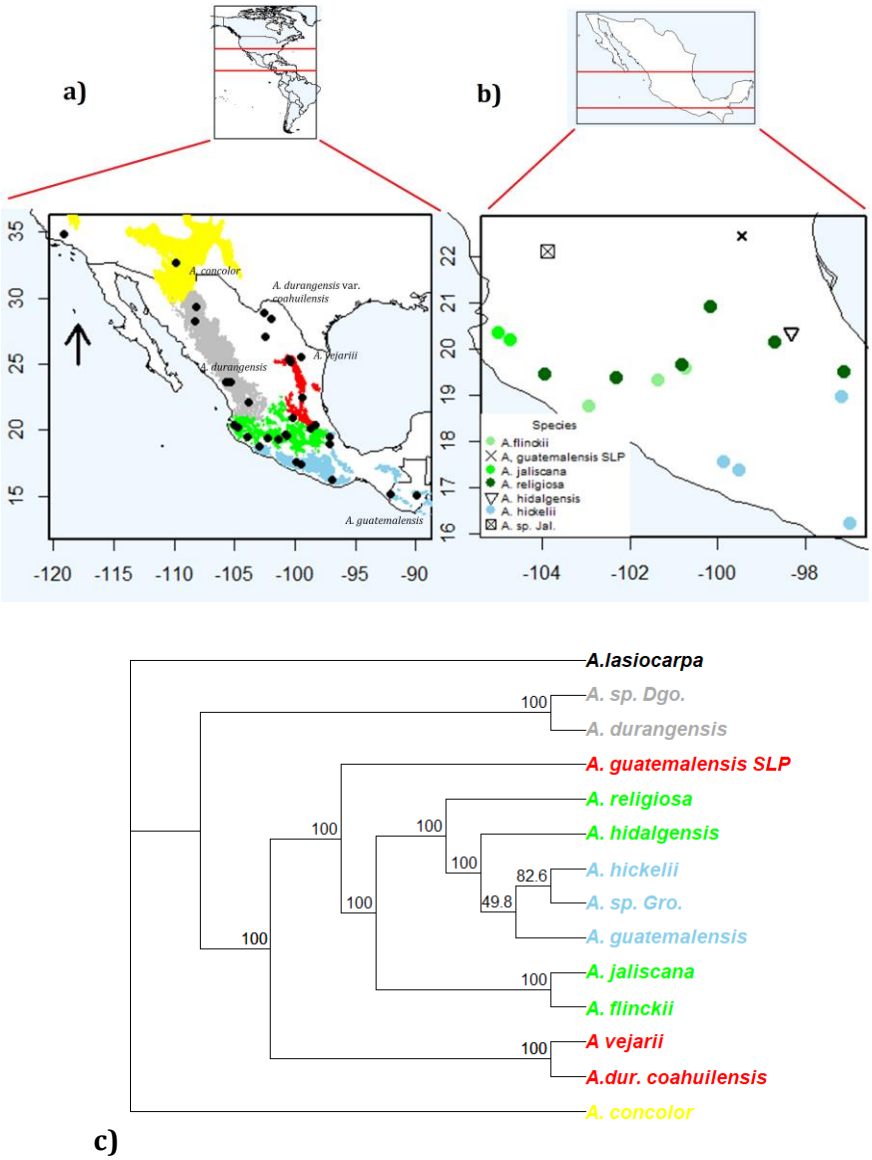

Fig. 1. (a) The 45 samples of the genus *Abies* (Pinaceae) from USA, Mexico and Guatemala included in this study. In yellow North region, in gray Sierra Madre Occidental, in red Sierra Madre Oriental, in green Transmexican Volcanic Belt, in blue Sierra Madre del Sur. North labels indicating species *Abies concolor, A. durangensis, A. durangensis* var. *coahuilensis* and the South *A. guatemalensis*. (b) Species complex in Mexico. (c) Majority-rule consensus of species tree obtained with SVDquartets analysis derived from 88,464 SNPs with values of support.

This gradient covered most of the fir natural range in the region and aimed accounting for both shared and taxa-specific polymorphisms. We assigned individuals to taxonomic species following the specifications of herbarium´s vouchers and Aguirre-Planter et al. (2012), excepting for some 'problematic' samples two from Durango (Northwestern Mexico), one from San Luis Potosí (Northeastern Mexico), one from Mezquitic in Jalisco state and two from Guerrero (Southern Mexico), which exhibited intermediate characters or were assigned to a species, but collected outside its known natural range; we referred them simply as *Abies* sp. To root our networks and phylogenies, we used and individual of *A. lasiocarpa* as outgroup (Table 1). This species, from section *Balsamea*, represents a divergence of ~20 Ma from our clade of interest (Sects. *Grandis* and *Oiamel*) according to previous phylogenetic calibrations (Aguirre-Planter *et al.*, 2012; Semerikova *et al.*, 2018).

We extracted DNA from frozen needles with either a DNeasy Plant Mini Kit (Qiagen), or a modified CTAB protocol (Vázquez-Lobo, 1996). In both cases, DNA was quantified with a Qubit™ V 3.0 fluorometer (Thermo Fisher Scientific). We double-digested individual DNA samples (200np) with enzymes *Msp*I (C|CGG) and *Pst*I (TGCA|G), and ligated unique barcodes and adapters to digestion products. Library preparation was done following the procedure described by Poland *et al.*, (2012), with the exception that a blue Pippin (SAGE sciences) was used to size libraries before PCR amplification. Single end sequencing (100x1) was done in an Illumina Hiseq 2500 lane among with other firs samples for a different study (totalling 368 individually barcodes samples). Both library preparation and sequencing were performed at the Institute of Integrative Biology and Systems at Université Laval (http://www.ibis.ulaval.ca/en/services-2/genomic-analysis-platform/).

Table 1. *Abies* spp. sampled in Mexico, Guatemala and USA. IDs = identification code, taxonomic identification, locality and longitude and latitude.

Phylogeny IDs	Taxa	Locality	Latitude	Longitude
AcCh01	*Abies concolor*	Pinos Altos, Chih.	28.2533	-108.3127
AcCh02	*Abies concolor*	Arroyo de las Garrochas, Chih.	29.3200	-108.2000
AcCh03	*Abies concolor*	Arroyo de las Garrochas, Chih.	29.3200	-108.2000
AcUs01	*Abies concolor*	Mount Graham Arizona, USA	32.6300	-109.8289
AcUs02	*Abies concolor*	Los Padres National Forest California. USA	34.8167	-119.1300
AuDg01	*Abies durangensis*	Mpio. Pueblo Nuevo Sta. Barbara, Dgo.	23.6525	-105.4358
AuDg02	*Abies durangensis*	Parque Nal. Puerto de los Ángeles, Dgo.	23.6072	-105.3572
AdCo01	*Abies durangensis* var. *coahuilensis*	Sierra la Madera Cuatro Ciénegas, Coah.	27.1100	-102.5100
AdCo02	*Abies durangensis* var. *coahuilensis*	Sierra la Madera Cuatro Ciénegas, Coah.	27.1100	-102.5100
AdCo03	*Abies durangensis* var. *coahuilensis*	Rincón de María, Muzquiz, Coah.	28.4500	-102.0300
AdCo04	*Abies durangensis* var. *coahuilensis*	Sierra del Carmen, Coah.	28.8700	-102.5900
AfJa2004	*Abies flinckii*	El Terrero, Col.	19.4640	-103.9470
AfMi1404	*Abies flinckii*	El Caracol, Mich.	19.5830	-100.7500
AfMi1606	*Abies flinckii*	Dos Aguas, Mich.	18.7670	-102.9500
AfMi1709	*Abies flinckii*	Los Sauces, Mich.	19.3410	-101.3650
AjJa1807	*Abies flinckii*	Cumbre de Guadalupe, Jal.	20.2160	-104.7210
AjJa1901	*Abies flinckii*	El Cuale, Jal.	20.3580	-104.9980
AgCp01	*Abies guatemalensis*	Volcán Tacaná, Chis.	15.1160	-92.1160
AgCp02	*Abies guatemalensis*	Volcán Tacanà, Chis.	15.1250	-92.1227
AgCp03	*Abies guatemalensis*	El Porvenir, Chis.	15.1160	-92.1160
AgGu01	*Abies guatemalensis*	Cerro Pinalon, Guatemala	15.0810	-89.9213
AgSp01	*Abies guatemalensis*	Sierra el Pino, S.L.P.	22.4539	-99.4562
AhOa04	*Abies hickelii*	San Jerónimo Coatlán, Oax.	16.2300	-97.0000
AhOa05	*Abies hickelii*	San Jerónimo Coatlán, Oax.	16.2300	-97.0000
AhOa06	*Abies hickelii*	San Jerónimo Coatlán, Oax.	16.2300	-97.0000
AhOa07	*Abies hickelii*	San Jerónimo Coatlán, Oax.	16.2300	-97.0000
AhVe01	*Abies hickelii*	Xometla, Ver.	18.9666	-97.2000
AlUs01	*Abies lasiocarpa*	Oregon Highway 242 west, USA	44.2243	-121.8718
AmJa2112	*Abies religiosa*	Sierra de Manantlán, Jal.	19.4640	-103.9470
ArHi5316	*Abies religiosa*	El Chico, Hgo.	20.1500	-98.7000

Table 1 (concluded).

Phylogeny IDs	Taxa	Locality	Latitude	Longitude
ArMi1519	*Abies religiosa*	Puerta Garnica, Mich.	19.6700	-100.8220
ArMi5502	*Abies religiosa*	Tancítaro, Mich.	19.3830	-102.3170
ArQuZa01	*Abies religiosa*	El Zamorano, Qro.	20.9290	-100.1820
ArVe4809	*Abies religiosa*	Cofre de Perote, Ver.	19.5170	-97.1500
AmCo01	*Abies vejarii*	Jamé, Coah.	25.3167	-100.5500
AvCo04	*Abies vejarii*	Mesa de Tablas, Coah.	25.2000	-100.3830
AvCo05	*Abies vejarii*	Mesa de Tablas, Coah.	25.2000	-100.3830
AvNl01	*Abies vejarii*	La Encantada Zaragoza, N. L.	25.5388	-99.4778
AvNl02	*Abies vejarii*	La Encantada Zaragoza, N. L.	25.5388	-99.4778
AvNl03	*Abies vejarii*	La Encantada Zaragoza, N. L.	25.5388	-99.4778
AfDg01	*Abies sp.*	Mpio Pueblo Nuevo Los Bancos, Dgo.	23.6500	-105.7300
AfDg02	*Abies* sp.	Mezquitic, Jal.	22.1172	-103.8814
AgGr01	*Abies* sp.	Leonardo Bravo, Tres Caminos, Gro.	17.5667	-99.8761
AgGr02	*Abies* sp.	Alquitrán, Chilpancingo, Gro.	17.4000	-99.5160
SpDg01	*Abies* sp.	Los Bancos, Pueblo Nuevo, Dgo.	23.6583	-105.7200
SpHi01	*Abies hidalgensis*.	Agua Blanca, Hgo.	20.3620	-98.3330

2.2. SNP database assembling

We used the pyRAD (Eaton, 2014) pipeline (https://github.com/dereneaton/ipyrad) to process raw sequence reads. In pyRAD, we used a *phred Qscore* offset of 33and we discarded samples with 100,000 reads. After quality-filtering, we demultiplexed samples based on unique barcodes and adapter sequences. These sequences were de-multiplexed allowing for one base mismatch in barcodes, which were trimmed afterwards in pyRAD. We then clustered reads for each sample with VSEARCH version 1.9.3 (https://github.com/torognes/vsearch), and aligned them with MUSCLE version 3.8.31 (Edgar, 2004). We first performed a clustering step for establishing homology among reads within samples. We determined the optimal value for the clustering parameter using a threshold series approach (Ilut, Nydam, & Hare, 2014). This method aims assembling reads into loci by minimizing both false homozygosity (splitting

reads from the same locus in different groups) and false heterozygosity (clustering different paralogs). After testing thresholds between 0.80 and 0.99 in pyRAD, we found optimal clustering at 0.90 (Fig. S1).

We assembled the final dataset after testing for different values for the maximum number of SNPs and insertions/deletions allowed at a locus, and the maximum proportion of samples allowed to share a heterozygous site. The number of retained loci plateaued at 12 SNPs, three insertions/deletions, 0.3 of shared heterozygosity, respectively (Fig. S1). Loci retrieved above these values were assumed to represent paralogs. After setting the minimum number of ingroup samples bearing data for a given locus at 50% (n=22), and discarding loci with more than four undetermined or heterozygous sites (default pyRAD settings) or less than two haplotypes (to filter paralogs), the final dataset for phylogeny construction was composed of 31,480 loci (88,464 characters; 46.24% of missing data).

2.3. Phylogenetic, reticulation, isolation by distance and genetic structure analyses

We used two approaches to investigate the phylogenetic relationships of Mesoamerican firs. First, we inferred a maximum likelihood phylogenetic tree with RAxML-HPC ver 8.2.12 (Stamatakis, 2014) using a GTR + GAMMA model of nucleotide substitution and searching for the best-scoring tree (-f a option). Node support was determined with 500 bootstrap samples. Analyses were carried out on the CIPRES Science Gateway version 3.3 (Miller, Pfeiffer, & Schwartz, 2010). Second, we built a species tree with SVDquartets (Chifman & Kubatko, 2014) implemented in PAUP* version 4a146 (Swofford, 2002), using QFM quartet amalgamation and multispecies coalescent options. This method infers relationships among all possible quartets from a species matrix under the coalescent model, and then estimates a species tree (without branch lengths) using a quartet assembly method. We used non-parametric bootstrapping with 500 replicates to assess variability in the estimated tree. Because of short read length, which results in little phylogenetic information within loci (Nieto-Montes de Oca *et al.*, 2017), we did not attempt estimating particular gene trees with any method.

Possible reticulations in the evolution of Mesoamerican firs were evaluated within a phylogeographic context. To do so, we first reduced our SNP dataset, with VCFtools (Danecek *et al.*, 2011), down to 9,277

markers, which were those that had minor allele frequencies higher than 5% and a proportion of missing data below 0.6. With this dataset, we first tested for isolation by distance, as expected under the no-diversification framework. We performed a Mantel test (with 1000 replicates) between a pairwise matrix of Nei's genetic distances estimated with StAMPP (Pembleton, Cogan, & Forster, 2013), and a geographic distance matrix obtained with data of longitude and latitude of each sample, with library *fossil* (Vavrek, 2011). We then looked for reticulations in the *Abies* genealogy (expected under the radiation scenarios) with a network analysis in SplitsTree 4.15.1 (Huson, 1998), using the same genetic distance matrix above.

We also visualized genetic structure with a PCA performed with SNPRelate (Zheng *et al.*, 2012), a clustering analysis carried out with genetic distances (Jombart, 2008) for building a network in *SplitsTree* 4.15.1 (http://www.splitstree.org/), and an admixture analysis performed with ADMIXTURE v. 1.23 (Alexander, Novembre, & Lange, 2009). For this last analysis, we used a cross-validation procedure to infer the most likely number of genetic lineages (*K*), after testing *K* values ranging between one and ten. Sample composition of lineages inferred with the last four methods (SplitsTre, PCA, genetic distances, admixture) was visually compared with those of the phylogenies above.

Data availability, SNP data, maps, metadata, admixture files and parameters used are available at the DRYAD repository xxxx.xxxx available upon acceptance.

3. Results

3.1. Sequencing and assembly results.

After filtering, the mean number of reads retained by individual was 46,788 (± 47,639; standard deviation), which were assembled in 15,536 (± 4848; standard deviation) loci per sample, with a mean depth of 33.49X (± 25.94; standard deviation). Samples having the lowest (AfDg02, AjJa1901 and AcUs02) and highest (ArQuZa01, AvNl02 and AcCh02; Figure S2) number of reads did not have any particular geographic origin, taxonomic identity or DNA extraction method. Assembled reads resulted in a data

matrix of 2,828,273 aligned nucleotides that contained 88,464 SNPs; 18,068 of which were parsimony-informative.

3.2. Phylogeny of Mesoamerican firs

Both phylogenetic methods, ML and SVDquartets, produced consensus trees with highly supported nodes; although bootstrap values were slightly higher for the ML tree. Excepting for the position of two samples (AfDg02 and AjJa1901), trees had very similar topologies (Fig. 2), with the Mesoamerican lineage being sister to *A. concolor*, and subdivided in four main subgroups. Subgroups coincided with and were allopatrically distributed along the main mountain chains of the region. They were nested from north to south and from west to east, suggesting a stepping-stone colonization pattern; that is, from Western USA (i.e., Arizona and California) to the Sierra Madre Occidental in the northwest (II), to the Sierra Madre Oriental in the northeast (III), then into Central Mexico (the Transverse Volcanic Belt; IV), and finally south, into the Sierra Madre del Sur and Guatemala (V).

The lineage of *A. concolor* was basal to the rest of the Mesoamerican firs, clades within subgroups mostly coincided with previously recognized taxonomical species or subspecies. Except for individuals of *A. durangensis* and 'problematic' samples from northwestern Mexico. For instance, subgroup III contained two clades that were respectively composed of individuals of *A. durangensis* var. *coahuilensis* and *A. vejarii*. Subgroup IV included two other clades mostly formed by samples from populations of *A. religiosa* and *A. flinckii* / *A. jaliscana*, respectively. Subgroup V contained two additional clades formed by individuals of *A. hickeli* and *A. guatemalensis*. This subgroup further contained some 'problematic' samples. For example, the northernmost individual of *A. hickeli* (AhVe01) was not grouped with its conspecifics, while some samples identified as *A. guatemalensis,* but collected north of its range were either grouped within the *A. hickeli* clade (AgGr01-02) or were sister to both subgroup III and IV (AgSp01). The position of this sample was shared with the sole individual of *A. hidalguensis* (SpHi01) included in our analyses (Fig. 2).

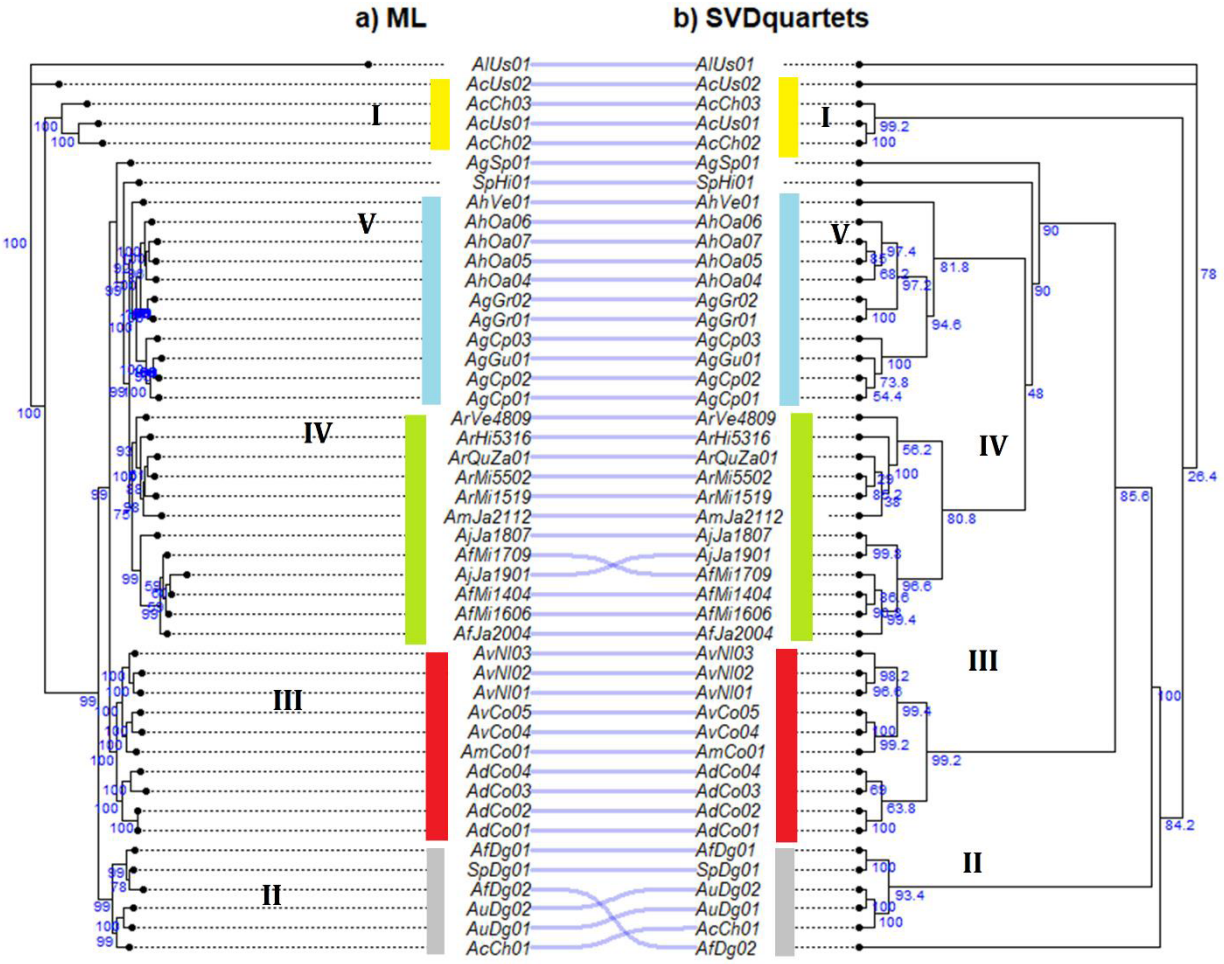

Fig. 2. Phylogenetic relationships of Mesoamerican firs (*Abies*, Pinaceae) derived from 88,464 SNPs obtained from Genotyping by sequence (GBS). (a) Maximum likelihood tree. (b) Majority-rule consensus tree from the SVDquartets analysis. Nodal support values are bootstrap proportions. See Table 1 for individual codes. I: North Region (NR); II; Sierra Madre Occidental (SMOCC); III: Sierra Madre Oriental (SMOR); IV Trans Mexican Volcanic Belt (TVB); Sierra Madre del Sur (SMS). Color bars according to Figure 1.

3.3. Evidence for reticulation

Most subgroups and clades within subgroups could be recovered with the Neighbor-Net network (Fig. 3). The most noteworthy features were that the two main clades from subgroup (central Mexico, Trans Mexican Volcanic Belt) were separated from each other, as were the samples of *A. durangensis* var.

coahuilensis, *A. vejarii*, and *A. durangensis*. Incongruences were evident in most internodes separating subgroups, clades or even samples within clades.

Further signals of reticulation were found in the multivariate analyses, which revealed low population genetic structure. The first four PC-axes explained 45.35% of the genetic variation (Fig. S3). The first component (20.70%) accounted for the separation of *A. concolor* and *A. lasiocarpa* from the Mesoamerican species, and the second (10.98%) and third (7.44%) ones, for the separation of species Sierra Madre Oriental and Sierra Madre Occidental (*A. durangensis, A. durangensis* var. *coahuilensis* and *A. vejarii*) from the rest; the fourth axis (6.23%) accounted for the division between samples from *A. flinckii*. When we excluded *A. concolor* and *A. lasiocarpa* of analysis. The first component accounted for the separation of *A. durangensis var. coahuilensis* and *A. vejarii*, the second and third ones for the separation of *A. durangensis* from the rest; the fourth axis accounted for the division between samples from *A. flinckii, A, religiosa* and *A. hickelii-A. guatemalensis*. The tree based on Nei's genetic distances showed a strong geographic structure from north to south located with *A. concolor* and *A. guatemalensis,* respectively (Fig. S4). However, separation between individuals and groups of individuals was gradual and suggested isolation by distance, which was confirmed by a Mantel test between genetic and geographic distances (*p-value* < 0.01; Fig. 4).

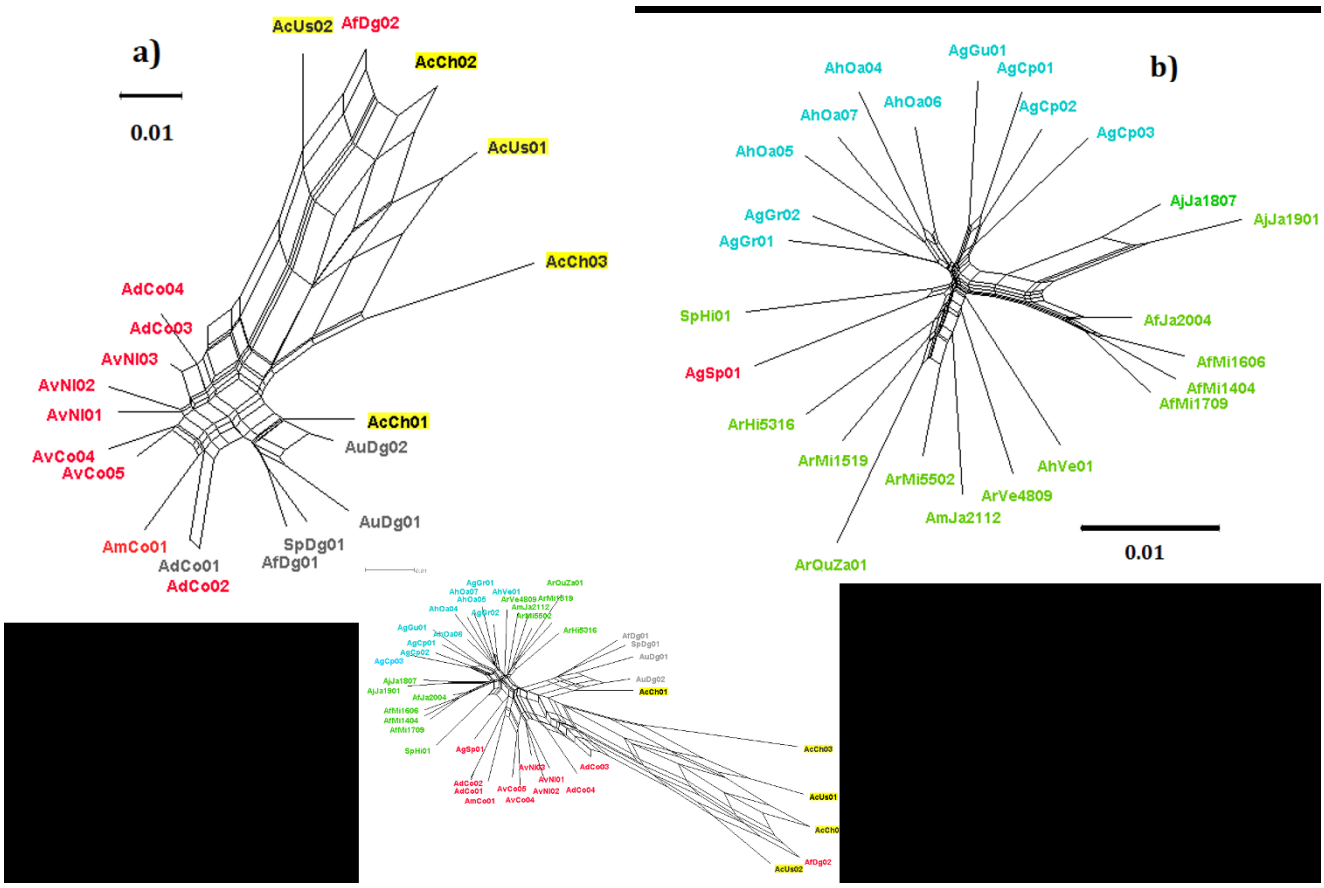

Fig. 3. Neighbor-Net network for Mesoamerican firs (*Abies*, Pinaceae) derived from 9,277 SNPs, codes in samples see (Table 1). (a) North region in yellow (I), Sierra Madre Occidental (II) in gray and Sierra Madre oriental in red (III). (b) Trans Mexican Volcanic Belt in green (IV) and Sierra Madre del Sur in blue (V). Inset with all species and colored by group.

Cross-validation on Admixture analyses suggested three main genetic lineages (*K*), although validation values for *K*=3 to 6 were similar (0.66-0.70; Fig. 5). The optimal cluster subdivision separated *A. concolor*, and individuals from Sierra Madre Oriental and Sierra Madre Occidental. When successively increasing *K*, individuals of *A. flinckii* and *A. jaliscana* (*K*=4), surprisingly individuals of *A. jaliscana* had ancestry of *A. flinckii* and *A. religiosa*. *K*=5 separated individuals of Sierra Madre Oriental and Sierra Madre Occidental. *K =6* separated individuals of *A. religiosa*. Significantly shared ancestry was observed for 'problematic' individuals, especially AgSp01 (*A. guatemalensis*) and SpHi01 (*A. hidalgensis*) and unassigned samples from northwestern Mexico (Fig. 5).

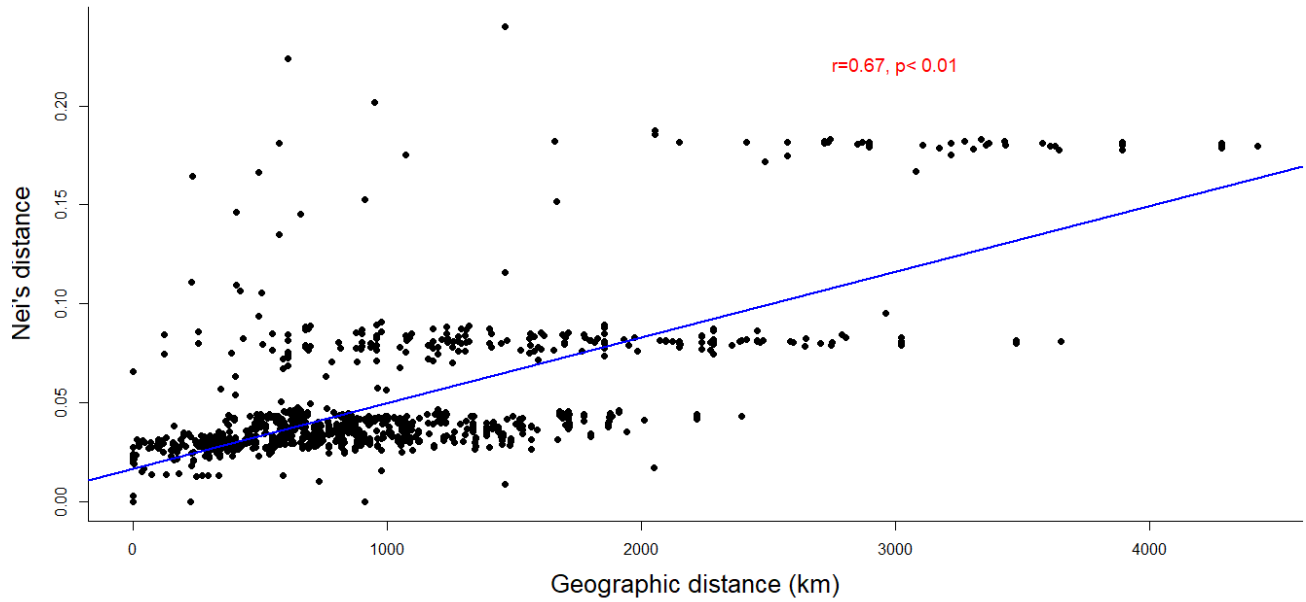

Fig. 4. Nei's genetic distances tree obtained with 9 277 SNPs and significant relationship between genetic distance and geographic distances for 46 samples of genus *Abies* (Pinaceae) from southern United States until Guatemala.

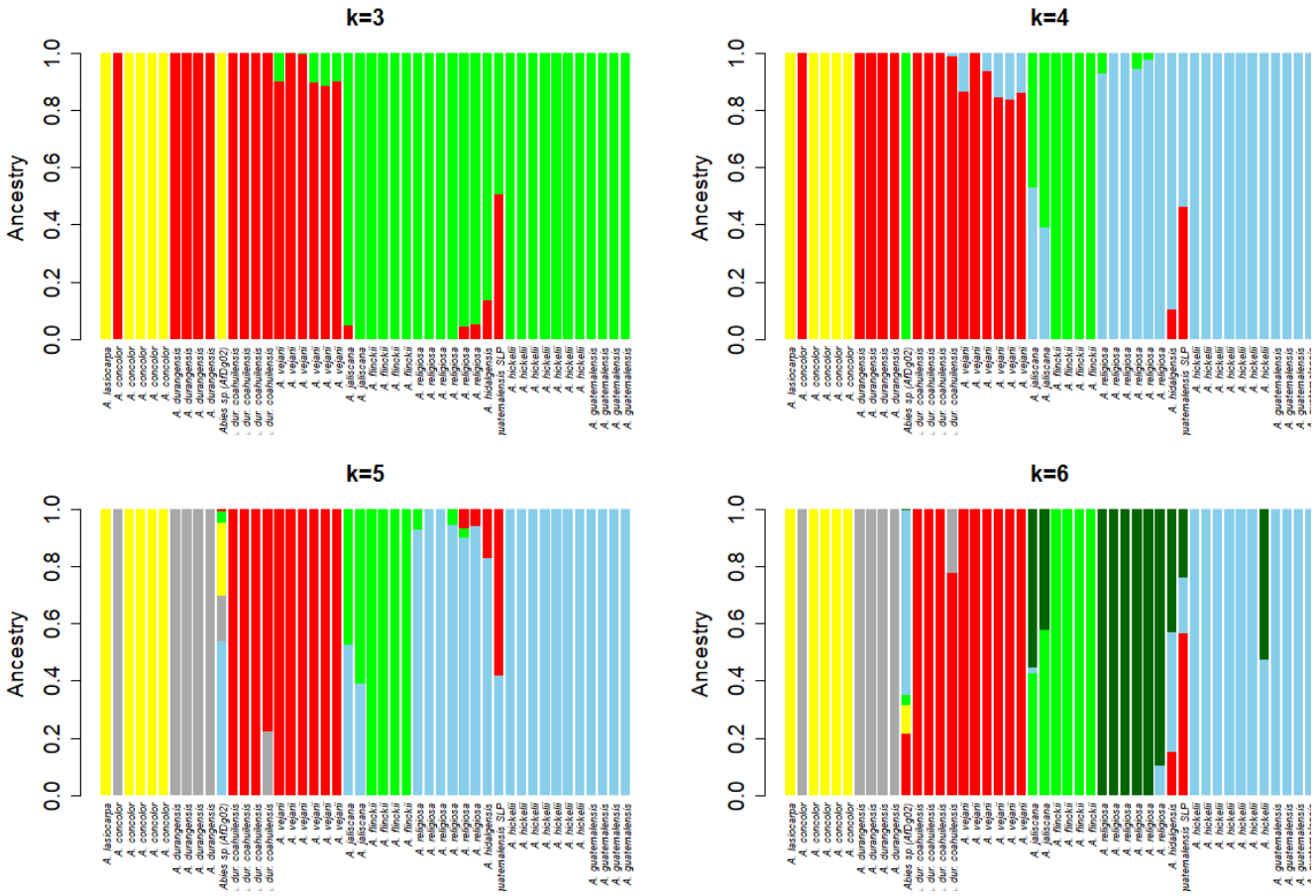

Fig. 5. ADMIXTURE clustering analysis for Mesoamerican firs obtained from variation at 9,277 SNPs assuming 3-6 genetic clusters (*K*) The samples are grouped by species, from left to right *A. concolor* (region I, in yellow), *A. durangensis* (region II, in gray), *A. durangensis* var. *coahuilensis* and *A. vejarii* (region III, in red), *A. jaliscana, A. flinckii* and *A. religiosa* (region IV, in green), *A. hickelii* and *A. guatemalensis* (region V, in blue).

4. Discussion

4.1. Isolation by distance, species radiation and reticulation

In this study, we report a largely resolved phylogeny for Mesoamerican firs, and propose a model of evolution based on non-adaptive allopatric processes, including reticulation and an isolation by distance framework. As in previous works, our phylogenies showed that Mesoamerican firs form a unique lineage related to the species from sect. *Grandis* from southwestern USA (*A. concolor* and *A. grandis*), which points

to a unique colonization of Mesoamerica following the main mountain ranges of the region. That is, from the Southern parts of the Rocky Mountains, into the Sierra Madre Occidental; then to the East, into the Sierra Madre Oriental, and South, into the Transmexican Volcanic Belt and the Sierra Madre del Sur and Guatemala (Fig. 1).

Both the palynological record and phylogenetic studies, including this one (Graham, 1976, 1999; Aguirre-Planter *et al.*, 2012; Xiang *et al.*, 2015; Semerikova *et al.*, 2018), suggest a recent colonization of Mesoamerica, likely taking place during the early Pliocene (~5 Ma). Given that such a recent migration would translate into a rapid species diversification, a sole fir lineage is likely to have occupied the whole modern range of the genus in the region, and later diverge into modern species after population isolation and allopatric differentiation. The climatic oscillations of the Pleistocene could have provided the ideal framework for such a radiation, through population fragmentation and isolation during the warmer periods, expansions and secondary contacts during the glacial periods; thus allowing for genetic exchanges between neighboring stands, and for differentiation among the more distanced ones (Mastretta-Yanes *et al.*, 2015).

An expected outcome of a conifer lineage undergoing such a rapid radiation is the retention of ancestral polymorphisms, and a further slow accumulation of new mutations among the resulting taxa (Bouillé & Bousquet, 2005; Loh *et al.*, 2008; Mims *et al.*, 2010). While testing for the second factor is out the scope of the present study, we were able to identify compelling evidence for the presence of shared polymorphisms among Mesoamerican with our SNP dataset (Figs. 3, 5 and S4). Previous studies with isozymes and cytoplasmic DNA markers and had reached similar conclusions but poorer resolution (Aguirre-Planter *et al.*, 2000; Jaramillo-Correa *et al.*, 2008). Conifers are indeed reputed for retaining ancient alleles because of both large ancestral effective population sizes and rampant interspecific gene flow (Hamrick *et al.*, 1992; Petit & Hampe, 2006; Mimura & Aitken, 2007).

Experiments in firs have revealed that viable hybrids are produced when crossing species from the same or closely related sections (Clair & Critchfield, 1988; Critchfield, 1988). For example, crosses

between *A. concolor* and *A. religiosa* produced 43% of viable seeds, which originated individuals exhibiting intermediate characters for needle morphology and monoterpene composition (Clair & Critchfield, 1988; Critchfield, 1988). Thus, if viable hybrids can be produced between two of the most divergent species from the region, it would be safe to assume that natural interspecific gene flow is, at least in part, responsible for the reticulations observed herein. Whether hybridization is also contributing adaptive alleles between neighboring species, such as observed in spruce and pine, is still a hypothesis to test; as it is its potential role for generating new species (Shen, Ran, & Wang, 2019).

4.2. Non-adaptive forces as main drivers of species divergence

Tropical mountains have been described as both 'museums' and 'cradles' of biodiversity (Mastretta-Yanes *et al.*, 2018). Indeed, the topographic complexity of these zones provide small regions of climatic and ecological stability that act as microrefugia for ancient taxa (Parra-Olea *et al.*, 2012; Hylander *et al.*, 2015), but also offer ideal conditions for the formation of new species, via allopatric and/or adaptive radiation prompted by landscape variability (Mastretta-Yanes *et al.*, 2015, 2018).

Theoretical and empirical studies have shown that the geographic distribution, and ecological and morphological breadth of species originated through adaptive or non-adaptive radiations can be quite different. For instance, when divergence is mostly driven by adaptive factors, species would be more environmentally and morphologically diverse than taxa derived from non-adaptive processes (Muschick *et al.*, 2014). On the other hand, non-adaptive radiations would result in mostly allopatric taxa, while adaptive processes can produce co-distributed species that exploit different environmental resources (Pinto *et al.*, 2008). Our phylogenies revealed several groups of allopatric species, which have previously shown little to no climate niche variation (Martínez-Méndez *et al.*, 2016) and small morphological differences that mostly adjust to an isolation by distance framework (Strandby, Christensen, & Sørensen, 2009). Evidence that altogether point to a non-adaptive species radiation.

Comparisons with other species-rich genera from the mountains of Mesoamerica, notably pines and oaks, help reinforce the point above. A recent phylogeny revealed a rapid diversification for both major

North American oak clades in Mesoamerica (Hipp *et al.*, 2018). However, contrary to firs, oaks colonized this region much earlier, 15-20 Ma (Cavender-Bares *et al.*, 2018; Hipp *et al.*, 2018); and went through a much more spectacular radiation, that produced *c*. 154 morphologically and ecologically diverse species (Hipp *et al.*, 2018). Pines, as oaks, are also very diverse *c*. 50 species (Gernandt & Pérez-De La Rosa, 2014), the pines arrived to Mexico late Cretaceous, their divergence is older than Mexican firs for subseccion Cembroides (27 Ma) and around of 5 Ma for subseccion *Ponderosae* and *Australes* (Gernandt *et al.*, 2008; Sánchez-González, 2008). Similarly to firs, species within the same section share a similar climatic niche and morphology after their diversification in Mesoamerica (López-Reyes *et al.*, 2015; Ramos-Dorantes *et al.*, 2017). The contribution of adaptive forces from the beginning of the diversification process appears thus more evident in oaks than in pines or firs, even if in all three cases, the same mountains of Mesoamerica acted as cradles for diversification. It would be interesting to test whether this also holds for other conifer lineages that moved into and diversified in the tropics, like junipers, cypress, or yellowwoods (*Podocarpus*).

4.3. Character displacements after secondary contact?

It must be noted that even if non-adaptive forces apparently were predominant during the divergence of Mesoamerican firs, with their concomitant effects on fitness (González-Martínez, Ridout, & Pannell, 2017), this does not rule that selective factors might have also been involved. Forest trees are particularly resilient to bottlenecks, which they can counter through facultative selfing for purging genetic load (Ledig, Hodgskiss, & Johnson, 2005; Petit & Hampe, 2006). Such a strategy may also facilitate the action of selective forces for more rapidly taking advantageous alleles to fixation. Within a scenario of hybridization after secondary contact, selective forces can act upon and reinforce incipient reproductive barriers or facilitate the introgression of adaptive alleles between taxa (Hamilton, Lexer, & Aitken, 2013; De La Torre, Roberts, & Aitken, 2014). The first case will result in character displacement between the hybridizing taxa along a few environmental or morpho-physiological axes (Ortiz-Medrano *et al.*, 2016). On the long term,

these species will begin exploiting different environments as if under sympatric speciation model (McCormack *et al.*, 2010). The second case will produce a tension hybrid zone where species display a mosaic genomic landscape, with scattered permeable and impermeable regions to gene flow (De Lafontaine *et al.*, 2015). To explore such a possibility, more developed genomic resources are necessary and larger sample size with studies at population level, such as currently available for spruce (Hamilton *et al.*, 2013; De La Torre *et al.*, 2014).

At the genus-wide scale, climate niche differences are minor among Mesoamerican fir taxa (Martínez-Méndez *et al.*, 2016), and morphological traits tend to adjust to an isolation by distance pattern (Strandby *et al.*, 2009). However, at the local scale, some of the species groups detected herein appear good candidates for exploring adaptive reinforcement. For instance, species within subgroup IV (*A. religiosa*, *A. flinckii* and *A. jaliscana*) have contrasting cone and needle morphologies (Vázquez-García *et al.*, 2014; Cruz-Nicolás *et al.*, 2020), are distributed at different elevation zones (Cuevas-Guzmán *et al.*, 2011), and exhibit non-overlapping pollen production times (Mantilla-Blandón, 2006). Similarly, *A. hickelii* and *A. hidalgensis* are the only two Mesoamerican firs that have more than two resin canals, a putatively adaptive trait against herbivores or related to drought (López, Climent, & Gil, 2010; Huang *et al.*, 2016; Jankowski *et al.*, 2017). Whether or not this is the result of character displacement after secondary contact with neighboring taxa is still a hypothesis to test, and the phylogeny obtained herein should provide the adequate framework to address this question using comparative methods.

4.4. Taxonomic implications

Obviously, our results also have relevant implications for the taxonomy and conservation of Mesoamerican firs. For instance, although the lineages detected in our phylogenies mostly fit the distribution of mountain ranges, they also support the recognition of some contentious taxa, like *A. coahuilensis* (Johnston, 1943), *A. flinckii* (Rushforth, 1989) or *A. jaliscana* (Vázquez-García *et al.*, 2014). The first case is particularly noteworthy, as taxonomists often group firs from the northern portion of the

Sierra Madre Oriental as a variety of *A. durangensis* (i.e., *A. durangensis* var. *coahuilensis*). This taxon is geographically separated from variety *durangensis*, which is distributed in the Sierra Madre Occidental, by the arid Central Plateau, which likely diminishes gene flow between them (Ledig, Hodgskiss, & Jacob-Cervantes, 2002). Here, these two taxa appeared clearly separated from each other, with var. *coahulensis* even being phylogenetically closer to *A. vejari* (Figs. 1 and 2) than to *A. durangensis* var. *durangensis* (Figs. 2 and 3). Whether this is the result of speciation or introgression from *A. vejari* is still to be tested, and the outcome should be considered for future conservation efforts.

Firs from the western portion of the TVB have been the source of a still ongoing taxonomic debate, with authors recognizing up to four different species (Martínez, 1948; Debreczy & Rácz, 1995; Aguirre-Planter *et al.*, 2000; Vázquez-García *et al.*, 2014). Our results point that two clearly defined evolutionary lineages are distributed in this region (*A. religiosa* and *A. flinckii*), and provide evidence for the recognition of a third one (*A. jaliscana*) as, at least, a different ESU within *A. flinckii*. *A. flinckii* has been described as a subspecies of both *A. religiosa* var. *emarginata* and *A. guatemalensis* var. *jaliscana* (Martínez, 1948). However, results from this and previous genetic, ecological and morphological studies (Aguirre-Planter *et al.*, 2000; Cruz-Nicolás *et al.*, 2020) indicate that it deserves a species status. For instance, when compared to *A. religiosa*, *A. flinckii* has distinctive needle length and apex form (Cruz-Nicolás *et al.*, 2020) and cone size (Vázquez-García *et al.*, 2014); it further produces pollen much earlier than *A. religiosa* i.e., February-April vs. April-June (Mantilla-Blandón, 2006). The distinction of its westernmost populations as a different (sub)species, i.e. *A. jaliscana,* and the dynamics of its contact zone with *A. religiosa* (where the recognition of another subspecies, *A. religiosa* subsp. *colimensis*, has been proposed (Silba 2008)) should be also carefully addressed in future ecological and landscape genomics surveys.

Our results further suggest that the description and geographic distribution of *A. guatemalensis* should be extensively revised. This species has been reported in northeastern (Sierra Madre Oriental), central (TVB) and southern Mexico (Sierra Madre del Sur), and in both Guatemala and Honduras (Aguirre-Planter *et al.*, 2000; Ávila Bello & López-Mata, 2001; Strandby *et al.*, 2009). However, samples assigned to this

species appeared paraphyletic in our analyses, with some individuals forming its own cluster, others grouped within *A. flinckii*, and an additional sample, from northeastern Mexico (*AgSp01*), being basal to subgroup of Sierra Madre del Sur (Figs. 2 and 3). This sample was collected at a significantly lower elevation (1770 m a.s.l.) than the rest of firs from the same region, except for the sole individual of *A. hidalgensis* included herein (2275m a.s.l), with which it shared its phylogenetic position (Fig. 2 and 5). This indicates that a distinctive divergence/speciation processes might be taking place below 2,000 m a.s.l. at the eastern slopes of the Sierra Madre Oriental, and that this region deserves further exploration.

4.5. Perspectives

Studies such as this one open the door for future phylogenomics surveys and detailed coalescence population analyses, especially for contentious groups of taxa, like tropical conifers (Moreno-Letelier, Mastretta-Yanes, & Barraclough, 2014). As previously shown for North American oaks (Cavender-Bares *et al.*, 2018; Hipp *et al.*, 2018), using the large number of markers that can be derived from massive sequencing provides unprecedented resolution for tackling these taxonomic/phylogenetic challenges.

The necessity of including more than one individual per taxon, and as many populations as possible must also be highlighted, as it allows accounting for both shared and not shared polymorphisms and have a better representation of the different evolutionary processes that modelled species divergence (Pham *et al.*, 2017). This is particularly important when working with long-lived species or species distributed dynamic environments like tropical mountains, in which reticulated evolution seems the rule (Padilla-González *et al.*, 2017). Highly resolved phylogenies, like those that can be generated with massive sequencing data will also provide ideal framework for understanding ecological and genomic trends within a comparative method framework.

Finally, tackling the various hypotheses generated herein for understanding the divergence of slowly-evolving taxa in the tropics using detailed coalescence analyses will help explaining how the huge

biodiversity of these regions was generated. For instance, it would be interesting to test if the non-adaptive radiation hypothesis holds for other conifer groups in the region.

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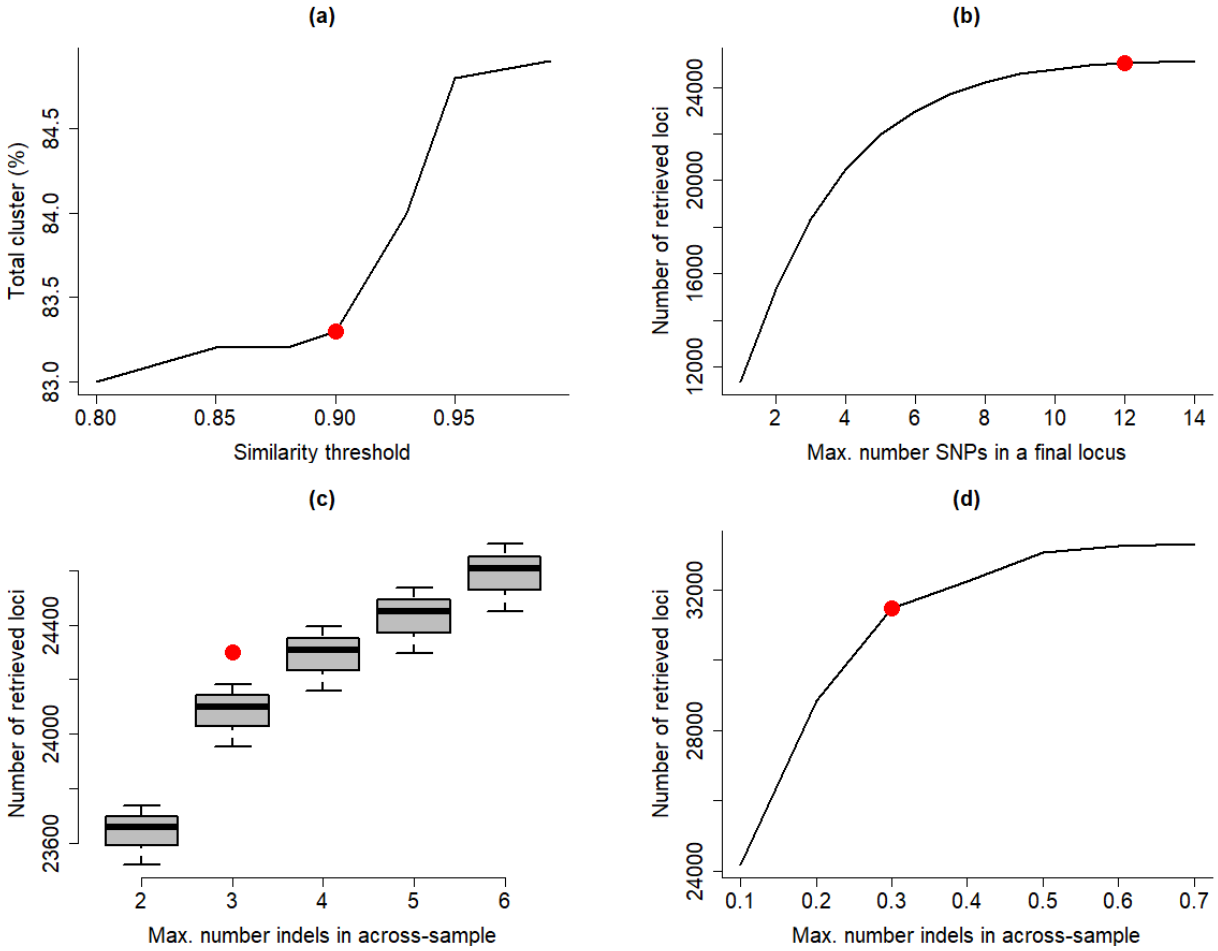

Fig. S1. (a) Variation in the proportion of clusters retrieved with different similarity thresholds ; (b) variation in the number of retrieved loci with different maximum number of SNPs in a final locus; (c) variation in the number of retrieved loci with different maximum numbers of insertions/deletions in across-sample clusters ; (d) variation in the number of retrieved loci with different maximum proportions of samples with a shared heterozygous site.

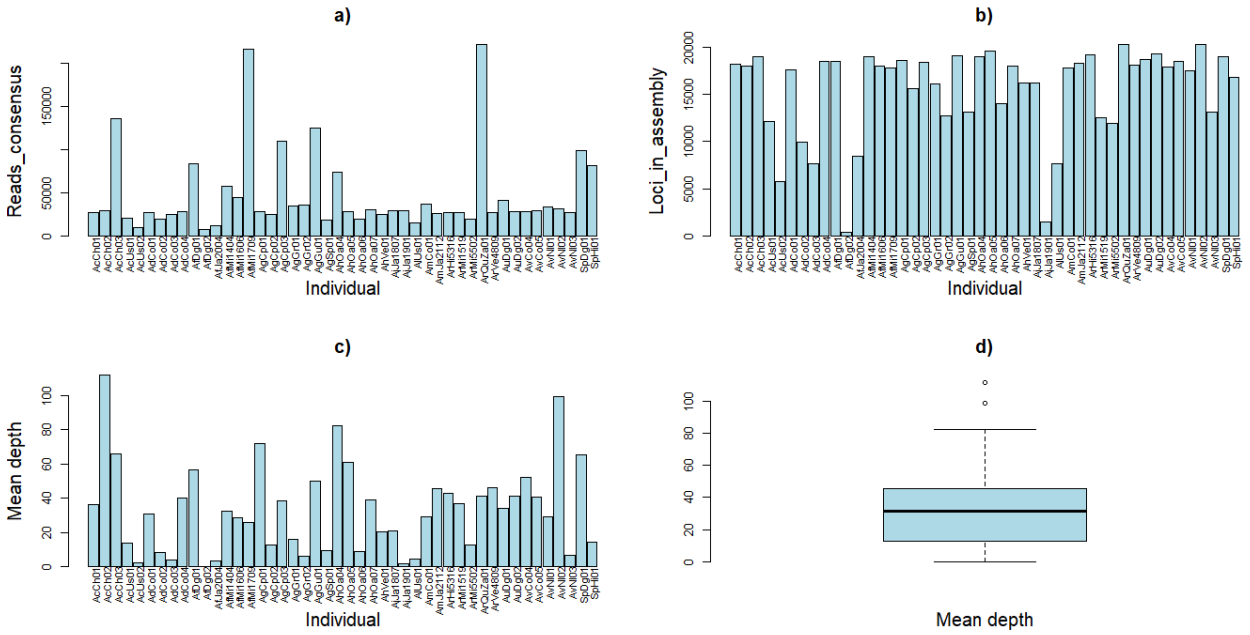

Figure S2. (a) Read consensus/individual; (b) Loci in assembly/individual; (c) Mean depth/individual; (d) boxplot of mean depth for 46 samples of genus *Abies* used in this study.

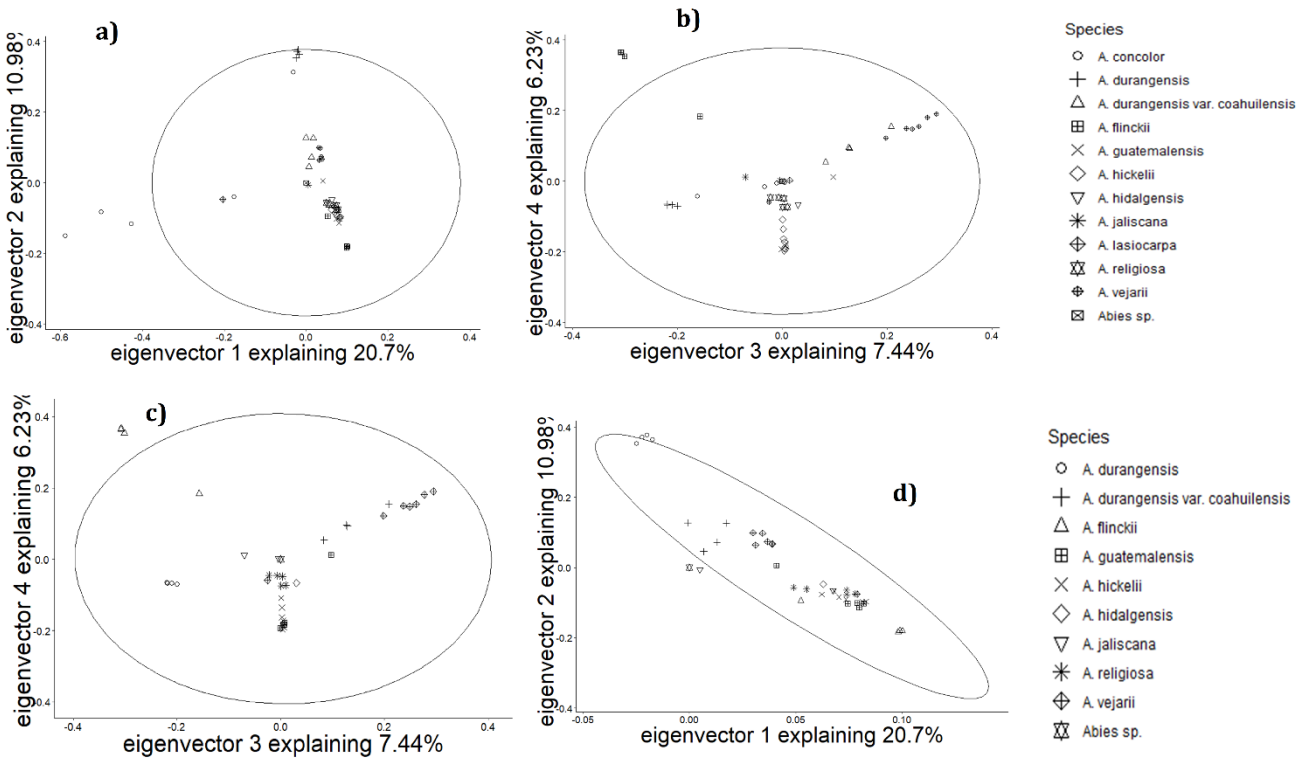

Fig. S3. Four principal components (PCA) of genetic variation (9 277 SNPs) with 46 samples of genus *Abies* (Pinaceae) in samples since southern of United States until Guatemala, considering overall species (a-b). Excluding *A. concolor* and *A. lasiocarpa* (c-d).

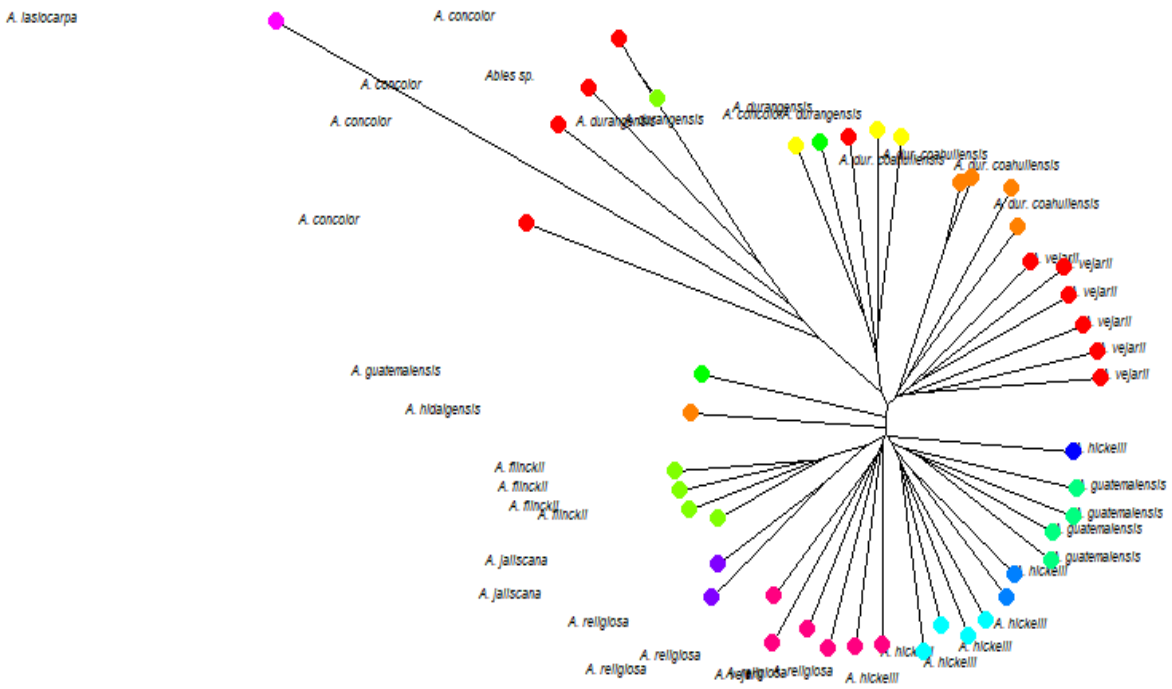

Fig. S4. Nei's genetic distances tree colored by species for 46 samples of genus *Abies* (Pinaceae) since southern of United States until Guatemala, with 9,277 SNPs.

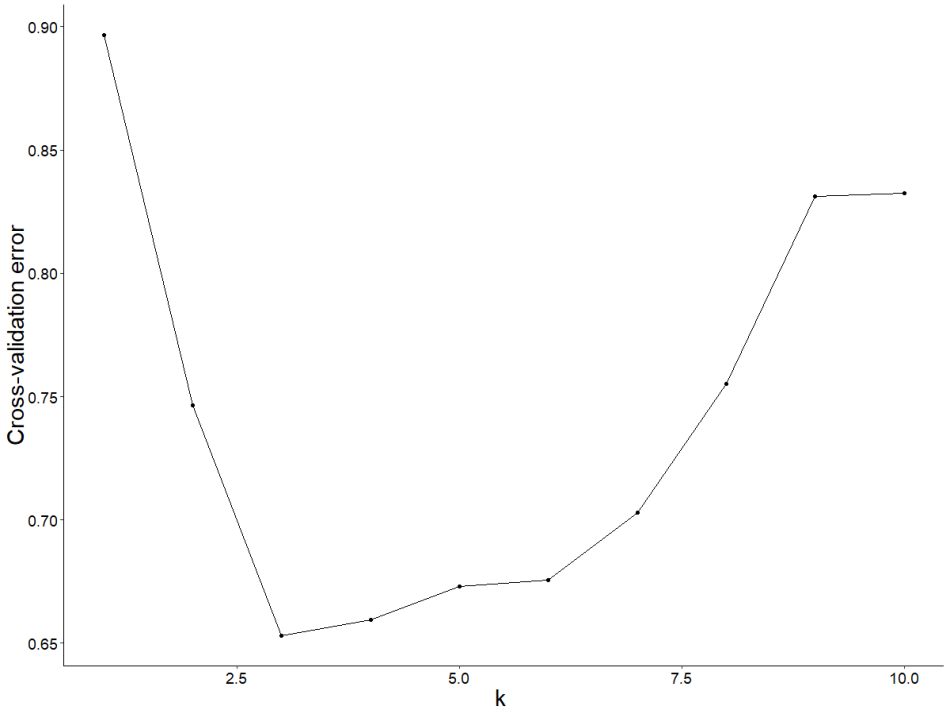

Figure S5. Cross-validation for K = 1-10 for ADMIXTURE analysis with 46 samples of genus *Abies* (Pinaceae) since Southern United States until Guatemala.

CAPITULO III: Contrasting evolutionary processes drive morphological and genetic differentiation in a subtropical fir (*Abies*, Pinaceae) species complex

RESUMEN

Es frecuente observar clinas entre caracteres morfológicos con geografía y/o clima en plantas. El origen de esos cambios en muchos casos permanece bajo cierto grado de incertidumbre. En este capítulo se propone una aproximación para probar hipótesis de aislamiento por adaptación y aislamiento por distancia en clinas fenotípicas bajo comparaciones P_{ST}-G'_{ST}, en poblaciones naturales de dos especies del centro de México (*A. flinckii* y *A. religiosa*). Se propusieron tres hipótesis, la primera donde la variación morfológica únicamente sería producto de un aislamiento por distancia y por tanto, $P_{ST} = G'_{ST}$, una segunda hipótesis donde la variación morfológica se correlaciona positivamente con alguna variable ecológica (ej., temperatura, precipitación) y en este caso $P_{ST} > G'_{ST}$ que sería una evidencia de aislamiento por adaptación, y una tercera hipótesis donde $P_{ST} > G'_{ST}$ pero solo en la especie con tamaños poblacionales grandes, lo que evidenciaría aislamiento por adaptación y la prevalencia de factores estocásticos en la especie con tamaños pequeños. A partir de estas comparaciones se concluyó que al menos en dos caracteres importantes (grosor de la hoja y posición del canal resinífero), puede invocarse a la selección direccional como conductor de esa diferenciación morfológica, mientras que en la especie de menor tamaño poblacional las clinas fenotípicas son producto de procesos estocásticos.

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Contrasting evolutionary processes drive morphological and genetic differentiation in a subtropical fir (*Abies*, Pinaceae) species complex

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Interacting stochastic and selective forces drive population and species divergence. Such interaction may generate contrasting clines between genetic and phenotypic factors, which can be related to either geographical or environmental variation depending on the predominant evolutionary force (which in its turn is partly determined by population size). Here, we investigated whether the morphological and genetic differentiation across a species complex in *Abies* in central Mexico fits isolation by distance (IBD) or isolation-by-adaptation (IBA) frameworks. This complex includes two species (*A. religiosa* and *A. flinckii*) with discernible morphological and environmental differences and dissimilar range sizes. After comparing variation at nuclear SSR loci and diagnostic morphological traits of needles with the climate variables contributing to ecological differentiation, we found that the widely distributed *A. religiosa* has more genetic diversity and is morphologically more heterogeneous than the geographically restricted *A. flinckii*. Morphological differentiation at three physiologically important traits (needle thickness, number of stomata rows and location of the resin duct) is significantly correlated with geography in *A. flinckii* (indicative of IBD), but is significantly associated with climate variation in *A. religiosa* (suggesting IBA). In agreement with quantitative genetics theory, P_{ST} (phenotypic differentiation)-G'_{ST} (genetic differentiation) comparisons indicate contrasting contributions of putatively adaptive (*A. religiosa*) and stochastic (*A. flinckii*) factors to the morphological differentiation of species related to their population size. The integration of such quantitative genetic/evolutionary aspects may reinforce species descriptions and help in disentangling resilient taxonomic discordance.

ADDITIONAL KEYWORDS: isolation by adaptation – isolation by distance – Mexico – P_{ST}-G'_{ST} – SSRs – Transmexican Volcanic Belt.

INTRODUCTION

Understanding how phenotypic, genetic and environmental variation integrate and fuel population divergence and speciation is one of the most important goals in evolutionary biology (Nosil, Funk & Ortiz-Barrientos, 2009; Gompert *et al.*, 2013). It is generally thought that local adaptation and ecological differentiation arise from a combination of favouring individuals in their local environments and selection against migrants (Camin & Ehrlich, 1958; Via, Bouck & Skillman, 2000; Lenormand, 2002; Hendry, 2004; Nosil, 2004). Such effects can be further amplified by physical, seasonal or physiological barriers that reduce gene flow, foster population divergence and, ultimately, result in reproductive isolation (Sakaguchi *et al.*, 2019). Because selection usually acts on phenotypes, a correlation is expected between the targets and the

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drivers of divergent selection (Shafer & Wolf, 2013; Karhunen *et al.*, 2014). Such a pattern is known as isolation by adaptation, IBA (Nosil, Egan & Funk, 2008; Nosil *et al.*, 2009). IBA gradients can be particularly prominent in contact zones between diverging taxa, especially when hybrids and introgressants are viable and have an intermediate fitness compared with their parents in transitional environments (Uribe-Salas *et al.*, 2008; Strandby, Christensen & Sørensen, 2009).

Given that environmental differences between localities increase with geographical distance, distinguishing IBA from patterns produced by neutral processes (e.g. IBD) is often difficult (Merilä & Crnokrak, 2001), although there are different approaches at hand to differentiate IBA from IBD (Shafer & Wolf, 2013; Dewoody, Trewin & Taylor, 2015; Ozerov *et al.*, 2015). One popular method is to compare the genetic differentiation of populations (as estimated with neutral markers, F_{ST}) with the differentiation determined for the quantitative traits potentially affected by selection, Q_{ST}, i.e. F_{ST}-Q_{ST} (Wright, 1965; Spitze, 1993). The idea is that F_{ST} is mostly driven by the interplay of stochastic processes, i.e. mutation, genetic drift and gene flow (Kimura, 1983), whereas Q_{ST} is the result of interacting adaptive and environmental effects (Lynch & Spitze, 1994). More specifically, Q_{ST} is the proportion of the total genetic variance explained by the variation between populations; it reflects the statistical association between the genes affecting a phenotype and the observed phenotypic variation among subdivided populations (Lynch & Spitze, 1994; Merilä & Crnokrak, 2001; Leinonen *et al.*, 2013). Consequently, a significantly higher Q_{ST} value provides evidence that selection may be implicated in the phenotypic differentiation of populations, whereas lower Q_{ST} estimates or similar Q_{ST} and F_{ST} values would suggest that the effects of drift, phenotypic plasticity and selection are indistinguishable for that particular character (Allendorf & Luikart, 2007; Whitlock, 2008).

A main constraint of this method is that to reliably estimate Q_{ST}, carefully designed tests under controlled conditions are required, which allow for the reduction of the environmental effects on phenotypes and increase the accuracy of Q_{ST} estimates (Yang, Yeh & Yanchuk, 1996). However, such tests have been applied for only a few species, and additional approaches have had to be developed to approximate Q_{ST} from the phenotypic variation observed in natural populations, an estimate called P_{ST} (Storz, 2002). Despite different limitations of these proxies (Pujol *et al.*, 2008; Brommer, 2011), F_{ST}-P_{ST} comparisons do provide evidence that natural selection contributes more to phenotypic differentiation than genetic drift (Ojeda *et al.*, 2016) or phenotypic plasticity (Chin & Sillett, 2016). These tests can also guide the further refinement of research hypotheses and the design of field experiments to test them (Leinonen *et al.*, 2008). Such evidence is particularly compelling given that phenotypes can vary rather quickly (within a few generations) after sudden changes in the selective regime (Kremer & Le Corre, 2012), implying that phenotypic effects can be observed before significant variation can be measured in the underlying gene polymorphisms (Le Corre & Kremer, 2012). This represents an advantage when studying non-model, perennial, sessile taxa for which no genomic resources have been developed, because under such a framework, the relative contribution of adaptive and demographic factors to population divergence can be easily scaled and compared through F_{ST}-P_{ST} (Leinonen *et al.*, 2008).

The Transmexican Volcanic Belt (TVB) provides an interesting setting for testing hypotheses related to IBA-IBD differentiation using F_{ST}-P_{ST} comparisons. It is a subtropical mountain range that crosses central Mexico from east to west, where conifer forests are distributed in a sky-island system (McCormack, Huang & Knowles, 2009) between 1700 and 4200 m a.s.l. These 'islands' are distributed almost linearly (Mastretta-Yanes *et al.*, 2015), conforming in general terms to a stepping-stone pattern (Gaggiotti & Foll, 2010). Two species of *Abies* Mill. (fir) are common along the TVB. *Abies flinckii* Rushforth (also called *A. religiosa* (Kunth) Mirb. var. *emarginata* Loock & Martínez or *A. guatemalensis* Rehder var. *jaliscana* Martínez by some authorities) is distributed in small isolated populations in the western parts of the TVB at elevations > 1700 m a.s.l., and *A. religiosa* (Kunth) Schltdl. & Cham. is widely distributed in the eastern and central parts of the TVB, mostly forming large, monospecific stands between 2400 and 4200 m a.s.l. (Rzedowski, 2006). Their phenotypic divergence increases with the distance separating their populations (Strandby *et al.*, 2009), with individuals of *A. religiosa* being generally taller (45–60 m) than those of *A. flinckii* (35–45 m), and bearing larger cones (11–15 cm vs. 8–11 cm) with lighter colours (Farjon, 2010). Needles of *A. flinckii* tend to be longer and narrower than those of *A. religiosa* and, depending on the zone, these two species further tend to initiate their growing season asynchronously (Mantilla-Blandón, 2006), indicating that their divergence may have an adaptive component (Vázquez-García *et al.*, 2014; Martínez-Méndez *et al.*, 2016).

Studies using genetic markers have shown that these two taxa form distinct genetic entities that show signs of introgression in their zone of contact, in the central region of the TVB (Jaramillo-Correa *et al.*, 2008; Aguirre-Planter *et al.*, 2012; Múgica-Gallart, 2013). Such introgression fits an IBD framework reasonably well, suggesting the predominance of

neutral factors (Aguirre-Planter, Furnier & Eguiarte, 2000). Further, palynological evidence indicates that these taxa faced the Holocene climatic variations *in situ* and only underwent elevational migrations after the Last Glacial Maximum (contrary to their boreal congeners, which underwent significant latitudinal shifts; Caballero *et al.*, 2010), which renders them ideal for F_{ST}-P_{ST} comparisons. Furthermore, no provenance-progeny tests covering the entire distribution are available for either *A. flinckii* or *A. religiosa*. To our knowledge, the management, improvement and reforestation programmes only cover local provenances (Muñoz *et al.*, 2011).

In this work, we explored whether the differentiation of these subtropical firs conforms better to an IBA or an IBD framework, and if these patterns are also observed within species. A stochastic null possibility is that this divergence is solely modelled by demographic factors that are maintained within species, i.e. morphological IBD, and no correlation with ecological variation (Fig. 1A), and two alternative hypotheses, with contrasting roles for adaptation, can be postulated. First, contrasting adaptive patterns could be driving species differentiation, i.e. morphological and ecological variation are correlated, translating into an IBA pattern between and within species (Fig. 1B). Second, because the balance between evolutionary forces mainly depends on the effective population size (Charlesworth, 2009), stochastic forces should predominate in the most restricted species, whereas selection is most likely to act in the most widely distributed species (Fig. 1C). Following this framework, we expect that the morphological variation in *A. flinckii* should conform to an IBD pattern, whereas an IBA pattern should be evident for at least a few characters in *A. religiosa*.

Our results will help understand how divergence originates among subtropical taxa with temperate origins, and how evolutionary processes generate the wide phenotypic variation observed within and between these species (Jaramillo-Correa *et al.*, 2015). They will further allow previous taxonomic proposals for *Abies* in Mexico (Eckenwalder, 2009; Farjon, 2010; Vázquez-García *et al.*, 2014) to be addressed from an evolutionary point of view and translated into new strategies for management and conservation.

MATERIAL AND METHODS

Sampling

Needles from the previous growing season were collected along vegetative branches of 399 individuals in 20 natural populations without signs of reforestation (120 trees from populations of *A. flinckii* and 279 individuals from localities of *A. religiosa*; Table 1, Fig. 2). All individuals were reproductively mature trees with diameters at breast height exceeding 20 cm (Aguirre-Planter *et al.*, 2000; Ávila-Bello, López-Mata & Mandujano, 2015). All were located at least 20 m away from the borders of populations to avoid atypical morphologies produced by increased light or wind exposure (Hoffmann & Blows, 1994). When possible, samples were taken from different parts of the lower third of the crowns to account for potential intra-individual morphological variation.

Given the scattered distribution of both species, populations were defined as independent forest patches. These were between *c.* 10 km (i.e. populations 4 and 7) and *c.* 450 km apart (1 and 5) for *A. flinckii*, and between *c.* 15 km (populations 24 and 25) and *c.* 715 km (11 and 14) for *A. religiosa*. Voucher specimens for two locations are stored in Mexican herbaria (Supporting Information, Appendix S1); complete samples are stored at the Institute of Ecology (UNAM) and are available from the authors upon request. Additional details on sampling and species assignment can be found elsewhere (Aguirre-Planter *et al.*, 2000; Méndez-González, Jardón-Barbolla & Jaramillo-Correa, 2017).

Morphological variation

We characterized morphologically all individuals for which needles could be collected from at least three different locations in the crown (i.e. repetitions) (76 trees of *A. flinckii* and 188 trees of *A. religiosa*) (Table 1, Fig. 2). Needles were fixed in formaldehyde-acetic acid-50% ethanol (FAA; Ruzin, 1999), and transverse sections were made at the medial part with razor blades. Samples were mounted on slides with Entellan and photographed with an Evolution LC digital camera fixed to an inverted microscope (OLYMPUS IX-81) with a 40× field. Images were analysed in the software Image Pro-Plus v.7.0 (Media Cybernetics, Silver Spring, USA) to measure 11 of the taxonomically diagnostic variables described for *Abies* (Panetsos, 1992; Strandby *et al.*, 2009; Ghimire *et al.*, 2015; Table 2, Fig. 3). Four additional ratios were calculated for some of these traits (see Table 2) to correct for co-variation with needle size (e.g. larger needles may have more stomata rows). For each continuous variable and ratio, we used the mean of all repetitions per individual for subsequent analyses.

As an initial inspection, morphological data were submitted to univariate analyses, followed by Kruskall–Wallis tests to detect differences between species and intraspecific populations. However, given its likely co-variation, mean values per population were used to estimate a Pearson's correlation matrix to identify groups of co-varying traits; only one variable per co-linearity group was retained to test for IBD and IBA (Robakowski *et al.*, 2004). This correlation matrix

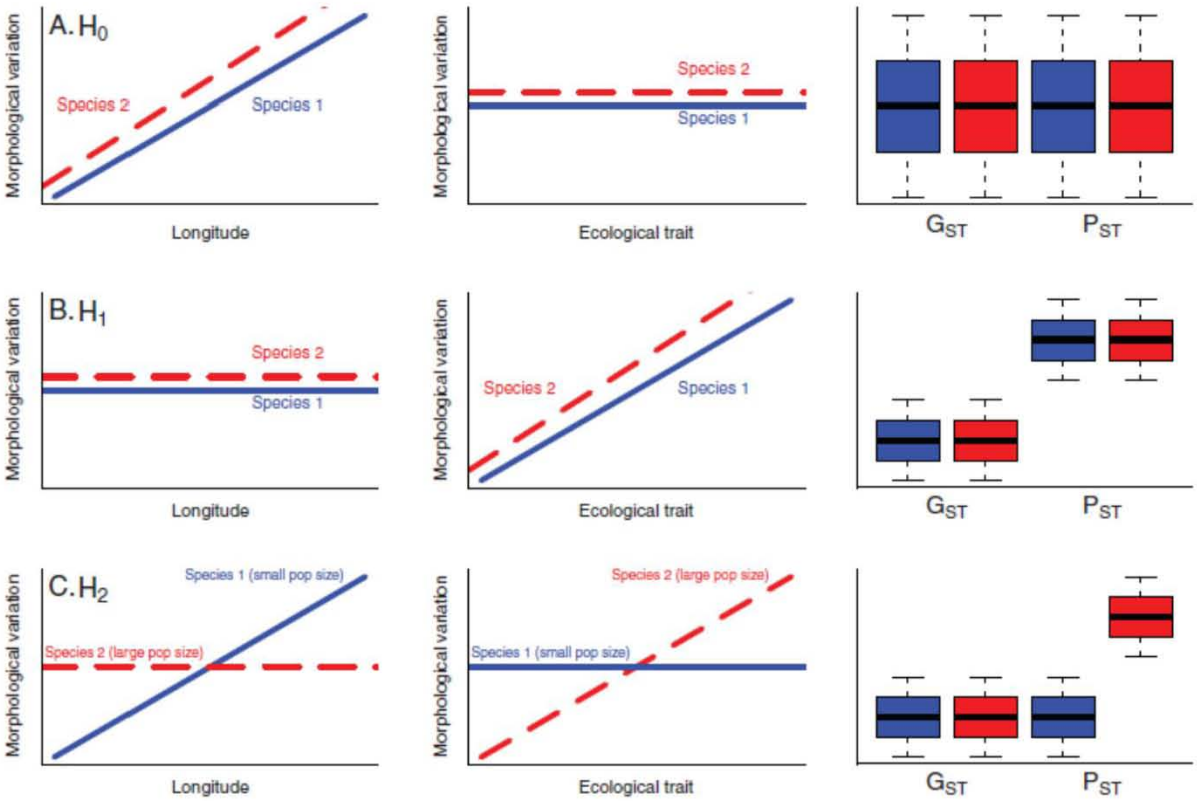

Figure 1. Hypothesized relationships for isolation by distance (IBD; A), and isolation by adaptation (IBA; B, C) for morphological traits under directional selection in species with similar (B) and different effective population sizes (C). Right: neutral differentiation measured as G'_{ST} and phenotypic divergence measured as P_{ST} for each scenario.

was further used to test for morphological similarities across individuals of the same species/population through a principal components (all variables) and a linear discriminant analysis (excluding qualitative variables) performed with the MASS package in R v.7.3–50 (Venables & Ripley, 2002).

Climate variation and correlation with morphological traits

Data from the mean annual temperature (Bio 1), isothermality (Bio 3) and precipitation of the driest month (Bio 14) and of the driest quarter (Bio 17) were obtained at a 30" arc resolution (*c.* 1 km) from the WorldClim portal (Fick & Hijmans, 2017) as a set of rasters from which bioclimatic variables were extracted for each sampled locality, using functions available in Niche Toolbox (Osorio-Olvera *et al.*, 2018). These four variables represent overall temperature and drought stress tolerance in trees (Booth *et al.*, 1988; Anfodillo *et al.*, 1998), and summarize the climate niche of Mexican firs relatively well (Martínez-Méndez *et al.*, 2016).

To test for IBD and IBA, we performed linear regressions between longitude, climate and the mean values per population of morphological traits. These correlations were performed for all populations (IBD and IBA within species). Only the uncorrelated morphological traits showing significant associations with either climate or geography in at least one species were kept for performing F_{ST}-P_{ST} comparisons. Significance was determined after applying a sequential Bonferroni correction on $P < 0.05$.

Genetic diversity

DNA was extracted for all individuals sampled (Table 1) with a modified 2% CTAB protocol (Vázquez-Lobo, 1996), and its concentration and quality surveyed with a Qubit and through electrophoresis on 0.8% agarose gels. Genetic variation was estimated with four microsatellites originally developed for *A. guatemalensis* (*Ab*07, *Ab*12, *Ab*20 and *Ab*27; Rasmussen *et al.*, 2008). These markers were selected because of their elevated number of alleles, absence of linkage disequilibrium and reduced frequency of null alleles (Rasmussen *et al.*, 2008; Múgica-Gallart,

Table 1. Reference name, number of individuals, location and elevation for populations of *Abies flinckii* (pop 1–7), and *A. religiosa* (pop 8–25) in central Mexico

Pop	Name	Phenotyped	Genotyped	Longitude	Latitude	Elevation (m a.s.l.)
1	El Caracol	15	20	−100.75	19.58	2340
2	Los Sauces	10	20	−101.35	19.33	2250
3	Dos Aguas	10	18	−102.95	18.77	2500
4	Cumbre de Guadalupe	11	22	−104.72	20.20	2100
5	Cuale	10	20	−105.00	20.35	2490
6	El Terrero	10	20	−103.93	19.45	2500
7	Atenguillo	10	–	−104.63	20.17	2359
	Total	**76**	**120**			
8	Nevado de Toluca	10	21	−99.80	19.18	3240
9	La Cañada	15	20	−100.18	19.43	2800
10	Puerta Garnica	12	21	−100.82	19.67	2880
11	Sierra Manantlán	10	19	−103.95	19.45	2500
12	Nevado de Colima	9	18	−103.58	19.58	3330
13	Volcán Atlitzin	10	22	−97.35	18.97	3060
14	Cofre de Perote	11	21	−97.15	19.52	3510
15	Tlaxco	10	19	−98.08	19.68	2760
16	El Chico	9	19	−98.70	20.15	2940
17	Volcán Popocatépetl	14	20	−98.68	19.08	3330
18	Tancítaro	10	20	−102.32	19.38	3030
19	Ajusco	8	20	−99.27	19.22	3369
20	La Malinche	10	19	−98.06	19.25	3358
21	Volcán Colima	15	20	−103.64	19.52	2928
22	Amanalco	10	–	−99.96	19.26	2796
23	Zamorano	11	–	−100.18	20.93	3156
24	El Verde	4	–	−98.61	19.26	3218
25	Río Frío	10	–	−98.68	19.37	3102
	Total	**188**	**279**			

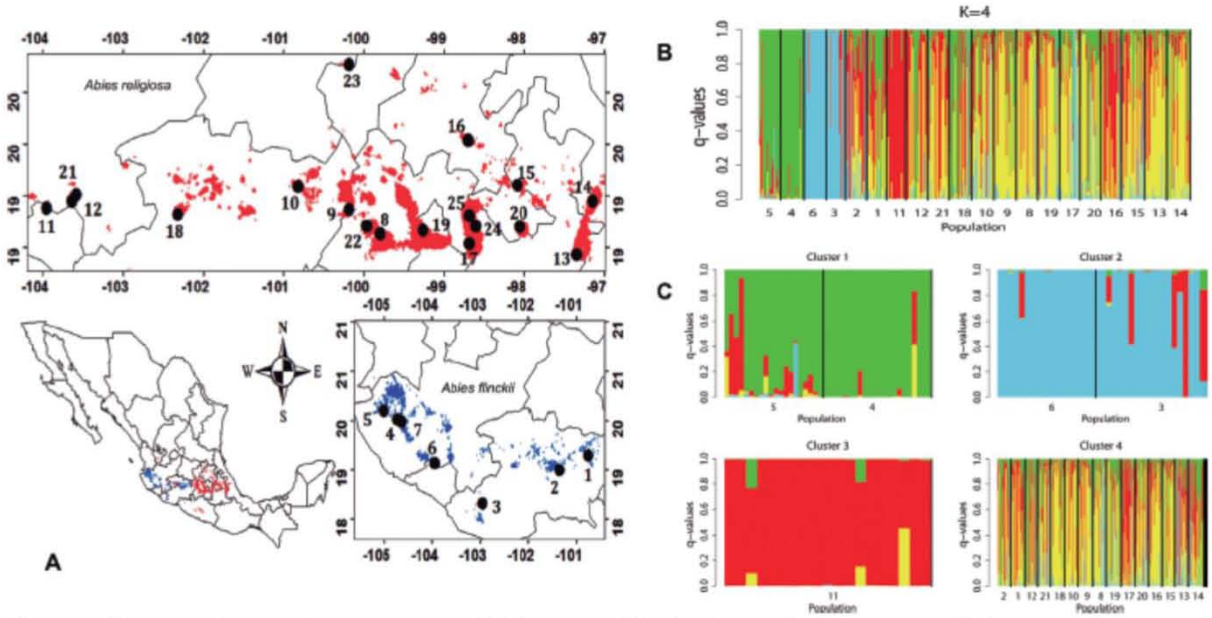

Figure 2. Projection of geographic range generated with a species distribution model (Cruz *et al.*, unpubl. data) for *A. flinckii* (pops 1-7) and *Abies religiosa* (pops 8-25; A); points indicate sampled populations. Bayesian clustering analyses for $K = 4$ obtained with Structure (B and C); populations are ordered by longitude from west to east. A zoom for each individual cluster is presented in (C).

Table 2. Morphological traits of needles evaluated for *Abies flinckii* and *A. religiosa* (diagnostic characters according to Panetsos, 1992; Strandby *et al.*, 2009; Ghimire *et al.*, 2015)

Variable	Units	Abbreviation
Shape of needle apex; acute (1), emarginate (2)	–	SNAP
Needle length	cm	NL
Number of stomata rows	count	RS
Needle width	µm	NW
Needle thickness	µm	NT
Depth of the notch	µm	DN
Thickness of the centre	µm	TC
Lateral thickness	µm	LT
Distance from the centre of the resin duct to the abaxial needle surface	µm	AB
Distance from the centre of the resin duct to the needle margin	µm	MD
Distance from the centre of the resin duct to the adaxial needle surface	µm	AD
Allometric variables		
By length of needle	–	NL/RS
By thickness of needle	–	NT/NW
By thickness of needle	–	DN/TC
Position of resin ducts	–	AB/AD

2013). They further revealed the same genetic signal previously obtained with isozymes and plastid SSRs for the same populations used here (Aguirre-Planter *et al.*, 2000; Jaramillo-Correa *et al.*, 2008), and thus should provide a good proxy for estimating G'_{ST} (Heller & Siegismund, 2009). Markers were amplified under conditions described in Supporting Information, Appendix S2 and in Múgica-Gallart (2013). PCR products were analysed on an ABI Prism 3730xl (Applied Biosystems) with molecular standard Liz 600 (Applied Biosystems), which was used as a reference for genotyping with the software GeneMarker v.2.2.0 (SoftGenetics).

After correcting genotypes for the putative presence of null alleles with Microchecker (Van Oosterhout *et al.*, 2004), we tested for departures from Hardy–Weinberg expectations in all populations, and for linkage disequilibrium between loci with GENEPOP v.4.7 (Rousset, 2008); we used 20 batches of 5000 iterations each, which followed a dememorization of 10 000 steps. We then estimated standard genetic diversity indices per population using GenAlex v.6.5. (Peakall & Smouse, 2012); these included the mean number of alleles A; the number of effective alleles, A_e and the expected heterozygosity, H_e. Population inbreeding (F_{IS}) was further calculated with Fstat v.2.9.3.2. (Goudet, 2002).

We initially examined population genetic structure through global, species, local (i.e. local population F_{ST}; Faubet & Gaggiotti, 2008) and individual F_{ST} values estimated with the *hierfstat* package for R. We determined individual values from a vector derived from a pairwise F_{ST} matrix; 99% confidence intervals were estimated using 10 000 bootstrap replicates. We further inferred standardized values of population differentiation (G'_{ST}; Hedrick, 2005) to account for possible underestimations of F_{ST}, given that we were employing highly polymorphic markers (SSRs) in outbreeding species with high heterozygosity (Leinonen *et al.*, 2008). Such values were calculated per species and population, with their respective 99% confidence intervals, with the *mmod* package (Winter, 2012). Species and local G'_{ST} values were retained for the G'_{ST}-P_{ST} comparisons. Then, to explore how well taxonomy explained our genetic data, we performed an analysis of molecular variance (AMOVA) using Arlequin v.3.5.2.2 (Excoffier & Lischer, 2010) by assuming two groups corresponding to each species (*A. flinckii* and *A. religiosa*) and using the groups obtained from the STRUCTURE analysis below.

We inferred the most likely number of gene pools (K-value) with STRUCTURE v.2.3.3 (Pritchard, Stephens & Donnelly, 2000). We performed ten runs for each K-value (set from 1 to 20), each consisting of 500 000 Markov chain Monte Carlo (MCMCs) iterations, which followed an initial 125 000 steps that were discarded as *burn-in*. Considering the number of loci employed, we selected a non-admixture model with correlated allele frequencies after removing the LOCPRIOR option (Hubisz *et al.*, 2009). The most likely K-value was chosen based on ΔK (Evanno, Regnaut & Goudet, 2005), which was computed in STRUCTURE HARVESTER (Earl & VonHoldt, 2012).

F_{ST}-P_{ST} COMPARISONS

Because field tests were unavailable, we approximated Q_{ST} from the phenotypic differentiation of populations (P_{ST}; Leinonen *et al.*, 2006; Brommer, 2011) for each retained morphological trait:

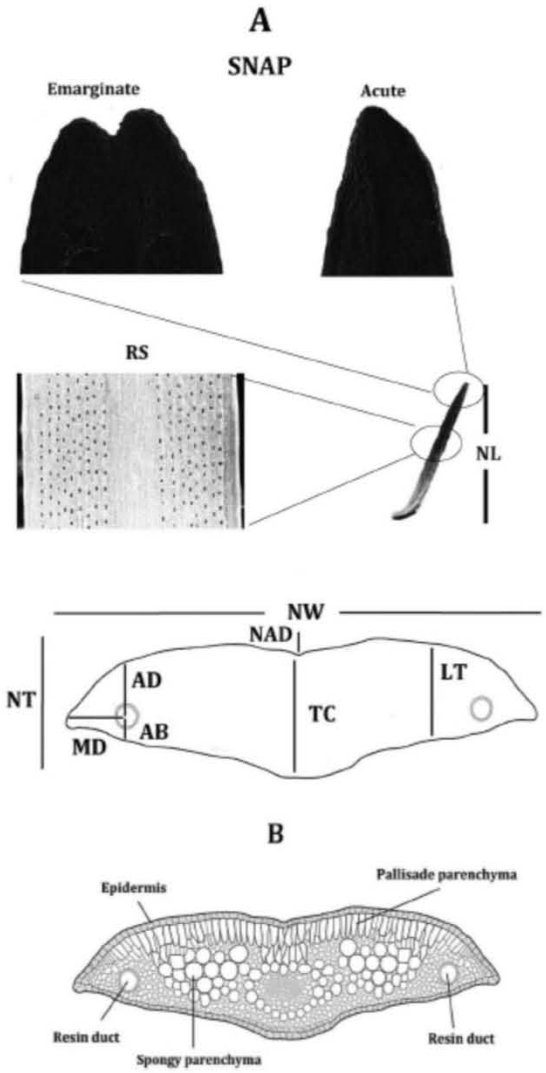

Figure 3. Morphological traits of needles evaluated for *Abies flinckii* and *Abies religiosa* (A), anatomical structure observed in transversal sections of these needles (B).

$$P_{ST} = \frac{(\frac{c}{h^2})\sigma_B^2}{(\frac{c}{h^2})\sigma_B^2 + 2\sigma_W^2} \quad (1)$$

where, σ_B^2 and σ_W^2, are, respectively, the between- and within-population phenotypic variance, h^2 is the heritability of the trait (not available) and c is a constant ascertaining the partitioning of additive genetic effects across populations (also not available); c determines the proportion of phenotypic differentiation that can be accounted for by differences in the distribution of additive genetic variation among localities or by phenotypic plasticity and non-additive effects (Pujol *et al.*, 2008).

In the absence of field tests, and therefore in the absence of estimates of heritability and additive variation, the accuracy of P_{ST} to approximate Q_{ST} depends on the c/h^2 ratio, which was estimated following Seeholzer & Brumfield (2018):

$$\frac{c}{h^2} = \frac{-2F_{ST(upper)}\sigma_{W(upper)}^2}{\sigma_{B(lower)}^2(F_{ST(upper)} - 1)} \quad (2)$$

where $\sigma^2_{W\,(upper)}$ is the upper confidence interval (CI) value for the within-populations phenotypic variance, $\sigma^2_{B(lower)}$ is the lower CI value for the between-populations phenotypic variance and $F_{ST\,(upper)}$ is the upper CI value estimated from the genetic markers (F_{ST} or G'_{ST}). c/h^2 ratios closer to zero are considered to provide robust evidence that P_{ST} exceeds F_{ST} and thus that phenotypic differentiation deviates from neutral expectations (Brommer, 2011). Following this rationale and taking into account results from a compilation on various taxa (Brommer, 2011), we considered c/h^2 ratios < 0.25 to indicate strong evidence for natural selection driving phenotypic differentiation, 0.26–0.50 to reflect moderate selection, 0.51–0.75 weak selection and values closer to or higher than one suggesting very weak or no selection; such values should further suggest that differentiation mostly results from random changes (i.e. genetic drift), plasticity and environmental effects (Brommer, 2011). P_{ST} was estimated globally, per species and per population.

Variance components (σ^2_B and σ^2_W) for P_{ST} and confidence intervals (99%) were obtained by extracting mean squares (MS) and sum of squares, respectively, from an ANOVA. The dependent variable on these models was the *ln* of each morphological trait (R Development Core Team, 2018). The confidence intervals were employed for calculating c/h^2. Finally, as our study system is distributed longitudinally, we explored, through linear regressions, the relationship between local P_{ST} for each trait and longitude to examine the possibility of a stepwise variation for these traits (Faubet & Gaggiotti, 2008).

RESULTS

Morphological variation

All sampled individuals had linear needles that were elliptically shaped in transverse section. All needles had a marked protrusion on the abaxial side and a distinct notch on de adaxial side. The spongy parenchyma of all samples was disrupted on the abaxial portion by two resin ducts (Fig. 3B). Stomata were mostly found on the abaxial face, although some rows were observed near the apex on the adaxial side in some individuals of both taxa. Stomata were elliptically distributed, forming parallel rows (Supporting Information, Appendix S3). Species were clearly differentiated by needle length (NL; longer in *A. flinckii*, Supporting Information, Appendix S4) and apex shape (emarginate in *A. flinckii* and acute in *A. religiosa*; Fig. 4A).

We found significant differences for almost all analysed traits at the inter- and intraspecific levels (Supporting Information, Appendices S4 and S5A, B). Morphological variation was considerably lower within populations, especially for traits related to the position of the resin duct in *A. flinckii* (Kruskall–Wallis test; $P < 0.05$, Supporting Information, Appendix S5C). Characters exhibiting higher variation within populations were those related to needle thickness (NT, TC and LT), and the number of stomata rows (RS; $P < 0.05$, Supporting Information, Appendix S5C). The first two components of the multivariate analyses captured 61.5% (PCA) and 75.4% (LDA) of the morphological variance, respectively. They allowed two groups coinciding with species identity to be distinguished (Fig. 4B). Several groups of correlated variables were identified with these multivariate analyses and the correlation matrix (Supporting Information, Appendix S6). Consequently, three morphological traits (NT, RS and AB) and their corrected ratios for co-variation with needle size (NT/NW, NL/RS, AB/AD) were retained for performing the correlations with climate and geography.

Climate variation and correlation with longitude and morphological traits

Results of all correlations between the six morphological variables retained, longitude and the four climate variables gathered (Bio 1, 3, 14 and 17) are listed in Table 3; the significant relationships found are illustrated in Fig. 5. In *A. religiosa*, morphology was correlated with climate in seven regressions out of 24, after Bonferroni correction (linear regressions, $P < 0.05$), but no correlation with longitude was observed. More specifically, NT and AB were both positively correlated with the precipitation of the driest month and the driest quarter, and with the mean annual temperature (Fig. 5F, H). In *A. flinckii*, only one morphological trait (RS) showed correlation with isothermality (Bio 3) and longitude (Fig. 5A, C), suggesting contrasting forces driving morphological differentiation for this species when compared with *A. religiosa*. Only two of the correlations with climate remained significant (in *A. religiosa*) when using corrected ratios for needle size (NT/NW with Bio14 and Bio 17), and one more of the corrected ratios, AB/AD, become significantly associated with Bio 14 in *A. flinckii* (Table 3). No corrected ratios were associated with longitude in any species.

Genetic diversity

Genetic diversity indices for all populations are listed in Table 4. Overall indices were not significantly different between species (Table 4), except for A_e (Mann–Whitney–Wilcoxon test; $P < 0.05$). Elevated inbreeding coefficients were observed for both species ($F_{IS} = 0.18$ for *A. religiosa*

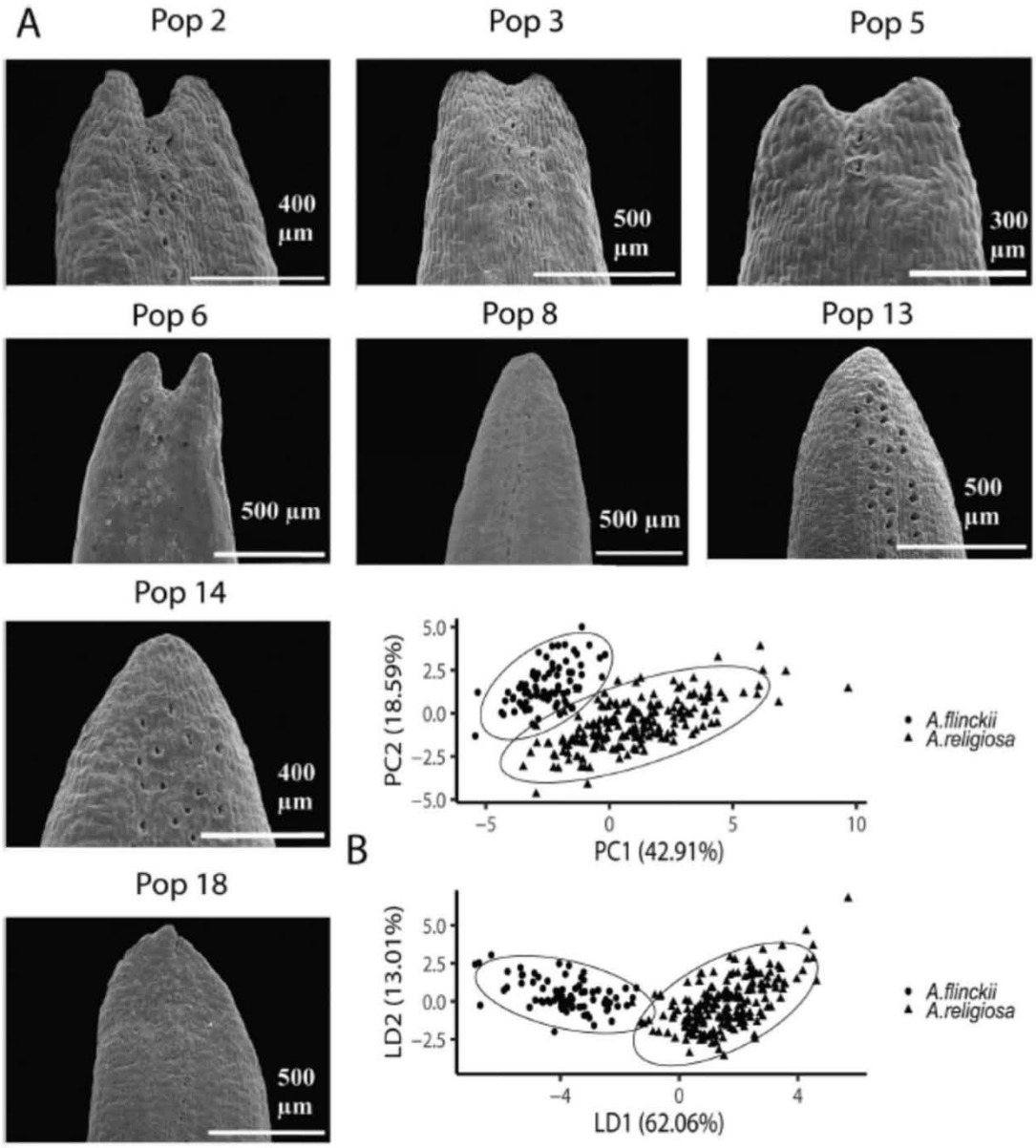

Figure 4. Examples of apex shape observed in individuals of *Abies flinckii* (pops 2, 3, 5, and 6) and *A. religiosa* (pops 8, 13, 14 and 18) (A). Scatter-plots for the first two principal components (top) and discriminant linear functions (bottom) obtained from the variation of 15 and 14 morphological traits, respectively (B).

and = 0.15 for *A. flinckii*), although these values were also similar between taxa ($P > 0.05$). Significant departure from Hardy–Weinberg expectations were observed in three populations of *A. flinckii* and ten populations of *A. religiosa*. Similar to previous results for the SSR surveyed, no linkage disequilibrium was observed.

Bayesian statistical modelling for clustering genotypes implemented by STRUCTURE returned the peaks for ΔK at $K = 2$ and $K = 4$ (i.e. four clusters) in analyses using all populations. In general, *A. flinckii* is composed of two genetic and geographically isolated clusters, one formed by populations 4 and 5 and one by populations 3 and 6. The westernmost population of *A. religiosa* (#11) was genetically distinct from the rest (Fig. 2A–C; Supporting Information, Appendix S7A, B). No further genetic subdivisions were observed at higher *K*-values for *A. religiosa*, whereas populations 3, 4, 5 and 6 of *A. flinckii* formed their own genetic

Table 3. Adjusted r^2 values obtained between morphological traits with longitude and climatic variables for seven natural populations of *Abies flinckii* and 18 natural populations of *A. religiosa*. $^*P < 0.05$, $^{**}P < 0.01$, after Bonferroni correction. *RS*, number of stomata rows; *NT*, needle thickness; *AB*, distance from the centre of the resin duct to the abaxial needle surface; *Bio 1*, annual mean temperature; *Bio 3*, isothermality; *Bio 14*, precipitation of driest month and *Bio 17*, precipitation of driest quarter

		Longitude		Bio 1		Bio 3		Bio 14		Bio 17	
Species	Variable	r^2 adj.	*P* value	r^2 adj.	*P* value	r^2 adj.	*P* value	r^2 adj.	*P* value	r^2 adj.	*P* value
A. flinckii	RS	**0.808**	**0.007****	0.000	1.000	**0.807**	**0.008****	0.237	0.303	0.000	1.000
	NT	0.565	0.063	0.000	1.000	0.242	0.297	0.036	0.638	0.000	1.000
	AB	0.575	0.059	0.000	1.000	0.285	0.250	0.202	0.347	0.069	0.567
	NL/RS	0.000	1.000	0.394	0.155	0.000	1.000	0.037	0.637	0.213	0.321
	NT/NW	0.000	1.000	0.000	1.000	0.000	1.000	0.185	0.369	0.000	1.000
	AB/AD	0.439	0.125	0.000	1.000	0.081	0.541	**0.611**	**0.046***	0.418	0.139
A. religiosa	RS	0.000	1.000	0.027	0.484	0.000	1.000	0.000	0.883	0.006	0.622
	NT	0.007	0.608	0.129	0.158	0.000	1.000	**0.327**	**0.016***	**0.329**	**0.016***
	AB	0.000	0.739	**0.381**	**0.008****	0.000	1.000	**0.335**	**0.014***	**0.379**	**0.008****
	NL/RS	0.000	1.000	0.055	0.356	0.000	0.859	0.203	0.069	0.110	0.196
	NT/NW	0.095	0.229	0.150	0.126	0.021	0.514	**0.387**	**0.007****	**0.344**	**0.012***
	AB/AD	0.008	0.603	0.000	1.000	0.000	1.000	0.054	0.357	0.034	0.450

pool at $K = 7$ (Supporting Information, Appendix S7B). A series of AMOVAs showed that differentiation was lower when grouping populations by taxonomy ($F_{CT} = 0.018$) than when using the groups provided by STRUCTURE at $K = 4$ ($F_{CT} = 0.062$; Table 5). However, in both cases, most of the variation was located within populations. Within species, analyses further revealed that local and mean genetic differentiation was higher for populations of *A. flinckii* ($F_{ST} = 0.125$; $G'_{ST} = 0.664$) than of *A. religiosa* ($F_{ST} = 0.039$; $G'_{ST} = 0.398$); local values were particular elevated for the isolated stands from the western part of the TVB (i.e. populations 3–6 and 11; Table 4).

COMPARING PHENOTYPIC DIFFERENTIATION AND NEUTRAL GENETIC DIFFERENTIATION

After establishing the presence of a linear relationship between geography and climate and the possibility of IBA with three phenotypic characters [the number of stomata rows (RS), NT, and distance from the centre of the resin duct to the abaxial needle surface (AB); Table 3; Figs 1 and 5] and estimating G'_{ST}, we tested for phenotypic divergence (P_{ST}) for these traits, while comparing with neutral genetic population differentiation. Global phenotypic divergence (P_{ST}) for these morphological traits was higher than G'_{ST} for both species, except for NT in *A. flinckii*. Neutral genetic differentiation among populations (G'_{ST}) ranged from 0.46 to 0.77 in *A. flinckii* and 0.20 to 0.51 in *A. religiosa*. When using G'_{ST} as a reference of genetic divergence to estimate c/h^2, this ratio was > 0.75 in all traits, indicating $P_{ST} \approx G'_{ST}$, except for NT and AB in *A. religiosa*. For these characters, c/h^2 values indicated $P_{ST} > G'_{ST}$. Thus, phenotypic and genetic divergence were within the same range for *A. flinckii*, but they were significantly different for *A. religiosa* in at least two traits (Table 6, Fig. 6A).

Only needle thickness consistently showed higher phenotypic than genetic differentiation when P_{ST} values were estimated locally in nine populations out of 14, three with moderate signal (c/h^2 ratios, 0.25–0.50) and the remaining ones with weak signal (c/h^2, 0.51–0.75). Abaxial distance showed moderate signals of selection in one population and weak evidence in three others (Fig. 6B, Supporting Information, Appendix S8). Such differences indicate putative conditional neutrality across populations. No relationship between P_{ST}/trait-longitude was detected; we discard a stepwise model for these traits (Supporting Information, Appendix S9).

DISCUSSION

ABIES RELIGIOSA AND *A. FLINCKII* ARE TWO DISTINCT, ANCIENT SPECIES THAT HAVE RECENTLY COME INTO CONTACT

In this study, we showed that the firs (*Abies*) from central Mexico clearly constitute two distinct morphological groups with abundant admixture in the zones of contact (Figs 2, 4; Table 5). Despite traits that are shared between species (such as the number and position of the resin ducts and shape and disposition of stomata), two main morphological groups coinciding with the species identity are evident (Fig. 4). These two groups could be mostly distinguished by needle length (longer in *A. flinckii*) and apex shape (emarginate in *A. flinckii* and acute in *A. religiosa*; Fig. 3A, Supporting Information,

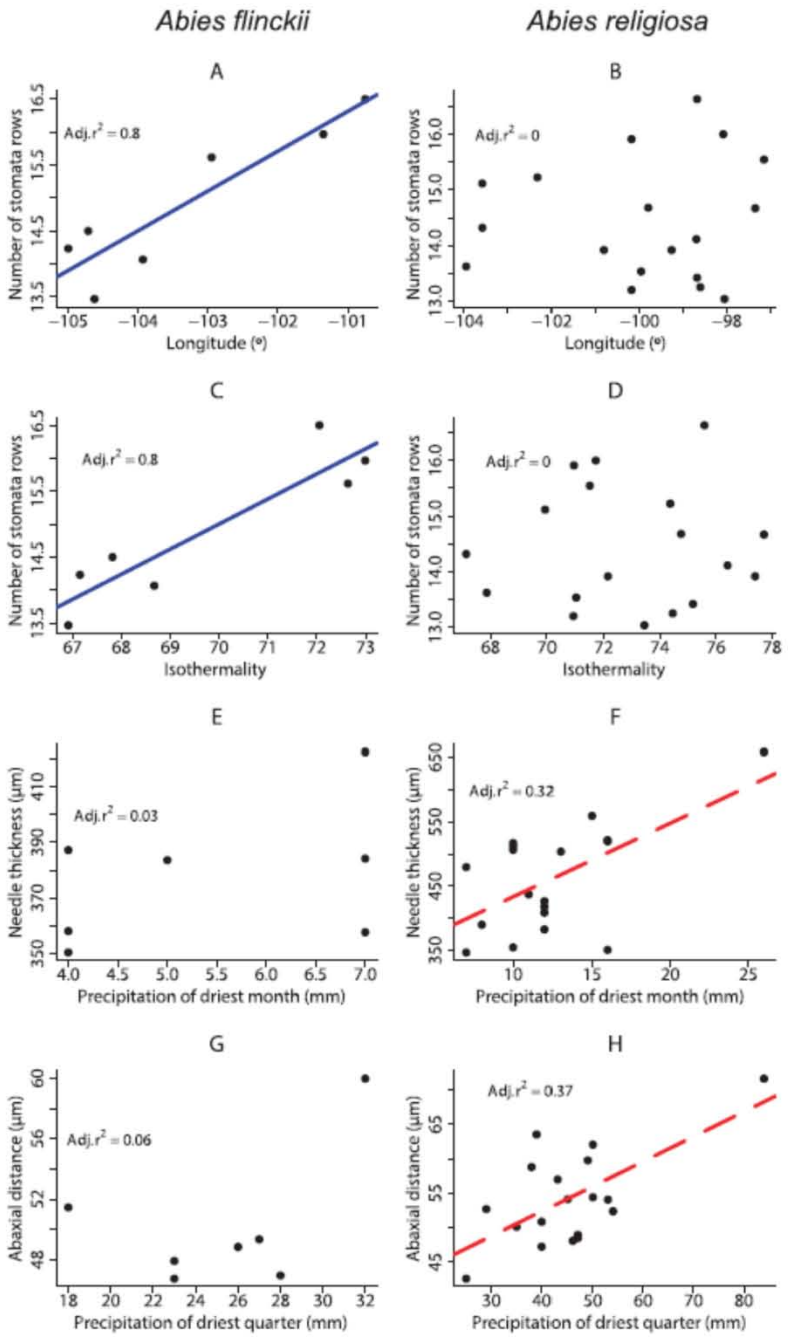

Figure 5. Correlations between the three most relevant morphological traits and longitude (A-B), isothermality (C-D), and precipitation (E-H) for populations of Abies flinckii (left) and A. religiosa (right).

Appendix S4). Consequently, and contrary to previous treatments of the genus (Eckenwalder, 2009; Farjon, 2010), our results support the recognition of *A. flinckii* and provide some evidence for further subdivision (i.e. the two gene pools found for $K = 4$; Fig. 2), as in the recognition of the westernmost populations (4 and 5) as

Table 4. Genetic diversity in populations *Abies flinckii* (pop 1–6) and *A. religiosa* (pop 8–21). *A*, mean number of alleles; A_e, effective number of alleles; H_e, expected heterozygosity; F_{IS}, inbreeding coefficient; F_{ST}, local genetic differentiation; G'_{ST}, standardized values of F_{ST} (Hedrick, 2005), † mean estimates per species, NS, not significant, *$P < 0.05$

Pop	*A*	A_e	H_e	F_{IS}	F_{ST}	G'_{ST}
1	15.5	10.3	0.902	+0.152*	0.062	0.579
2	10.5	6.3	0.835	+0.231*	0.070	0.584
3	5.3	3.3	0.674	+0.182^{NS}	0.070	0.690
4	13.8	7.5	0.864	+0.246*	0.077	0.681
5	10.0	6.4	0.826	+0.042^{NS}	0.074	0.640
6	4.5	2.6	0.601	+0.027^{NS}	0.119	0.711
Mean	**9.9**	**6.1**	**0.780**	**0.150**	**0.125**†	**0.664**†
8	14.3	9.4	0.891	+0.248*	0.028	0.273
9	13.8	7.9	0.864	+0.243*	0.032	0.311
10	15.0	8.7	0.878	+0.277*	0.030	0.296
11	6.0	3.7	0.710	+0.192^{NS}	0.083	0.709
12	13.8	8.6	0.883	+0.163*	0.031	0.322
13	14.8	8.4	0.868	+0.080*	0.033	0.333
14	18.0	12.8	0.915	+0.217*	0.027	0.283
15	13.5	8.3	0.866	+0.100^{NS}	0.032	0.297
16	13.3	8.7	0.881	+0.249^{NS}	0.033	0.364
17	13.0	8.5	0.878	+0.270*	0.031	0.301
18	16.5	10.5	0.901	+0.151*	0.034	0.410
19	13.0	8.9	0.884	+0.149*	0.030	0.292
20	12.8	6.7	0.847	+0.156*	0.038	0.387
21	11.5	7.2	0.857	+0.136^{NS}	0.037	0.393
Mean	**13.5**	**8.4**	**0.866**	**0.188**	**0.039**†	**0.398**†

Table 5. Analyses of molecular variance (AMOVA) on SSR frequencies considering different groupings of populations of *Abies flinckii* and *A. religiosa*. Top, populations grouped by species. Bottom, stands separated according to a Structure clustering analysis for $K = 4$ (see Fig. 2C)

Source of variation	d.f.	Sum of squares	Variance components	Percentage variation	*F*
Among species	1	18.341	0.034	1.83	$F_{CT} = 0.018$
Among populations within species	18	120.907	0.125	6.62	$F_{SC} = 0.067$
Within populations	778	1344.758	1.728	91.55	$F_{ST} = 0.018$
Total	797	1484.006	1.888		
Among groups	3	55.600	0.121	6.28	$F_{CT} = 0.062$
Among populations within groups	16	83.648	0.087	4.52	$F_{SC} = 0.048$
Within populations	778	1344.758	1.728	89.20	$F_{ST} = 0.108$
Total	797	723.446	1.906		

A. jaliscana, as proposed elsewhere (Vázquez-García *et al.*, 2014). The recognition of *A. flinckii* has a genetic and climatic basis that translates into a contrasting needle morphology (Fig. 5). Such genetic isolation could further justify its threatened/special protection status (SEMARNAT, 2010; Thomas, 2013) and indicate that additional measures, such as promoting gene flow between populations, are necessary to increase its chances of survival.

The differential genetic assignment of individuals corresponding to *A. religiosa* or *A. flinckii* (Fig. 2) suggests that the divergence of these taxa is rather ancient and probably occurred in allopatry. Although this still has to be tested with more adequate molecular and bioinformatics data, e.g. using genotyping-by-sequencing with approximate Bayesian computation to infer demographic histories (Cornille *et al.*, 2016), the complex biogeographical, climatic and geological history of the study area (a sky-island dynamic that promotes divergence and speciation; Gugger *et al.*, 2011; Mastretta-Yanes *et al.*, 2015), further reinforces this hypothesis.

Table 6. Results of P_{ST}-G'_{ST} comparisons for the number stomata rows (RS), needle thickness (NT) and distance from the centre of the resin duct to the abaxial needle surface (AB) for *Abies flinckii* and *A. religiosa*. G'_{ST}: standardized values of population genetic differentiation; σ^2_B, σ^2_W: the variance components of each morphological trait; P_{ST}: phenotypic differentiation of populations under the null assumption of $c/h^2 = 1$. All these estimates are followed by 99% confidence intervals. c/h^2 is the critical value at which the lower confidence interval for P_{ST} exceeds the upper 99% quantile of G'_{ST}. Values of c/h^2 lower than one are in bold; this is a signal that natural selection may have shaped the variation of this trait

Species	Trait	G'_{ST}	lower	upper	σ^2_B	lower	upper	σ^2_W	lower	upper	P_{ST}	lower	upper	c/h^2
A. flinckii	RS	0.664	0.461	0.770	0.144	0.048	1.298	0.031	0.021	0.050	0.698	0.323	0.968	6.992
	NT				0.173	0.057	1.559	0.049	0.033	0.078	0.639	0.268	0.959	9.107
	AB				0.261	0.087	2.357	0.032	0.022	0.052	0.801	0.455	0.982	3.994
A. religiosa	RS	0.398	0.204	0.515	0.146	0.068	0.462	0.046	0.035	0.062	0.613	0.356	0.867	1.921
	NT				0.941	0.442	2.980	0.054	0.042	0.073	0.897	0.751	0.973	**0.351**
	AB				0.585	0.275	1.852	0.055	0.042	0.074	0.841	0.649	0.956	**0.574**

Moreover, the genetic differences between *A. flinckii* and *A. religiosa* are also apparent with other types of genetic marker (Aguirre-Planter *et al.*, 2000; Jaramillo-Correa *et al.*, 2008).

If our hypothesis of an ancient divergence is correct, then the complex hybrid zone observed in central Mexico is the result of secondary contact (Fig. 2B, C), and the current or historical gene flow that has existed among stands is not compromising the phenotypic differentiation of species (Fig. 4). Furthermore, the relatively low rates of cytoplasmic DNA introgression reported in previous studies (Jaramillo-Correa *et al.*, 2008) and the palynological evidence suggesting that these taxa only experienced elevational migrations during the glaciations of the Pleistocene (Caballero *et al.*, 2010) may indicate that this contact zone has been geographically stable over a long period of time. Accordingly, independent in-depth surveys with genomic datasets are necessary to examine the evolutionary history of this region, and test hypotheses about the direction and putative adaptive role of these genetic exchanges (Barton & Hewitt, 1985; Cinget *et al.*, 2015; Abbott, 2017).

SELECTION VS. DRIFT IN MORPHOLOGICAL TRAITS

The correlations found here suggest that demographic and adaptive factors are not equally affecting the populations of both species. Indeed, the contrasting associations observed across taxa between their genetic and morphological differentiation and both climate and geography support our third hypothesis of phenotypic evolution, i.e. the morphological/climatic variation of the geographically secluded *A. flinckii* showed mostly an IBD pattern, whereas that of the widely distributed *A. religiosa* conformed better to an IBA pattern, at least for two key morphological traits [needle thickness and distance from the centre of the resin duct to the abaxial needle surface (abaxial distance)], Figs 1, 5, 6).

The P_{ST}-G'_{ST} comparisons for the retained morphological traits (number of stomata rows, needle thickness and abaxial distance) further suggest the predominant action of stochastic forces over selection in shaping the phenotypic variation of the restricted *A. flinckii* (Fig. 6A). Theory predicts that in small populations with reduced additive variance where divergent selection can act, genetic drift in combination with reduced gene flow is expected to produce such geographical phenotypic clines (Savolainen, Pyhäjärvi & Knürr, 2007; Le Corre & Kremer, 2012). Although our results for *A. flinckii* fit these expectations reasonably well, other factors like phenotypic plasticity can also be invoked. Indeed, partially adaptive plasticity can alleviate maladaptation in marginal populations, preventing them for becoming sinks (Chevin & Lande, 2011). However, controlled tests in contrasting habitats are necessary to account for the amount of phenotypic variation that can be explained by such plastic responses (Trussell, 2000). Such tests are, however, currently unavailable for Mexican firs (Muñoz *et al.*, 2011).

In contrast, the correlations between phenotypic variation and climate in *A. religiosa*, together with the significant P_{ST}-G'_{ST} differences, indicate that adaptive components could be more important than genetic drift and/or phenotypic plasticity in accounting for the morphological variation of this species (Le Corre & Kremer, 2003), at least for two morpho-physiologically important characters (Fig. 6A, B; see below). If we assume that this species reached its current elevation range *c.* 9000 years BP (Lozano-García *et al.*, 1993; Caballero *et al.*, 2010) and that its generation time is *c.* 50–60 years (Ávila-Bello *et al.*, 2015), 150–180 generations would have been available for selection to act, which is more than enough time to generate measurable phenotypic

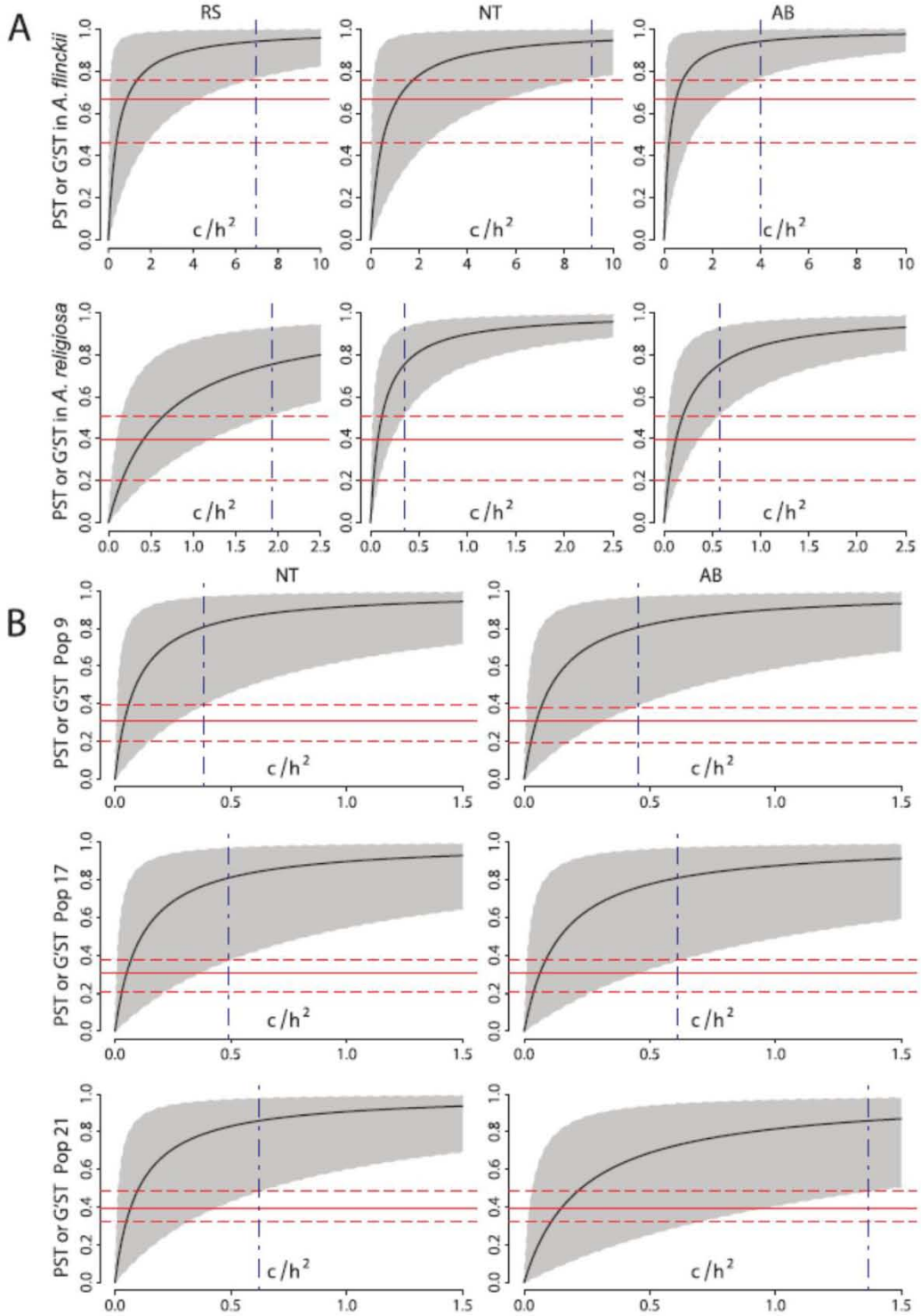

Figure 6. Comparison of phenotypic divergence (P_{ST}; black curve line) for the three most relevant traits driving the morphological divergence between Abies flinckii and A. religiosa and neutral genetic differentiation (G'_{ST}; horizontal red line). Estimates are presented for each separate taxon and for each trait within species (A). Illustrative examples for three locations of A. religiosa are shown in (B). The gray zone denotes the lower and upper 99% confidence intervals for P_{ST}, and the dashed red lines the same

differences between populations, even under moderate to weak selective regimes such as those hypothesized here (Kremer & Le Corre, 2012), and when using conservative estimates to approximate P_{ST} from c/h^2 (Leinonen *et al.*, 2006; Brommer, 2011).

Possible morpho-physiological adaptations in *A. religiosa*

The two morphological traits for which selection could be invoked in *A. religiosa* (i.e. NT and AB; Fig. 6) are linked to physiological processes related to water use and photosynthetic efficiency, as needle thickness increased with precipitation (Fig. 5F, H). In evergreen species this could represent a trade-off between decreasing leaf photosynthesis and nitrogen concentration for thicker leaves, so higher precipitation sites should be less palatable to herbivores (Turner, 1994; Cunningham, Summerhayes & Westoby, 1999; Santiago *et al.*, 2004; Santiago & Mulkey, 2005). The correlation between NT and the precipitation during the driest quarter in *A. religiosa* (Fig. 5F) hints at either a plastic response or a putative adaptive role for water use in these trees; thus, performing directed tests to disentangle these two hypotheses is necessary (Chin & Sillett, 2016). Recent studies suggest that increased leaf thickness and reduced water content might have an adaptive role against frost in Mediterranean taxa, including conifers (González-Zurdo *et al.*, 2016; Niinemets, 2016). Whether this also holds for subtropical firs, which are only episodically confronted with frost and environmental heterogeneity, is still a hypothesis worth testing.

Other than water availability, changes in needle morphology can also result from differences in the amount of light captured, which can affect cell volume and content in the palisade parenchyma (Knapp & Carter, 1998; Robakowski *et al.*, 2004); such changes would allow adjusting photosynthetic capacity (Wise, Sassenrath-Cole & Percy, 2000). Needle thickness is actually correlated with light availability within individuals of *A. amabilis* Douglas ex J.Forbes (Sprugel, Brooks & Hinckley, 1996), which implies a plastic response in this trait. However, our sampling strategy was aimed at minimizing such plastic effects. For character measurements, we only took into account trees located far away from population borders and for which we had three or more samples from different locations in the crown. Besides, we always sampled on the lower portion of the crown to avoid light-exposed needles, which are usually two to three times thicker than their shadow counterparts (Sprugel *et al.*, 1996). Dissecting P_{ST} values into local estimates also helped to disentangle plastic from putatively adaptive effects, and showed that such adaptive factors could be invoked in more than half of the populations, which is more than the number expected by chance.

A character for which adaptive responses cannot be safely invoked in *A. religiosa* is the distance from the centre of the resin duct to the abaxial needle surface (AB). Although this trait showed a significant correlation with precipitation, local P_{ST} estimates were significantly higher than G'_{ST} in only four locations (9, 10, 14 and 17; Fig. 6B, Supporting Information, Appendix S8); even the global P_{ST} value was only marginally significant (Table 6, Fig. 6A). This character is seldom measured in quantitative genetic/evolutionary surveys in conifers, and its adaptiveness is still uncertain.

Studies in pines have shown that the number, area and position of resin ducts may have an adaptive component, and be related to climate, drought and herbivore defence (López, Climent & Gil, 2010; Huang *et al.*, 2016; Jankowski *et al.*, 2017). Indeed, more and larger canals that are closer to the needle margins might provide more effective protection against freezing temperatures (Jankowski *et al.*, 2017); they may also mobilize and liberate more easily defensive compounds against defoliators and endophytes (Phillips & Croteau, 1999). However, plastic responses have also been inferred for resin canal-related traits across trial sites in a subtropical pine (López *et al.*, 2010), which complicates any inference based solely on phenotypic differences across populations by approximating Q_{ST} from P_{ST}.

Field tests are thus necessary to confirm the adaptive nature of these characters. However, our results allow for more specific experimental designs to be developed and for local testing of adaptive hypotheses under a quantitative genetics framework that takes individual relatedness into account (Troth *et al.*, 2018). Given the biological features of conifers and the nature of the evaluated phenotypic traits, it should come as no surprise that these characters are plastic to a certain degree (Willis & Niklas, 2004; López *et al.*, 2010; Scheepens, Frei & Stöcklin, 2010). Indeed, *A. concolor* (Gordon & Glend.) Lindl. ex Hildebr. (a close relative of the firs studied here) has shown plastic responses in ecophysiological traits related to water use over time (Grulke, 2010), which indicates that phenotypic plasticity must be addressed in future studies with Mexican firs.

intervals for G'_{ST}. The vertical blue line indicates the critical c/h^2 value at which the lower confidence limit of P_{ST} equals to upper confidence limit of G'_{ST}; above this point, P_{ST} no longer exceeds G'_{ST}. The lower this critical value, the more solid inferences of selection are to environmental effects (because there is low confidence interval overlap; indicating $P_{ST} \neq G'_{ST}$).

PERSPECTIVES: HOLISTIC PHENOMICS AND PHYLOGENOMICS

Other than performing field trials to test for the putatively adaptive nature of the analysed traits, a more holistic, functional view of phenotypes (phenomics) might be necessary to understand the biological drivers of morphological variation and speciation of firs and conifers in general. For instance, all traits discussed above can be linked to water use efficiency or herbivore defence; thus, its integration with ecophysiological and high throughput phenotypic measures should provide a neural view of biological processes that could be linked to fitness at both organism and population scales (Negin & Moshelion, 2017; Ubbens & Stavness, 2017). They could further be combined to recently developed genome-wide association tools to pinpoint gene networks involved in these processes (Bazakos *et al.*, 2017), and into predictive methods to forecast individual/population performances under possible future environmental conditions (Jaramillo-Correa *et al.*, 2015; Benito Garzón et al., 2018a, b).

In the particular case of *A. religiosa*, we could predict that populations bearing individuals with thicker needles will be more tolerant to future drier conditions, and that such tolerance should be incorporated into conservation and management programmes (Benito Garzón *et al.*, 2018a). In *A. flinckii*, it would be important to evaluate other traits (e.g. drought resistance, cold tolerance) in field experiments for elucidating the role of divergent selection, overshadowed here by the high genetic differentiation between populations.

Functional phenotypic views can be further combined with phylogenomics approaches to improve our knowledge and resolution of species boundaries and taxonomy (Younis *et al.*, 2018). For the particular case of the Mexican *Abies*, it should not only allow testing the taxonomic propositions available, particularly in the west of the TVB (Eckenwalder, 2009; Farjon, 2010; Vázquez-García *et al.*, 2014), but also to clarify the ecological and evolutionary basis of species subdivision and adaptation. In addition, as can be seen from our results, a holistic species subdivision not only facilitates a better design of conservation units, but allows for a better management of genetic resources.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

CAPITULO IV: Testing for niche centrality within the scope of the nearly neutral theory of evolution in a subtropical conifer species-pair

En este capítulo se probó la hipótesis de la centralidad del nicho ecológico. Se partió del supuesto según el cual en el centroide del nicho ecológico se dan las condiciones óptimas para el desarrollo de las especies, de tal suerte que en ese punto se espera mayor abundancia, tamaños poblacionales más grandes y por ende mayor diversidad genética. Para ello se calcularon las distancias al centroide del nicho ecológico para un par de especies del centro de México (*A. flinckii* y *A. religiosa*). Estas distancias junto con el área de las poblaciones fueron utilizadas como variables predictoras de diversidad genética calculada a partir de SSRs nucleares y 11 secuencias codificantes. Siguiendo esta misma idea se realizó una extensión, hacia la teoría casi neutral, donde se espera que en poblaciones más pequeñas (más lejanas al centroide del nicho ecológico), la selección purificadora tiende a relajarse. También determinamos si cada especie tiene diferentes preferencias ambientales lo que pudiera indicarnos una mayor divergencia entre especies, para ello se determinó la similitud de los nichos ecológicos. No se encontró relación con las variables predictoras, es decir, las distancias al centroide del nicho ecológico y el área con la cantidad de diversidad genética neutral/eficiencia de la selección purificadora. Excepto en la variación neutral con *A. flinckii*, donde al parecer marcadores altamente polimórficos y una relativa estabilidad demográfica contribuyen a observar una relación con la centralidad del nicho. Los nichos ecológicos de estas especies fueron similares; sin embargo, el traslape ambiental fue moderado.

Testing for niche centrality within the scope of the nearly neutral theory of evolution in a subtropical conifer species-pair

Running title: Niche centrality and genetic diversity in Mexican firs

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Abstract

Aim: There is growing interest in understanding how climate factors account for evolutionary processes that shape genetic diversity at both the population and species levels. The niche centrality hypothesis (NCH) predicts that genetic diversity accumulates more when ecological conditions are optimal (i.e. near the niche centroid). From the point of view of the nearly neutral theory (NTT) of evolution, this implies that marginal populations should accumulate more partially deleterious variants (and have higher π_N/π_S) than stands near the centroid. However, this may hold only under historical centrality (i.e. centrality have remained despite of post-glacial dynamics) and mutation-drift/drift-migration equilibrium.

Location: Transmexican Volcanic Belt, central Mexico.

Taxon: A fir sister species-pair endemic to central Mexico, *Abies flinckii* and *A. religiosa.*

Methods: We compared, between and within species, patterns of genetic diversity derived from nuclear SSRs (A, H_e) and gene-coding sequences (π_N, π_S), also we estimated the efficacy of purifying selection (π_N/π_S). After testing for niche overlap, we correlated indices with distance to the niche centroid under both current and Last Glacial Maximum (LGM) climatic conditions. Finally, we estimated the individual contribution of climatic and geographic variables to genetic diversity for each species.

Results: Narrowly distributed *A. flinckii* had lower genetic diversity than widespread *A. religiosa*. Populations of *A. flinckii* are currently closer to their niche centroid than they were during the LGM; current distances to the centroid were further negatively correlated with diversity at SSRs. Genetic diversity for *A. flinckii* could be largely accounted for by soil potassium content. On the other hand, populations of *A. religiosa* are currently at similar distances to the centroid as they were during the LGM. There was no relationship between distance to the centroid and genetic diversity at any time frame for this species. However, temperature, elevation and soil pH explained large parts of the variation of its neutral genetic diversity between populations.

Main conclusions: Other elements (like outcrossing rate, seed output, selection against inbred seedlings and microenvironmental variation) seem more important than niche centrality to account for the accumulation of genetic variation in long-lived species, like forest trees. The NCH should only hold at early life or successional stages in these plants.

Key words: *Abies*, ecological marginality, effective population size, niche centroid, microsatellites, neutral genetic variation.

1 | INTRODUCTION

A central goal in evolutionary biology is to understand how genetic diversity accumulates within and between species. The classic *neutral theory* (NT) of molecular evolution predicts that the rate at which variants appear and are maintained within a population is mostly determined by the interaction between mutation and random genetic drift (Kimura, 1968). The *nearly neutral theory* (NNT; Ohta, 1973) expanded this view and included weakly deleterious mutations, which should be more effectively eliminated in large populations than in small ones, where these mutations could be maintained over long periods of time, and even reach fixation as a result of a reduced efficiency of purifying selection. Such a premise translates, in the absence of gene flow and assuming constant mutation rates, in a negative relationship between effective population size and the ratio of nonsynonymous to synonymous polymorphism, π_N/π_S (Chen, Glémin, & Lascoux, 2017; Ellegren & Galtier, 2016; Henn, Botigué, Bustamante, Clark, & Gravel, 2015). However, because intrinsic demographic, geographic and biological factors indirectly affect effective population size, the rate at which species' accumulate genetic diversity should correlate with these factors and condition their survival and adaptability (Glémin, Bazin, & Charlesworth, 2006; Lowe, Kovach, & Allendorf, 2017).

Among the factors above, environmental heterogeneity and connectivity are of particular interest (Austerlitz, Mariette, Machon, Gouyon, & Godelle, 2000; Hamrick, Godt, Sherman, & Susan, 1992; Hewitt, 2000; Kremer & Le Corre, 2012; Nybom, 2004), as they are related to the size and geographic breadth of a species ecological niche and, ultimately, to the niche centrality hypothesis (NCH; Maguire, 1973; Martínez-Meyer, Díaz-Porras, Townsend Peterson, & Yáñez-Arenas, 2013; Yañez-Arenas, Martínez-Meyer, Mandujano, & Rojas-Soto, 2012). This hypothesis postulates that species should be more abundant (and thus more genetically diverse) when they occur in optimal environmental conditions, and rarer (and more genetically depauperate) when they grow in marginal environments (Lira-Noriega & Manthey, 2014; Manthey et al., 2015).

Limitations of the NCH include that it only holds under certain demographic and population genetics premises. For instance, species are expected to be at migration-drift and mutation-drift equilibrium and have experienced environmental stability over time (Duncan *et al.*, 2015; Pironon *et al.*, 2015; Osorio Olvera *et al.*, 2016). Consequently, complex historical or demographic events, like the rapid migratory waves during the Pleistocene glacial cycles (Excoffier & Ray, 2008; Hewitt, 2004), can make patterns of genetic variation stray off the NCH expectations (Guo, 2012). Likewise, estimates of genetic diversity are assumed to reflect current demographic conditions, which might be problematic when working with long-lived or rapidly expanding species that may not be at gene flow-drift equilibrium (Petit & Hampe, 2006). Finally, the NCH indirectly assumes genetic variation to be strictly neutral, while the accumulation of

partially deleterious or the fixation of adaptive variants in marginal populations can bias NCH expectations (Aguirre-Liguori *et al.*, 2017). Indeed, specific variables and environment heterogeneity could better explain the accumulation of genetic diversity through either a direct effect on population growth and purifying selection efficacy and/or by driving local adaptation through partial selective sweeps in marginal stands (Ortego *et al.*, 2012; Gugger, Ikegami, & Sork, 2013; Sork *et al.*, 2016). These limitations are often invoked to explain why only some species fit NCH predictions, while many others show no or opposite patterns of genetic diversity (Lira-Noriega & Manthey, 2014).

Temperate species from Mesoamerica are interesting models to test for NCH. Palynological studies indicate that they survived the Pleistocene glacial cycles *in situ*, experiencing only elevational migrations (Lozano-García *et al.*, 1993; Caballero *et al.*, 2010). They are further distributed in a sky-island system with limited gene flow (McCormack, Huang, & Knowles, 2009; Aguirre-Planter et al., 2012), in which each individual population has its own demographic history and has likely had enough time to adapt to its local environmental conditions and possibly reach migration-drift and mutation-drift equilibrium. Herein, we focus on two endemic firs (*Abies flinckii* Rushforth and *A. religiosa* (Kunth) Schltdl. & Cham.) distributed along the Transmexican Volcanic Belt (TVB) in central Mexico, at elevations of approximately 2,000 and 3,000 m. asl, respectively. These two species are genetically, phenologically and morphologically distinct (Mantilla-Blandón, 2006; Jaramillo-Correa *et al.*, 2008; Vázquez-García *et al.*, 2014; Cruz-Nicolás *et al.*, 2020), and have geographic ranges with contrasting sizes. *A. flinckii* is restricted to small fragmented populations in the west of the TVB, while *A. religiosa* forms large forests in the central and eastern portions of this mountain range (Figure 1).

In this study, we explore the niche centrality hypothesis within the framework of the nearly neutral theory of molecular evolution. We aimed to understand how ecological factors might condition the accumulation of genetic diversity by indirectly affecting effective population size. We first compared the levels of genetic diversity of *Abies flinckii* and *A. religiosa* with two types of genetic markers with contrasting mutation rates, microsatellites and gene-coding sequences. Having higher mutation rates, microsatellites should better reflect present or relatively recent demographic events, while gene coding sequences should be more adequate to explore past population size changes through their effect on the site frequency spectrum, and the differential accumulation of neutral and partially harmful variants (Zhai, Nielsen, & Slatkin, 2009). Then, following the NCH, we tested whether populations near the centroid (representing putatively optimal ecological conditions; Lira-Noriega & Manthey, 2014; Manthey et al., 2015) have higher levels of genetic diversity than those growing in marginal environments and, following the NNT, whether these marginal stands had higher π_N/π_S ratios than populations

inhabiting more optimal conditions. Given the contrasting biological features of the surveyed species, we also expected differences between taxa (*A. flinckii* having lower diversity and higher π_N/π_S than *A. religiosa*). However, as environmental stasis is anything but granted in long-lived species, we analysed how historical centrality affected the amount of genetic diversity between and within taxa by performing projections into the LGM and comparing them with niche models from the present (Pironon *et al.*, 2015). Finally, we analysed if genetic variability can be best explained by specific environmental factors, rather than purely demographic events, and discuss the implications for forest conservation and management.

2 | MATERIALS AND METHODS

2.1 | Sampling and estimates of genetic and nucleotide diversity

We collected needles from 399 trees in six and 14 populations of *A. flinckii* and *A. religiosa*, respectively (Figure 1 and Table 1). Populations were selected to cover as much as possible the natural range of each taxa. Sampled individuals were at least 30 m from each other (for more details see Aguirre Planter, Furnier, & Eguiarte, 2000; Méndez-González, Jardón-Barbolla, & Jaramillo-Correa, 2017). DNA was extracted with a modified 2% CTAB protocol (Vázquez-Lobo, 1996; Doyle and Doyle, 1990), and its concentration and quality evaluated with a Qubit™ V 3.0 (Thermo Fisher Scientific) and through electrophoresis on 0.8% agarose gels. We first amplified four nuclear SSRs (*Ab*07, *Ab*12, *Ab*20, *Ab*27) according to conditions described elsewhere (Cruz-Nicolás *et al.*, 2020). PCR products were analysed on an ABI Prism 3730xl Analyzer (Applied Biosystems) along with molecular standard Liz 600 (Applied Biosystems), which was used as reference for genotyping with GeneMarker v.2.2.0 (SoftGenetics). Genotypes were analysed for putative presence of null alleles with Microchecker (Van Oosterhout *et al.*, 2004), and both the mean number of alleles per locus (A) and the expected heterozygosity (H_e) were calculated for each population with GenAlex v. 6.5. (Peakall & Smouse, 2006).

We further amplified and sequenced a subset of individuals (N = 37 for *A. flinckii*; N = 84 for *A. religiosa;* see Table 1) for eleven gene-coding loci with primers retrieved from transcriptome and re-sequencing studies in *Abies alba* (Mosca et al., 2012; Roschanski, Fady, Ziegenhagen, & Liepelt, 2013; see Table 2 for details). Sequences were also obtained for two individuals of the boreal North American fir, *A. balsamea*, which were used as outgroup for diversity estimates (Ramírez-Lerma, 2012; Giles-Pérez, 2017). Sanger sequencing was performed at the High-Throughput Genomics Unit (HTGU) in the Department of Genome Sciences of University of Washington. The resultant sequences were edited and aligned with BioEdit v. 7.2.6 (Hall, 1999), and polymorphic sites corroborated through visual inspection of the

chromatograms in CodonCode Aligner (CodonCode Corporation). Identification of coding and non-coding regions was performed by comparison with transcriptomes of *A. alba* and *Picea glauca*. Haplotypes were 'phased' with the Bayesian algorithm PHASE implemented in PHASE v. 2.1.1 (Stephens, Smith, & Donnelly, 2001); we performed five independent runs, each consisting of 10,000 iterations, which followed an initial 1,000 steps that were discarded as burn-in. We assumed a general model of recombination (Ramírez-Lerma, 2012; Giles-Pérez, 2017).

We used aligned sequences to estimate summary statistics for each locus including *D* (Tajima, 1989), *H* (Fay & Wu, 2000), H_{norm} (Zeng *et al.*, 2006) and F_s (Fu, 1997). We then used these statistics and coalescence simulations to test different demographic hypotheses for each species. Demographic frameworks were simulated with *mlcoalsim* v. 1.42 (Ramos-Onsins & Mitchell-Olds, 2007) and included stable populations through time, and various expansion-contraction combinations with contrasting strength and duration (See Figure S1.1 for details). These last models were based on sky-island migratory hypotheses previously proposed for the TVB (Mastretta-Yanes *et al.*, 2015).

Coalescent simulations were parameterized using the observed θw and the population recombination parameter. The same four summary statistics above were collected from 10,000 MCMC iterations for each species. Compatibility with a demographic scenario was assumed when all observed statistics did not show significant differences from their posterior distribution (averaged across all 11 genes). Overall likelihoods were computed when more than one model was compatible (Ramos-Onsins & Mitchell-Olds, 2007; Grivet *et al.*, 2009). Then, individual gene values for each summary statistic were compared with the distribution of expected values under the most compatible demographic model. Genes exhibiting significant differences for any statistic were considered possible candidates for selection and were removed from the dataset.

For the retained genes, we estimated the nucleotide diversity, π (Tajima, 1983) for synonymous and non-synonymous positions in coding regions (π_S and π_N, respectively) and their ratio (π_N/π_S; McDonald & Kreitman, 1991). This ratio is a proxy for the efficacy of purifying selection, with values close to or greater than 1.0 indicating the accumulation of partially deleterious mutations because of relaxed purifying selection (Böndel et al., 2015; Chen et al., 2017). Average values per population were used for the linear regressions below.

2.2 | Ecological niche modelling

We estimated the Grinellian niche for each species separately by focusing on climatic dimensions. We assumed that these variables provide a good proxy for the fundamental niche, although we recognize that having physiological measures from field tests should increase resolution (Soberón, 2007b; Peterson *et al.*, 2011). We first obtained coordinates of occurrence for each taxa

from our own field collections (Aguirre-Planter *et al.*, 2000; Cruz-Nicolás *et al.*, 2020), and a public database available from Mexico's National Commission for Biodiversity (CONABIO; http://www.conabio.gob.mx/institucion/cgi-bin/datos.cgi?Letras=JM&Numero=15; see also Martínez-Méndez *et al.* 2016). To avoid redundancy and model overfitting, we restricted datasets to 19 points for *A. flinckii* and 51 occurrences for *A. religiosa.* We then gathered information for each retained location for 19 bioclimatic variables from the WorldClim database (Hijmans *et al.*, 2005) at *c.* 1 km^2 spatial resolution. We performed projections to Last Glacial Maximum conditions (LGM, *c.* 20 ka) to examine variations in distances to the niche centroid in the present and at 20 ka. Our argument is that the historical dynamics of species ranges might have a more direct impact on genetic diversity than current climate (Duncan *et al.*, 2015; Pironon *et al.*, 2015). We performed such projections using past climate layers for the LGM (2.5 arc-minutes pixel size *c.* 21.62 km^2) based on CCSM4 and MIROC-ESM global models (Hijmans *et al.*, 2005; Braconnot *et al.*, 2007).

We initially constructed Ecological Niche models that were projected onto LGM with MAXENT v. 3.3. (Phillips, Anderson, & Schapire, 2006). However, because different algorithms may perform differently depending on each species' particularities (Qiao, Soberón, & Peterson, 2015), and following the idea that fundamental niches are convex in shape and may be viewed as multi-dimensional ellipsoids, we further predicted and compared the performance of niche models using ellipsoids, as implemented in Niche ToolBox v. 0.4.1.5 (Osorio-Olvera *et al.*, 2018). Given that the calibration area (*M*) may influence the outcome of niche model predictions, particularly for those algorithms like MAXENT that are sensitive to an environmental background (Barve *et al.*, 2011; Soberón, Peterson, & Osorio-Olvera, 2018), we used two different *M*'s that could be biogeographically relevant for the two focal species: a 80 km and a 400 km buffer around the terrestrial ecoregion comprising the TVB (Olson *et al.*, 2001). In total, we obtained four niche models (M1-M4) per species (two methods × two *M* areas) both for the present and for the LGM. M1 (e.g., MAXENT with a buffer area of 80 km around the TVB) showed the most consistent results; thus, most analyses and interpretations were based in this model. Results for other models (M2-M4) are available as Supporting information (Appendix S1: Tables S1.1-1.4 and Figures S1.2-S1.4). Niche overlap and similarity between species were respectively evaluated following Broennimann et al. (2012) and the framework proposed by Warren, Glor and Turelli (2008), after correcting for spatial autocorrelation. Further details for these tests and ecological niche modelling are provided in Appendix S1.

We calculated niche centroids for both the present and the LGM following Martínez-Meyer et al. (2012, 2013). Following these authors, the niche centroid was assumed to be the mean of the multidimensional space for each model. To get the vector of values of each model's, we extracted values for all environmental variables from binary distribution maps constructed from

each species with a threshold of minimum training presence output, and then standardized them (mean = 0 and standard deviation =1) to set niche centroids at zero. Then, we calculated multidimensional Euclidean distances to the niche centroid for each population in the present and the LGM, to explore how population's centrality fluctuated over the last 20ka. To estimate the niche breadth for each species, we assumed a correlation between marginality and tolerance for each population. To do so, we measured the maximum departure of the centroid of the niche from the global centroid (Dolédec, Chessel, & Gimaret-Carpentier, 2000; Peterson *et al.*, 2011). We used the Euclidean distances the centroid for each presence point, calculated as a standardized difference between the values for each point and the mean values for all points within the climate space; these calculations were performed using the first six axes from a PCA (Velasco *et al.*, 2016), see Appendix S1 for details. To test for possible effects of postglacial altitude migration on distances to the centroid, we performed a correlation between elevation and distance to the niche centroid. Our hypothesis here was that populations at higher elevations were at farther distances from the niche centroid than populations at lower elevations (Caballero *et al.*, 2010; Sáenz-Romero *et al.*, 2012).

2.3 | Correlations between genetic diversity, niche centrality and population area

We tested whether locations closer to the ecological niche centroid had more optimal conditions for the development of the focal species than marginal locations, which result in differential accumulation of genetic diversity (Maguire, 1973; Martínez-Meyer *et al.*, 2013; Lira-Noriega & Manthey, 2014). For each species, linear regressions were performed in R (R Development Core Team, 2018) between estimates of genetic diversity (A and H_e for the nuclear SSRs and π_N, π_S for the gene-coding sequences) and the distance to the niche centroid per population. Following the NNT, regressions were also performed between π_N/π_S and the distance to the niche centroid, to evaluate whether the more marginal populations exhibited less efficient purifying selection than those closer to the optimal environmental conditions. In all cases, significance was evaluated after Bonferroni correction ($P < 0.05$).

Classic conservation programs propose that effective population size can be approximated by current population size and used as a proxy for inferring genetic variation (Frankham, 1996; Leimu & Fischer, 2008). We evaluated this possibility by correlating diversity estimates with current population area (km^2). Given that *Abies* populations in central Mexico are mostly monospecific (Hernández, 1985; Rzedowski, 2006), we retrieved a land use and vegetation cover layer from the Instituto Nacional de Estadística Geografía e Informática database (INEGI, 2003) serie II; scale 1:1 000,000. This layer was originally built from satellite images (Landsat TM), and crosschecked with field observations. We selected field/type/fir forest in Arc Gis 10.5 and, with

the *raster* calculator function, estimated the area of the selected polygons. Map resolution and natural population size only allowed us to estimate areas and perform analyses for *A. religiosa.* The smaller population sizes of *A. flinckii*, and its trend to form mixed forests with pines and oaks prevented us for estimating areas for this species.

2.4 | Redundant Discriminant Analysis (RDA)

To disentangle how climate and spatial factors may account for the genetic variation between and within species (Ortego *et al.*, 2012; Riordan *et al.*, 2016; Harrison *et al.*, 2019), we performed a series of partial redundancy analyses (RDA) with variance partitioning using '*vegan*' (Oksanen *et al.*, 2015) in R (R Development Core Team, 2018). Redundancy analyses are the multivariate equivalent of a linear model; they are similar to the canonical correspondence analysis, but are more powerful for detecting complex relationships between (species or genetic) diversity and environmental variables, while taking into account the spatial components of population structure (Legendre & Legendre, 2012).

We separately used neutral diversity estimates (log of H_e and π_S) as the response matrix for the RDAs and divided the explanatory variables into environmental and spatial, all variables were previously standardized. The environmental matrix consisted of the raw climate variables previously used in the ecological niche models above and estimates of two edaphic factors, soil potassium (K) content and pH, that account for forest soil productivity in the mountains of central Mexico (Peña-Ramírez, Vázquez-Selem, & Siebe, 2009). The K and pH values for each population were obtained from layers available in Cruz-Cárdenas et al. (2014) at a *c.* 1 km^2 spatial resolution; these layers were generated from 4400 soil samples across Mexico. The spatial matrix included longitude, elevation and population area in km^2 (when available). We ran two different RDAs for each species: a partial model of environmental variables controlling for spatial effects, and a partial spatial model controlling for climate effects. We then used variance partitioning to calculate the proportion of the genetic variation that could be explained by the independent contribution of each environmental and spatial factor. For each model, we determined the overall significance of each explanatory variable using 999 permutations. We finally calculated the adjusted coefficient of multiple determination (Adj. R^2), and identified the most significant components of spatial and environmental variance for each model by using a stepwise backward model selection process with the *leaps* package (Lumley & Miller, 2017) for R.

3 | RESULTS

3.1 | Genetic and nucleotide diversity

All SSR loci were polymorphic; collectively, they exhibited 124 size variants. The number of alleles per locus ranged from 27 for Ab07 to 35 for Ab27, with a mean of 31 alleles per locus. Inferred null alleles had low frequencies at all loci < 0.2 (Appendix S2: Table 2.1), and no linkage disequilibrium was detected between marker pairs. Average SSR genetic diversity was significantly lower (*ANOVA, P* < 0.05) in *A. flinckii* (*A* = 9.92, SE = 0.93; H_e = 0.78, SE = 0.03) than in *A. religiosa* (*A* = 13.50, SE = 0.43; H_e = 0.86, SE = 0.01; Table 1 and Figures 2a and 3). Within species, *A. flinckii* exhibited higher heterogeneity among populations than *A. religiosa*, with *A* values ranging from 4.5 to 15.5, and H_e estimates between 0.60 and 0.90; such values ranged between 6.0 and 18.0 (*A*), and 0.710 and 0.915 (H_e) in *A. religiosa* (Table 1 and Figure 2a).

All gene-coding loci were successfully amplified and sequenced in both focal species and in the species used as outgroup (*A. balsamea*). Altogether, these eleven loci covered over 6.31 Kbp, and contained 721 variable sites, which represent one SNP every 9 bp. In terms of number of segregating sites, the most variable loci were *CesA1* and *COBRA-like*, and the least variable were *ArMybIX* and a *heat shock protein* gene (Tables 2, Appendix S2.2-2.3). However, three loci (*CesA1*, *COBRA-like* and *Porin Mip1a*) were excluded because they consistently showed an excess of high-frequency variants (Appendix S2: Table S2.3), especially for non-synonymous mutations in *A. flinckii* and the westernmost populations of *A. religiosa.* These values could reflect selective pressures unique to significant genes or confounding effects between selective and demographic forces (Akey *et al.*, 2004). Consistent with SSR variation, gene-sequence nucleotide diversity was lower in *A. flinckii* than in *A. religiosa*, both at synonymous (π_S = 0.00077, SE = 0.002 for *A. flinckii*; and 0.00184, SE = 0.002 for *A. religiosa; ANOVA, P* < 0.05) and non-synonymous sites (π_N = 0.00035, SE = 0.0009 for *A. flinckii*; and 0.0005, SE = 0.0008 for *A. religiosa; ANOVA, P* > 0.05). Interestingly, such values translated to overall π_N/π_S ratios that were lower (although non-significantly) in *A. flinckii* (mean = 0.0257, SE = 0.0749) than in *A. religiosa* (mean = 0.0991, SE = 0.1919; Table 1, Fig. 2).

The most likely demographic scenarios in *A. flinckii* consisted of a strong and ancient population collapse (down to 15% of its ancestral size), and a constant size until present. The most likely demographic framework for *A. religiosa* was more complex; it included an ancient population contraction (down to 50% of its ancestral size) coinciding with the Last Interglacial, which was followed by a significant expansion (three times the ancestral size) during the Last Glacial Maximum, and a more recent population decline that has diminished its effective size by one third during the Holocene (Appendix S1: Figure S1.1). These two frameworks were consistent with the significant differences observed between species for three of the four

summary statistics. Both, Tajima's D (P = 0.003) and Fu's F_S (P = 0.001) were higher in *A. flinckii* (mean = 0.293 and 0.518, respectively) than in *A. religiosa* (mean = -0.596 and -0.174, respectively; Table 1), while Zheng's H_{norm} was lower in the first species (mean =-0.265) than in the second one (mean =-0.124; P = 0.008). No differences were observed for Fay and Hu's H (mean = -0.161 for *A. flinckii* and -0.157 for *A. religiosa*; P = 0.226; Table 1).

3.2 | Ecological niche models

All models tested (M1-M4) for both species were better than null random expectations, according to the binomial and partial ROC tests for both the present and the LGM (Appendix S1: Table S1.2). Current Euclidean distances projected on geographical space showed that the centroid for *A. flinckii* was mostly located in the western parts of TVB (with some isolated patches in the center), and in the central and eastern portions of this mountain chain (and to a less extent, in isolated locations in the west; Figures 3 and Appendix S1: Figure S1.2) for *A. religiosa*. Present niche overlap, as evaluated with Shoener' D, was moderate (0.47) but significant ($P < 0.05$), which implies that both species are currently occupying climatically similar environments. According to the PCA (first and second components explained 62.66% of the environmental variance; Appendix S1: Figure S1.3), small niche differences were driven by variables related to precipitation (*bio*12, *bio*14, *bio*19) and seasonality (*bio*4 and *bio*15). According to Euclidean distances, populations of *A. religiosa* tend to inhabit more marginal conditions than stands of *A. flinckii*, which are all presently located close to the niche centroid (Appendix S1: Table S1.3 and Figure S1.4; *ANOVA, $P < 0.01$*). When we visualized the niche models in a bivariate environmental space (Fig. 4a and 4b), we detected a higher point aggregation towards the niche centroid for both species; however, *A. religiosa* exhibited more presence points towards the niche periphery and thus a wider niche breadth than *A. flinckii* (*ANOVA, $P < 0.01$*; Figure 4c).

According to most of our projections to the LGM (M1, M2 and M4), populations of *A. flinckii* are now inhabiting more optimal conditions than in the past, when they were at larger average distances from the niche centroid; these differences (i.e., present-LGM) were, however, not significant (*ANOVA, $P > 0.05$*). On the other hand, stands of *A. religiosa* are located at similar distances from the centroid than they were 20 ka years ago (Figure 5a, Appendix S1: Table S1.4 and Figure S1.4). Interestingly, there was a positive and significant correlation between distances to the centroid and the current elevation of *A. religiosa* stands (Pearson's R = 0.803; $P < 0.001$), while no such a correlation was observed for *A. flinckii* (Pearson's R = 0.656; $P > 0.10$).

3.3 | Relationship between genetic diversity and environmental surrogates

The mean number of SSR alleles (*A*) was negatively and significantly correlated with the present distance of populations to the niche centroid (model M1) in *A. flinckii* (R^2 = 0.73; p < 0.05; Figure 5b). A similar trend was detected for the expected heterozygosity (H_e), but in this case the correlation was not significant (P > 0.05; Table 3). In *A. religiosa*, only H_e showed a significant correlation with the current distance of populations to the niche centroid (Table 3, Figure 5C and Appendix S1: Table S1.5) but, unexpectedly, this correlation was positive (R^2 = 0.31, P < 0.05). No significant correlation was observed for any species between the average nucleotide diversity at synonymous (π_S) or non-synonymous sites (π_N), and their ratios (π_N/π_S), and the present distance of populations to the niche centroid (Table 3 and Appendix S1: Table S1.5 and Figure S1.5).

Estimated areas for the sampled populations of *A. religiosa* ranged between 4.9 and 121 km^2 (Appendix S1: Table S1.3). These areas were not correlated to their longitude, elevation, estimates of genetic diversity (for both SSRs and gene-coding sequences) or the current distances to the niche centroid (P > 0.1; Table S1.5). As mentioned above, it was not possible to determine area for *A. flinckii*, due to small population size and extensive association with pines and oaks.

RDA models that best explained H_e included only environmental variables (soil K content; $R^2_{adj.}$ = 0.65, P = 0.019) in *A. flinckii*, and both environmental (*bio*1 and soil *pH*; R^2_{adj} = 0.51, P = 0.017) and spatial variables (longitude and elevation; R^2_{adj} = 0.35, P = 0.022) in *A. religiosa*. Models constructed for explaining π_S variation indicated only a marginal contribution (*P > 0.1*) of some variables (latitude and soil K content; Table 4) in *A. flinckii*. No significant model could be built for explaining π_S in *A. religiosa*. Different RDA models were consistent in variable contribution, particularly of soil *K* content for explaining H_e in *A. flinckii*, and *bio*1 and elevation for accounting for H_e variation in *A. religiosa* (Table 4).

4 | DISCUSSION

We found contrasting amounts of genetic variation and, according to the values of π_N/π_S, similar efficiency of purifying selection in two firs from central Mexico with contrasting range sizes. We further detected a significant relationship between current population niche centrality and the mean number of alleles per locus (*A*) for the rarer and geographically restricted *A. flinckii*, and no correlation for the more geographically widespread *A. religiosa*. Redundancy analyses provided some putative drivers for this accumulation, showing that mean *H*e values could be explained by soil *K* content in *A. flinckii*, and a combination of soil pH, elevation and mean annual temperature could account for such variation in *A. religiosa*. Our results indicate that differential environmental factors intrinsic to each species are conditioning the rate at which they are

accumulating neutral genetic diversity, which should be carefully considered in future surveys. This study highlights the resilience and versatility of forest trees (particularly conifers) to retain genetic diversity over long periods of time and provides some key environmental variables that might help disentangle patterns of evolutionary rate in subtropical conifers.

4.1 | Contrasting patterns of genetic diversity

Consistent with basic population genetics expectations, the rarer and more geographically isolated *A. flinckii* had lower genetic diversity than the more widespread and abundant *A. religiosa*, at both nuclear SSRs and gene-coding sequences (Table 1). Such trends coincide with those reported in previous studies with allozymes and cytoplasmic DNA markers for the same species (Aguirre-Planter *et al.*, 2000; Jaramillo-Correa *et al.*, 2008) and are indicative of a larger effective population size in *A. religiosa*.

After discarding gene coding regions with atypical values and bearing a potentially adaptive signal (i.e., *CesA1, COBRA-like* and *Porin Mip1a*; Appendix S2: Table S2.3), the average summary statistics and best-fitting demographic scenarios simulated herein for gene-coding sequences suggest contrasting population size changes between these two taxa. For instance, most Tajima's *D* and Fu's *F*s values were either close to or greater than zero in *A. flinckii*, indicating demographic stasis and decline, while these estimates were negative in *A. religiosa*, suggesting demographic expansion (Figure 2e-f). The best-fitting demographic scenarios provide further details (Appendix S1: Figure S1.1). They suggested an ancient population collapse, followed by a relative stability for *A. flinckii*, and a complex combination of contractions and expansions for *A. religiosa*. Both the volcanic activity and climate changes during the Pleistocene in the TVB could be invoked to account for such differences. Indeed, the western portions of the this mountain chain are older, and have experienced less volcanic activity during the last ~500 ka than the central and eastern ones (Ferrari *et al.*, 2012; Mastretta-Yanes *et al.*, 2015), which should have granted more environmental stability for *A. flinckii* than for *A. religiosa*. However, this western region also has less suitable surface for the development of fir forests than the central/eastern parts of the TVB.

Interestingly, π_N/π_S was not significantly different between species (although it was slightly higher in *A. flinckii*), roughly indicating that both taxa are equally effective in eliminating partially deleterious variants despite such contrasting demographic histories. While it must be noted that these differences were evaluated with only a modest number of genes and caution is advisable, several hypotheses can be drawn from our results. For instance, they indicate that the efficiency of purifying selection has somehow been decoupled from demographic and

evolutionary history in these taxa, and that the mechanisms for purging slightly deleterious mutations are similarly effective in both species. This idea was also suggested for two European pines with contrasting demography, and which showed no differences in selection efficiency (Grivet *et al.*, 2017). On the other hand, such similarities could be merely incidental, and the be the result of a long demographic stasis in *A. flinckii* (which provided more time for eliminating partially deleterious variants) and a less drastic population contraction in *A. religiosa* (which allowed for a lower accumulation of mildly harmful alleles). In any case, our results illustrate that fixation of (partially) deleterious mutations is also reduced in subtropical conifers. Explanations may include a predominantly outcrossing mating system, and strong selection against inbreed individuals at early life stages (Buschiazzo *et al.*, 2012; Chen *et al.*, 2017). However, a wider genome sampling, together with more specific estimates of adaptive evolution and gene load (Eyre-Walker & Keightley, 2009) are still necessary to understand how differential biological factors affect the accumulation of genetic diversity in these subtropical conifers.

4.2 | Ecological niche modelling

Agreeing with results from a previous survey that includes *A. flinckii* and *A. religiosa*, and which compares environmental overlap (Martínez-Méndez *et al.*, 2016), we observed no significant differences in the ecological niche of these species, although both taxa did have contrasting niche breadths, as *A. religiosa* occupied a more diverse ecological hyperspace than *A. flinckii* (Figure 4). Narrow niche breadths have been associated with increased diversification rates, likely prompted by allopatric speciation (Velasco *et al.*, 2016). Such a possibility is worth testing in the western parts of the TBV, where *A. flinckii* occurs, and where other morpho-species of *Abies* have been described, leading to an ongoing taxonomic debate (Eckenwalder, 2009; Farjon, 2010; Vázquez-García *et al.*, 2014). Even if a previous phylogenetic study suggested that diversification of Mesoamerican firs best fits an environmental stasis model with decreased extinction rates (Aguirre-Planter *et al.*, 2012), results from this and a recent morphological survey (Cruz-Nicolás *et al.*, 2020) indicate the need to re-evaluate this hypothesis with more advanced phylogenomic tools (i.e., only six cpDNA markers were available for estimating diversification rates in Mexican firs; Aguirre-Planter et al., 2012). Indeed, studies in Mexican pines (some of which are co-distributed with the focal species of this study) suggest that niche and morphological differentiation are correlated (Aguirre-Gutiérrez *et al.*, 2015; Ortiz-Medrano *et al.*, 2016), likely reflecting increased diversification.

Independently of the evolutionary consequences of niche differentiation, both *A. religiosa* and *A. flinckii* appear to occupy only a small portion (relatively near to the niche centroid) of the potentially available climatic space, although some exceptions were observed for *A. religiosa* (Fig.

4). This might indicate that either realized niches are narrower than estimated or that biological or demographic factors are preventing species from fully exploiting their climatic breadths. We performed several preliminary calibrations using various edaphic and physiographic variables, but none of them seemed to improve niche resolution (data not shown). However, we do recognize that including physiological measures and biotic interactions might improve niche estimate. For instance, on a finer scale, Peguero-Pina et al. (2007) observed differences in physiological and growth variables in shoots and needles of *Abies alba* from populations located in climatically contrasting sites.

Potential demographic and biological factors that are worth exploring in future studies include insufficient time for niche diversification (Morlon, Potts, & Plotkin, 2010; Moen & Morlon, 2014), niche fragmentation during isolation in refugia (Serra-Varela *et al.*, 2015), inability to adapt to extreme conditions because of small effective population size (especially in *A. flinckii*; Aguirre-Liguori et al., 2017), or unavailability of biotic interactions that facilitate seedling establishment, like nurse plants (Carbajal-Navarro *et al.*, 2019) or mycorrhiza (Argüelles-Moyao & Garibay-Orijel, 2018).

4.3 | Niche centrality and genetic diversity

We assumed the niche centroid to be a proxy for predicting "optimal conditions for species development", such that populations in the niche margins were inhabiting harsher habitats, which potentially affect reproductive success, individual establishment, longevity, outcrossing rate, and long-term population viability (Morrison, Marcot, & Mannan, 1998). Conforming to these expectations, and fitting the neutral theory, mean population SSR diversity for *A. flinckii* diminished as the distance to the centroid increased (Fig. 5b). Interestingly, populations of this species are also currently nearer to the species niche centroid than they were during the LGM (Fig. 5a). This implies a rapid response to better conditions in the accumulation of neutral genetic diversity, particularly at regions with high mutation rates, like SSRs. Indeed, such a relationship was not observed at regions with lower mutations rates, like nuclear gene-coding sequences, which fitted a more stable demographic scenario (Appendix S1: Figure S1.1). These results open the door to future demographic surveys. For instance, with a warmer climate, and populations inhabiting closer to optimal conditions than in the past 20 ka, individual establishment and reproductive success could be increasing in *A. flinckii*, which should provide higher chances of survival for this endangered species.

On the other hand, for *A. religiosa*, whose populations have remained at a relatively similar distance to the niche centroid during the last 20 ka (Fig. 5a), there was only a marginally

significant and positive relationship between niche centrality and genetic diversity (*H*e) at SSRs. That is, populations at the niche margins had higher *H*e than stands near the centroid. As for *A. flinckii*, no correlations were observed between niche centrality and nucleotide diversity. Such results could be reflecting the complex demographic scenario disclosed for this species. That is, current (and past) distances to the niche centroid are not adequately reflecting effective population size, and thus the accumulation of genetic diversity. The lack of a relationship between population area and diversity estimates also points in that direction (Appendix S1: Table S1.5), which seems to be recurrent in long-lived species (Suárez-Montes, Chávez-Pesqueira, & Núñez-Farfán, 2016). Recent evidence suggest that dispersal capacity, outcrossing rate, Allee effects, and a heterogeneous spatial structure of suitability, including the structure of the associated biotic community, may be more important for regulating population size than environment itself (Abeli, Gentili, Mondoni, Orsenigo, & Rossi, 2014; Osorio-Olvera et al., 2016; Dallas, Decker, & Hastings, 2017; Osorio-Olvera, Soberón, & Falconi, 2019). Surveying these factors is thus necessary to understand the drivers of evolutionary rate in long-lived taxa.

The lack of a relationship between π_N/π_S and niche centrality in both species, somehow mirrors the results above. Differences in this ratio have been observed between populations of a single species only when comparing recently colonized and more ancestral areas in annual plants (Böndel *et al.*, 2015; González-Martínez *et al.*, 2017). However, the lack of fast-migrating colonization fronts in subtropical conifers (as observed in their more boreal congeners) might preclude such differences from arising between populations of these species. Such differences, if present, should be only visible along elevation gradients within the same mountain (Ortiz-Bibian *et al.*, 2017), which would require a different sampling strategy than the one used here. In any case, it appears that the elevated outcrossing rates of conifers should be diminishing the influence of environmental marginality in the accumulation of genetic diversity (Chen *et al.*, 2017). That is, historical seed outputs and selection against recessive homozygotes during their long lifespan are elevated enough to ensure globally high outcrossing rates within populations (Mápula-Larreta *et al.*, 2008).

4.3 | RDAs

If environmental marginality cannot explain how Mexican firs differentially accumulate genetic variation, more specific variables may be implicated in such differentiation. We explored such a possibility through RDA analyses, which evidenced specific spatial and environmental traits associated with genetic diversity (H_e) within each species. Heterozygosity in *A. flinckii* was mostly explained by soil potassium concentration, while a significant contribution of temperature, elevation and soil pH was evidenced in *A. religiosa* (Table 4). Both taxa grow in rough and highly

heterogeneous mountain landscapes, which implies that the accumulation of diversity might be modelled at a finer scale than the one studied herein, where physiographic gradients might be more important than global niche characteristics. Soil differences have already been shown to significantly drive population structure at the local scale in *A. religiosa* (Méndez-González *et al.*, 2017), and our results suggest that they should be considered in more detailed surveys aiming to explain how genetic diversity is distributed in this and other congeneric species. Indeed, the contribution of local conditions to effective population size, and therefore viability, appears largely important in many taxa (Sletvold *et al.*, 2013). Such conditions and their local and general effects in genetic diversity, especially H_e, should be the object of future heterozygosity-fitness correlations. A previous study with conifers (Rodríguez-Quilón *et al.*, 2015) highlighted that local effects are more important for driving H_e than more general ones (like niche distance to the centroid), but this contribution was locus-specific, which is contrary to the wider genomic effects hinted by our results.

The putative effect of potassium concentration in the soil on genetic diversity is particularly compelling. This element has been associated with physiological processes regulating internal water flow, like stomatal closure, in various plants (Fournier *et al.*, 2005; Arquero, Barranco, & Benlloch, 2006). We recently found significant morphological differences between and within the species surveyed herein in characters related to water use, including the number of stomata rows, needle thickness, and the mean distance of the resin ducts to the needle's abaxial border (Cruz-Nicolás *et al.*, 2020), which hints that morpho-physiological adaptations might be important drivers for species thriving, and the accumulation of genetic diversity. Physiological studies in the field are however necessary to establish causality (Fournier *et al.*, 2005; Arquero *et al.*, 2006; Cruz-Nicolás *et al.*, 2020).

Another important aspect, especially for *A. religiosa*, is elevation. Mexican montane conifers have been migrating towards higher elevations since the end of the LGM (Caballero, Lozano-García, Vázquez Selem, & Ortega, 2010; Lozano-García, Beatriz, Margarita, & Urrutia-Fucugauchi Jaime, 1993), and they are expected to move even higher during the next decades, following ongoing climate change (Sáenz-Romero *et al.*, 2012). Given that differentiation along elevational gradients has an adaptive component in *A. religiosa* (Ortiz-Bibian *et al.*, 2017), the correlations observed herein between elevation and both H_e and the distance to the niche centroid might indicate that uphill migration and adaptation has been faster than anticipated for this species. Such a possibility should facilitate the proposed assisted migration programs for managing this species (Ortiz-Bibian *et al.*, 2017), but the contribution of local conditions should be carefully considered.

4.4 | Perspectives and final considerations

It appears that variables other than environmental marginality are necessary to account for the accumulation of genetic diversity and purifying selection efficiency in subtropical montane conifers. Unlike more temperate plants, for which range expansions from glacial refugia are a fundamental component for explaining these factors (Hewitt, 2000; 2004), local forces seem more important in subtropical regions (Ramírez-Barahona & Eguiarte, 2013; Mastretta-Yanes *et al.*, 2015, 2018). For instance, the atypical H_{norm} values observed for three genes (*Ces A1, Cobra-like, Porin Mip1a*) in the western most populations of both taxa suggest the action of differential selective forces, or at least conditional neutrality, between regions. Thus, after accounting for phylogeographic structure and landscape effects on gene flow (e.g., Gugger, Gonzalez-Rodriguez, Rodriguez-Correa, Sugita, & Cavender-Bares, 2011; Sork, Gugger, Chen, & Werth, 2016), it seems necessary to explore the putative action of local adaptation at the genome-wide level, and its effect in the differential accumulation of genetic diversity between populations.

Another factor that seems worth exploring when testing for niche centrality is the impact of differential mutations rates, both among genomic regions within species and between species. This seems particularly important for long-lived taxa (Buschiazzo *et al.*, 2012). Longevity implies that perennial plants are accumulating fewer mutations per unit of time than annuals (Petit & Hampe, 2006), which indicates that the effect of a rapid environmental shift that changes the distance to the niche centroid of a population, and thus its N_e, could be only observed through more rapidly evolving markers, like SSRs, than with markers harboring slower mutation rates, like gene-coding sequences. Our study points in that direction. However, differences in mutation rate between species should also be considered. Indeed, taxa accumulating more mutations per unit of time could exhibit more readily any effect of niche shift on N_e than species with lower mutation rates. Such differences have not been reported so far between congeneric conifers, but, although unlikely, they could constitute an explanation for the contrasting diversity figures observed herein between *A. flinckii* and *A. religiosa*.

Finally, our study also informs on the conservation status of the two focal species. *A. flinckii*, and all fir populations from the western portion of the TVB, are currently listed as threatened (SEMARNAT, 2010b); our results provide further evidence to justify this status. Populations #3, 6, and 11 should be of especial concern, as they bear the lowest amount of genetic diversity among the studied stands. *A. religiosa* is currently considered of least concern, and it is widely exploited for timber in the central and eastern portions of the TVB (SEMARNAT, 2016). However, our results urge for a more active species management, and maybe a status change. According to our demographic models, populations of this species have been declining since the end of the LGM, and are accumulating significant amounts of partially deleterious

mutations, which may compromise their viability. This species could become quickly threatened if exploitation is not accompanied by an adequate management aiming to mitigate the accumulation of these potentially harmful mutations.

Tables

Table 1. Genetic diversity in natural populations of *Abies flinckii* (AF) and *A. religiosa* (AR in gray) in central Mexico. Estimates were calculated with four nuclear SSRs and eight gene-coding sequences. *N* = Sampled individuals for SSR screening, *A*, number of alleles per population; H_e, expected heterozygosity. *N** = Sampled individuals for sequencing screening (4,763 pb) is followed by mean values of nucleotide diversity, π (Tajima, 1983) for non-synonymous (π_N) and synonymous positions (π_S) in coding regions and a measure to assess the efficacy of the selection against weakly deleterious alleles ($\pi_N/\ \pi_S$); *D* (Tajima, 1989), F_s (Fu, 1997), *H* (Fay & Wu, 2000) and H_{norm} (Zeng *et al.*, 2006).

Pop	Spp	*N*	*A*	H_e	*N**	π_N	π_S	π_N/π_S	*D*	*Fs*	*H*	H_{norm}
1	AF	20	15.50	0.900	4	0.00062	0.00019	0.00000	0.261	-0.162	-0.333	-0.829
2	AF	20	10.50	0.835	7	0.00075	0.00151	0.10838	0.046	0.333	-0.110	0.381
3	AF	18	5.25	0.674	7	0.00057	0.00152	0.04614	-0.045	-0.168	0.310	0.389
4	AF	22	13.75	0.864	5	0.00000	0.00031	0.00000	-0.191	0.520	-0.442	-0.746
5	AF	20	10.00	0.826	7	0.00018	0.00028	0.00000	0.675	1.131	-0.410	-0.842
6	AF	20	4.50	0.601	7	0.00000	0.00082	0.00000	1.015	1.454	0.022	0.059
		Mean	**9.92**	**0.783**		**0.00035**	**0.00077**	**0.02575**	**0.293**	**0.518**	**-0.161**	**-0.265**
8	AR	21	14.25	0.891	7	0.00029	0.00137	0.05450	-0.844	-0.309	-0.102	0.007
9	AR	20	13.75	0.864	7	0.00022	0.00103	0.01515	-0.679	-0.631	0.147	0.119
10	AR	21	15.00	0.878	7	0.00044	0.00196	0.09329	-0.783	-0.282	-0.125	0.060
11	AR	19	6.00	0.710	7	0.00046	0.00177	0.04657	0.122	0.664	-0.388	-0.625
12	AR	18	13.75	0.883	7	0.00040	0.00173	0.20964	-0.706	-0.011	-0.619	-0.493
13	AR	22	14.75	0.868	7	0.00104	0.00296	0.10150	-0.618	0.254	0.341	0.265
14	AR	21	18.00	0.915	7	0.00069	0.00103	0.17071	-0.935	-0.607	-0.672	-0.957
15	AR	19	13.50	0.866	7	0.00098	0.00311	0.26064	-0.163	0.454	-0.212	0.061
16	AR	19	13.25	0.881	7	0.00060	0.00332	0.12889	-0.741	-0.865	-0.097	-0.020
17	AR	20	13.00	0.878	7	0.00072	0.00207	0.10883	-0.226	-0.030	-0.207	0.014
18	AR	20	16.50	0.901	7	0.00028	0.00131	0.00000	-0.855	-0.229	-0.013	-0.028
19	AR	20	13.00	0.884	7	0.00032	0.00038	0.00000	-0.722	-0.504	0.062	0.109
20	AR	19	12.75	0.847								
21	AR	20	11.50	0.857								
		Mean	**13.50**	**0.866**		**0.00054**	**0.00184**	**0.09914**	**-0.596**	**-0.174**	**-0.157**	**-0.124**

Table 2. Name, ID in the transcriptome of *Abies alba* (Roschanski *et al.*, 2013), length of sequenced fragment (base pairs; bp), number of non-coding regions and biological function of eleven gene-coding regions sequenced in populations of *Abies flinckii* and *A. religiosa* in central Mexico.

Gen-coding loci	ID	bp	Intron	Biological function
porin Mip1	8248	728	1	Response to water deprivation, water transport
*Lh*ca4	8855	850	2	Photosynthesis, light harvesting
α-1,6 xilosiltransferase	14455	759	0	Root hair elongation
fructose-1,6 dihosphate aldolase	14514	308	0	Response to salt stress
heat shock protein	27033	422	0	Response to heat, response to bacterium
ArMybVI		721	1	Transcription regulation
*ArMyb*IX		409	1	Transcription regulation
ArMybSTR		566	1	Transcription regulation
CesA1	3918	332	0	Cellulose biosynthetic process
COBRA-like	9652	468	1	Cellulose synthesis
porin Mip1a	23660	749	1	Water transport

Table 3. Relationships between indices of genetic diversity obtained with four nuclear SSRs (*A,* number of alleles; H_e, expected heterozygosis), mean values of eight nuclear genes ($\pi_S, \pi_N, \pi_N/\pi_S$) and distances to the niche centroid in *Abies flinckii* and *A. religiosa* (light gray color) in central Mexico. Significant values after Bonferroni correction are in bold.

A. flinckii **Index**	**M1** r^2 **adj.**	***p-value***
A	**0.73**	**0.037**
H_e	0.49	0.150
π_S	0.67	0.059
π_N	0.00	1.000
π_N/π_S	0.00	0.750
A. religiosa		
A	0.23	0.090
He	**0.31**	**0.045**
π_S	0.00	0.954
π_N	0.00	1.000
π_N/π_S	0.01	0.635

Table 4. Results of redundancy analyses (RDAs) on H_e and π_S in *Abies flinckii* and *A. religiosa* in central Mexico (a) Partitioning of variance with pure environmental and pure spatial components are shown. Proportion constrained corresponds to the partitioned variance relative to the constrained variance of the full RDA model. (b) Significance of individual variables in simple RDAs of genetic variation with environment and space. Bold face text indicates variables that are still significantly associated with genetic variation; *P-values* in italics were marginally significant

	a) Summary of RDA results for genetic variation				
Species	**Index**	**Partitioned variance**	**Proportion constrained**	**Adj. R^2**	***P***
	H_e				
A. flinckii	Environmental (K)	0.018	0.723	**0.654**	**0.019**
	Spatial (Longitude + Elevation)	0.011	0.465	0.109	0.409
A. religiosa	Environmental (bio1 + pH)	0.002	0.589	**0.514**	**0.017**
	Spatial (Longitude + Elevation)	0.001	0.453	**0.353**	**0.022**
	π_S				
A. flinckii	Environmental (K)	0.668	0.809	0.762	*0.051*
	Spatial (Latitude)	0.472	0.573	0..466	*0.091*
A. religiosa	Environmental (pH + K)	0.072	0.199	0.021	0.375
	Spatial (Latitude)	0.038	0.106	0.017	0.286
	b) Significance of individual variables				
	Variable		**Variance**	***F***	***P***
A. flinckii (H_e)	**K**		**0.018**	**10.487**	**0.019**
A. religiosa	**Bio 1**		**0.001**	**14.323**	**0.013**
H_e	pH		0.0009	4.2046	*0.05*
	Longitude		0.0007	2.95	*0.082*
	Elevation		**0.001**	**8.546**	**0.01**

pH = soil pH; K = potassium in soil.

Figure legends

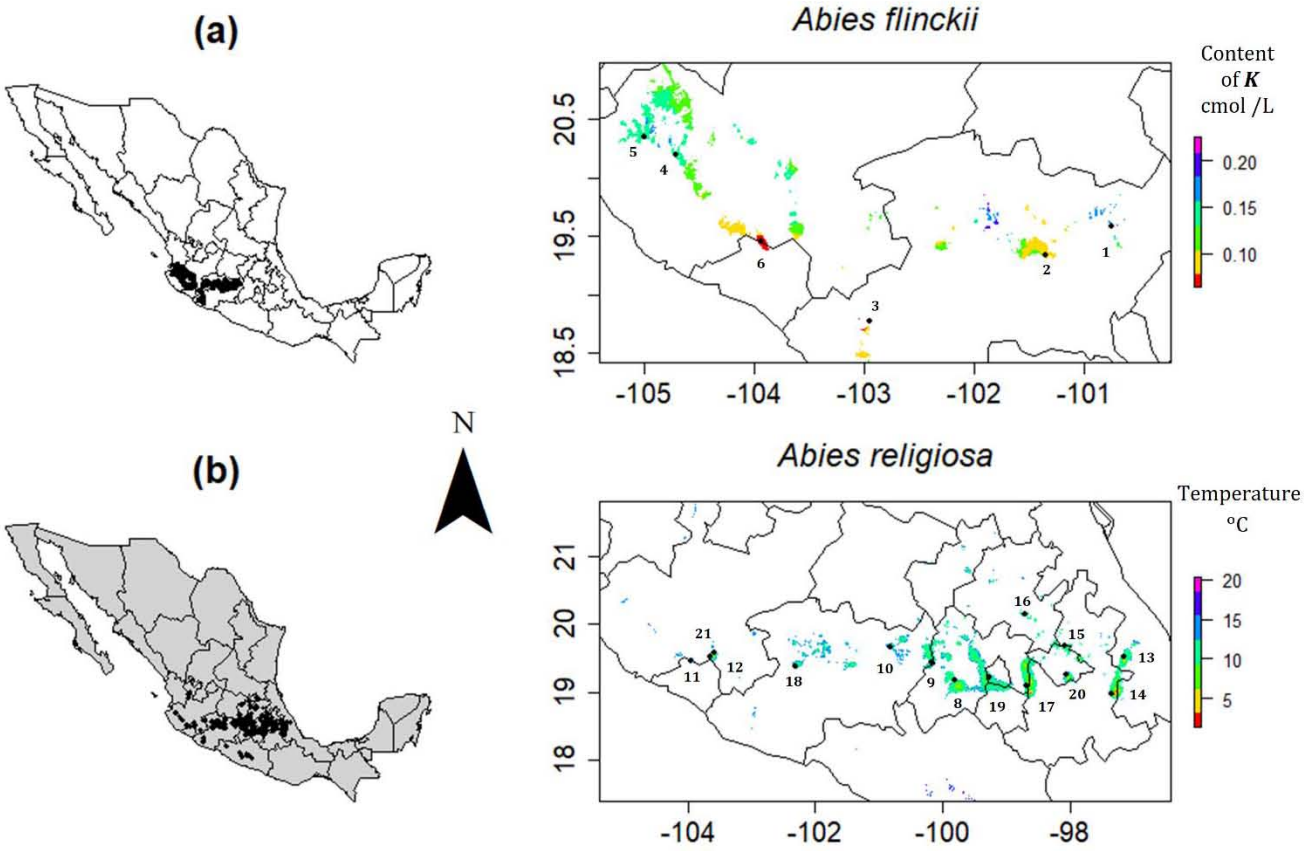
Figure 1. Map of natural distribution of two Mexican firs across central Mexico: *Abies flinckii* (a) and *A. religiosa* (b); points indicate sampled populations. Figures in the right panel show the distribution of two variables relevant for explaining variation of expected heterozygosity (H_e) between populations in a Redundant Discriminant Analysis: soil potassium (*K*) content (cmol/L) for *Abies flinckii* and mean annual temperature (°C) for *A. religiosa*.

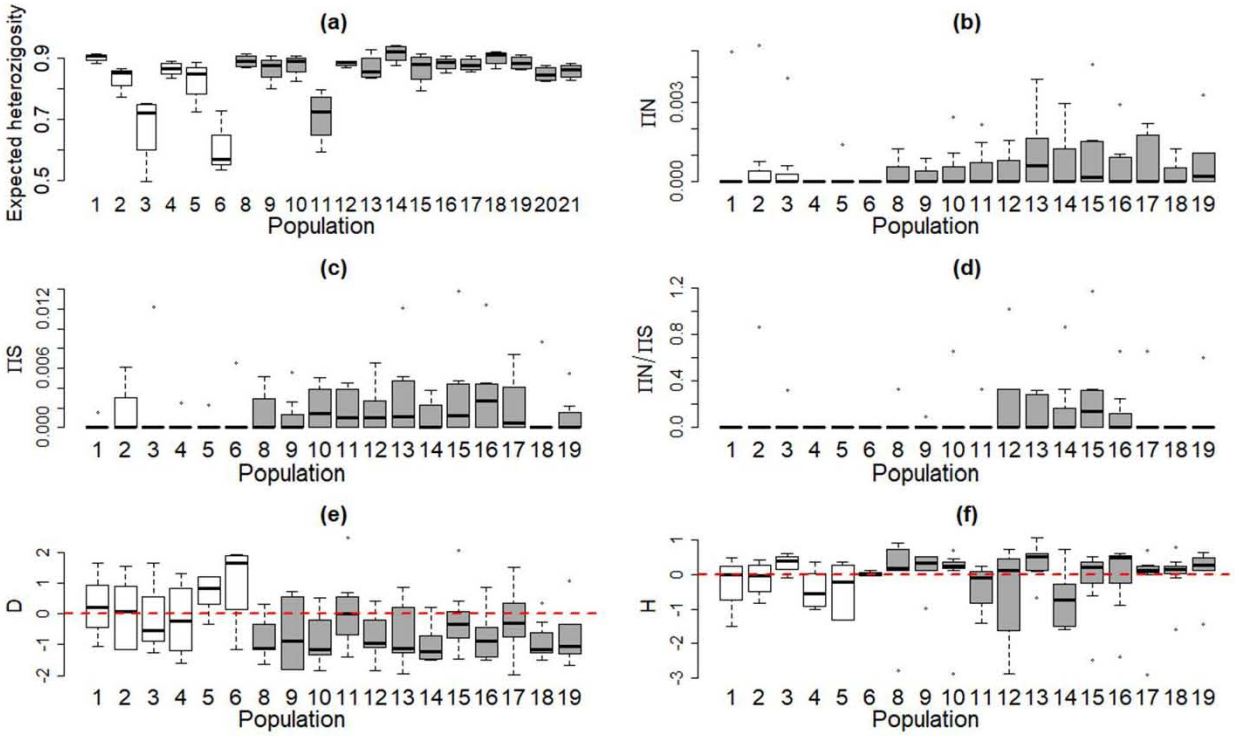
Figure 2. Boxplots displaying summary statistics of genetic variation in populations of *Abies flinckii* (white) and *A. religiosa* (gray) in central Mexico. Expected heterozygosity obtained with four microsatellites (a), Nucleotide diversity at nonsynonymous sites: π_N (b), nucleotide diversity at synonymous sites: π_S, (c), ratio of nonsynonymous versus synonymous nucleotide diversity: π_N/π_S (d), Tajima´s *D* (Tajima, 1989, e); *H* (Fay & Wu, 2000, f). Estimates b-f obtained with eight gene-coding regions.

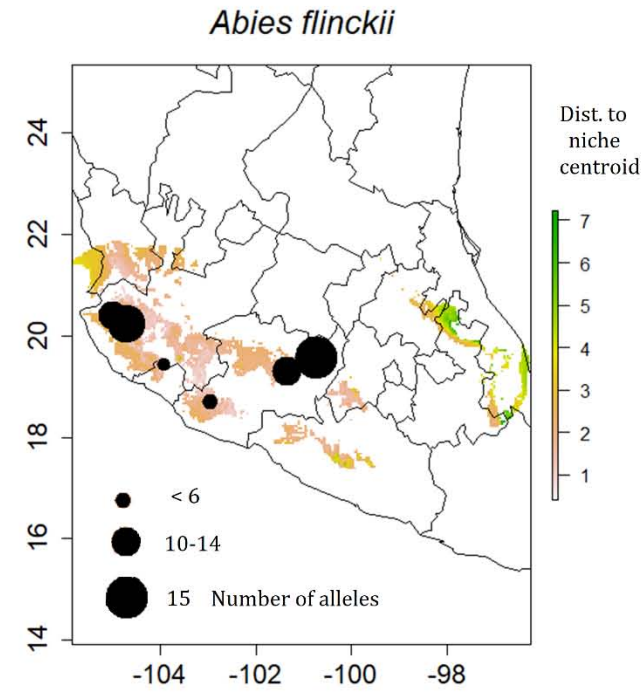
Figure 3. Euclidean distances to the niche centroid obtained with Model M1 for *Abies flinckii* and *A. religiosa* in central Mexico. The map is colored with pink representing nearer distances to the niche centroid and green farther from centroid. Circle size indicates mean number of alleles per population as estimated with four nuclear SSRs.

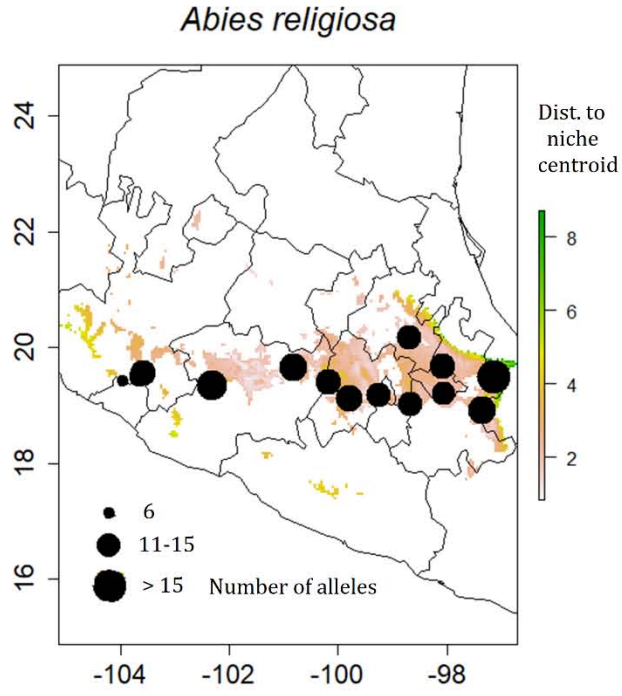
Figure 4. Bivariate (x = 2) environmental space constructed from environmental variables, PC1 and PC2 (light gray points), for *Abies flinckii* (a) and *A. religiosa* (b) in central Mexico. Dark gray points denote the extant niche model as a proxy for the species fundamental niche. Black points show the occupied climate niche space for each species. The white point indicates the centroid for each species. The panel "c" shows boxplots of niche breadth estimated with Euclidean distances to niche centroid for six PCAs in a multidimensional climatic niche space.

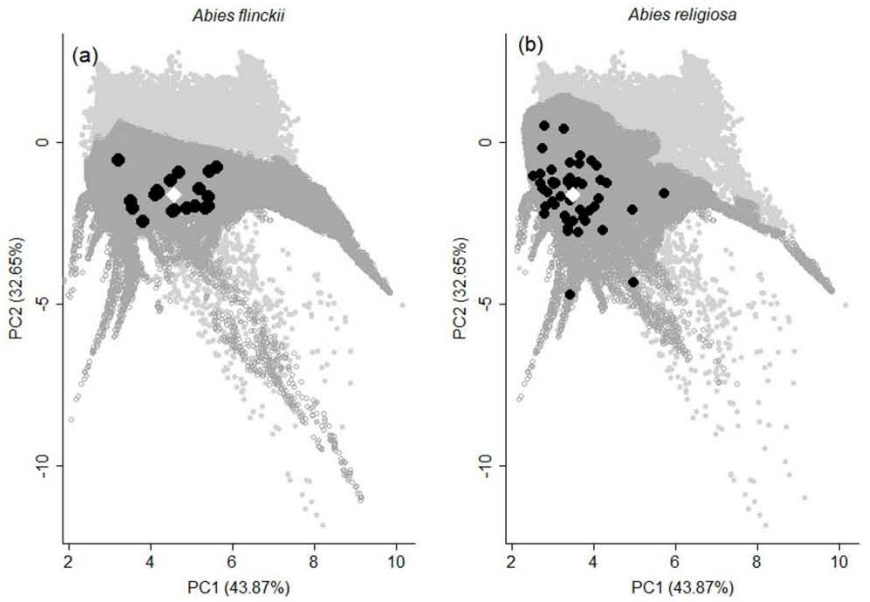
Figure 5. Boxplots of distances to niche centroid in the present and in Last Glacial Maximum (LGM) with two models of circulation, CCSM4 and MIROC (a); in white, *Abies flinckii* and in gray, *A. religiosa* in central Mexico. Relationship between distance to niche centroid and number of alleles (estimated with nuclear SSRs) in *Abies flinckii* (*P-value* < 0.05; b) and *A. religiosa* (*P-value* > 0.05; c).

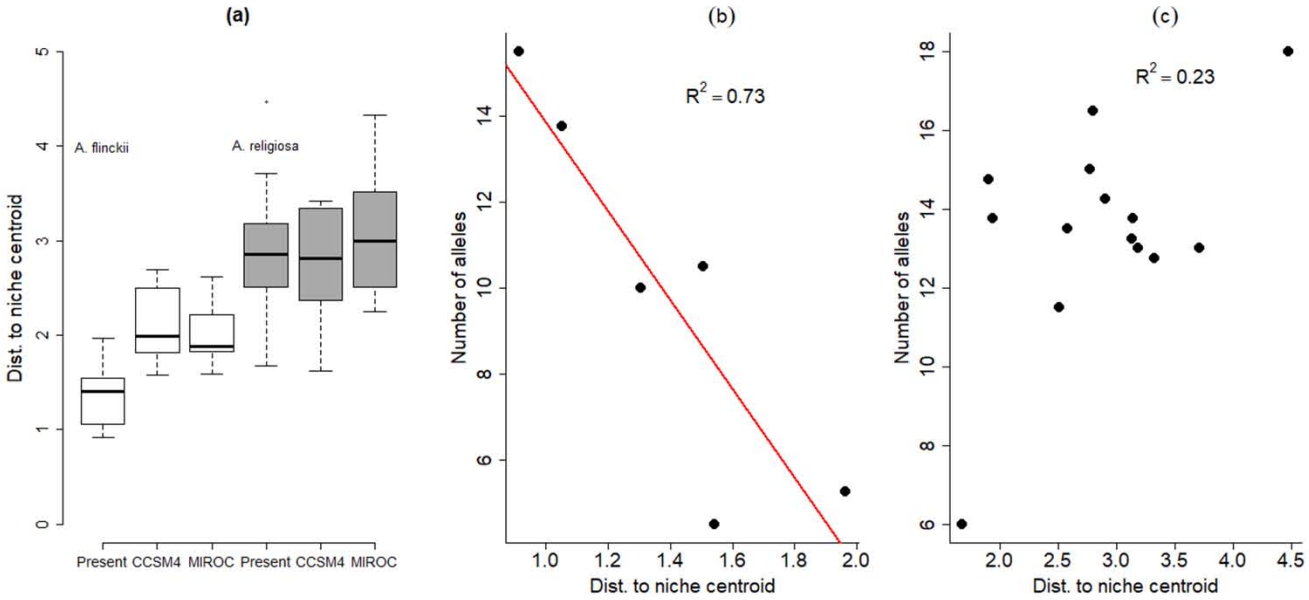

(a)
(b)
N
Abies flinckii
Content of K cmol /L
0.20
0.15
0.10
20.5
19.5
18.5
-105
-104
-103
-102
-101
1
2
3
4
5
6
Abies religiosa
Temperature °C
20
15
10
5
21
20
19
18
-104
-102
-100
-98
8
9
10
11
12
13
14
15
16
17
18
19
20
21


(a)
Expected heterozigosity
Population
(b)
ΠIN
Population
(c)
ΠIS
Population
(d)
ΠIN/ΠIS
Population
(e)
D
Population
(f)
H
Population


Abies flinckii
Dist. to niche centroid
< 6
10-14
15 Number of alleles


Abies religiosa
Dist. to niche centroid
6
11-15
> 15 Number of alleles


Abies flinckii
(a)
PC2 (32.65%)
PC1 (43.87%)
Abies religiosa
(b)
PC2 (32.65%)
PC1 (43.87%)
(c)
Niche breadth
A. flinckii
A. religiosa


(a)
Dist. to niche centroid
A. flinckii
A. religiosa
Present CCSM4 MIROC Present CCSM4 MIROC
(b)
$R^2 = 0.73$
Number of alleles
Dist. to niche centroid
(c)
$R^2 = 0.23$
Number of alleles
Dist. to niche centroid

Data availability statement

Microsatellite data available from the Dryad Digital Repository and points of presence for each species used for ecological niche modelling available at: (XXXXX).

DNA sequences GenBank Accession number: (XXXXX)

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BIOSKETCH

Jorge Cruz Nicolás is broadly interested in ecology and evolution of plants, especially forest trees. This study is part of the doctoral research conducted by JC-N. He is part of a large working group of collaborators that test evolutionary hypotheses using Mexican firs (*Abies*, Pinaceae) as models.

Author contributions: J.C.N. and J.P.J.C. conceived the study; G.G.P., E.A.P., N. M.M. collected environmental and molecular data; J.C.N., J.P.J.C., A.L.N. and L.E.E. analysed the data and interpreted results; J.C.N. and J.P.J.C. drafted the manuscript and led the writing process; all other authors contributed revisions.

This manuscript has two additional files sup_0001_AppendixS1.docx and sup_0002_AppendixS2.docx (At the end of this document).

CAPITULO V: DISCUSIÓN GENERAL Y CONCLUSIONES

En este trabajo se infirieron los procesos evolutivos de los oyameles mexicanos. Los resultados obtenidos permitieron detectar linajes independientes para el género *Abies* en México. La diferenciación de estos linajes sería el resultado de procesos que actúan en diferentes escalas de tiempo y espacio. Estos procesos fueron inferidos con herramientas filogenéticas y estudios poblacionales. El patrón de distribución en zonas de alta montaña geográficamente aisladas (Hernández, 1985; Rzedowski, 2006; Martínez-Méndez *et al.*, 2016), evidencia que la dispersión y el flujo génico son limitados entre poblaciones/especies. Se propone a la especiación alopátrida como el factor más importante de especiación, debido a que, con algunos casos excepcionales como en la región central de México, no se detectaron zonas de traslape entre especies, dentro de las que se pudieran generar híbridos. La especiación simpátrida y parapátrida parecieron tener una influencia mucho menor en la diferenciación de oyameles en México.

En esta disertación se obtuvieron datos de Genotipado por secuenciación (GBS) para generar una filogenia. Esta aproximación mejoró considerablemente el nivel de resolución con respecto a estudios previos (Aguirre-Planter *et al.*, 2012) y mostró que la secuenciación de nueva generación es una herramienta útil en especies de reciente divergencia (Escudero *et al.*, 2014; Massatti *et al.*, 2016). Las relaciones filogenéticas sugieren que los sistemas montañosos de México son "*hotspots*" de endemismo y diversificación, como ya se ha visto con otros grupos de organismos (Mastretta-Yanes *et al.*, 2018; Sosa, De-Nova, & Vásquez-Cruz, 2018).

Siguiendo esta línea, los capítulos III y IV ejemplifican casos de estudio a nivel poblacional para el género *Abies* en la región central de México. Se mostró que existe un grado de diferenciación genética importante, así como una deficiencia de heterocigotos sobre todo para la especie con distribución más restringida y fragmentada (F_{ST} = 0.125 y F_{IS} 0.150 en *Abies flinckii*; F_{ST} = 0.039 y F_{IS} = 0.188 para *Abies religiosa*). En la misma dirección, se encontró una diferenciación fenotípica importante en tres caracteres morfológicos de las hojas (P_{ST} = 0.639-0.801 en *A. flinckii* y 0.613-0.897 en *A. religiosa*). Lo cual nos remitió a detectar fuerzas evolutivas contrastantes operando sobre esta variación, donde los tamaños poblacionales parecen tener un factor relevante. También se determinó que la centralidad del nicho y la variación genética están relacionadas en *A. flinckii* pero no en *A. religiosa*, la cual parece estar más influenciada por procesos históricos. Estos hallazgos sugieren que las especies, a pesar de su cercanía geográfica, tienen rutas evolutivas

contrastantes, lo que eventualmente ha tenido consecuencias importantes en el proceso de divergencia y posible especiación.

Esta tesis doctoral tiene las siguientes contribuciones: 1) Establecimiento de las relaciones filogenéticas del género *Abies* en México. 2) Determinación de cuáles son los procesos evolutivos predominantes en un par de especies del género *Abies* a partir de la comparación de la variación genética y morfológica. 3) Evaluación de la centralidad del nicho ecológico bajo la lupa de la teoría neutral y casi neutral de evolución para este mismo par de especies. 4) A partir de estas herramientas se propone a la especiación alopátrida como el modelo de especiación prevaleciente. A continuación, se discuten cada uno de estos apartados.

5.1. Especiación alopátrida inferida a partir de las relaciones filogenéticas del género Abies en México.

Los nueve linajes identificados para el género *Abies* en México, corroboran que las zonas montañosas por sus características climáticas son "*hotspots*" de biodiversidad con numerosos endemismos (Parra-Olea *et al.*, 2012; Schneeweiss, Winkler, & Schönswetter, 2017; Mastretta-Yanes *et al.*, 2018; Sosa *et al.*, 2018). Las zonas de alta montaña fungen como refugios para estas especies de afinidad templada, y la presencia contigua de zonas bajas funciona como barrera climática, permitiendo la divergencia. De esta forma, la topología de los árboles presentó una cierta afinidad geográfica, lo que también apoya la especiación alopátrida (Wiley & Lieberman, 2011). Al norte de México y sur de Estados Unidos se identificó a *Abies concolor* como el linaje más divergente del resto. En la Sierra Madre Occidental *Abies durangensis*, en la Sierra Madre Oriental *Abies durangensis* var. *coahuilensis* y *A. vejarii*. En la Faja Volcánica Transmexicana *A. flinckii*, *A. jaliscana*, *A. religiosa* y en la Sierra Madre del Sur *A. hickelli* y *A. guatemalensis.*

Las longitudes cortas de las ramas de la filogenia son probable resultado de eventos de especiación reciente, posiblemente a finales del Plioceno y principios del Pleistoceno (Jaramillo-Correa *et al.*, 2008; Aguirre-Planter *et al.*, 2012; Giles-Pérez, 2017). El registro fósil para este género en México es muy escaso hasta ahora solo es conocido hacia finales del Plioceno (i.e., 5 Ma) en el estado de Veracruz lo que sugiere una rápida diversificación del género después de su llegada al territorio que hoy es México (Graham, 1976, 1999). Los nueve linajes observados y la similitud de sus requerimientos ambientales (Martínez-Méndez *et al.*, 2016) apoyan la hipótesis de radiación alopátrida no adaptativa. Es decir, que en alopatría surgió alguna incompatibilidad reproductiva al menos parcial y que se mantuvo después en un contacto secundario en parapatría

o simpatría. En algunos casos como *A. religiosa* y *A. flinckii*, la selección divergente habría desplazado algunos caracteres disminuyendo las posibilidades de hibridación (Rundell & Price, 2009; Czekanski-Moir & Rundell, 2019). Otras zonas de simpatría donde sería factible probar esta hipótesis incluyen aquellas entre *A. religiosa* y *A. hickelii* y una más entre *A. religiosa* y *A. hidalgensis*.

Si bien es cierto que el aislamiento ha contribuido de manera importante para la divergencia de estos linajes, no debe perderse de vista que las relaciones filogenéticas vienen a ser una representación imperfecta de las relaciones entre las especies a lo largo de su evolución y que el proceso de especiación no necesariamente es dicotómico, si no que tiende a ser más complejo (Wiley, 2010). En el capítulo II, se observó que conforme se avanza de norte a sur, el patrón tiende a ser más reticulado, al mismo tiempo que se observó un patrón de aislamiento por distancia. Esto implica que en el caso de los linajes del centro-sur de México, la especiación es aún más incipiente.

Si partimos de un modelo de aislamiento en pequeñas "islas", podemos suponer que la acción de la deriva génica es importante en el proceso de divergencia de los oyameles en México, probablemente iniciándose en forma de cuellos de botella drásticos producto de efectos fundadores (Lande, 1980; Turelli, Barton, & Coyne, 2001; Coyne & Orr, 2004; Wang *et al.*, 2016). Esto derivaría en la reducción del tamaño poblacional y un aumento en los procesos estocásticos. Estos a su vez llevarían a la fijación de distintas mutaciones, aunque probablemente esto no se traduciría en aislamiento reproductivo. Uno de los principales argumentos a favor de esta hipótesis es que los caracteres reproductivos están sujetos a la selección natural y que es complicado que la deriva génica por si misma afecte estos caracteres. Por el contrario, el intercambio genético es latente en plantas relativamente cercanas y con barreras reproductivas porosas (Coyne & Orr, 2004; Sobel *et al.*, 2010). Este hecho fue corroborado con el análisis de *admixture* para varios linajes mexicanos.

El intercambio genético en poblaciones naturales del género *Abies* es frecuente entre especies cercanamente emparentadas (Liepelt *et al.*, 2010; Semerikova & Semerikov, 2014). Aunque de forma experimental las cruzas entre individuos de distintas secciones no han sido exitosas, sí ha sido posible obtener individuos híbridos de especies más cercanas, como *A. concolor x A. religiosa* (Clair & Critchfield, 1988; Critchfield, 1988), lo que nos habla de un reducido aislamiento reproductivo en estas especies cercanas o con una divergencia muy reciente. Siguiendo esta línea, queda abierta la posibilidad para estudios posteriores de evaluar el grado aislamiento reproductivo entre especies; por ejemplo, entre especies cercanas geográficamente y

divergentes como *A. flinckii* x *A. religiosa,* o entre tres especies muy divergentes y geográficamente separadas como *A. concolor* con *A. flinckii* o *A. hickelii*, o entre *A. durangensis* x *A. guatemalensis*. Este tipo de estudios serían una referencia importante para determinar la existencia y la eficiencia de barreras reproductivas y comprender mejor los mecanismos de divergencia.

Es posible que gran parte de la discordancia en las descripciones taxonómicas del género *Abies* se deba a la divergencia reciente y al flujo génico ancestral. En este sentido, el presente estudio es una valiosa aportación, pues puntualiza que la geografía es importante en la delimitación de linajes, por lo que la filogenia y la morfología deberían utilizarse de forma simultánea en el reconocimiento de las especies. Sería recomendable tratar a *A. durangensis* var. *coahuilensis* como una especie distinta de *A. durangensis*, retomando el nombre *A. coahuilensis* que ya se había propuesto hace varias décadas (Johnston, 1943). El análisis de cuartetos también confirmó que *A. jaliscana*, ubicado geográficamente en el extremo oeste de la FVT, es un linaje distinto de *A. flinckii*, lo que también es sustentado con caracteres morfológicos y patrones fenológicos (Mantilla-Blandón, 2006; Vázquez-García *et al.*, 2014). Los ejemplares del estado de Guerrero analizados e inicialmente descritos como *A. guatemalensis*, pero agrupados dentro del clado de *A. hickelii,* merecen una especial atención. Si, como dijimos previamente, la especiación en estas regiones es incipiente, es muy posible que el número de caracteres que tradicionalmente utilizamos para su clasificación no sea "suficiente". Por lo que considerar un estudio taxonómico más a profundidad incluyendo, por ejemplo, características de la cutícula o del polen, posiblemente nos brinden una mejor resolución del status taxonómico de estas especies/poblaciones.

Merecen también una mención especial el caso de *A. hidalgensis* (elevación = 2275 msnm), y el de una muestra geográficamente cercana, e identificada como *A. guatemalensis* (elevación = 1770 msnm), en el estado de San Luis Potosí (Aguirre-Planter *et al.*, 2000). Estas muestras no se agruparon con ninguno de los nueve linajes previamente identificados. Las dos localidades de estas muestras se encuentran en una zona de transición entre la Sierra Madre Oriental y la Faja Volcánica Transmexicana, pero a menores elevaciones de las que usualmente se distribuyen los oyameles (< 2300 msnm). Hasta donde se sabe, *A. hidalgensis* está restringido a dos localidades cercanas (Debreczy & Rácz, 1995; Hernández-Álvarez, 2018). Esta zona tendría que ser otro foco potencial de exploración para futuros estudios. La idea sería identificar poblaciones nuevas de *A. hidalgensis,* si es que existen, o potenciales regiones de hibridación con *A. religiosa*. Incluso es posible que el

ejemplar de *A. guatemalensis* de San Luis Potosí, forme parte de la distribución de esta o una nueva especie.

5.2. Fuerzas evolutivas contrastantes conducen la diferenciación genética y morfológica de un complejo de oyameles del centro de México.

Bajo un modelo de especiación alopátrida, el aislamiento reproductivo puede o no desarrollarse; esto solo se puede verificar hasta que las especies entran en contacto. En el capítulo III el análisis con Structure entre *A. flinckii* y *A. religiosa* evidenció una gran proporción de mezcla genética en el área de traslape geográfico, reforzando la hipótesis de un débil aislamiento reproductivo, un denominador común en coníferas (Ledig *et al.*, 2004; Shen *et al.*, 2019). En cambio, las poblaciones en alopatría y con tamaño poblacional pequeño de *A. flinckii* y *A. jaliscana* (poblaciones 5 y 6) formaron grupos genéticos distintos, estructurados geográficamente y con menor diversidad genética.

En este capítulo se observó que la variación neutral influenciada por procesos estocásticos y la variación morfológica en estas especies no se comportó igual. Mientras que fuimos capaces de distinguir cuatro grupos en estas dos especies con marcadores genéticos, solo pudimos diferenciar dos con los caracteres morfológicos de las hojas, que correspondieron con la taxonomía de las dos especies (*A. flinckii* y *A. religiosa*). Esto sugiere que los cambios genéticos se fijan más rápidamente entre especies que los cambios morfológicos. Una posible explicación es que estos últimos son producto de procesos complejos de expresión espacial y temporal donde interactúan muchos genes (Nei, 2013). También en ocasiones los fenotipos pueden presentar un cierto nivel de plasticidad fenotípica ante ciertas condiciones ambientales (Des Marais, Hernandez, & Juenger, 2013).

En un estudio anterior en donde se incluyeron características del cono y de las hojas se observó un gran traslape morfológico, principalmente entre *A. hickelii*, *A. guatemalensis* y *A. religiosa* (Strandby *et al.*, 2009). También hay evidencia previa de que los híbridos obtenidos de forma experimental entre *A. concolor* x *A. religiosa* presentan características intermedias en el largo y grosor de las hojas, pero no muestran variación considerable en el número de hileras de estomas (Clair & Critchfield, 1988). A diferencia de estos estudios, en la zona de traslape geográfico entre *A. flinckii* (con hojas más largas) y *A. religiosa* (con hojas más gruesas), no se observó ningún fenotipo intermedio ni ninguna clina fenotípica, lo que no aporta algún indicio de especiación parapátrica entre estos taxones. Sin embargo, para descartar este tipo de especiación de una forma

más formal sería necesario un muestreo más exhaustivo y dirigido hacia la búsqueda de potenciales híbridos con marcadores genéticos y morfológicos entre los que se pueden incluir caracteres reproductivos.

Siguiendo con el contraste entre las dos especies del centro de México, el análisis comparativo P_{ST}-G'_{ST} evidenció que las clinas entre caracteres morfológicos y geográficos/ambientales pueden ser atribuidos a diferentes fuerzas evolutivas. Para *A. flinckii*, nuestros datos sugirieron un modelo de aislamiento por distancia. Al igual que con la divergencia genética, hay una predominancia de procesos estocásticos ($P_{ST} = G'_{ST}$), lo que se atribuyó a los pequeños tamaños poblacionales, mayor fragmentación y estructura genética ($G'_{ST} = 0.66$). En contraste, *A. religiosa*, especie con mayor distribución y tamaño poblacional, mostró aislamiento por adaptación ($P_{ST} > G'_{ST}$); en este caso, la selección natural sería la fuerza evolutiva predominante, al menos para un par de caracteres (grosor de la hoja y posición del canal resinífero). Esta comparación indica que los factores demográficos y adaptativos no afectaron de la misma forma a ambas especies luego de su diferenciación. La comparación dentro de *A. religiosa* mostró que las poblaciones con tamaños más grandes tienden a presentar mayores valores de P_{ST} que las poblaciones pequeñas, y por tanto más evidencia de que el carácter está influenciado por la selección natural. Es posible entonces que estemos frente a un caso de "neutralidad condicional" en estas poblaciones, es decir, que en algunas poblaciones ciertos alelos pueden tener efectos en la adecuación en algunos ambientes y en otros ser "invisible" a la selección natural (Anderson *et al.*, 2013).

Podemos proponer que esta evolución fenotípica también es parte importante de la zona difusa del proceso de especiación, y evidentemente tiene implicaciones a nivel taxonómico. Algunos de los caracteres diagnóstico que se utilizan en la taxonomía del género (ej. la posición del canal resinífero), pueden variar con la geografía o el clima. Por lo tanto, es oportuno determinar qué fuerzas evolutivas predominan en determinadas poblaciones. Por ejemplo, en el presente trabajo encontramos la prevalencia de procesos estocásticos en poblaciones con tamaños poblacionales pequeños. La gran cantidad de variación local que genera discordancias taxonómicas es un fenómeno recurrente en coníferas mexicanas, muchas de ellas con distribución restringida (Strandby *et al.*, 2009; Gernandt & Pérez-De La Rosa, 2014). La aproximación aquí realizada podría extenderse a estos y otros taxones. Lo cual, sin duda puede contribuir a disminuir discordancias taxonómicas, a entender la dinámica evolutiva poblacional y proponer medidas

adecuadas de conservación. Muchas de estas poblaciones no solo de oyamel se encuentran distribuidas de forma restringida, con un número reducido de individuos, con ciertas características morfológicas particulares y en general sometidas a presiones antropocéntricas. Con datos empíricos sería posible proponer unidades evolutivamente significativas si la situación lo amerita y reducir de forma significativa la presión sobre estas poblaciones.

5.3. Centralidad del nicho ecológico y diversidad genética/selección purificadora: dos historias evolutivas contrastantes en la Faja Volcánica Transmexicana

Los escenarios demográficos aquí analizados sugirieron que *A. flinckii* tuvo un cuello de botella ancestral (reducción al 15% de su N_e original), para después mantenerse con tamaños poblacionales pequeños; lo que explica su menor nivel de variación genética y supone una relativa estabilidad demográfica y aislamiento por largos periodos de tiempo. Es muy posible que la divergencia entre *A. jaliscana* y *A. flinckii* inferida en la filogenia sea producto del aislamiento de sus poblaciones. Podemos suponer entonces que la capacidad de dispersión en estas especies ha sido limitada por largos periodos de tiempo. Esto explicaría por qué, al menos con marcadores altamente polimórficos, encontramos una relación negativa entre la centralidad del nicho ecológico y la diversidad genética (Osorio Olvera *et al.*, 2016). Por el contrario, *A. religiosa* presentó mayores fluctuaciones demográficas, ajustándose a un escenario de contracción en el último interglacial y de expansión en el Último Máximo Glacial, seguida de una contracción durante el Holoceno. A pesar de esta demografía tan compleja, *A. religiosa* mostró valores elevados de diversidad genética. Aun cuando las poblaciones difirieron considerablemente en su área (medida en km^2), los valores de diversidad fueron superiores a los de otras coníferas mexicanas con mayor distribución geográfica (Dvorak *et al.*, 2009), por lo que podemos suponer que a lo largo de su historia evolutiva *A. religiosa* ha mantenido tamaños efectivos relativamente grandes.

Al parecer en *A. religiosa* los factores históricos son más determinantes para la acumulación de diversidad genética que la distancia de las poblaciones al centro nicho ecológico. Las poblaciones del extremo este de la FVT, especialmente la población Cofre de Perote, presentó mayores valores de diversidad genética, pero fue la más lejana del centroide. Al contrario, la población ubicada en el extremo oeste de la FVT, sin estar tan alejada del centroide del nicho, presentó menores valores de variación genética que las demás. De ahí que en algunos casos la relación con el centroide del nicho fuera positiva, es decir se observó mayor diversidad genética

en las poblaciones más alejadas del centroide. Estudios recientes con secuenciación de nueva generación y grandes tamaños muestrales han mostrado que esta aproximación de centralidad del nicho ecológico, por sí misma solo tiene una contribución pequeña para explicar patrones de diversidad genética, se ha observado que frentes de expansión así como la localización de refugios históricos contribuyen en gran medida para explicar patrones de diversidad genética (Hewitt, 2004; Gougherty *et al.*, 2020). Más allá de los argumentos a favor o en contra de la hipótesis de la centralidad (Abeli *et al.*, 2014; Dallas *et al.*, 2017; Soberón *et al.*, 2018; Santini *et al.*, 2019), el presente trabajo sugiere que si no existe una relación con la centralidad del nicho actual e histórica, ni con el área como en *A. religiosa*, es muy probable que los valores de diversidad nos están remitiendo a procesos históricos de esas poblaciones (ej. grandes poblaciones ancestrales) o que la fragmentación resulto en valores estocásticos de diversidad. En este sentido, por ejemplo, existe evidencia de que en poblaciones recientemente fragmentadas, los valores de diversidad no muestran diferencias con poblaciones que no han sufrido fragmentación; más bien, estos valores pudieran estar reflejando tamaños poblacionales históricos (Chávez-Pesqueira *et al.*, 2014). Es decir, hay un desfase entre los cambios demográficos y los valores de diversidad de las poblaciones.

Se han observado también pocas diferencias en los niveles heterocigosis en coníferas cuando se comparan efectos pre-cosecha y post-cosecha (e.g., la extracción de individuos) en intervalos cortos de tiempo, ya que los alelos eliminados en primera instancia son los alelos raros (Buchert, Rajora, & Hood Bruce P Dancik, 1997). Es decir, aún en presencia de fluctuaciones demográficas, los valores de heterocigosis no necesariamente serán contrastantes, esto podría explicar porque no encontramos una relación entre la diversidad genética y área.

Los valores de π_N/π_S tampoco variaron entre especies (π_N/π_S = 0.02 en *A. flinckii* y 0.01 en *A. religiosa*), lo que indicó una eficiencia similar para eliminar mutaciones deletéreas a pesar de sus historias demográficas contrastantes (π_N/π_S = 0.02 en *A. flinckii* y 0.01 en *A. religiosa*). En el caso de *A. flinckii* se podría postular que el aislamiento más prolongado de sus poblaciones ha sido una posible causa de la eliminación de esas mutaciones deletéreas pero sería una hipótesis a probar en trabajos próximos. Las diferencias en π_N/π_S suelen ser muy sutiles, aun con un considerable número de genes o utilizando métodos de nueva generación (Marsden *et al.*, 2016; Chen *et al.*, 2017), razón por la cual no podemos ser totalmente conclusivos, más allá de la tendencia de más mutaciones deletéreas en *A. flinckii*. Adicionalmente, no debe perderse de vista que si se observó una diferencia en las tasas de consanguinidad (F_{IS}). Esto sugiere la necesidad de

explorar otros indicadores adicionales, como la cantidad de semillas vanas, la tasa de polinización cruzada, el número de semillas por cono o la viabilidad de los embriones (Ledig *et al.*, 2005; Ledig, Hodgskiss, & Johnson, 2006; Mápula-Larreta *et al.*, 2008), que eventualmente pudieran dar más elementos del estado de las poblaciones para proponer medidas adecuadas de conservación.

Tratando de resumir algunas de las ideas de este capítulo, podemos suponer que, si bien los argumentos detrás de la hipótesis de centralidad parecen válidos a nivel teórico, no encontramos un acoplamiento entre estas dos variables (e.g., diversidad genética/centralidad) sobre todo en *A. religiosa*. Es probable que para esta especie, el número de generaciones desde el final del último máximo glacial (i.e. el inicio de su actual declive) no sea suficiente para observar un efecto en la disminución de diversidad genética, por lo menos a escala poblacional. El Cuadro 3 trata de resumir los principales hallazgos de este trabajo.

Cuadro 3. Evidencias en el análisis filogenético, de nicho climático, genético y morfológico que soportan la hipótesis de especiación alopátrida, en dos especies endémicas del centro de México, *Abies flinckii* y *A. religiosa*.

Especie	Filogenia	Nicho ecológico	Genético	Morfológico
Abies flinckii	Unido a su grupo hermano *Abies jaliscana* y junto con *A. religiosa*, formaron un clado en la Faja Volcánica Transmexicana.	Nicho similar con *A. religiosa.* Aunque el traslape fue moderado.	Presento tres grupos genéticos estructurados geográficamente.	Se comportó como un grupo morfológico. En la variación clinal predominaron los procesos estocásticos.
Abies religiosa	Se definió como un linaje estructurado geográficamente.	Nicho similar a *A. flinckii,* aunque con traslape moderado.	Presentó dos grupos genéticos, uno de ellos representado por una población aislada. Se infiere que la zona de traslape con *A. flinckii* es una zona de contacto secundario.	Un único grupo morfológico. En la variación clinal, la selección natural parece ser la fuerza predominante.

5.4. Especiación y montañas tropicales.

La corteza terrestre que caracteriza al territorio mexicano es una de las más accidentadas de la tierra, con excepción de la Península de Yucatán se pueden percibir una gran cantidad de montañas a lo largo del territorio nacional, cuyas elevaciones oscilan en promedio entre los 1 000 y 4 000 msnm, aunque algunas de ellas sobrepasan esta elevación. A pesar de esta cierta similitud entre montañas, en cada sistema montañoso hay ciertas peculiaridades que generan una gran diversidad de climas y microhábitats. La Sierra Madre Occidental se caracteriza por ser el más largo y continuo de los sistemas montañosos de México (> 200 km de ancho), cuenta con numerosas barrancas y variaciones de temperatura extremas (Rzedowski, 2006). En el extremo opuesto encontramos a la Sierra Madre Oriental, con una longitud de 800 km y una amplitud de 80 a 100 km, abarca la parte noreste de México y está caracterizada por numerosos barrancos y valles. En su parte sur está cubierta por la Faja Volcánica Transmexicana y en su parte más sureña está configurada en diferentes edades de formación (Eguiluz de Antunaño, Aranda García, & Marret, 2000). La Faja

Volcánica Transmexicana (TVB) es un sistema montañoso discontinuo, que alberga las prominencias topográficas más altas de México (> 4000 msnm). Debido a la actividad volcánica reciente es común observar una gran cantidad de cerros y un substrato permeable en el que no se desarrollan vías de drenaje superficial aun con pendientes pronunciadas (Rzedowski, 2006). La TVB es el sistema montañoso más joven sus elevaciones datan del Plioceno y el Pleistoceno (Ferrari *et al.*, 2012). La Sierra Madre del Sur corre desde la parte oeste de la Faja Volcánica Transmexicana y también esta enlazada por medio de montañas con el norte de Oaxaca y sureste de Puebla. Algunos consideran que una parte de la Sierra Madre del Sur puede considerarse como una prolongación de la Sierra Madre Oriental, como en los otros sistemas montañosos la continuidad se ve interrumpida por valles y una serie de ríos (Rzedowski, 2006; Morrone, 2017).

A partir de determinados de puntos de conexión entre los sistemas montañosos, algunos estudios sugieren que históricamente estos sistemas han funcionado como corredores biológicos para la dispersión de muchos mamíferos desde Norteamérica sobre todo durante el Pleistoceno. Además de la dispersión, los procesos de extinción local han jugado un papel importante en la fauna que se observa actualmente. Al día de hoy se cuenta un número importante de especies hermanas separadas en forma de islas y separadas por decenas de kilómetros, donde sin duda el proceso de especiación está operando (Ceballos, Arroyo-Cabrales, & Ponce, 2010). En organismos sésiles las discontinuidades se acentúan de forma más notable, aunque sin duda, la duración de los periodos de aislamiento y la capacidad de dispersión será determinante. Es conocido que gran parte de la diversificación del género *Pinus* en México, obedece en algunos casos al menos inicialmente a este aislamiento en pequeñas islas (Farjon, 1996). A la fecha se siguen reportando nuevos endemismos importantes que no han sido esclarecidos de forma contundente, por ejemplo *Pinus yecorensis* en la localidad de Yecora, Sonora (López-Reyes *et al.*, 2015). Cuando se estudian algunos casos con un poco más de profundidad la diferenciación genética es más evidente que la morfológica, así lo muestran los casos incipientes de especiación en las Sierras Madre Oriental y Occidental entre *Pinus johannis* y *Pinus discolor*, respectivamente (Flores-Rentería *et al.*, 2013). Otro caso documentado es el de la chara transvolcánica (*Aphelocoma ultramarina*) donde la separación geográfica ha jugado un papel importante (McCormack *et al.*, 2008).

Si bien es cierto que puede invocarse a la especiación alopátrida al menos en un principio, también es importante reconocer que hay un proceso de oportunidades ecológicas para la

especiación dadas las condiciones ambientales contrastantes que pueden existir en distancias cortas en montañas subtropicales (Mastretta-Yanes *et al.*, 2018). En el caso del ave *Aphelocoma ultramarina* las oportunidades ecológicas no se han descartado en el proceso de especiación (McCormack *et al.*, 2008). En *Pinus johannis* y *Pinus discolor* la variación morfológica hasta hoy no se sabe si es producto de procesos adaptativos o plasticidad fenotípica (Flores-Rentería *et al.*, 2013). El estudio de la especiación en montañas subtropicales es todavía muy incipiente, si bien es cierto que las herramientas genómicas nos han brindado una perspectiva objetiva del estado de las poblaciones (Mastretta-Yanes *et al.*, 2018), un aspecto muy descuidado, poco profundizado y sin embargo muy necesario son los estudios de jardín común para vislumbrar procesos adaptativos. En gran parte de los trabajos solo se abordan los aspectos de nicho climático, simulaciones demográficas y estudios comparativos de genética de poblaciones, la variación cuantitativa o procesos fisiológicos importantes como la dinámica de apertura y cierre de estomas en plantas están ausentes en la mayoría de los casos. Finalmente, vale la pena mencionar que la influencia humana sobre la vegetación natural ha tenido un impacto considerablemente negativo, provocando un grado de fragmentación considerable en muchas poblaciones naturales, principalmente debido a la expansión de la agricultura, el desarrollo de la ganadería, los incendios forestales (Rzedowski, 2006) y más recientemente de los asentamientos humanos, de forma directa o contaminando el aire y el agua.

5.5. Consideraciones finales

Tratando de resumir y brindar una perspectiva general a partir de los resultados, y para entender la dinámica evolutiva del género *Abies* en México, se encontró una dicotomía entre la diferenciación genética y morfológica, tanto a gran escala (el género en México), como a nivel regional (FTV). A partir de estos datos fue claro que la divergencia genética es más rápida, seguida por la diferenciación morfológica (al menos con los caracteres aquí estudiados), mientras que las características ambientales se conservan similares. Es decir, es más probable encontrar más diferencias genéticas que morfológicas, lo que no necesariamente implica que unos marcadores sean mejores que otros, simplemente evidencía que estos organismos están en proceso de superar la etapa difusa de especiación, tal vez este sea el caso de muchas especies mexicanas con discordancia taxonómica. Según se observó en el presente trabajo los datos de GBS pueden

brindarnos una resolución más fina, que adicionalmente puede complementarse con datos morfológicos para entender mejor el proceso de especiación.

Este trabajo subraya que para entender el proceso de especiación en plantas y en este caso del género *Abies*, los datos genéticos son necesarios, pero no son suficientes. Más allá de la resolución que puedan brindarnos los distintos tipos de datos genéticos no debe perderse de vista, que los datos genéticos también nos brindan un acercamiento a la historia evolutiva de las especies y en determinadas situaciones el estado actual de las poblaciones. Las diferencias morfológicas fueron perceptibles y además mostraron que aquella entidad que llamamos especie puede estar sometida a diferentes presiones de selección a lo largo de su distribución y así mostrar cambios graduales en ciertos caracteres. A pesar de la similitud entre especies (*A. flinckii* y *A. religiosa*) los datos de nicho climático sugirieron diferencias sutiles, posiblemente relacionadas con la elevación a la cuál se encuentran muchas de sus poblaciones, lo que eventualmente con el transcurrir de las generaciones podría o no traducirse en nichos climáticos distintos para estas especies y poblaciones. Aunque como se anotó el cambio en las preferencias climáticas no es un prerrequisito bajo un modelo de especiación alopátrida, en determinadas localidades si podrían existir oportunidades de adaptación lo que eventualmente haría más interesante el estudio de la especiación. La aproximación global de estos aspectos puede ayudar a discernir mejor las discordancias taxonómicas entre especies y poblaciones.

VI. LITERATURA CITADA

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SUPPORTING INFORMATION (Chapter IV)

Testing for niche centrality within the scope of the nearly neutral theory of evolution in a subtropical conifer species-pair

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APPENDIX S1: Supplementary methods of details of ecological niche modelling in *Abies flinckii* and *A. religiosa.* Tables S1.1.-S1.5 and Figures S11.-S1.5.

Supplementary Information

MATERIALS AND METHODS

Points of presence

We obtained occurrence data (latitude and longitude) from our own field collections (Aguirre-Planter *et al.*, 2000; Cruz-Nicolás *et al.*, 2020), and from a public database available from Mexico's National Commission for Biodiversity, CONABIO (http://www.conabio.gob.mx/institucion/cgi-bin/datos.cgi?Letras=JM&Numero=15; see also Martínez-Méndez *et al.* 2016). After verification, we eliminated, with the package '*spThin'* (Aiello-Lammens *et al.*, 2015), points with dubious taxonomic identification or which were less than 10 km apart to avoid overfitting the model with spatially autocorrelated occurrences. The final database consisted of 19 points for *A. flinckii* and 51 occurrences for *A. religiosa* (available in https://github.com/jorgecruzn/Presence_records). We used 70% of the presence records as training data and the remaining 30% for evaluating the model. The presence records for evaluation were selected in such a way that they uniformly covered each species natural range.

Bioclimatic layers

For ecological niche modelling in the present we obtained 19 bioclimatic variables from the WorldClim database (Hijmans *et al.*, 2005), at *c.* 1 km^2 spatial resolution. We then performed

a principal component analysis, PCA for avoiding correlation and to detect all possible variation these PCAs were generated with '*ENMGadgets*' (Barve & Barve, 2018), see Table S1 for details. To perform transferences to last Glacial Maximum conditions (LGM, *c.* 20 ka) we did not use Principal Components, we used the raw bioclimatic variables compiled at a spatial resolution of 2.5 arc-minutes (pixel size *c.* 21.62 km^2) from the WorldClim database (Hijmans *et al.*, 2005; Braconnot *et al.*, 2007), using the Community Climate System (CCSM4) and the interdisciplinary Research on Climate (MIROC-ESM) global circulation models. In this case, our selection criteria included showing Pearson's correlation coefficients below 0.70, being ecologically important for describing the Mexican firs distribution according to a previous study (Martínez-Méndez *et al.*, 2016); and showing significant correlations with phenotypic variation in these species (Cruz-Nicolás *et al.*, 2020). This resulted in six bioclimatic variables retained for *A. flinckii* (*bio1, 2, 3, 12, 14, 19*) and eight for *A. religiosa* (*bio1, 2, 3, 4, 12, 14, 15, 18*).

Calibration area

Given that the calibration of area *M* may influence the outcome of niche model predictions (Barve *et al.*, 2011; Soberón *et al.*, 2018), we used two different *M*'s that could be biogeographically relevant for the two species: a 80 km and a 400 km buffer around the terrestrial ecoregion comprising the TVB (Olson *et al.*, 2001). We used polygons containing these two regions to mask environmental layers and calibrate niche models both in the present and in the LGM.

We first predicted each species distribution with MAXENT (Phillips *et al.*, 2006). Parameters included a raw output, linear characteristics, and ten bootstrap replicates (Merow, Smith, & Silander, 2013); we omitted the options *extrapolate* and *do clampling*, and left all others by default. These same options were used for the present and LGM estimates, because we assumed a certain stasis in the climatic niche dimensions of both species (Alba-Sánchez *et al.*, 2010). We further predicted niche models using *Ellipsoids* as implemented in Niche ToolBox v. 0.4.1.5 (Osorio-Olvera *et al.*, 2018). This method fits an ellipsoid model using the shape matrix (covariance matrix) of the niche variables. In total we obtained four niche models (M1-M4) per species (two methods × two *M* areas) both for the present and for the LGM (Table S1).

Performance of models

To transform model's outputs into discrete binary presence/absence maps, we used a threshold of minimum training presence. We evaluated the performance of each model using the binomial cumulative probability, and the partial receiver operating characteristics (partial ROC; Peterson, Papes, & Sober, 2008; Saupe, Papes, Selden, & Vetter, 2011). The first one is a test that incorporates dimensions of correct predictions for both presence (success in predicting independent test data) and absence points based on proportion of the area predicted (Williams & Peterson, 2009). The second one aims to compare the ROC/AUC ratios produced by each model with those expected under a random distribution. We performed 500 bootstraps of each dataset and set the omission error at 5%. All tests were performed using Niche Toolbox v. 0.4.1.5 (Osorio-Olvera *et al.*, 2018).

Niche overlap

We evaluated environmental niche overlap between species following (Broennimann *et al.*, 2012) and using Shoener's *D* (Schoener, 1970) after correcting for spatial autocorrelation. We then used the framework proposed by (Warren *et al.*, 2008) to test for niche similarity. This analysis includes a multivariate environmental grid, based on the first two axes of a PCA summarizing all the selected environmental variables, in which each cell represents a unique combination of the environmental conditions available in the study area, to evaluate niche divergence. To create the PCA axes, we used nine bioclimatic layers of current conditions to perform such (bio 1, 2, 3, 4, 12, 14, 15, 18, 19). To do so, we delimited a convex polygon with a buffer of 20 km around the presence records of each species in Arc Gis 10.5 and used a mask a as dummy variable for background data.

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Table S1.1. Characteristics of four ecological niche modellings used in *Abies flinckii* and *A. religiosa* in central Mexico.

Model	Algortihm	Enviromental layers (present)	*M*
M1	MaxEnt	PC1, PC2, PC3, PC4, PC5, PC6	TMVB + 80 Km
M2	(Phillips *et al.*, 2006)	(96 % of variance explained)	TMVB + 400 Km
M3	Ellipsoids	PC1, PC2, PC3	TMVB + 80 Km
M4	(Osorio-Olvera *et al.*, 2018)	(85% of variance explained)	TMVB + 400 Km

Table S1.2. Results obtained in the analyses used to evaluate four ecological niche modellings (M1-M4) in *Abies flinckii* and *A. religiosa* in central Mexico.

Model	Species	Binomial test	Partial receiver operating characteristic (ROC)	
		P	Mean area under the curve ratio	*p*
M1	*Abies flinckii*	0.0004	1.99	0
	Abies religiosa	< 0.00001	1.99	0
M2	*Abies flinckii*	0.00002	1.99	0
	Abies religiosa	< 0.00001	1.64	0
M3	*Abies flinckii*	0.0006	1.94	0
	Abies religiosa	0.011	1.79	0
M4	*Abies flinckii*	0.00002	1.94	0
	Abies religiosa	0.023	1.88	0

Table S1.3. Euclidean distances to the niche centroid in the present with four models (M1-M4) and estimated area of *Abies flinckii* (Pops. 1-6 white color) and *A. religiosa* (Pops 8-21, in gray color) in central Mexico.

Pop	Longitude	Latitude	Elevation (m a.s.l.)	M1	M2	M3	M4	Area (km^2)
1	-100.75	19.583	2340	0.913	0.917	2.096	2.287	-
2	-101.35	19.333	2250	1.505	1.668	3.559	3.495	-
3	-102.95	18.767	2500	1.963	2.098	5.021	3.834	-
4	-104.716	20.2	2100	1.052	1.191	4.829	3.560	-
5	-105.000	20.35	2490	1.305	1.501	6.163	4.569	-
6	-103.933	19.45	2500	1.541	1.320	1.869	1.691	-
8	-99.80	19.183	3240	2.906	2.510	8.980	11.507	121.640
9	-100.18	19.433	2800	1.939	1.685	3.256	4.391	33.166
10	-100.817	19.667	2880	2.776	2.354	6.956	7.937	42.097
11	-103.95	19.45	2500	1.679	1.567	2.817	2.499	4.999
12	-103.583	19.583	3330	3.135	2.672	16.476	12.972	16.658
13	-97.35	18.967	3060	1.905	1.765	6.771	9.194	33.663
14	-97.15	19.517	3510	4.474	3.664	24.140	28.167	15.716
15	-98.083	19.683	2760	2.579	2.399	6.115	7.186	4.999
16	-98.7	20.15	2940	3.128	2.810	6.653	9.106	52.849
17	-98.683	19.083	3330	3.710	3.337	8.097	8.126	93.433
18	-102.317	19.383	3030	2.797	2.539	11.912	8.797	4.999
19	-99.267	19.223	3369	3.179	2.762	8.010	8.562	14.758
20	-98.055	19.253	3358	3.325	2.983	7.726	8.413	5.499
21	-103.642	19.521	2928	2.513	2.094	11.624	8.991	17.487

Table S1.4. Euclidean distances to the niche centroid for four models in *Abies flinckii* (Pops. 1-6 in white) and *A. religiosa* (Pops 8-21 gray color) in central Mexico, for the Last Glacial Maximum models: M1-M4; ccl: CCSM4 Model and mrl with MIROC model.

Pop	M1_ccl	M1_mrl	M2_ccl	M2_mrl	M3_ccl	M3_mrl	M4_ccl	M4_mrl
1	2.688	1.882	2.520	2.106	2.035	1.652	1.802	1.940
2	2.082	NA	2.187	NA	4.762	3.671	1.694	3.238
3	1.573	1.592	1.621	1.684	NA	NA	NA	NA
4	1.819	2.221	1.978	1.831	1.933	2.391	2.709	2.848
5	2.498	2.619	2.310	2.057	0.881	1.977	2.572	3.106
6	1.894	1.824	1.877	1.390	2.177	1.983	2.287	2.295
8	3.412	2.856	4.262	2.632	8.712	4.976	2.886	1.600
9	2.519	2.248	3.540	2.359	2.421	2.536	3.544	1.316
10	2.787	2.513	3.252	2.572	3.057	1.868	2.226	1.184
11	3.344	2.958	2.140	2.342	2.288	3.609	2.414	2.376
12	2.859	2.380	2.342	1.915	1.987	2.319	2.161	2.139
13	2.528	4.331	3.280	3.439	4.199	3.812	4.747	3.878
14	5.364	5.940	NA	5.040	1.152	3.177	4.083	4.620
15	2.354	3.181	2.970	2.837	2.707	2.683	1.818	2.640
16	1.622	3.681	2.831	2.829	1.968	3.471	1.662	2.133
17	2.365	3.521	3.450	3.071	3.173	4.716	1.885	1.725
18	3.421	2.438	3.171	2.533	2.690	4.511	2.188	3.117
19	2.831	3.041	3.655	2.832	4.207	4.873	2.498	2.175
20	2.343	3.427	3.222	3.006	4.617	3.453	2.161	2.764
21	2.978	2.547	2.405	1.985	1.396	2.426	2.166	2.153

Table S1.5. Relationships between indices of genetic diversity obtained with four nuclear SSRs (*A,* number of alleles; H_e, expected heterozygosis), synonymous polymorphism (π_S), non-synonymous polymorphism (π_N,) and ratio (π_N/π_S) obtained with eight nuclear genes and distances to the niche centroid and area in *Abies flinckii* and *A. religiosa* (in gray color) in central Mexico. The results are shown for models (M2-M4). Significant values after Bonferroni correction are in bold.

A. flinckii	**M2**		**M3**		**M4**		**Area**	
Index	**r^2 adj.**	***p-value***	**r^2 adj.**	***p-value***	**r^2 adj.**	***p-value***	**r^2 adj.**	***p-value***
A	0.33	0.271	0.00	1.000	0.00	1.000		
He	0.05	0.657	0.00	1.000	0.00	1.000		
π_S	-0.23	1.000	0.00	1.000	0.00	1.000		
π_N	0.61	0.084	0.00	1.000	0.00	1.000		
π_N/π_S	0.00	1.000	0.00	1.000	0.00	1.000		
A. religiosa								
A	0.20	0.123	**0.32**	**0.041**	0.26	0.069	0.00	1.000
He	0.28	0.060	0.17	0.155	0.12	0.246	0.00	0.922
π_S	0.00	0.676	0.00	1.000	0.00	1.000	0.01	0.665
π_N	0.00	1.000	0.00	0.836	0.00	0.657	0.00	1.000
π_N/π_S	0.00	1.000	0.00	1.000	0.00	1.000	0.00	1.000

Figure S1.1. Demographic scenarios.

Scenarios of divergence between population´s *Abies* central Mexico 1-4. Unstable demographic models (altitudinal migration hypothesis of "*sky islands*" during Pleistocene). Four consecutive events were simulated with these parameters: **1)** A period of stable population size of duration tpast = 0.5, with an initial population size (npast = 0.5, $npast_{Scenario4}$ = 1.5) and final (nrec= 0.5, $npast_{Escenario4}$ = 1.5); **2)** A bottleneck of duration tpast = 0.261 (Since formation stratovolcanoes in TVB) with four different intensities ($npast_{Scenario1}$ = 0.025, $npast_{Scenario2}$ = 0.05, $npast_{Scenario3}$ = 0.15, $npast_{Scenario4}$ = 0.5; $nrec_{Scenario1}$ = 0.025, $nrec_{Scenario2}$ = 0.05, $nrec_{Scenario3}$ = 0.15, $nrec_{Scenario4}$ = 0.5); **3)** A population expansion tpast = 0.231, N_e growing at least three times its initial population size (npast = 3, $npast_{Scenario4}$ = 5; nrec = 3, $npast_{Scenario4}$ = 5); **4)** A bottleneck of duration tpast = 0.009 (Holocene), where popualtion´s N_e reduces at least a third in relation of population expansion. The times are in *4N* generations.

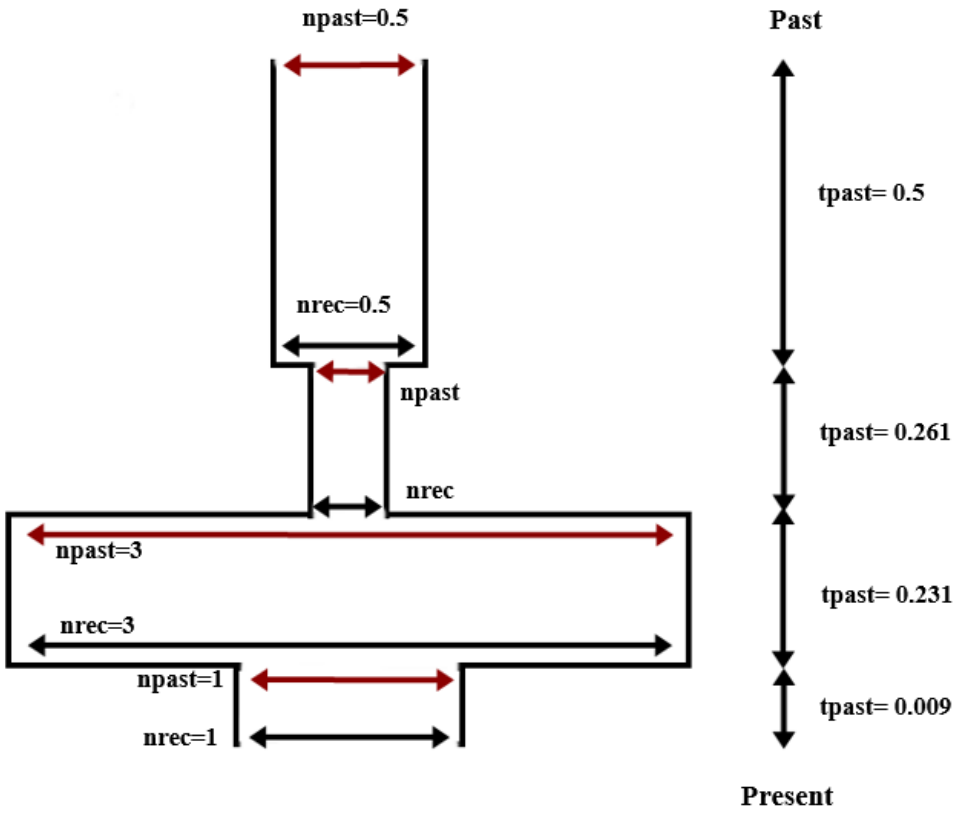


Figure S1.1. Demographic scenarios (continued…).

Scenarios 5-7; Unstable demographic models (hypothesis of altitudinal migration of *sky-islands* during Pleistocene). Four events were simulated with these parameters: **1)** A period of stable population size of duration tpast = 0.5, initial population size (npast = 0.5) and nrec =0.5; **2)** A bottleneck of duration tpast = 0.489 (since formation of stratovolcanoes in TVB until LGM) with three intensities $npast_{Scenario4}$ = 0.025, $npast_{Scenario5}$ = 0.05, $npast_{Scenario6}$ = 0.15; $nrec_{Scenario4}$ = 0.025, $nrec_{Scenario5}$ = 0.05, $nrec_{Scenario6}$ = 0.15); **3)** An expansion population tpast = 0.003 (LGM), population size increasing six times its initial population size (npast=3; nrec=3); **4)** A bottleneck of duration tpast= 0.009 (Holocene), N_e reduces a third in relation to period of population expansion.

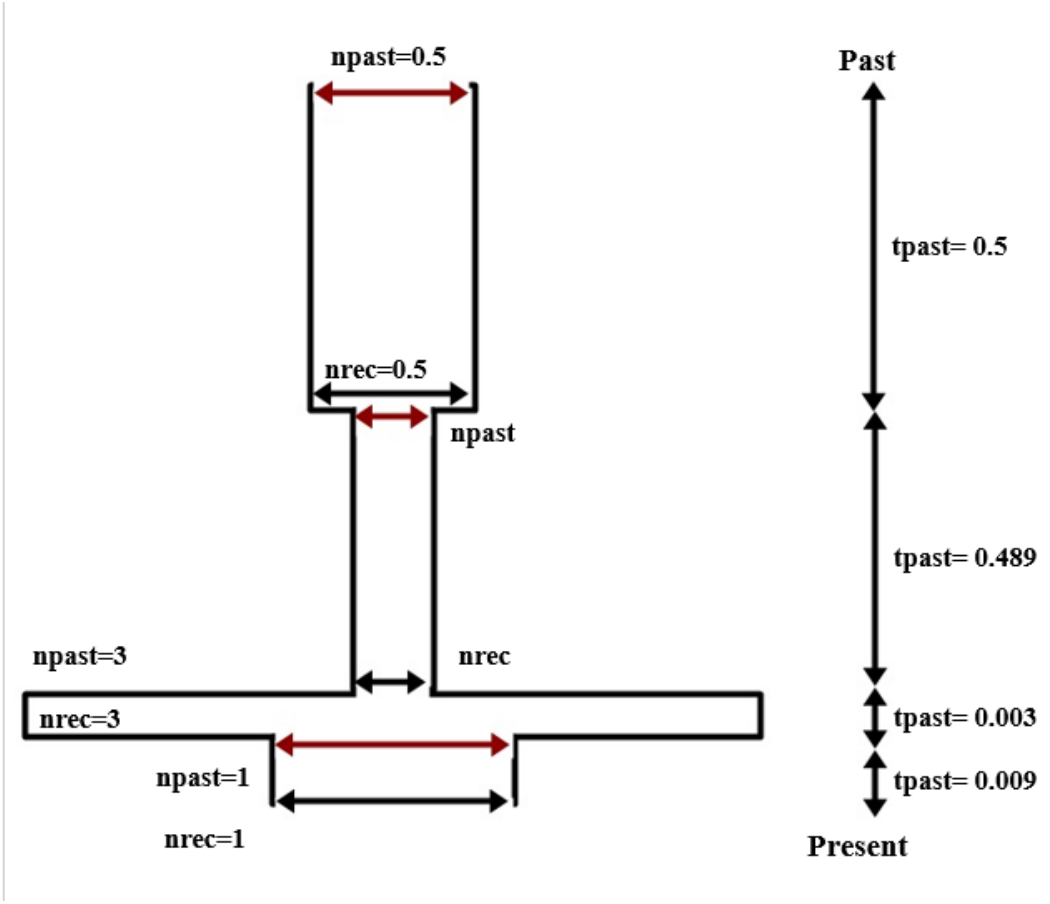


Figure S1.1. Demographic scenarios (continued…).

Scenarios 8 and 9: Bottleneck following by expansion population, Three events were simulated with these parameters: 1) A period of stable population size tpast= 0.5, initial population size (npast= 0.5) and final (nrec =0.5); **2)** A bottleneck of duration $tpast_{Scenario7}$ = 0.261 (since formation of stratovolcanoes in TVB) or $tpast_{Scenario8}$ = 0.489 (since formation of stratovolcanoes until LGM) that reduce population until 10% of population size (npast = 0.05, nrec= 0.05); **3)** An expansion population of duration $tpast_{Scenario7}$ = 0.239 or $tpast_{Scenario8}$ = 0.011 (LGM-present), the population size increases two times of initial population size (npast = 3; nrec=3). The times are in *4N* generations.

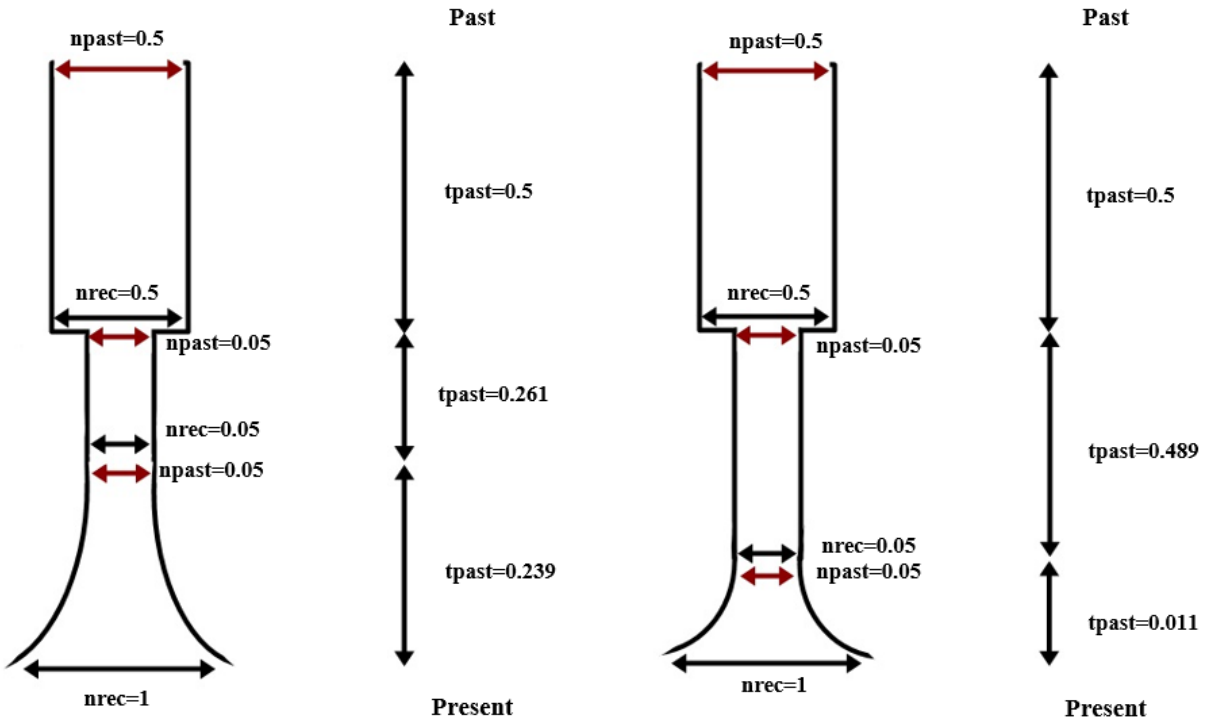


Figure S1.1. Demographic scenarios (continued…).

Scenarios 11 and 12: "Population expansion or colonization". Two events were simulated : **1)** A period of stable population size ($npast_{Scenario10}$ = 0.5, $npast_{Scenario11}$ = 0.2; $nrec_{Scenario10}$ = 0.5, $nrec_{Scenario11}$ = 0.2), with duration tpast = 0.5; **2)** An expansion population tpast = 0.5 (since formation of stratovolcanoes in TVB until present), the population growth two times (Scenario 10) or five times (Scenario 11) its initial size. The times are in *4N* generations.

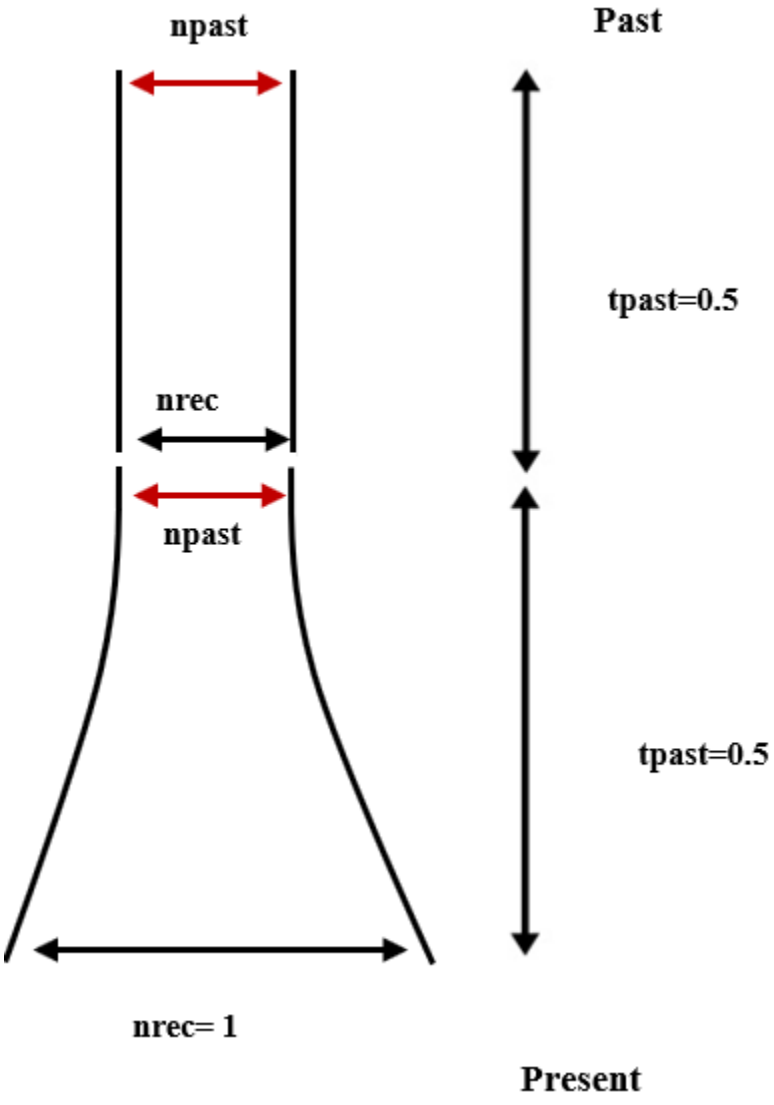


Figure S1.2. Euclidean distances to niche centroid in the present and Last Glacial Maximum (LGM) in two Mexican firs in central Mexico projected in the geography. The maps are colored as distance to niche centroid a pink-gray color indicates nearer to centroid and green color indicates farther to centroid. AF = *Abies flinckii;* AR = *A. religiosa;* CCSM = CCSM Model; MIROC = MIROC model; M1 = Model 1; M4 = Model 4.

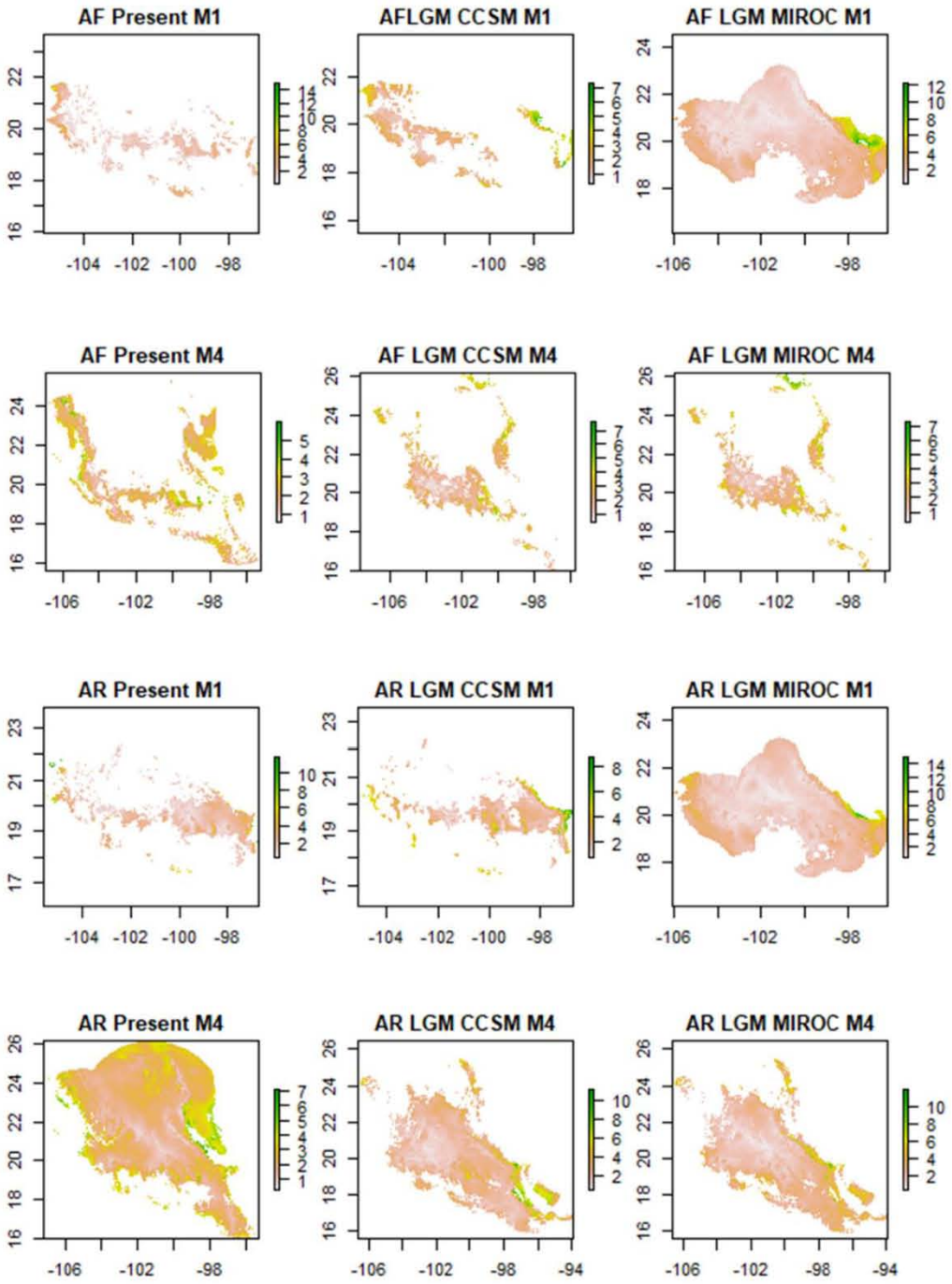


Figure S1.3. The contribution of the climatic variables on the two axes of the PCA and the percentage of inertia explained by the two axes. The PCA-environmental representing the niche of the species along the two first axes for *Abies flinckii* and *A. religiosa* across central Mexico. Gray shading shows the density of the occurrences of the species by cell. The solid and dashed contour lines illustrate, 100% and 50% of the available (background) environment (a). Histograms show the observed niche overlap *D* between *Abies flinckii* and *A. religiosa* (bars with a diamond) and simulated niche overlaps (gray bars) calculated from 100 iterations (b). The test was significant.

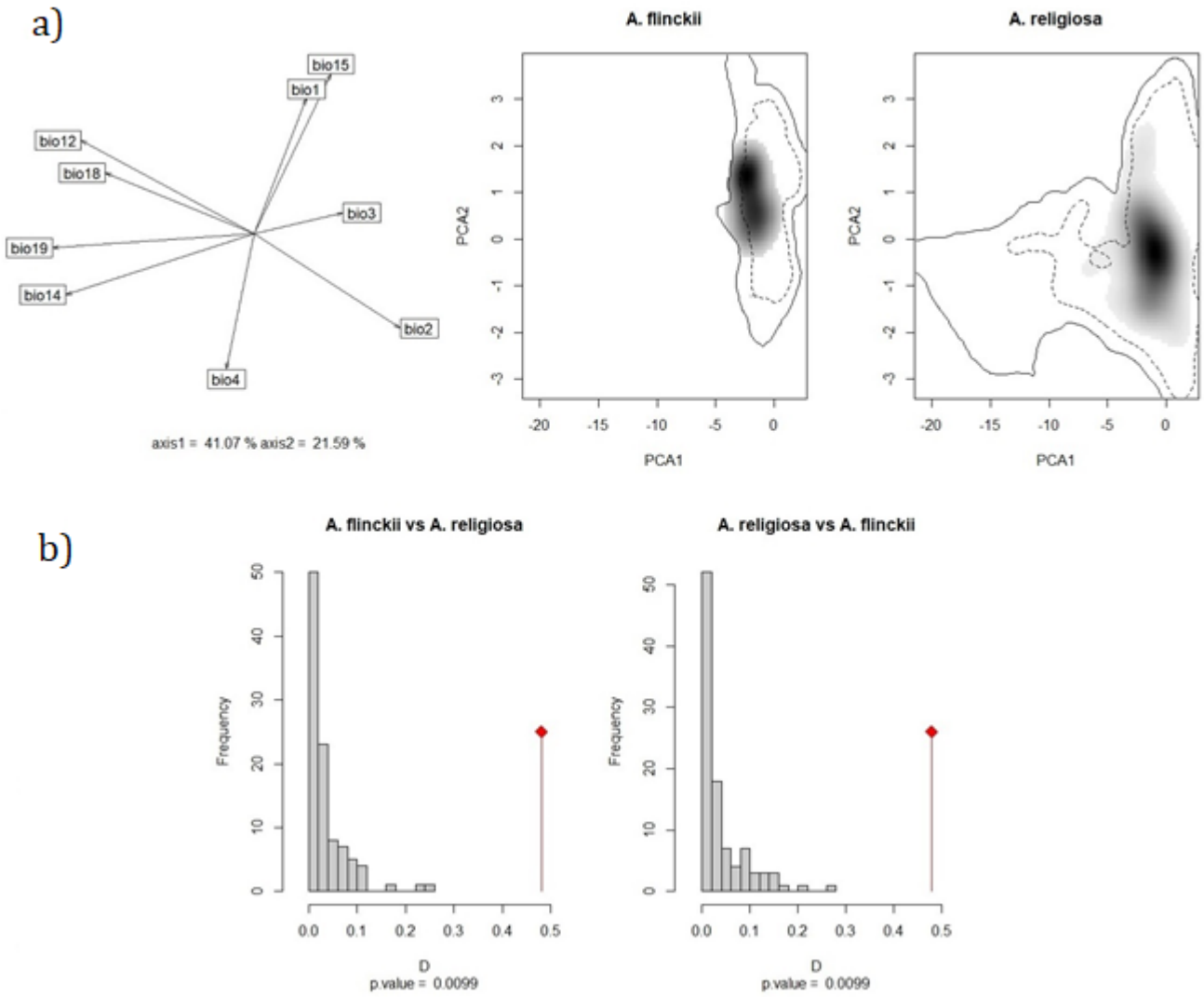


Figure S1.4. Boxplots of Euclidean distances to niche centroid for four models (M1-M4) in present and the Last Glacial Maximum (LGM) of 6 populations of *Abies flinckii* (white) and 14 of *A. religiosa* (gray) in central Mexico.

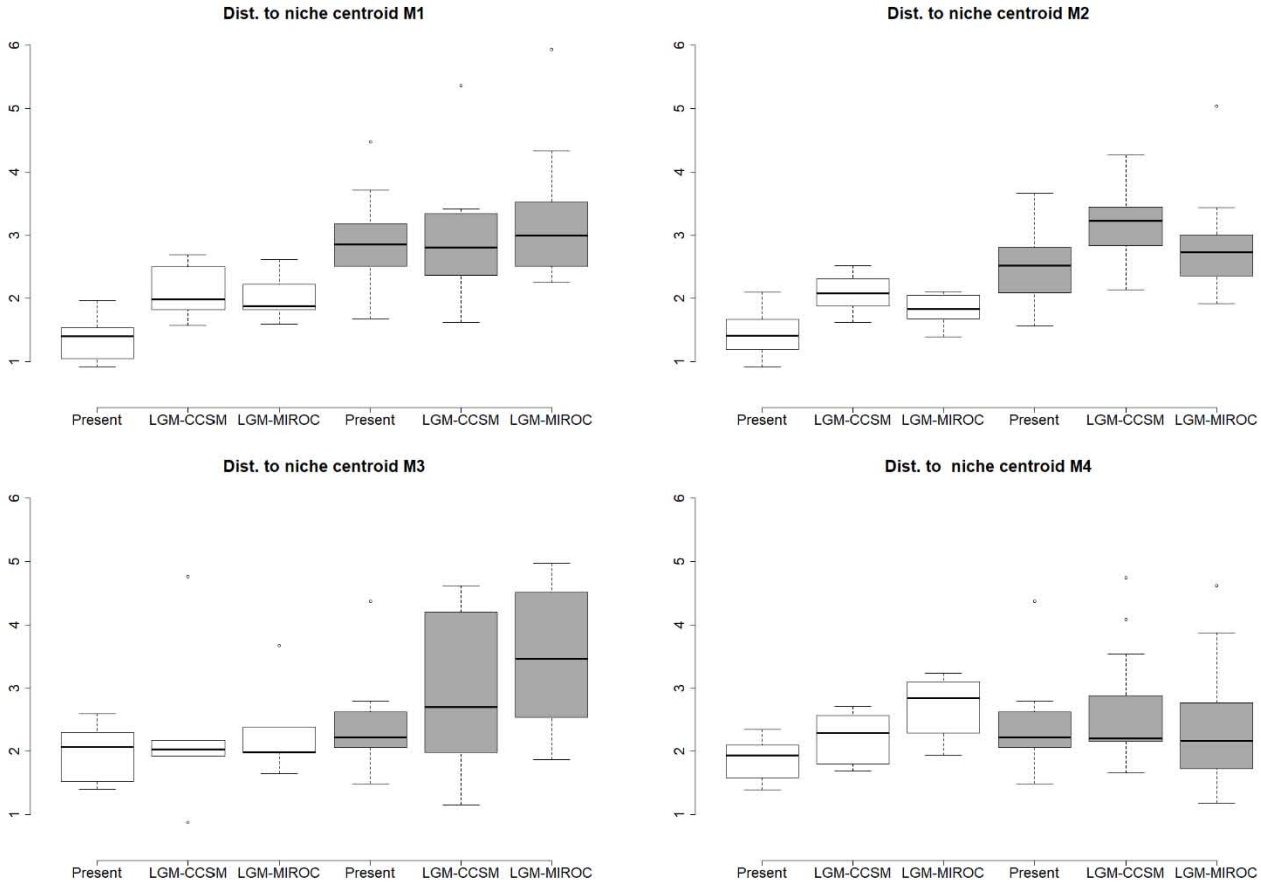


Figure S1.5. Relationships between π_N and π_S with distances to niche centroid in *Abies flinckii* (a) and *A. religiosa* (b) for models M1-M4 in central Mexico.

a)

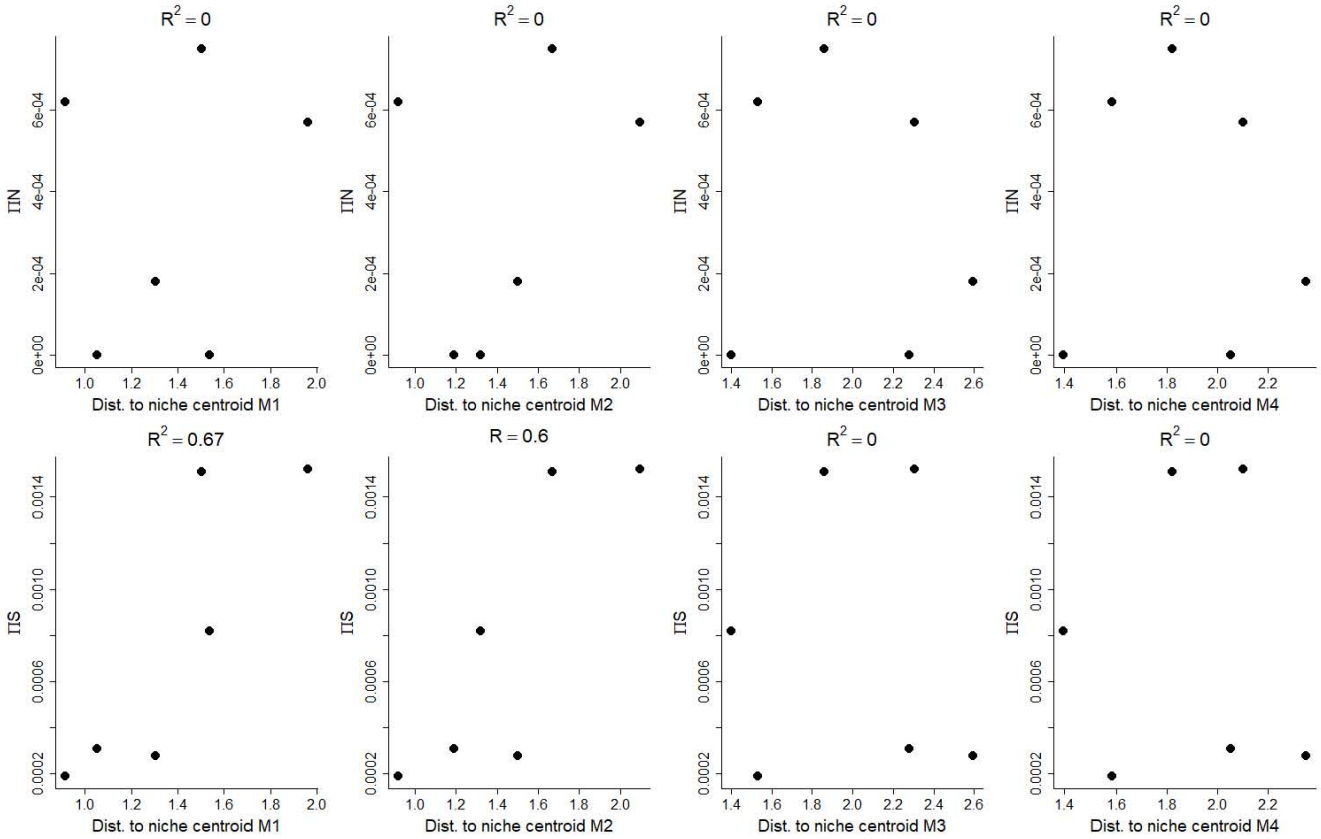
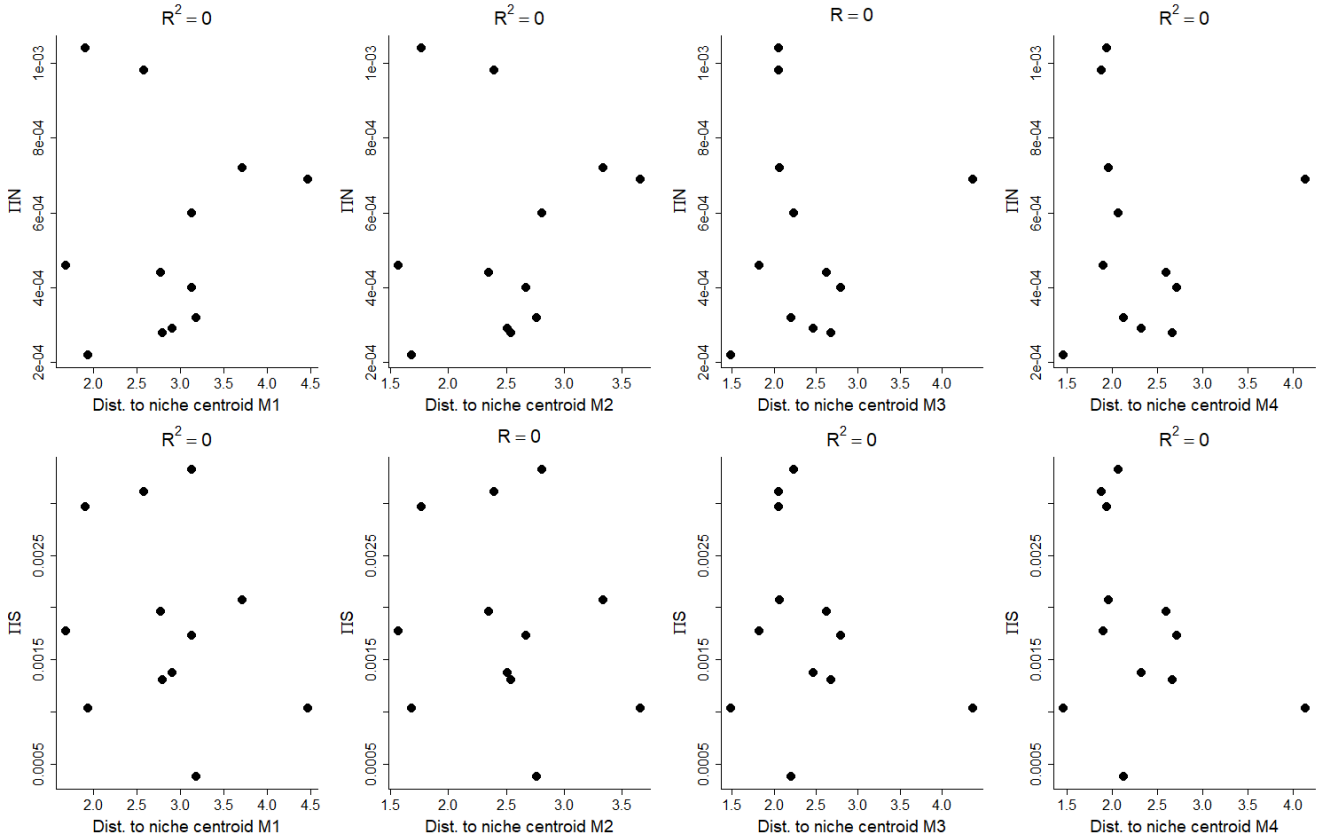


Figure S1.5. (continued…)

b)



SUPPORTING INFORMATION

Testing for niche centrality within the scope of the nearly neutral theory of evolution in a subtropical conifer species-pair

Jorge Cruz-Nicolás, Gustavo Giles-Pérez, Andrés Lira-Noriega, Norberto Martínez-Méndez, Erika Aguirre-Planter, Luis E. Eguiarte, Juan P. Jaramillo-Correa

APPENDIX S2: Tables S2.1, 2.2 and 2.3.

Table S2.1. Frequency of null alleles and β value associated to other sources of error, calculated for each locus in nuclear SSRs for *Abies flinckii* and *A. religiosa* in central Mexico.

Locus	β	Null alleles
Ab07	0.000	0.083
Ab12	0.000	0.030
Ab20	0.000	0.048
Ab27	0.004	0.171

Table S2.2. Sampled individuals; *N*; name of each gene-coding loci; Segregating sites: *S*; Nonsynonymous polymorphism: π_N; synonymous polymorphism: π_S; ratio of nonsynonymous and synonymous polymorphism: π_N/π_S; *D* (Tajima, 1989), F_s (Fu, 1997), *H* (Fay & Wu, 2000) and H_{norm} (Zeng *et al.*, 2006) for each gene/population in *Abies flinckii* (pops. 1-6) and *A. religiosa* (pops. 8-19) in central Mexico.

Pop	N	Gene-coding loci	S	π_N	π_S	π_N/π_S	D	Fs	H	H_{norm}
1	**4**	Porin Mip1	3	0.00000	0.00000	0.00000	0.204	-0.844	0.500	0.707
		Lhca4	0	0.00000	0.00000	0.00000	NA	NA	NA	NA
		α-1,6 xylosyltransferase	1	0.00000	0.00154	0.00000	-1.055	-0.182	-1.500	-3.195
		Fructose 1,6 biphosphate aldolase	0	0.00000	0.00000	0.00000	NA	NA	NA	NA
		Heat shock	0	0.00000	0.00000	0.00000	NA	NA	NA	NA
		ArMybIX	1	0.00493	0.00000	NA	1.633	0.540	0.000	0.000
		ArMybSTR	0	0.00000	0.00000	0.00000	NA	NA	NA	NA
		ARMybVI	0	0.00000	0.00000	0.00000	NA	NA	NA	NA
		Mean	**0.625**	**0.00062**	**0.00019**	**0.00000**	**0.26067**	**-0.162**	**-0.3333**	**-0.8293**
2	**7**	Porin Mip1	0	0.00077	0.00000	0.00000	0.908	-0.466	0.44	0.513
		Lhca4	0	0.00000	0.00000	0.00000	-1.155	-0.595	0.132	0.3
		α-1,6 xylosyltransferase	2	0.00000	0.00612	0.00000	1.554	2.538	-0.176	-0.266
		Fructose 1,6 biphosphate aldolase	0	0.00000	0.00000	0.00000	NA	NA	NA	NA
		Heat shock	0	0.00000	0.00000	0.00000	NA	NA	NA	NA
		ArMybIX	1	0.00520	0.00599	0.86700	0.077	0.783	-0.835	0.975
		ArMybSTR	0	0.00000	0.00000	0.00000	NA	NA	NA	NA
		ARMybVI	0	0.00000	0.00000	0.00000	-1.155	-0.595	NA	NA
		Mean	**0.375**	**0.00075**	**0.00151**	**0.10838**	**0.0458**	**0.333**	**-0.1098**	**0.3805**
3	**7**	Porin Mip1	4	0.00059	0.00000	NA	-1.245	-2.377	0.622	0.697
		Lhca4	0	0.00000	0.00000	0.00000	NA	NA	NA	NA
		α-1,6 xylosyltransferase	0	0.00000	0.00000	0.00000	NA	NA	NA	NA
		Fructose 1,6 biphosphate aldolase	0	0.00000	0.00000	0.00000	NA	NA	NA	NA
		Heat shock	0	0.00000	0.00000	0.00000	NA	NA	NA	NA
		ArMybIX	1	0.00394	0.01218	0.32300	1.641	2.338	-0.089	-0.129
		ArMybSTR	0	0.00000	0.00000	0.00000	-0.532	-0.465	0.396	0.598
		ARMybVI	0	0.00000	0.00000	0.00000	NA	NA	NA	NA
		Mean	**0.625**	**0.00057**	**0.00152**	**0.04614**	**-0.0453**	**-0.168**	**0.30967**	**0.38867**

Table S2.2. (Continued…)

Pop	N	Gen	S	π_N	π_S	π_N/π_S	D	Fs	H	H_{norm}
4	**5**	Porin Mip1	1	0.00000	0.00000	0.00000	0.334	0.536	-0.857	-1.826
		Lhca4	0	0.00000	0.00000	0.00000	NA	NA	NA	NA
		α-1,6 xylosyltransferase	2	0.00000	0.00247	0.00000	-1.587	0.586	0.356	0.516
		Fructose 1,6 biphosphate aldolase	1	0.00000	0.00000	0.00000	1.303	1.029	-0.267	-0.583
		Heat shock	0	0.00000	0.00000	0.00000	NA	NA	NA	NA
		ArMybIX	0	0.00000	0.00000	0.00000	NA	NA	NA	NA
		ArMybSTR	3	0.00000	0.00000	0.00000	-0.812	-0.071	-1	-1.092
		ARMybVI	0	0.00000	0.00000	0.00000	NA	NA	NA	NA
		Mean	**0.875**	**0.00000**	**0.00031**	**0.00000**	**-0.1905**	**0.52**	**-0.442**	**-0.7463**
5	**7**	Porin Mip1	1	0.00000	0.00000	0.00000	-0.341	0.186	-1.319	-2.998
		Lhca4	3	0.00000	0.00000	0.00000	1.218	3.293	-1.319	-1.993
		α-1,6 xylosyltransferase	1	0.00000	0.00223	0.00000	0.324	0.643	0.264	0.6
		Fructose 1,6 biphosphate aldolase	1	0.00000	0.00000	0.00000	0.842	0.944	-0.659	-1.499
		Heat shock	0	0.00000	0.00000	0.00000	NA	NA	NA	NA
		ArMybIX	0	0.00000	0.00000	0.00000	NA	NA	NA	NA
		ArMybSTR	4	0.00140	0.00000	NA	0.793	0.581	0.352	0.338
		ARMybVI	1	0.00000	0.00000	0.00000	1.212	1.139	0.22	0.5
		Mean	**1.375**	**0.00018**	**0.00028**	**0.00000**	**0.67467**	**1.131**	**-0.4102**	**-0.842**
6	**7**	Porin Mip1	2	0.00000	0.00000	0.00000	1.933	2.749	0.000	0.000
		Lhca4	1	0.00000	0.00000	0.00000	-1.155	-0.595	0.132	0.300
		α-1,6 xylosyltransferase	2	0.00000	0.00652	0.00000	1.838	2.697	-0.044	-0.066
		Fructose 1,6 biphosphate aldolase	0	0.00000	0.00000	0.00000	NA	NA	NA	NA
		Heat shock	0	0.00000	0.00000	0.00000	NA	NA	NA	NA
		ArMybIX	1	0.00000	0.00000	0.00000	1.444	0.966	0.000	0.000
		ArMybSTR	0	0.00000	0.00000	0.00000	NA	NA	NA	NA
		ARMybVI	0	0.00000	0.00000	0.00000	NA	NA	NA	NA
		Mean	**0.750**	**0.00362**	**0.01259**	**0.08437**	**1.8**		**0.119**	

Table S2.2. (Continued…)

Pop	N	Gen	S	πN	πS	$\pi N / \pi S$	D	Fs	H	Hnorm
8	**7**	Porin Mip1	1	0.00000	0.00000	NA	-1.141	-0.476	0.152	0.338
		Lhca4	9	0.00000	0.00203	0.00000	0.320	0.153	0.923	0.535
		α-1,6 xylosyltransferase	5	0.00000	0.00516	0.00000	-1.623	-2.100	0.747	0.615
		Fructose 1,6 biphosphate aldolase	0	0.00000	0.00000	0.00000	NA	NA	NA	NA
		Heat shock	0	0.00000	0.00000	0.00000	NA	NA	NA	NA
		ArMybIX	5	0.00123	0.00377	0.32700	-1.123	0.979	-2.788	-2.247
		ArMybSTR	1	0.00000	0.00000	0.00000	-1.155	-0.595	0.132	0.300
		ARMybVI	1	0.00108	0.00000	NA	-0.341	0.186	0.220	0.500
		Mean	**2.750**	**0.00029**	**0.00137**	**0.05450**	**-0.8438**	**-0.3088**	**-0.1023**	**0.00683**
9	**7**	Porin Mip1	2	0.00089	0.00000	NA	-0.248	-0.269	-0.970	-1.438
		Lhca4	9	0.00000	0.00560	0.00000	0.708	-0.945	0.394	0.224
		α-1,6 xylosyltransferase	4	0.00024	0.00264	0.09091	-1.798	-1.640	0.527	0.508
		Fructose 1,6 biphosphate aldolase	0	0.00000	0.00000	0.00000	NA	NA	NA	NA
		Heat shock	0	0.00000	0.00000	0.00000	NA	NA	NA	NA
		ArMybIX	1	0.00000	0.00000	0.00000	0.541	0.735	0.273	0.609
		ArMybSTR	4	0.00000	0.00000	0.00000	-1.798	-3.143	0.527	0.508
		ARMybVI	2	0.00059	0.00000	NA	-1.481	1.475	0.132	0.300
		Mean	**2.888**	**0.00022**	**0.00103**	**0.01515**	**-0.679**	**-0.631**	**0.147**	**0.119**
10	**7**	Porin Mip1	18	0.00000	0.00000	0.00000	-0.184	-0.272	0.444	0.645
		Lhca4	6	0.00000	0.00288	0.00000	0.529	1.400	0.220	0.181
		α-1,6 xylosyltransferase	4	0.00000	0.00502	0.00000	-1.164	-2.170	0.703	0.677
		Fructose 1,6 biphosphate aldolase	1	0.00000	0.00402	0.00000	-1.155	-0.595	0.132	0.300
		Heat shock	0	0.00000	0.00000	0.00000	NA	NA	NA	NA
		ArMybIX	5	0.00246	0.00377	0.65300	-1.831	0.325	-2.879	-2.321
		ArMybSTR	2	0.00000	0.00000	0.00000	-1.481	-1.475	0.264	0.399
		ARMybVI	1	0.00108	0.00000	NA	-0.195	0.816	0.242	0.541
		Mean	**4.625**	**0.00044**	**0.00196**	**0.09329**	**-0.783**	**-0.282**	**-0.125**	**0.060**

Table S2.2. (Continued…)

Pop	N	Gen	S	πN	πS	$\pi N/\pi S$	D	Fs	H	Hnorm
11	**7**	Porin Mip1	3	0.00000	0.00000	0.00000	0.422	0.923	-0.576	-0.854
		Lhca4	6	0.00000	0.00374	0.00000	2.453	2.528	-0.088	-0.072
		α-1,6 xylosyltransferase	1	0.00000	0.00187	0.00000	-0.195	0.297	0.242	0.541
		Fructose 1,6 biphosphate aldolase	0	0.00000	0.00000	0.00000	NA	NA	NA	NA
		Heat shock	1	0.00000	0.00400	0.00000	0.015	0.417	-1.067	-2.331
		ArMybIX	2	0.00148	0.00453	0.32600	-1.401	0.586	-1.422	-2.063
		ArMybSTR	1	0.00000	0.00000	0.00000	-1.141	-0.476	0.152	0.338
		ARMybVI	2	0.00217	0.00000	NA	0.700	0.375	0.044	0.066
		Mean	**2.000**	**0.00046**	**0.00177**	**0.04657**	**0.122**	**0.664**	**-0.388**	**-0.625**
12	**7**	Porin Mip1	5	0.00156	0.00153	1.01961	0.422	0.923	0.727	0.686
		Lhca4	7	0.00039	0.00118	0.33051	-1.023	0.559	-1.727	-1.219
		α-1,6 xylosyltransferase	1	0.00000	0.00088	0.00000	-1.155	-0.595	0.132	0.300
		Fructose 1,6 biphosphate aldolase	0	0.00000	0.00000	0.00000	NA	NA	NA	NA
		Heat shock	2	0.00000	0.00649	0.00000	-0.382	-0.362	0.424	0.629
		ArMybIX	5	0.00123	0.00377	0.32700	-1.831	0.325	-2.879	-2.321
		ArMybSTR	2	0.00000	0.00000	0.00000	-0.959	-0.855	-1.495	-2.258
		ARMybVI	2	0.00000	0.00000	0.00000	-0.011	-0.072	0.484	0.731
		Mean	**3.000**	**0.00040**	**0.00173**	**0.20964**	**-0.706**	**-0.011**	**-0.619**	**-0.493**
13	**7**	Porin Mip1	2	0.00120	0.00000	NA	-0.382	-0.362	-0.667	-0.988
		Lhca4	7	0.00000	0.00215	0.00000	0.844	0.612	0.545	0.385
		α-1,6 xylosyltransferase	0	0.00000	0.00000	0.00000	NA	NA	NA	NA
		Fructose 1,6 biphosphate aldolase	1	0.00000	0.00000	0.00000	-1.155	-0.595	0.132	0.300
		Heat shock	2	0.00202	0.00000	NA	-1.132	0.952	0.533	0.734
		ArMybIX	3	0.00389	0.01205	0.32300	0.826	1.252	0.088	0.103
		ArMybSTR	7	0.00000	0.00512	0.00000	-1.944	1.007	1.061	0.667
		ARMybVI	4	0.00124	0.00434	0.28600	-1.385	-1.088	0.697	0.657
		Mean	**3.250**	**0.00104**	**0.00296**	**0.10150**	**-0.618**	**0.254**	**0.341**	**0.265**

Table S2.2. (Continued...)

Pop	N	Gen	S	π_N	π_S	π_N/π_S	D	Fs	H	H_{norm}
14	**7**	Porin Mip1	5	0.00129	0.00000	NA	-1.236	-1.606	-0.264	-0.254
		Lhca4	6	0.00000	0.00101	0.00000	0.195	0.061	-0.747	-0.615
		α-1,6 xylosyltransferase	0	0.00000	0.00000	0.00000	NA	NA	NA	NA
		Fructose 1,6 biphosphate aldolase	0	0.00000	0.00000	0.00000	NA	NA	NA	NA
		Heat shock	0	0.00000	0.00000	0.00000	NA	NA	NA	NA
		ArMybIX	2	0.00123	0.00377	0.32600	-1.451	0.432	-1.515	-2.246
		ArMybSTR	2	0.00000	0.00000	0.00000	-1.481	-1.475	-1.582	-2.391
		ARMybVI	4	0.00298	0.00343	0.86900	-0.704	-0.445	0.747	0.719
		Mean	**2.375**	**0.00069**	**0.00103**	**0.17071**	**-0.935**	**-0.607**	**-0.672**	**-0.957**
15	**7**	Porin Mip1	5	0.00155	0.00132	1.17424	-0.268	-1.85	0.44	0.362
		Lhca4	5	0.00000	0.00000	0.00000	2.04	4.591	-0.622	-0.574
		α-1,6 xylosyltransferase	2	0.00028	0.00103	0.27184	-1.451	0.432	0.303	0.449
		Fructose 1,6 biphosphate aldolase	1	0.00000	0.00402	0.00000	-1.155	-0.595	0.132	0.3
		Heat shock	0	0.00000	0.00000	0.00000	NA	NA	NA	NA
		ArMybIX	8	0.00448	0.01380	0.32500	-0.385	0.211	-2.485	-1.41
		ArMybSTR	1	0.00000	0.00000	0.00000	-0.341	0.186	0.22	0.5
		ARMybVI	2	0.00149	0.00473	0.31400	0.416	0.206	0.527	0.797
		Mean	**3.000**	**0.00098**	**0.00311**	**0.26064**	**-0.1634**	**0.45443**	**-0.2121**	**0.06057**
16	**7**	Porin Mip1	2	0.00104	0.00000	NA	-0.691	-0.594	-0.889	-1.289
		Lhca4	7	0.00000	0.00215	0.00000	0.844	0.612	0.545	0.385
		α-1,6 xylosyltransferase	4	0.00000	0.00427	0.00000	-1.481	-2.604	0.615	0.592
		Fructose 1,6 biphosphate aldolase	2	0.00000	0.01243	0.00000	-0.201	-0.207	0.396	0.598
		Heat shock	3	0.00080	0.00321	0.24922	-1.278	-1.727	0.484	0.565
		ArMybIX	6	0.00295	0.00452	0.65300	-1.493	-0.242	-2.400	-1.655
		ArMybSTR	3	0.00000	0.00000	0.00000	-0.886	-1.290	0.571	0.667
		ARMybVI	0	0.00000	0.00000	0.00000	NA	NA	NA	NA
		Mean	**3.375**	**0.0006**	**0.00332**	**0.12889**	**-0.741**	**-0.865**	**-0.097**	**-0.020**

Table S2.2. (Continued…)

Pop	N	Gen	S	π_N	π_S	π_N/π_S	D	Fs	H	H_{norm}
17	**7**	Porin Mip1	4	0.00145	0.00000	NA	-0.301	-0.128	0.132	0.127
		Lhca4	4	0.00000	0.00500	0.00000	0.649	-0.621	0.703	0.677
		α-1,6 xylosyltransferase	1	0.00000	0.00088	0.00000	-1.155	-0.595	0.132	0.300
		Fructose 1,6 biphosphate aldolase	1	0.00000	0.00743	0.00000	-0.341	0.186	0.220	0.500
		Heat shock	0	0.00000	0.00000	0.00000	NA	NA	NA	NA
		ArMybIX	6	0.00211	0.00323	0.65300	-1.959	-0.756	-2.901	-2.091
		ArMybSTR	1	0.00000	0.00000	0.00000	0.015	0.417	0.267	0.583
		ARMybVI	1	0.00221	0.00000	NA	1.508	1.287	0.000	0.000
		Mean	**2.250**	**0.00072**	**0.00207**	**0.10883**	**-0.226**	**-0.030**	**-0.207**	**0.014**
18	**7**	Porin Mip1	2	0.00042	0.00000	NA	-1.481	-1.475	-1.582	-2.391
		Lhca4	6	0.00000	0.00000	0.00000	-0.279	1.955	-0.089	-0.070
		α-1,6 xylosyltransferase	4	0.00000	0.00864	0.00000	0.361	0.322	0.791	0.761
		Fructose 1,6 biphosphate aldolase	0	0.00000	0.00000	0.00000	NA	NA	NA	NA
		Heat shock	1	0.00000	0.00187	0.00000	-1.330	-0.476	0.152	0.338
		ArMybIX	1	0.00123	0.00000	NA	-1.141	-0.476	0.152	0.338
		ArMybSTR	1	0.00000	0.00000	0.00000	-0.959	-0.855	0.352	0.531
		ARMybVI	1	0.00059	0.00000	NA	-1.155	-0.595	0.132	0.300
		Mean	**2.000**	**0.00028**	**0.00131**	**0.00000**	**-0.855**	**-0.229**	**-0.013**	**-0.028**
19	**7**	Porin Mip1	3	0.00042	0.00000	NA	-1.671	-0.761	-1.451	-1.694
		Lhca4	7	0.00000	0.00215	0.00000	1.075	0.730	0.636	0.449
		α-1,6 xylosyltransferase	1	0.00000	0.00088	0.00000	-1.155	-0.595	0.132	0.300
		Fructose 1,6 biphosphate aldolase	0	0.00000	0.00000	0.00000	NA	NA	NA	NA
		Heat shock	0	0.00000	0.00000	0.00000	NA	NA	NA	NA
		ArMybIX	3	0.00105	0.00000	NA	-1.278	-1.727	0.484	0.565
		ArMybSTR	2	0.00000	0.00000	0.00000	-0.959	-0.855	0.352	0.531
		ARMybVI	1	0.00108	0.00000	NA	-0.341	0.186	0.220	0.500
		Mean	**2.125**	**0.00032**	**0.00038**	**0.00000**	**-0.722**	**-0.504**	**0.062**	**0.109**

Table S2.3. Sampled individuals: *N*; name of each gene-coding loci; Segregating sites: *S*; Nonsynonymous polymorphism: π_N; synonymous polymorphism: π_S; ratio of nonsynonymous and synonymous polymorphism: π_N/π_S; *D* (Tajima, 1989), F_s (Fu, 1997), *H* (Fay & Wu, 2000) and H_{norm} (Zeng *et al.*, 2006) for each gene/population in *Abies flinckii* (pops. 1-6) and *A. religiosa* (pops. 8-19) in central Mexico.

Pop	N	Gen	S	π_N	π_S	π_N/π_S	D	Fs	H	H_{norm}
1	**5**	CesA1	17	0.02644	0.04375	0.60400	2.073	4.809	0.292	0.070
		COBRA-like	22	0.01886	0.08831	0.21400	2.520	11.077	0.267	0.076
		Porin Mip1a	10	0.00000	0.01097	0.00000	2.338	4.442	0.214	0.097
		Mean	**16**	**0.01510**	**0.04768**	**0.27267**	**2.310**	**6.776**	**0.258**	**0.081**
2	**7**	CesA1	17	0.02298	0.03944	0.58300	2.633	9.545	0.132	0.041
		COBRA-like	22	0.01571	0.08232	0.19100	2.999	15.354	0.000	0.000
		Porin Mip1a	10	0.00047	0.01012	0.04644	2.338	4.442	0.214	0.097
		Mean	**16**	**0.01305**	**0.04396**	**0.27348**	**2.657**	**9.780**	**0.115**	**0.046**
3	**7**	CesA1	17	0.02337	0.03994	0.58500	2.516	8.622	0.152	0.046
		COBRA-like	22	0.01592	0.08179	0.19500	2.824	10.466	-0.485	-0.118
		Porin Mip1a	13	0.00000	0.01634	0.00000	1.465	1.356	0.643	0.235
		Mean	**17**	**0.01310**	**0.04602**	**0.26000**	**2.268**	**6.815**	**0.103**	**0.054**
4	**5**	CesA1	17	0.02374	0.04913	0.48300	2.456	8.622	0.152	0.046
		COBRA-like	25	0.02039	0.09145	0.22300	1.807	10.466	-0.485	-0.118
		Porin Mip1a	15	0.00154	0.00902	0.17073	1.417	1.356	0.643	0.235
		Mean	**19**	**0.01522**	**0.04987**	**0.29224**	**1.893**	**6.815**	**0.103**	**0.054**
5	**7**	CesA1	18	0.02236	0.05287	0.42300	2.913	5.534	0.352	0.105
		COBRA-like	28	0.02033	0.08602	0.23600	2.229	7.355	0.176	0.035
		Porin Mip1a	14	0.00345	0.00532	0.64850	1.874	0.274	-0.352	-0.130
		Mean	**20**	**0.01538**	**0.04807**	**0.43583**	**2.339**	**4.388**	**0.059**	**0.003**
6	**7**	CesA1	16	0.02238	0.03944	0.56700	2.923	12.781	0.000	0.000
		COBRA-like	22	0.01571	0.08232	0.19100	2.999	15.354	0.000	0.000
		Porin Mip1a	11	0.00174	0.01023	0.17009	2.620	3.237	0.747	0.336
		Mean	**16**	**0.01328**	**0.04400**	**0.30936**	**2.847**	**10.457**	**0.249**	**0.112**
8	**7**	CesA1	19	0.02161	0.02871	0.75200	1.128	-2.286	1.363	0.387
		COBRA-like	25	0.01637	0.07685	0.21300	1.962	8.698	-5.451	-1.215
		Porin Mip1a	18	0.00338	0.00499	0.67735	1.174	-2.437	2.374	0.707
		Mean	**21**	**0.01379**	**0.03685**	**0.54745**	**1.421**	**1.325**	**-0.571**	**-0.040**

Table S2.3. Continued…

Pop	N	Gen	S	π_N	π_S	π_N/π_S	D	Fs	H	H_{norm}
9	**7**	CesA1	24	0.02369	0.02740	0.86500	0.248	-1.029	2.286	0.528
		COBRA-like	26	0.01764	0.08607	0.20500	2.403	2.344	0.044	0.009
		Porin Mip1a	14	0.00315	0.00569	0.55360	1.729	-0.355	2.377	0.707
		Mean	**21**	**0.01483**	**0.03972**	**0.54120**	**1.460**	**0.320**	**1.569**	**0.415**
10	**7**	CesA1	18	0.02143	0.02383	0.89900	1.102	-0.367	1.099	0.327
		COBRA-like	24	0.01644	0.07958	0.20700	2.418	6.648	-1.670	-0.386
		Porin Mip1a	15	0.00301	0.00813	0.37023	1.108	0.093	1.600	0.531
		Mean	**19**	**0.01363**	**0.03718**	**0.49208**	**1.543**	**2.125**	**0.343**	**0.157**
11	**7**	CesA1	11	0.01797	0.01875	0.95900	2.808	10.124	0.000	0.000
		COBRA-like	26	0.00606	0.02833	0.21400	-2.218	7.622	-25.152	-0.854
		Porin Mip1a	6	NA	NA	NA	2.453	2.528	-0.088	-0.072
		Mean	**14**	**0.01202**	**0.02354**	**0.58650**	**1.014**	**6.758**	**-8.413**	**-0.309**
12	**7**	CesA1	13	0.01852	0.02053	0.90200	2.074	3.256	0.396	0.155
		COBRA-like	25	0.00872	0.03907	0.22300	-0.875	3.555	-19.780	-4.409
		Porin Mip1a	18	0.00319	0.00246	1.29675	0.309	-1.060	3.286	0.910
		Mean	**19**	**0.01014**	**0.02069**	**0.80725**	**0.503**	**1.917**	**-5.366**	**-1.115**
13	**7**	CesA1	22	0.02083	0.02955	0.70500	0.337	2.873	1.714	0.428
		COBRA-like	30	0.01865	0.08147	0.22900	1.297	4.987	-3.560	-0.673
		Porin Mip1a	21	0.00242	0.00549	0.44080	-0.067	-0.910	2.933	0.698
		Mean	**24**	**0.01397**	**0.03884**	**0.45827**	**0.522**	**2.317**	**0.362**	**0.151**
14	**7**	CesA1	17	0.02123	0.02569	0.82600	1.480	2.768	1.143	0.357
		COBRA-like	26	0.01750	0.08315	0.21000	2.197	2.238	-2.681	-0.577
		Porin Mip1a	19	0.00230	0.00479	0.48017	0.556	-1.198	2.909	0.808
		Mean	**21**	**0.01368**	**0.03788**	**0.50539**	**1.411**	**1.269**	**0.457**	**0.196**
15	**7**	CesA1	22	0.02137	0.02958	0.72200	0.418	1.754	1.670	0.417
		COBRA-like	28	0.01944	0.08169	0.23800	1.671	2.590	-1.848	-0.364
		Porin Mip1a	21	0.00359	0.00434	0.82719	0.902	-3.443	3-121	0.812
		Mean	**24**	**0.01480**	**0.03854**	**0.59573**	**0.997**	**0.300**	**-0.089**	**0.288**
16	**7**	CesA1	19	0.02351	0.03129	0.75100	1.588	0.718	1.275	0.362
		COBRA-like	29	0.01614	0.06936	0.23300	0.729	6.068	-8.044	-1.568
		Porin Mip1a	23	0.00335	0.00464	0.72198	0.312	0.216	3.576	0.839
		Mean	**24**	**0.01433**	**0.03510**	**0.56866**	**0.876**	**2.334**	**-1.064**	**-0.122**
17	**7**	CesA1	27	0.02653	0.03762	0.70500	0.494	-1.583	3.253	0.677
		COBRA-like	24	0.01622	0.08863	0.18300	2.479	4.571	-0.622	-0.137
		Porin Mip1a	20	0.00265	0.00434	0.61060	1.247	-0.966	3.253	0.884
		Mean	**24**	**0.01513**	**0.04353**	**0.49953**	**1.407**	**0.674**	**1.961**	**0.475**

Table S2.3. Continued…

Pop	N	Gen	S	π_N	π_S	π_N/π_S	D	Fs	H	H_{norm}
18	**7**	CesA1	20	0.02335	0.02233	1.04600	0.809	4.337	1.319	0.358
		COBRA-like	14	0.01892	0.08373	0.22600	2.589	8-017	0.424	0.096
		Porin Mip1a	15	0.00215	0.00479	0.44885	0.814	0.411	1.273	0.433
		Mean	**16**	**0.01481**	**0.03695**	**0.57362**	**1.404**	**2.374**	**1.005**	**0.296**
19	**7**	CesA1	23	0.02352	0.02929	0.80300	0.506	0.922	1.890	0.454
		COBRA-like	26	0.01837	0.08616	0.21300	2.391	5.174	0.000	0.000
		Porin Mip1a	15	0.00330	0.00548	0.60219	1.455	-2.562	1.956	0.649
		Mean	**21**	**0.01506**	**0.04031**	**0.53940**	**1.451**	**1.178**	**1.282**	**0.368**