UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO

POSGRADO EN CIENCIAS BIOLÓGICAS

FACULTAD DE CIENCIAS

Esclareciendo la historia evolutiva de aves de los bosques tropicales de México mediante la integración de información genómica, ecológica y biogeográfica

Tesis que para obtener el grado de

DOCTOR EN CIENCIAS

Presenta:

M.C. Alexander Llanes Quevedo

Tutor

Dr. Adolfo Gerardo Navarro Sigüenza/Facultad de Ciencias, UNAM

Comité Tutor

Dra. Alicia Mastretta Yanes/CONAHCYT-CONABIO

Dr. Luis Antonio Sánchez González/Facultad de Ciencias, UNAM

Ciudad Universitaria, CD. MX. **Octubre 2023**

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COORDINACIÓN GENERAL DE ESTUDIOS DE POSGRADO
COORDINACIÓN DEL POSGRADO EN CIENCIAS BIOLÓGICAS
FACULTAD DE CIENCIAS
OFICIO: CGEP/CPCB/FC/0732/2023

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 Comité de Posgrado en Ciencias Biológicas, celebrada el día **27 de febrero de 2023** se aprobó el siguiente jurado para el examen de grado de **DOCTOR EN CIENCIAS** del estudiante **LLANES QUEVEDO ALEXANDER** con número de cuenta **517493999** con la tesis titulada: **"Esclareciendo la historia evolutiva de aves de los bosques tropicales de México mediante la integración de información genómica, ecológica y biogeográfica",** realizada bajo la dirección del **DR. ADOLFO GERARDO NAVARRO SIGÜENZA:**

Presidente:	**DR. JUAN JOSÉ MORRONE**
Vocal:	**DR. LUIS ENRIQUE EGUIARTE FRUNS**
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Vocal:	**DR. LEOPOLDO DANIEL VÁZQUEZ REYES**
Secretario:	**DRA. ALICIA MASTRETTA YANES**

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"
Ciudad Universitaria, Cd. Mx., a 14 de septiembre de 2023

COORDINADOR DEL PROGRAMA

POSGRADO EN CIENCIAS BIOLOGICAS
COORDINACIÓN

DR. ADOLFO GERARDO NAVARRO SIGÜENZA

c. c. p. Expediente del alumno

AGNS/AAC/GEMF/EARR/mnm

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ÍNDICE

Resumen

La identificación y explicación de los patrones y procesos que generan la biodiversidad son objetivos centrales de la biología evolutiva y la sistemática, pero su estudio es complicado por la multiplicidad de factores que influyen en los eventos de dispersión, diferenciación y extinción de linajes que moldean la distribución actual de las especies. Las aves de los bosques tropicales de México (BTM) constituyen interesantes sistemas para tales estudios pues, en numerosos casos, presentan poblaciones diferenciadas morfológica y/o genéticamente, aun cuando las áreas que ocupan son aparentemente continuas y se esperaría mayor dispersión y menor estructuración genética. En esta tesis analicé los patrones distribución y variación genética de aves co-distribuidas en los BTM, para inferir los procesos históricos, ecológicos y demográficos que han determinado su evolución, usando un enfoque filogeográfico comparativo e integrando información distribucional, genómica y ecológica. En el primer capítulo realicé análisis de la distribución histórica y actual (últimos 10 años) de la riqueza (*alfa* y *beta*) y de parsimonia de endemismos de la avifauna de los BTM, basados en datos de ocurrencia y mapas de distribución de 260 especies residentes permanentes. Históricamente la mayor riqueza específica se ha localizado en la vertiente del Golfo de México y la Península de Yucatán, mientras que el mayor número de especies endémicas se distribuye en los bosques estacionalmente secos de la costa del Pacífico y la Cuenca del Balsas. Los datos actuales indican una disminución del número de especies en todas las áreas, especialmente en Veracruz, la Cuenca del Balsas y algunas partes de la Costa Pacífica. La diversidad beta (recambio de especies) es elevada, tanto a nivel regional como dentro de cada una de las áreas por separado. La regionalización biogeográfica muestra dos grupos principales: 1) Los bosques secos de la vertiente Pacífica de México y la Cuenca del Balsas; 2) Las áreas al norte de la provincia Veracruzana, que forman el grupo hermano de las áreas del norte del Istmo de Tehuantepec y la Península de Yucatán, que a su vez forman agrupaciones independientes. El segundo capítulo es una revisión de los enfoques genómicos en la ornitología Neotropical y su utilidad en biogeografía. En este, encontré una tendencia a incorporar métodos genómicos, aunque los análisis comparativos son escasos y hay sesgos importantes hacia el estudio de las selvas tropicales, principalmente en la subregión Brasileña y de las especies del Orden Passeriformes. Los estudios genómicos se han centrado en los factores históricos que determinan patrones espaciales y temporales en la diversidad, el papel del flujo de genes en la diversificación y persistencia de las especies, así como en la influencia de la ecología en los procesos de adaptación y diferenciación local. El tercer capítulo está enfocado al estudio una especie politípica de los BTM, el Carpintero de Velázquez (*Melanerpes santacruzi)* y sus relaciones evolutivas con otras especies filogenéticamente cercanas, *M. carolinus* y *M. aurifrons*. Los análisis realizados con polimorfismos de nucleótidos simples (SNPs) indicaron que las tres especies son grupos recíprocamente monofiléticos, aunque la relación de *M. aurifrons* con su grupo hermano es ambigua. *Melanerpes santacruzi* está estructurada en tres grupos genética y ecológicamente diferenciados, con mezcla genética entre ellos, especialmente en sus zonas de contacto. Los modelos ecológicos y demográficos sugieren áreas intermitentes de simpatría y conectividad entre sus poblaciones desde el último período interglacial. Hay flujo génico desigual entre *M. aurifrons* y las poblaciones adyacentes de *M. santacruzi*, con predominio en la dirección *M. aurifrons* a *M. s. grateloupensis.* El cuarto capítulo es un análisis comparativo de patrones de diferenciación genética, ecológica y biogeográfica de cuatro especies co-distribuidas en los BTM. Los datos genómicos empleados (SNPs) indicaron una congruencia espacial parcial en la estructura genómica de las especies en el Istmo de Tehuantepec y humedales de Tabasco. Sin embargo, los tiempos de divergencia y las dinámicas poblacionales fueron diferentes para cada especie. Además, se emcontraron diferencias en cuanto a los efectos de la distancia y el ambiente en la diversidad genética de cada especie: en *Melanerpes santacruzi* y *Saltator atriceps* hay una relación entre la variación genómica y ambiental, mientras que en *Xiphorhynchus flavigaster* e *Icterus gularis*, la diferenciación genética es consecuencia de la distancia geográfica y limitado flujo genético. Esta tesis aporta evidencia sobre los procesos históricos que han modelado la evolución de las aves del BTM y de la influencia de procesos de adaptación local a diferentes condiciones ambientales, que en conjunto han promovido la elevada diversificación de la avifauna Neotropical.

Abstract

The identification and explanation of the patterns and processes generating biodiversity are central objectives of evolutionary biology and systematics, but their study is complicated by the multiplicity of processes that influence the events of dispersal, differentiation and extinction of lineages that shape the current distribution of the species. The birds of the Mexican tropical forests (MTF) constitute interesting systems for such studies because, in many cases, they present morphologically and/or genetically differentiated populations, even when the areas they occupy are apparently continuous and would be expected a greater dispersion and less genetic structuring. In this thesis, I analyzed the distribution patterns and genetic variation of co-distributed birds in the MTF, to infer the historical, ecological, and demographic processes that have determined their evolution, using a comparative phylogeographic approach and integrating distributional, genomic, and ecological information. In the first chapter, we performed analyses of the historical and current distribution (last 10 years) of species richness (alpha and beta) and parsimony of endemicity of the BTM avifauna, based on occurrence data and distribution maps of 260 permanent resident species. Historically, the highest species richness has been distributed on the slopes of the Gulf of Mexico and the Yucatan Peninsula, while the largest number of endemic species is distributed in the seasonally dry forests of the Pacific coast and the Balsas Basin. Current data indicates a decrease in the number of species in all areas, especially in Veracruz, the Balsas Basin, and some regions of the Pacific Coast. Beta diversity (species turnover) is high, both regionally (MTF as a whole) and within each of the areas separately. The biogeographic regionalization yields two main groups: 1) dry forests of the Pacific slope of Mexico and the Balsas Basin; 2) north of the Veracruz province, which is the sister group of the areas to the north of the Isthmus of Tehuantepec and the Yucatan Peninsula, which in turn constitute independent groups. The second chapter is a review of genomic approaches in Neotropical ornithology and their applications in biogeography. In this review, I found a trend to incorporate genomic methods, although comparative analyzes are scarce and there are important biases towards the study of tropical forests, specially in the Brazilian subregion, and of the species of the Order Passeriformes. Genomic studies have focused on the historical factors determining spatial and temporal patterns in diversity, gene flow in diversification, persistence of species, and the influence of ecology on local adaptation and differentiation processes. The third chapter is focused on the study of a polytypic species inhabiting the MTF, the Velazquez's Woodpecker (*Melanerpes santacruzi*) and its evolutionary relationships with other phylogenetically close species, *M. carolinus* and *M. aurifrons*. Our analyses, based on single nucleotide polymorphisms (SNP), indicated that all three species are reciprocally monophyletic groups, although the relationship of *M. aurifrons* to its sister group is ambiguous. *Melanerpes santacruzi* is structured into three genetically and ecologically differentiated groups, with genetic admixture between them, especially in their contact zones. Ecological and demographic models suggest intermittent areas of sympatry and connectivity among their populations since the last interglacial period. There is uneven gene flow between *M. aurifrons* and adjacent populations of *M. santacruzi*, with prevalence in the direction of *M. aurifrons* to *M. s. grateloupensis*. The fourth chapter is a comparative analysis of genomic, ecological and biogeographic patterns of four species co-distributed in the MTF. Our dataset, constituted by SNP markers, indicated that there is a partial spatial congruence in the genomic structure of the species in the Isthmus of Tehuantepec and Tabasco wetlands. However, divergence times and population dynamics were different for each species. We found differences in the effects of distance and environment on the genetic diversity of each species: in *Melanerpes santacruzi* and *Saltator atriceps* there is a significant relationship between genomic and environmental variation, while in *Xiphorhynchus flavigaster* and *Icterus gularis*, genetic differentiation is consequence of geographical distance and limited gene flow. This thesis provides evidence about the historical processes that have shaped the evolution of MTF birds and the influence of local adaptation processes to different environmental conditions, which together have promoted the high diversification of Neotropical avifauna.

INTRODUCCIÓN GENERAL

El Neotrópico es una región biogeográfica que incluye casi toda América del Sur, toda Centroamérica, las Antillas y parte de México (Morrone, 2019). Esta región, definida hace más de un siglo y medio por Sclater (1858) a partir del estudio de la distribución de varios grupos de aves, presenta los mayores valores de diversidad alfa y gamma de especies entre todas las unidades ecogeográficas del mundo (Terborgh, 1980; Terborgh *et al*., 1990; Stotz *et al*., 1996). Debido a esto, la identificación y explicación de los patrones y procesos que han generado la elevada biodiversidad del área ha sido de interés permanente en biología evolutiva y ecología desde el siglo XIX. Sin embargo, el entendimiento de estos elementos es complicado por la multiplicidad de fenómenos que influyen en los eventos de dispersión, diferenciación y extinción de los linajes. Por tanto, el estudio de la evolución biológica en el Neotrópico todavía tiene mucho que aportar, especialmente considerando el avance contemporáneo en los métodos de estudio de la biodiversidad.

El Neotrópico alberga aproximadamente una de cada tres especies de aves descritas en la Tierra, y ha sido propuesto como el centro de origen de la mayoría de las aves modernas (Stotz *et al*., 1996; Orme *et al*., 2006). La actividad tectónica y cambios paleogeográficos del Neógeno (*e.g*., Chapman, 1917), así como las reorganizaciones de las comunidades vegetales por los ciclos glaciales del Cuaternario (*e.g*., Haffer, 1969) han sido sugeridos como los principales impulsores de la diversificación de la avifauna en la región. Asimismo, se han propuesto dos grandes hipótesis para explicar los patrones de diferenciación de la biota Neotropical: la hipótesis de los refugios y la de disturbio-vicarianza (Rull, 2011). De acuerdo con la primera, los procesos de especiación en el Neotrópico han estado principalmente determinados por la ocurrencia de ciclos de aislamiento/diferenciación y de flujo genético entre los parches de bosques tropicales, debidos a contracciones y expansiones poblacionales durante los periodos glaciares e interglaciares, respectivamente (Haffer, 1969). Según la hipótesis de disturbio-vicarianza, se propone un papel preponderante de las migraciones bióticas desde las tierras altas a las tierras bajas, seguida de expansiones poblacionales y procesos de especiación durante el Pleistoceno a través de las tierras bajas. Las montañas neotropicales, según esta hipótesis, habrían sido decisivas para la evolución biológica del Cuaternario, ya que podrían haber actuado como “bombas de especies” para las tierras bajas y medias circundantes (Rull, 2011).

Entre los biomas más biodiversos del Neotrópico, se encuentran los bosques tropicales (Cayuela y Granzow-De la Cerda, 2012). En México, estos ecosistemas, que se distribuyen típicamente desde el nivel del mar hasta los 1900 m, son continuos en las

vertientes Atlántica y Pacífica de México en el eje norte-sur. En la mayor parte de su rango, los bosques tropicales de México (BTM) están separados por los sistemas montañosos que conforman la Zona de Transición Mexicana a la Región Neártica (Fig. 1a), entrando en contacto en el sureste del país, en el Istmo de Tehuantepec. Desde el punto de vista ecológico, los BTM se clasifican como bosques cálido-secos (principalmente hacia la costa del Pacífico, sur de Tamaulipas-norte de Veracruz y la porción noroeste del Estado de Yucatán) y bosques cálido-húmedos (vertiente del Golfo de México y del Mar Caribe y costas de los Estados de Nayarit y Chiapas; INEGI, CONABIO e INE, 2008) (Fig. 1b y c).

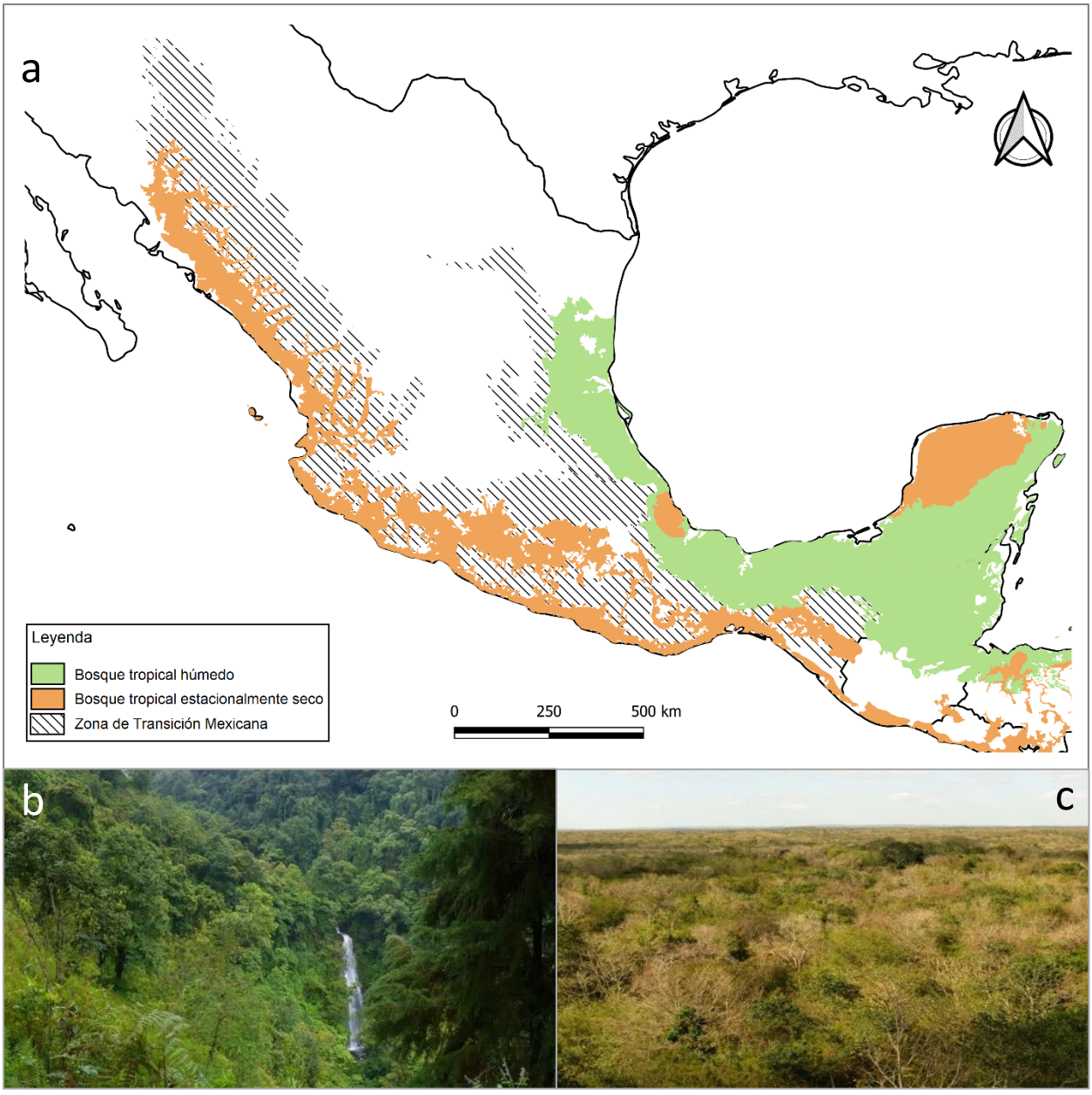

Figura 1. Bosques Tropicales de México (BTM).(a) Distribución geográfica de los bosques neotropicales en México de acuerdo con Olson *et al*. (2001) y principales cadenas montañosas del país que representan la Zona de Transición Mexicana, de acuerdo con Morrone (2019). (b) Vista de

bosque tropical húmedo en Coatepec, Veracruz; (c) Vista de bosque tropical estacionalmente seco tomada en Uxmal, Península de Yucatán. Fotografías tomadas por K. Mjia y K. Schulz respectivamente, distribuidas bajo licencia CC-BY2.0.

Los BTM presentan una alta diversidad de aves, con más de 500 especies incluyendo residentes permanentes (de las cuales más del 10% presenta alguna categoría de endemismo), residentes temporales y de tránsito (Berlanga *et al*., 2015, Navarro-Sigüenza *et al*., 2014). Sin embargo, el estudio de los procesos evolutivos y patrones actuales de diferenciación morfológica/genética de las aves de los BTM ha recibido relativamente poca atención en comparación con otros taxones, como los distribuidos en bosques mesófilo de montaña (*e.g*., Cortés-Rodríguez *et al*., 2008; Barber y Klicka, 2010; Ornelas *et al*., 2013). La mayoría de los trabajos filogeográficos sobre aves de bosques tropicales se encuentran limitados a especies distribuidas en la costa Pacífica mexicana (*e.g*., Cortés-Rodríguez *et al*., 2013; Castillo-Chora *et al*., 2021), y pocos de ellos abarcan un enfoque comparativo e integrador, lo que limita las inferencias que pueden ser realizadas sobre la historia compartida entre los taxones y las áreas que ocupan.

Las aves de los BTM constituyen sistemas muy interesantes para los estudios evolutivos, pues en numerosos casos presentan poblaciones morfológica y/o genéticamente diferenciadas, aun cuando las áreas que ocupan son aparentemente continuas y sería esperable una mayor dispersión y menor estructuración genética (Sánchez-González *et al*., 2015). Esta diferenciación morfológica -muchas veces reconocida con la descripción de subespecies- generalmente se ha detectado hacia las costas del Atlántico y del Pacífico o asociadas a “rupturas” geográficas o ecológicas en la costa de Jalisco y el Istmo de Tehuantepec respectivamente (Howell y Webb, 1995; Fig. 2).

En biogeografía y filogeografía, la existencia de patrones de distribución y variación entre los taxones es reconocida como una evidencia de la historia compartida entre las biotas y las áreas que estos ocupan (*e.g*., Bermingham y Moritz, 1998; Avise, 2000; Arbogast y Kenagy, 2001). Teniendo esto en consideración, con la presente tesis pretendo analizar los patrones espaciales de ocurrencia y variación genética de aves co-distribuidas en los BTM mediante la integración de información biogeográfica, genómica y ecológica. La conjunción de estas líneas de evidencia permitirá una aproximación más detallada a los procesos históricos, ecológicos y demográficos que han determinado la evolución de las aves y los ecosistemas que ocupan.

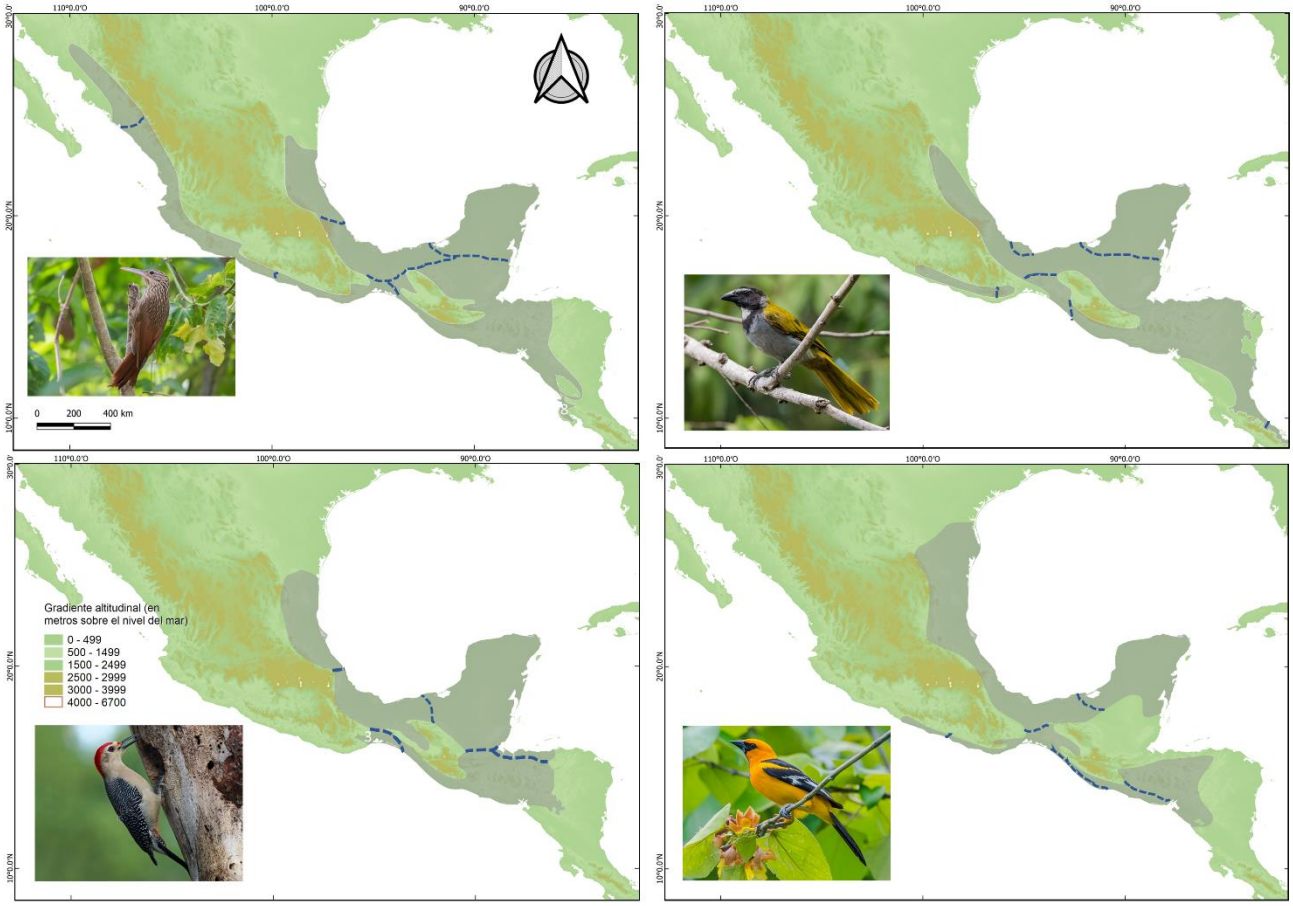

Figura 2. Patrones de distribución y variación morfológica de cuatro especies de aves co-distribuidas en los bosques tropicales de México. En gris se representan las áreas de ocurrencia de cada especie y en líneas discontinuas azules, los límites aproximados de distribución de subespecies distinguidas en base a diferencias morfológicas. De izquierda a derecha, y de arriba hacia abajo se representan: *Xiphorhynchus flavigaster*, *Saltator atriceps*, *Melanerpes santacruzi* e *Icterus gularis* (Fotos ilustrativas de las especies tomadas de eBird [http://ebird.org] por J. M. Artigas Azas, D. Danko, I. Davies y J. M. Artigas Azas, respectivamente). El mapa base es el modelo digital de terreno de Hydro1k (https://www.usgs.gov/centers/eros/science/usgs-eros-archive-digital-elevation-hydro1k).

De forma más específica este proyecto se centra en las siguientes preguntas: (1) ¿Existe congruencia espacial y temporal entre los agentes que han propiciado los patrones de diferenciación de las aves de los BTM? y, (2) ¿Cuál es la dinámica genética actual de las áreas de contacto entre las poblaciones de esas especies, principalmente en el Istmo de Tehuantepec? Para abordar las problemáticas antes mencionadas, utilicé como principal hipótesis que la presencia de patrones de distribución similares de poblaciones diferenciadas de aves de los BTM (en ausencia de barreras aparentes a su dispersión) es producto de la divergencia genética derivada de eventos históricos de vicarianza por la formación de barreras coincidentes espacio-temporalmente, que en el presente han desaparecido.

Para poner a prueba esta hipótesis, propuse como objetivo general el analizar la congruencia de la estructura filogeográfica entre especies de aves co-distribuidas en los BTM, con las características actuales del paisaje y con los modelos climáticos al pasado. A su vez, subdividí este objetivo en los siguientes objetivos específicos:

(1) Analizar los patrones biogeográficos de riqueza de especies de las aves residentes en los BTM;

(2) Estimar la diversidad genética de las poblaciones de algunas especies de aves co-distribuidas en los BTM a lo largo de sus áreas de distribución -enfatizando sus posibles zonas de contacto- usando marcadores moleculares generados por secuenciación masiva;

(3) Determinar la estructura filogeográfica de las poblaciones de dichas especies de aves;

(4) Reconstruir la historia geográfica de las entidades genéticas recuperadas a través de modelos de nicho ecológico proyectados al pasado;

(5) Identificar polimorfismos asociados a genes candidatos que indiquen adaptación local de las poblaciones de las especies a diferentes condiciones ambientales.

Esta tesis contiene cuatro capítulos y una sección de discusión y conclusiones generales. En el primer capítulo presento un análisis de los patrones biogeográficos y de riqueza histórica y actual de las especies de aves residentes en los BTM. En el segundo capítulo realizo una revisión bibliográfica acerca del impacto del desarrollo de las tecnologías actuales de secuenciación masiva del DNA en los estudios biogeográficos de las aves neotropicales. El tercer capítulo se enfoca en el estudio, a nivel poblacional, de una especie politípica distribuida en los BTM, el Carpintero de Velázquez *Melanerpes santacruzi* y sus relaciones evolutivas con otros taxones filogenéticamente cercanos (*M. carolinus* y *M. aurifrons*). Finalmente, en el cuarto capítulo presento un análisis comparativo e integrador de los patrones genómicos, ecológicos y biogeográficos de cuatro especies co-distribuidas en los BTM.

Con la presente tesis pretendo ofrecer un acercamiento integrador de los procesos ecológicos y genéticos que han moldeado la diversidad avifaunística de los BTM, lo que permitirá mejorar el entendimiento actual de la complejidad de los patrones de distribución de las especies y la diversidad genética de la biota Mesoamericana en general. Además, la información generada podrá constituir una fuente adicional para sustentar las decisiones taxonómicas sobre límites de especies, su correspondencia con los patrones biogeográficos y las prioridades de conservación.

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CAPÍTULO 1. Patrones históricos y actuales de diversidad y relaciones biogeográficas de la avifauna residente de los bosques tropicales de México

Alexander Llanes-Quevedo[1,2], Luis E. Sánchez-Ramos[3] y Adolfo G. Navarro-Sigüenza[2]

[1] Posgrado en Ciencias Biológicas, Universidad Nacional Autónoma de México

[2] Museo de Zoología, Facultad de Ciencias, Universidad Nacional Autónoma de México, Apartado Postal 70-399, 04510. Ciudad de México, México

[3] Centro de Geociencias, Universidad Nacional Autónoma de México. Juriquilla, Querétaro, México

Patrones históricos y actuales de diversidad y relaciones biogeográficas de la avifauna residente de los bosques tropicales de México

RESUMEN.- El estudio de los patrones de distribución de la biodiversidad es esencial para reconstruir su historia evolutiva y planificar adecuadamente estrategias de manejo y conservación. En este trabajo presentamos un análisis de la diversidad y de los patrones biogeográficos de la avifauna de los bosques tropicales de México (BTM), ecosistemas con valores excepcionales de biodiversidad, que se encuentran sometidos a crecientes presiones antrópicas. Para realizar esto compilamos mapas de distribución generados a partir de datos históricos (de especímenes colectados entre el siglo XVIII y 2007) y registros de ocurrencia de GBIF de los últimos 10 años (2013-2023) de 260 especies residentes permanentes de los BTM. A partir de estos datos, realizamos análisis comparativos del número y cambio de composición de especies (considerando las matrices de distribución "histórica" y "actual") y un análisis de parsimonia de endemismos en los BTM. De acuerdo con los datos históricos, la mayor riqueza de especies se localiza en la vertiente del Golfo de México y la Península de Yucatán, mientras que los bosques estacionalmente secos de la costa del Pacífico y la Cuenca del Balsas presentaron los valores más altos de especies endémicas. Sin embargo, los datos recopilados en la última década indican una disminución notable del número de especies en todas las áreas analizadas, especialmente en Veracruz, la Cuenca del Balsas y algunas regiones de la Costa Pacífica. El cálculo de los índices de diversidad *beta* de las aves en los BTM arrojó elevados valores de recambio de especies para las dos matrices de datos evaluadas, tanto a nivel regional como dentro de cada provincia biogeográfica analizada. La hipótesis biogeográfica obtenida en este trabajo muestra que la avifauna de los BTM se agrupa en dos grandes clados: i) un grupo que contiene los bosques secos de la vertiente pacífica de México y la Cuenca del Balsas y ii) otro grupo que contiene las áreas al norte de la provincia Veracruzana y es el clado hermano de las áreas del norte del Istmo de Tehuantepec y la Península de Yucatán, que a su vez forman agrupaciones independientes.

Palabras clave: análisis de parsimonia de endemismos, cambios antrópicos, conservación de aves, distribución histórica, distribución actual, diversidad beta, Neotrópico

Historical and current patterns of diversity and biogeographic relationships of the resident avifauna of the tropical forests of Mexico

ABSTRACT.- The study of biodiversity distribution patterns is essential to reconstruct its evolutionary history and adequately plan management and conservation strategies. In this work, we present an analysis of diversity and biogeographic patterns of the avifauna of the Mexican tropical forests (MTF), ecosystems with exceptional values of biodiversity, highly threatened by the increasing anthropic pressures. To do this, we compiled distribution maps generated from historical data (of specimens collected between the 18th century and 2007) and GBIF occurrence records from the last 10 years (2013-2023) of 260 permanent resident species of the MTF. Based on these data, we performed comparative analyzes of the number and composition change of species (considering the "historical" and "current" distribution matrices) and a parsimony analysis of endemicity in the MTF. According to historical data, the highest species richness is located on the slopes of the Gulf of Mexico and the Yucatan Peninsula, while the seasonally dry forests of the Pacific coast and the Balsas Basin presented the highest values of endemic species. However, the data collected in the last decade indicate a notable decrease in the number of species in all the areas analyzed, especially in Veracruz, the Balsas Basin, and some regions of the Pacific Coast. Beta diversity indices calculation yielded high values of species turnover for the two data matrices evaluated, both at the regional level and within each analyzed biogeographic province. The biogeographic hypothesis obtained in this work shows that the MTF avifauna is grouped into two major clades: i) a group that contains the dry forests of the Pacific slope of Mexico and the Balsas Basin and ii) another group that contains the areas north of the Veracruz province and is the sister clade of the northern areas of the Isthmus of Tehuantepec and the Yucatán Peninsula, which in turn form independent groupings.

Key words: anthropic changes, beta diversity, bird conservation, current distribution, historical distribution, Neotropics, Parsimony analysis of endemicity

INTRODUCCIÓN

El estudio de los patrones de ocurrencia de las especies es esencial para reconstruir su historia evolutiva y potenciar los esfuerzos de conservación en el contexto contemporáneo de pérdida acelerada de la biodiversidad. El arreglo espacial de la biodiversidad es el complejo producto de la historia compartida entre las biotas y las áreas que estos ocupan. Uno de los mejores ejemplos de esta complejidad lo constituye la biota mexicana, cuya diversidad es un reflejo de la posición geográfica, historia geológica, relieve y clima de México (Ramamoorthy *et al*., 1993). En México pueden distinguirse hasta diecisiete provincias fisiográficas, y dos regiones climáticas separadas por el trópico de Cáncer, lo que junto con una posición geográfica limítrofe entre las regiones biogeográficas Neártica y Neotropical, ha producido una diversificación biológica notable a nivel global (Morrone, 2019). La diversidad de México tanto a nivel de ecosistemas como de especies se distribuye heterogéneamente, formando patrones espaciales complejos de paisajes con diferente composición biótica, aun entre áreas relativamente cercanas (Koleff *et al.,* 2008).

La posición geográfica de los bosques tropicales de México (BTM) en el extremo septentrional del Neotrópico y el confinamiento (durante gran parte de su extensión) entre las costas Atlántica y Pacífica de México y los sistemas montañosos que conforman la Zona de Transición a la Región Neártica, han generado condiciones particulares para la evolución de los componentes bióticos que los caracterizan. La estrecha faja de área ocupada por estos bosques y la influencia ambiental y biótica de las serranías adyacentes han potenciado el efecto de las oscilaciones climáticas del Pleistoceno en el aislamiento y reconexión de las comunidades forestales neotropicales (Prance, 1982; Toledo, 1982: Metcalfe *et al*., 2000). Como resultado de estos procesos, los BTM están caracterizados por la presencia de un gran número de taxones endémicos y poblaciones diferenciadas morfológica y/o genéticamente (*e.g*., aves: Rocha-Moreira *et al*., 2020; Castillo-Chora *et al*., 2021; Llanes-Quevedo *et al*., 2022; anfibios: Mulcahy y Mendelson, 2000; mamíferos: Hernández-Canchola y León-Paniagua, 2017). Además, los BTM se encuentran entre los ecosistemas más amenazados de toda Mesoamérica (Dinerstein *et al*., 1995; Ceballos y Valenzuela *et al*., 2010; García-Oliva y Jaramillo, 2011).

Los BTM han sido clasificados en dos tipos principales: bosques húmedos y bosques estacionalmente secos, atendiendo a la abundancia de las precipitaciones y las características de su vegetación dominante (Villaseñor y Ortiz, 2013). Los primeros constituyen comunidades dominadas por árboles perennifolios (de entre 35-40 m de altura generalmente), en zonas con precipitación abundante (preciptación media anual superior los

2,000 mm) y entre el nivel del mar y los 1,000 msnm (CONABIO, 2022; https://www.biodiversidad.gob.mx/ecosistemas/). Por otra parte, los bosques estacionalmente secos presentan árboles relativamente más bajos (5-15 m), en zonas cálidas con precipitaciones medias anuales que oscilan entre 300−1,200 mm y largas temporadas de sequía de entre cinco a ocho meses. Generalmente estos ecosistemas se distribuyen entre el nivel del mar y los 1,900 msnm (CONABIO, 2022; https://www.biodiversidad.gob.mx/ecosistemas/). Estos últimos son más abundantes en la vertiente Pacífica del país, en las provincias biogeográficas Cuenca del Balsas y Tierras Bajas del Pacífico, mientras que los bosques húmedos se distribuyen principalmente en las provincias Veracruzana y Península de Yucatán (Morrone, 2019).

A la fecha se han realizado varios estudios evolutivos y biogeográficos que han estado encaminados a comprender los patrones de ocurrencia de los BTM y la fauna asociada a ellos. En algunos casos, estos ecosistemas han sido incluidos como parte del análisis de regiones geográficas específicas, como la Península de Yucatán (Cortés-Ramírez, 2012), o el oeste de México (en conjunto con bosques de montaña pertenecientes a la Zona de Transición Mexicana; García-Trejo y Navarro-Sigüenza, 2004). En otros casos, los trabajos se han realizado analizando por separado los bosques húmedos o los estacionalmente secos en México (*e.g*., plantas del género *Bursera*: Becerra, 2005; aves de la costa del Pacifico Mexicano: Ríos-Muñoz y Navarro-Sigüenza, 2012; Castillo-Chora *et al*., 2020), incluyendo toda Mesoamérica (mamíferos de bosques húmedos: Olguín-Monroy *et al*., 2013; aves de bosques húmedos: Miller *et al*., 2011; Castillo-Chora *et al*., 2021), o abarcando el Neotrópico (*e.g*., aves: Prieto-Torres *et al*., 2019a, b).

Sin embargo, a pesar de que las provincias neotropicales mexicanas (que incluyen los bosques tropicales de México y América Central) constituyen una unidad biogeográfica natural (*i.e*., el dominio Mesoamericano; Morrone, 2014), ningún trabajo ha integrado el estudio de faunas asociadas a todas ellas para la obtención de patrones biogeográficos conjuntos. Por ello, en el presente trabajo examinamos la distribución geográfica de la riqueza histórica y actual de especies de aves residentes en los BTM y generamos una hipótesis de regionalización biogeográfica basada en un Análisis de Parsimonia de Endemismos (Rosen y Smith, 1988; PAE, por sus siglas en inglés: *Parsimony Analysis of Endemicity*).

Debido a su gran diversidad y a la atención que históricamente ha recibido su estudio, las aves se han convertido en modelos para los trabajos de evolución biológica y biogeografía (Brumfield, 2012). Las aves cuentan con un buen estado de conocimiento, una

taxonomía relativamente estable, un alto porcentaje de endemismo en el área evaluada (y en todo el país en general), así como gran diversidad ecológica, etológica y de vagilidad.

Este trabajo resulta relevante para comprender los cambios en los patrones históricos y actuales de distribución de la riqueza de las aves de México, lo que tiene implicaciones para la planeación de su conservación y manejo. Además, la regionalización biogeográfica aquí propuesta permite mejorar la comprensión de la historia evolutiva de los BTM, especialmente considerando que la mayoría de los estudios biogeográficos previos parten de la separación de los bosques tropicales húmedos de los estacionalmente secos, y generalmente los análisis abarcan solamente de alguno de ellos (*e.g*., Prieto-Torres *et al*., 2019). La generación de hipótesis de relación entre las áreas ocupadas por los BTM permitirá contar con un marco de referencia útil para contrastarlos en el futuro con información de la variación genética de las poblaciones y las especies, para evaluar la congruencia de los patrones recuperados.

MÉTODOS

Área de estudio.- El área de estudio incluye las cuatro provincias neotropicales ubicadas en México (*sensu* Morrone, 2019; Fig. 1): Tierras bajas del Pacífico (localizada en una franja angosta e ininterrumpida que recorre la costa de los estados de Sinaloa, Nayarit, Jalisco, Colima, Michoacán, Guerrero, Oaxaca y Chiapas), Cuenca del Balsas (ubicada en el centro del país, en parte de los estados de Guerrero, México, Jalisco, Michoacán, Morelos, Oaxaca y Puebla), Veracruzana (costa del Golfo de México de los estados de Veracruz, Tabasco, Tamaulipas y parte de San Luis Potosí, Hidalgo, Puebla, Oaxaca, Chiapas y Campeche) y Península de Yucatán (sureste de México, abarcando los estados de Campeche, Yucatán y Quintana Roo). Sobre esta área se construyó una gradilla conformada por hexágonos de 0.33 grados de diámetro (Fig. 1), a partir de la cual se realizaron los análisis subsecuentes de diversidad y patrones biogeográficos.

Muestreo taxonómico.- Se analizaron datos de presencia y mapas distribucionales de 260 especies de aves (pertenecientes a 82 familias y 179 géneros) residentes en los BTM (Tabla S1; Materiales Suplementarios). Las especies fueron seleccionadas de acuerdo con los siguientes criterios: (1) Especies nativas; (2) Especies con datos de ocurrencia disponibles en el portal del Servicio de Información sobre Biodiversidad Global (GBIF por sus siglas en inglés, *Global Biodiversity Information Facility*) y mapas distribucionales en el Atlas de las Aves de México (Navarro-Sigüenza y Peterson, 2007); (3) Especies para las que se ha

descrito la ocupación de los BTM de acuerdo con el compendio ornitológico *Birds of the World* (Cornell University, 2023, https://birdsoftheworld.org/bow); (4) Especies residentes permanentes en el área analizada; y (5) Categoría específica de acuerdo a la AOU (Chesser *et al*., 2022). De esta forma, fueron excluidas del análisis especies introducidas, migratorias y aves acuáticas (e.g., Anseriformes, Gruiformes y Aequorlitornithes, *sensu* Prum *et al*., 2015).

Recopilación de mapas de distribución y datos de presencia.- Construimos dos bases de datos de ocurrencia de las especies con la intención de recuperar los patrones históricos y actuales de la distribución de la avifauna de los BTM. Para obtener los patrones históricos, compilamos los mapas de distribución de las especies seleccionadas del Atlas de las Aves de México (Navarro-Sigüenza y Peterson, 2007). Dichos mapas fueron construidos con información de más de 750 mil registros georreferenciados de aves de México a partir de los cuales se realizaron modelos predictivos de la distribución de las especies con la aplicación del algoritmo GARP (Navarro-Sigüenza y Peterson, 2007; Navarro-Sigüenza *et al.,* 2009). Los registros provienen de especímenes de museos (colectados entre el siglo XVIII y 2007), literatura e información original de diferentes especialistas de más de 80 colecciones científicas en México, Estados Unidos, Canadá y varios países de Europa (Navarro-Sigüenza y Peterson, 2007). A partir de estos mapas, se extrajeron los puntos de coincidencia con las celdas definidas dentro de la gradilla del área de estudio, y se generaron matrices de presencia (1, presencia; 0, ausencia) de las especies (columnas) por cada celda (filas).

Por otra parte, para obtener un panorama más actualizado de la diversidad de la avifauna de los BTM, recopilamos datos primarios de ocurrencia de este grupo (combinaciones de especies-latitud y longitud-localidad-año de registro) en GBIF en la última década, es decir, en el rango temporal comprendido entre 2013-2023 (Occurrence Downloads: https://doi.org/10.15468/dl.xceppm, doi.org/10.15468/dl.cbxx4j, doi.org/10.15468/dl.b5y3qg, doi.org/10.15468/dl.rc73kz, doi.org/10.15468/dl.e6bh6c, doi.org/10.15468/dl.9knvwp, doi.org/10.15468/dl.gr2t9j, doi.org/10.15468/dl.577f73, doi.org/10.15468/dl.bjqw2a, doi.org/10.15468/dl.prpy8u). A partir de los datos recopilados, se realizaron filtrados manuales para eliminar las especies excluidas de acuerdo con los criterios antes establecidos y los registros duplicados por celda.

Patrones de riqueza de las aves de los BTM.- Para visualizar los patrones de distribución de las especies de aves de los BTM, construimos mapas de riqueza en QGIS v.3.18 Zurich (QGIS.org, 2022) empleando ambas bases de datos. Para cada matriz, superpusimos los puntos únicos de presencia de cada especie por hexágono en la gradilla y posteriormente proyectamos en el mapa los valores de la suma de especies registradas en cada celda. Además, realizamos proyecciones incluyendo únicamente las especies endémicas y cuasiendémicas de México, de acuerdo con Berlanga *et al*. (2015). En ese trabajo, se consideran como endémicas a aquellas especies cuya distribución geográfica se encuentra restringida a los límites políticos del territorio mexicano y como cuasiendémicas, aquellas con áreas de distribución que se extienden ligeramente fuera de México (≤ 35 000 km2), debido a la continuidad de los hábitats (González García y Gómez de Silva, 2002). Para estos tres sets de datos, calculamos la significación estadística de la diferencia de los valores de riqueza entre las matrices histórica y actual mediante pruebas de U de Mann-Whitney, luego de la comprobación de la no normalidad de los datos con la prueba de Kolmogórov-Smirnov. Los cálculos fueron realizados con el software GraphPadPrism v8.0.2 (San Diego, California, USA, www.graphpad.com).

Adicionalmente, recopilamos la información concerniente al riesgo de extinción de cada especie de acuerdo con la Lista Roja de Especies Amenazadas de la Unión Internacional para la Conservación de la Naturaleza (2022; IUCN, por sus siglas en inglés) y la Norma Oficial Mexicana (NOM-059-Semarnat, 2010). Agrupamos las especies en las categorías de IUCN: “En Peligro Crítico”, “En Peligro” y “Vulnerable”, y de acuerdo con las categorías de la NOM-059: “Probablemente extintas en el medio silvestre”, “En Peligro de extinción”, “Amenazadas” y “Sujetas a protección especial” y proyectamos en el mapa su suma para cada celda de la gradilla. Para la generación de estos mapas, no se incluyeron las especies clasificadas como de menor preocupación y aquellas no evaluadas o con datos insuficientes.

Finalmente, estimamos el cambio en la composición de especies de aves en los BTM (diversidad β; Whittaker, 1972) mediante el cálculo del índice de Sørensen (β_{SOR}) con el paquete *betapart* (Baselga y Orme, 2012) en R v.4.1 (R Development Core Team, 2021). Ese índice de disimilitud está dado por dos componentes: el recambio de especies medido con el índice de Simpson (β_{SIM}) y el anidamiento, medido con el índice β_{NES} (Baselga, 2010). Esto permite cuantificar las diferencias asociadas con las especies no compartidas, independientemente de la riqueza específica de las áreas (Koleff *et al.*, 2003) y, reducir los sesgos que pueden generar tamaños de biotas diferentes en el análisis (Baselga, 2010). Los

cálculos de β -representada por el promedio de las combinaciones pareadas entre todas las celdas de la gradilla- se efectuaron con ambas matrices para la totalidad del área de estudio, y por separado para cada una de las provincias biogeográficas que la componen. Además, construimos un dendrograma a partir de un análisis de clasificación de la matriz de disimilitud de datos históricos para visualizar las relaciones entre las áreas e inferir los procesos que han generado los patrones de distribución de las especies.

Análisis de parsimonia de endemismos y regionalización biogeográfica.- Para evaluar las relaciones entre las áreas donde se distribuyen los BTM, implementamos un PAE (Rosen y Smith, 1988; Morrone y Escalante, 2002) con base en la matriz de datos históricos y la gradilla de 0.33°. Los análisis fueron realizados con el software NONA 2.0 (Goloboff, 1999) a través de WinClada versión 1.00.08 (Nixon, 2002). Para el enraizamiento del cladograma incluimos un área hipotética con solo ausencias, de acuerdo con lo recomendado por Rosen y Smith (1988) y Morrone (1994). Utilizamos la opción de búsqueda de *ratchet* (Nixon, 1999) con 500 iteraciones por repetición, una retención de árbol por iteración y un 40 % de muestreo de los caracteres. De los árboles obtenidos seleccionamos los más parsimoniosos y a partirde estos, calculamos el consenso estricto. Para este árbol se calcularon los índices de consistencia (CI), de retención (RI) y longitud (L). Las agrupaciones obtenidas mediante el PAE se visualizaron como conjuntos de celdas en mapas construidos en el sistema de información geográfica QGIS v.3.18.

RESULTADOS

Para las 260 especies seleccionadas, recopilamos sendos mapas de distribución a partir del Atlas de Aves de México (Navarro-Sigüenza y Peterson, 2007) y 37,289 registros recientes únicos georreferenciados en la zona de estudio. Estas especies representan aproximadamente el 24% de la avifauna total registrada para México (1,107 especies; Berlanga *et al*., 2015) e incluyen 33 especies endémicas y 22 cuasiendémicas, (Tabla S1, Materiales Suplementarios).

Patrones de riqueza de las aves de los BTM.- Los números de especies por celda variaron significativamente según la matriz empleada: para la matriz de datos históricos los valores fueron entre 21 y 207 con un promedio de 82; para la matriz de datos actuales los números fueron menores, entre 1-178, con un promedio significantivamente menor, 52 especies (Mann-Whitney, $p \leq 0.0001$; Fig. 2a). Al considerar los datos históricos, las áreas con

mayores valores de riqueza de especies por celda fueron los bosques húmedos de las planicies del norte del Istmo de Tehuantepec, las inmediaciones de la Selva Lacandona y la Península de Yucatán. Las celdas con menores números de especies se encontraron hacia los extremos septentrionales del área de estudio, en las vertientes del Golfo de México y del Golfo de California. De acuerdo con los registros de ocurrencia de la última década, el este de la Península de Yucatán, Chiapas y algunas áreas del centro y sur Veracruz, albergan los mayores números de especies del país (con entre 80 y 200 especies) aunque el número de celdas es significativamente menor que el obtenido a partir de la matriz de datos históricos.

Por otra parte, la riqueza histórica de especies endémicas varió entre 0-12 especies por hexágono (Fig. 2b), alcanzando los mayores valores en las áreas ocupadas por los bosques estacionalmente secos del oeste del país en Jalisco, Nayarit y Sinaloa, y los de la Cuenca del Balsas, en Guerrero, Puebla y Oaxaca. Las áreas correspondientes a la provincia Veracruzana y Península de Yucatán presentaron bajos valores endemismo, generalmente por debajo de tres especies endémicas por hexágono. Al incluir las especies cuasiendémicas, se encontraron los mayores valores en la vertiente Pacífica de México (generalmente entre 6-17 taxones por celda), aunque también aparecieron como áreas importantes los bosques húmedos de la península de Yucatán y de la porción norteña de la provincia Veracruzana (Fig. 2c). La evaluación de los registros de presencia más actuales reveló, tanto para las especies endémicas como para las cuasiendémicas, un número significativamente menor de especies por celda (Mann-Whitney, $p \leq 0.0001$; Fig. 2b y c). Estas disminuciones son más apreciables hacia las costas de Michoacán y Guerrero y el oeste de la Cuenca del Balsas para las especies endémicas, mientras que para las cuasi-endémicas también se obtienen esas áreas, además de prácticamente la totalidad de la provincia Veracruzana. Por otra parte, para Yucatán se obtuvo que los registros de GBIF entre 2013-2023 indican disminuciones puntuales en algunas áreas de la Península y un aumento respecto al número de cuasi-endémicas obtenido por la matriz histórica. Estos incrementos se han reportado cerca de importantes asentamientos humanos y enclaves turísticos como el área comprendida entre Holbox-Cancún-Playa del Carmen-Tulum, por ejemplo.

Respecto a la distribución de aves con algún grado de amenaza según la IUCN y la NOM-059 (Fig. 3), se encontró un patrón actual de reducción en la distribución de especies en los BTM. De acuerdo con la matriz histórica, las áreas del norte del Istmo de Tehuantepec presentaron los mayores números de especie por celda con valores de 5-9 y 42-70,

respectivamente. Los bosques húmedos de la porción norteña de la provincia Veracruzana, los de la Península de Yucatán y los bosques secos del Pacífico (en los estados Jalisco, Colima, Michoacán, Guerrero y Oaxaca) también presentaron valores relativamente altos de especies de aves residentes con riesgo de extinción (generalmente por encima de tres especies amenazadas según UICN y 28 según las NOM-059). Sin embargo, de acuerdo con la matriz de datos actuales, el número de estas áreas es sensiblemente menor, así como el número de especies distribuidas en ellas. Para las especies dentro de las listas de amenaza de IUCN y de la NOM-59, las áreas más críticas parecen ser el norte y sur de la Península de Yucatán, el este de Chiapas, el Istmo de Tehuantepec y algunas localidades de Veracruz, Nayarit y Sinaloa.

El cálculo de los índices de diversidad *β* para el área de estudio indicó un alto recambio taxonómico en la avifauna de los BTM tanto con la matriz de datos históricos como la de datos actuales (Tabla 1). A nivel regional la β_{SOR} fue en ambos casos superior a 0.99, con $\beta_{SIM} = 0.93$ y $\beta_{NES} = 0.06$. Al descomponer el área de estudio en las provincias biogeográficas, los valores de diversidad obtenidos también fueron similares. Para ambas matrices, la Península de Yucatán presentó los menores índices (histórica: $\beta_{SIM} = 0.7736$, $\beta_{NES} = 0.1298$; actual: $\beta_{SIM} = 0.8320$, $\beta_{NES} = 0.0928$) y la provincia Tierras Bajas del Pacífico los mayores (histórica: $\beta_{SIM} = 0.9366$, $\beta_{NES} = 0.0465$; actual: $\beta_{SIM} = 0.9117$, $\beta_{NES} = 0.0712$).

El análisis de clasificación a partir de la matriz histórica generó dos agrupaciones principales (Fig. 4). La primera incluyó a las áreas correspondientes a los bosques tropicales de la vertiente Atlántica de México (que contiene 235 de las 260 especies analizadas y 25 únicas de esta área). Este conjunto se dividió en dos subconjuntos, uno que contiene las celdas correspondientes a las áreas del sur de Veracruz (aproximadamente desde las inmediaciones del Eje Volcánico Transmexicano) hasta los límites orientales del Estado de Tabasco y otro, subdividido en agrupaciones de celdas del norte de Veracruz y la Península de Yucatán. La segunda agrupación principal incluyó las celdas localizadas en la costa del Pacífico (231 especies, 21 especies únicas) y a su vez se subdividió en dos grupos. El primero formado por los hexágonos ubicados desde Sinaloa hasta la porción media del estado de Jalisco, mientras que el segundo subgrupo se organizó en otros dos conjuntos de celdas, uno que contiene la gran mayoría de hexágonos del área de la Cuenca del Balsas y el otro con las localizadas desde el centro-este de Jalisco hasta Guatemala, incluyendo aquellas localizadas al sur del Istmo de Tehuantepec.

Análisis de parsimonia de endemismos y regionalización biogeográfica.- A partir de la matriz histórica, se obtuvieron tres árboles igualmente parsimoniosos (CI=6, RI=80, L=4311) a partir del cual se calculó el cladograma de consenso estricto, (Fig. 5, Fig. S1, Materiales Suplementarios; CI=6, RI=88 y L=5184). Este reveló la existencia de dos agrupamientos principales: uno que contiene la mayoría de las celdas de los BTM de la vertiente del Pacífico y otro, las de la vertiente del Golfo de México y el Mar Caribe. Dentro del primero, están contenidas las celdas correspondientes a los bosques secos localizados desde Sinaloa hasta la porción media del estado de Jalisco aproximadamente y un grupo de celdas que a su vez se subdivide en dos: un conjunto que contiene todas las áreas restantes de los bosques secos del Pacífico Mexicano (desde Jalisco hasta Chiapas, por toda la costa occidental) y otro que contiene los bosques de la Cuenca del Balsas, con algunas celdas aledañas pertenecientes a la provincia Tierras Bajas del Pacífico. Por otra parte, el grupo que contiene los bosques húmedos y secos de la vertiente Atlántica presenta los hexágonos correspondientes a las áreas más septentrionales de la distribución de los bosques húmedos en la vertiente del Golfo de México hasta el centro de Veracruz y un grupo que incluye al resto de las celdas. Este último grupo se subdivide a su vez en dos agrupaciones resueltas: una que contiene las celdas localizadas en la Planicie Costera del Golfo de México (según Morrone, 2019) en la porción centro-oriental del norte del Istmo de Tehuantepec y otra con las celdas correspondientes a la parte norte y centro de la provincia Península de Yucatán.

DISCUSIÓN

Los bosques neotropicales presentan valores excepcionales de diversidad y complejos patrones de distribución, como resultado de múltiples eventos geológicos, ecológicos y evolutivos (Duellman, 1982; Steadman *et al*., 2017; Brumfield, 2012), especialmente en el territorio mexicano, donde entran en contacto con los componentes bióticos de la región Neártica (Morrone 2019; Escalante *et al*., 1993). Las aves representan una parte importante de esa diversidad (Haffer, 1987; Stotz, 1996), por lo que el estudio de sus patrones biogeográficos constituye una aproximación relevante para entender los procesos históricos y ecológicos que han moldeado la biodiversidad de los BTM. Estos bosques se consideran entre los ecosistemas más amenazados a nivel mundial principalmente por las altas tasas de deforestación, cambio de uso de suelo, minería y tráfico de vida silvestre, entre otros (Dinerstein *et al*., 1995; Ceballos *et al*., 2010; García-Oliva y Jaramillo, 2011). Por tanto, el entendimiento de la historia evolutiva de los BTM es esencial no solo desde el punto de vista teórico, sino para la planeación eficiente de su conservación,

especialmente dado el panorama actual de degradación de la biodiversidad debido a las presiones antropogénicas y el cambio climático (Cuervo-Robayo *et al*., 2020; Prieto-Torres *et al*., 2020).

Patrones de riqueza de las aves de los BTM.- Los patrones históricos que encontramos fueron congruentes con la literatura, tanto para los datos de riqueza de especies total como para las endémicas (*e.g*., Ríos-Muñoz y Navarro-Sigüenza, 2012; Navarro-Sigüenza *et al*., 2014; Peterson y Navarro-Sigüenza, 2016). La mayor concentración de especies en total se presenta al norte del Istmo de Tehuantepec a lo largo de la vertiente del Golfo de México y la península de Yucatán, mientras que el mayor número de especies endémicas se concentra a lo largo de la planicie costera del Pacífico. Al incluir las especies cuasiendémicas, la Península de Yucatán y el norte de Veracruz emergen como áreas de agregación de especies con un alto valor de conservación si se considera que México constituye la mayor parte de su área distribucional.

La conformación de la avifauna de México es resultado de diferentes procesos históricos que han afectado a diferentes escalas espaciales y temporales a este territorio. Así, la avifauna de Península de Yucatán se ha conformado a partir de elementos de procedencia continental, antillana y endémica del área, aunque este es el componente minoritario (Paynter, 1955; Cortés-Ramírez *et al*., 2012; Escobar-Luján *et al*., 2021). Esta área es geológicamente joven, lo que aunado a las características del paisaje que carece de accidentes geográficos notables que puedan funcionar como barreras, ha propiciado la dispersión de taxones mesoamericanos y de las islas del Caribe (Vuilleumier, 1985), así como mantener el flujo de genes entre sus poblaciones, limitando los procesos de diferenciación y especiación. El endemismo de la península se ha conformado por especies de distribución relictual que se han diferenciado por haber colonizado tempranamente el área, o por presentar elevadas tasas de evolución (Paynter, 1955). Por otra parte, la conformación de la avifauna de los bosques secos del occidente de México ha estado afectada principalmente por el aislamiento producido por el Eje Volcánico Transmexicano y la Sierra Madre del Sur, y por los cambios paleoclimáticos. Estas barreras geográficas a la dispersión junto con los ciclos climáticos del Pleistoceno han generado efectos de aislamiento y contacto periódicos, que a su vez derivaron en procesos de diferenciación de poblaciones de aves y la formación de taxones endémicos (Becerra, 2005; Morrone, 2014; Arbeláez-Cortés *et al*., 2014; Navarro-Sigüenza *et al*., 2017; Castillo-Chora *et al*., 2021).

Los patrones generales de riqueza histórica total de especies en México descritos en este trabajo son congruentes con los encontrados en otros grupos de vertebrados como mamíferos, anfibios y reptiles (Koleff *et al*., 2008). Las áreas que contienen altos valores de riqueza de especies de aves (mayores de 60%) coinciden, con algunas diferencias, con las zonas de mayor riqueza de especies de mamíferos, y en una medida menor con las de anfibios y reptiles (Koleff *et al*., 2008). Respecto a la concentración de especies endémicas, la costa del Pacifico, el Eje Volcánico Transmexicano y la Sierra Madre del Sur son las áreas más importantes para anfibios y reptiles (Flores-Villela, 1993; Ochoa-Ochoa y Flores-Villela, 2006), mientras que para los mamíferos son la Cuenca del Balsas y el Eje Volcánico (Ceballos *et al.,* 2005; Escalante *et al*., 2020).

Respecto al escenario actual de diversidad de la avifauna de los BTM, nuestros análisis indican una disminución de la riqueza de especies en comparación con los datos históricos y los modelos de distribución potencial del Atlas de las Aves de México (Navarro-Sigüenza y Peterson, 2007; Navarro-Sigüenza *et al.,* 2009). Esta diferencia podría estar determinada en parte por las propias fuentes de datos. La metodología empleada para elaborar los mapas de distribución de esta obra podría tener un efecto de sobreestimación de las áreas reales de presencia de los taxones, mientras que los registros de presencia en GBIF podrían ofrecer una sub-representación la distribución actual real debido de diversos factores (como la accesibilidad de las áreas, la experiencia de los observadores en la identificación de especies, y los hábitos y detectabilidad de estas, entre otras). Sin embargo, es poco probable que estas sean las únicas fuentes de discordancia entre las matrices obtenidas.

Además de lo previamente expuesto, la diferencia entre los patrones de riqueza histórica y actual de las aves de los BTM podría estar fuertemente influenciada por los cambios de uso de suelo y vegetación que han ocurrido sistemáticamente en México desde el siglo XVI (*e.g*., González, 1999). En este país las tasas de deforestación anual entre 1976 y 2015 han oscilado entre los 92,000 - 776,000 hectáreas de bosque (Velázquez *et al*., 2002; FAO 2015). Esto a conllevado, por ejemplo, a una disminución de las selvas húmedas de 12,600,000 ha en 1976 a aproximadamente 8,800,000 ha en 2015, y de los bosques estacionalmente secos, de más de 25,000,000 a menos de 22,500,000 ha en el mismo periodo (SEMARNAT, 2023). Las zonas más críticas de deforestación se encuentran en Veracruz, Campeche, Quintana Roo, Yucatán, Chiapas, Michoacán y Jalisco (CONAFOR, 2020), lo cual concuerda con las áreas de mayor disminución de la riqueza de aves por celda para todas las categorías evaluadas en este trabajo.

La distribución de las especies con algún grado de amenaza según las listas de IUCN y la NOM-059 tuvo un comportamiento similar al de los conjuntos de datos anteriores. Si bien al considerar los datos históricos, se podría esperar que se distribuyeran mayormente en grandes extensiones en la Planicie Costera del Golfo de México, el norte de Veracruz y la porción sureste de la Península de Yucatán (con algunos hexágonos concentrando más de 60 especies amenazadas según la NOM-059 y hasta ocho según la IUCN), este panorama es bastante diferente al emplear la matriz con datos recientes. Con ésta se obtiene que esas especies quedan confinadas a escasas celdas (generalmente agrupadas en números de dos a tres en prácticamente toda el área evaluada, lo que correspondería con áreas de 60 a 90 km^2), en su mayoría no contiguas, con la excepción de Yucatán. La mayor parte de hexágonos con alta concentración de especies en peligro no se encuentra representada en el sistema de Áreas Naturales Protegidas Federales de México; fundamentalmente aquellos ubicados en Veracruz y en la costa del Pacífico (Fig. 3; http://sig.conanp.gob.mx/), lo que constituye un factor de riesgo adicional para la conservación de varias especies de aves y sus hábitats.

La baja cobertura de áreas de importancia para las aves dentro del sistema Nacional de Áreas naturales Protegidas -menos del 15% de la superficie ocupada actualmente por los BTM- ya había sido señalada anteriormente por otros autores (*e.g*., Peterson y Navarro-Sigüenza, 2016; Navarro-Sigüenza *et al*., 2011; Ramírez-Albores *et al*., 2021). Sin embargo, esta situación persiste, a pesar de la definición de al menos siete nuevas áreas protegidas en la última década, ninguna de las cuales está enfocada a la preservación de los BTM. Este escenario es más dramático si se considera que las agregaciones de especies endémicas o con áreas de distribución restringida no son coincidentes para todos los grupos de vertebrados, por lo que para los planes de manejo no es factible usar unos grupos para diseñar la conservación de otros (Koleff *et al*., 2008).

Los análisis de diversidad *β* indicaron altos valores de cambio en la composición de especies entre las diferentes áreas para cada una de las matrices evaluadas, tanto en el análisis regional como a nivel de las provincias biogeográficas (*sensu* Morrone, 2019). Esto concuerda con otros resultados previamente obtenidos para diferentes grupos, como los mamíferos, aves y reptiles no aviares en áreas geográficas como Europa, el este de China y el Bosque Atlántico de Brasil (Svenning *et al*., 2011; Rodríguez-Da Silva *et al*., 2014; Si *et al*., 2015), donde se ha encontrado que el aumento de la diversidad β está relacionado con factores como con la heterogeneidad topográfica y valores relativamente altos de temperatura media anual y evapotranspiración. A nivel de las bioprovincias, estos elevados

valores de *β* pueden estar determinados por las diferencias ecológicas internas de cada unidad biogeográfica. Esto puede provocar que celdas cercanas de diferentes provincias tengan valores de recambio similares, mientras que son más diferentes de los valores de diversidad de las otras celdas dentro de la misma provincia biótica, pero que están más alejadas geográfica y ecológicamente. Esto ocurre, por ejemplo, con los hexágonos del sureste de Veracruz y suroeste de Yucatán, que presentan valores de *β* más similares entre sí, que respecto a los del resto de las provincias biogeográficas a las que pertenecen (Fig. 4). Por lo tanto, estos resultados podrían ser el reflejo de la hetergeneidad interna en los valores de recambio dentro de cada bioprovincia y que responde a factores ecológicos a pesar de su identidad biogeográfica.

Los índices de disimilitud indican un alto recambio y bajo anidamiento en todas las áreas analizadas. Aunque el patrón general de alta disimilitud se mantiene al comparar las diversidades *β* para cada una de la bioprovincias, las proporciones explicadas por el recambio de especies son variables, yendo desde el 80% en la provincia de Yucatán hasta el 95% en las Tierras Bajas del Pacífico. Asimismo, la Península de Yucatán mostró los menores valores de diversidad β, presentando el mayor valor en cuanto al componente de anidamiento, llegando casi al 13%. Estos resultados no apoyan la hipótesis de preponderancia del anidamiento sobre el recambio para comunidades muy afectadas por las oscilaciones climáticas (Dobrovolski *et al*., 2012; Baselga *et al*., 2012), como las que se han inferido para los BTM durante Pleistoceno. Según Baselga *et al*. (2012), este debería ser el patrón para los taxones que han experimentado contracciones en sus áreas de distribución, debido a las oscilaciones paleoclimáticas, y que luego del cambio de las condiciones ambientales, han tenido dinámicas de expansiones poblacionales y colonización de nuevas áreas. En cambio, nuestros resultados indican un mayor valor relativo del recambio sobre el anidamiento, lo que sugiere la existencia de fuertes presiones selectivas del ambiente y de procesos de diferenciación sobre las especies (Baselga *et al*., 2012).

El análisis de la diversidad β de los BTM, además permitió la clasificación de las áreas de acuerdo con su similitud. Los bosques estacionalmente secos del Pacifico se agregaron, formando un grupo único, subdividido en: (1) áreas desde Sinaloa hasta el estado de Jalisco, (2) la Cuenca del Balsas y (3) áreas localizadas desde el centro-este de Jalisco hasta Guatemala. Por otra parte, los bosques húmedos y los parches de bosque estacionalmente secos de la Vertiente Atlántica se concentraron en un mismo clúster. La disimilitud en la composición de especies de plantas y animales entre diferentes áreas ocupadas por bosques secos ha sido previamente documentada (*e.g*., Ríos-Muñoz y Navarro-Sigüenza,

2012; Banda *et al*., 2016; Oswald *et al*., 2017; Prieto-Torres *et al*., 2019), y se ha sugerido que se debe a una compleja historia evolutiva y eventos de diferenciación que han ocurrido de forma independiente en los parches formados por estas comunidades. Nuestro trabajo corrobora estos hallazgos y además indica que, en el caso de la comparación entre los bosques secos de México, la disimilitud no solo se debe los procesos de divergencia evolutiva que han caracterizado la historia biogeográfica del Pacífico, sino también por un efecto de la colonización de los parches distribuidos en el Golfo de México por taxones presentes en los bosques húmedos aledaños.

Análisis de parsimonia de endemismos y regionalización biogeográfica.- Los PAE permiten el descubrimiento de áreas con patrones distribucionales congruentes, y por tanto, en la obtención de hipótesis de regionalización biogeográfica a través del reconocimiento de conjuntos particulares de biotas (Morrone y Escalante, 2002; Morrone, 2004). Aun cuando han recibido críticas como método en biogeografía histórica (*e.g*., Brooks y van Veller, 2003; Peterson, 2008), los PAE son adecuados y útiles para inferir relaciones históricas entre las áreas en ausencia de información filogenética para sus especies (Humphries y Parenti, 1999; Morrone, 2004).

La flora y fauna de los BTM ha sido estudiada con diferentes de marcadores moleculares y métodos de inferencia filogenética. Al igual que sucede con la mayoría de las regionalizaciones biogeográficas realizadas, la mayor parte de los estudios filogeográficos y sistemáticos han estado enfocados en especies distribuidas en solo un tipo de bosque (*e.g*., bosque estacionalmente seco: Arbeláez-Cortez *et al*., 2014; Castillo-Chora *et al*., 2020; o bosque húmedo: Miller *et al*., 2011; Castillo-Chora *et al*., 2021). Los pocos trabajos que incluyen taxones de ambos tipos de bosque distribuidos en las cuatro provincias neotropicales de México (*e.g*., Ortiz-Ramírez *et al*., 2020; Rocha-Moreira *et al*., 2020; Llanes-Quevedo *et al*., 2022) no incluyen completamente esas unidades biogeográficas, ya sea porque las especies de interés no ocupan completamente esas áreas, o por deficiencias en el muestreo. Dadas estas limitaciones, el empleo del PAE en base a los datos distribucionales de aves residentes en los BTM es útil porque ofrece un panorama más completo, aunque con una limitada resolución, de las relaciones jerárquicas entre las áreas Neotropicales de México.

Nuestros resultados muestran una correspondencia con la regionalización fisiográfica de México de Morrone (2019). Sin embargo, de las cuatro provincias neotropicales del país en ese trabajo, solo la de la Cuenca del Balsas constituye un grupo monofilético. Las

provincias Tierras Bajas del Pacífico y Veracruzana se encontraron como agrupaciones parafiléticas, conteniendo celdas correspondientes a esas provincias más las de la Cuenca del Balsas y Península de Yucatán. Esta última provincia biogeográfica se subdividió en dos grupos. La mayor parte de las celdas correspondientes al centro y norte de la península formaron un grupo, que es hermano del que que contiene los hexágonos de la Planicie Costera del Golfo de México (sureste de Veracruz y Tabasco) y otros del suroeste de Campeche.

La evolución biótica de la Cuenca del Balsas ha estado marcada por el efecto aislante de las provincias fisiográficas que la delimitan (Eje Volcánico Transmexicano al norte, la Sierra Madre del Sur al sur, y la Sierra Norte de Oaxaca al oriente) y la continuidad con los bosques tropicales de la costa del Pacífico (Morrone, 2014). Esta provincia biogeográfica comprende amplias y disyuntas áreas de bosque tropical estacionamente seco y constituye uno de los *hotspots* más importantes para el endemismo en México (Vázquez-Reyes *et al*., 2018). Nuestros resultados apoyan la identidad biogeográfica de esta región recuperada a través de análisis previos de distribución de las especies endémicas y variables ambientales (Morrone, 2019) así como de otros que han hipotetizado su relación cercana con los bosques de las Tierras Bajas del Pacifico, específicamente aquellos distribuidos desde el sur de Jalisco hasta Chiapas (e.g., Morrone *et al.*, 1999; Montaño-Rendón *et al*., 2015. Arbeláez-Cortés *et al*., 2014).

Para la bioprovincia de Tierras Bajas del Pacífico, por otra parte, ha sido documentada la diferenciación -tanto a nivel de composición de especies como a nivel genético- en varios grupos de animales (*e.g*., García-Trejo y Navarro-Sigüenza, 2004; Arbeláez-Cortez *et al*., 2014; Prieto-Torres *et al*., 2019). En aves, García-Trejo y Navarro-Sigüenza (2004) encontraron que tanto taxones de montaña como de tierras bajas apoyan una separación del área comprendida desde Sonora hasta el sur de Nayarit (*Euptilotis neoxenus, Campephilus imperialis*, *Aphelocoma wollweberi*, *Cyanocorax beecheii, Melanerpes uropygialis* y *Corvus sinaloae*) del resto de las Tierras Bajas del Pacífico. La zona aledaña a la Bahía de Banderas -donde el Eje Volcánico llega prácticamente hasta la costa en la parte sur de la Bahía- podría constituir una barrera geográfica, limitando la distribución de especies como *Callipepla douglasii, Ortalis wagleri, Calocitta colliei, Cyanocorax beecheii* y *Vireo paluster* hacia el sur, y *Calocitta formosa, Ortalis poliocephala* y *Cyanocorax sanblasianus* hacia el norte (García-Trejo y Navarro-Sigüenza, 2004).

Por otra parte, las bioprovincias Veracruzana y de Yucatán han sido reconocidas como unidades biogeográficas en estudios a partir de la regionalización propuesta por

Rzedowski (1978) (*e.g*., Morrone, 2005, 2006, 2017, 2019). Sin embargo, otros trabajos basados en diversos taxones (*e.g*., *Sceloporus:* Smith, 1941; vertebrados de México y Centroamérica: Stuart, 1964; Monroy-Olguín *et al*., 2012) han indicado una regionalización algo diferente, reflejando la complejidad fisiográfica, historia geológica y evolución biótica de estas áreas. Los patrones de la biodiversidad de la Península de Yucatán son consecuencia de su "reciente" formación geológica (Paynter, 1955), así como de los episodios de regresiones y transgresiones marinas que han llegado a producir variaciones en su área de superficie emergida de hasta el 50% (Vázquez-Domínguez y Arita, 2010). Además, los cambios climáticos durante el Pleistoceno han provocado que las condiciones xéricas en la Península actuaran como barrera ecológica, aislando a las poblaciones en la región de Petén (Lavin *et al*., 1991; Licona-Vera *et al*., 2018; Ortiz-Ramírez *et al*., 2020). La provincia Veracruzana también ha tenido una evolución geológica heterogénea: su parte más norteña ha estado emergida desde al menos el Mioceno tardío (12-10 millones de años), mientras que la región al sur del Macizo ígneo de Palma Sola es bastante más reciente, quedando completamente emergida durante la transición del Plioceno al Pleistoceno (Ferrari *et al*., 2005; Jácome Paz *et al*., 2022).

Las celdas correspondientes a la Planicie Costera del Golfo de México (que incluye las porciones sureñas de Veracruz, Campeche y Tabasco) formaron un grupo aparte del resto de hexágonos de las provincias Veracruzana y Península Yucatán. Esta agrupación es consecuencia del patrón distribucional de diversos taxones de aves como *Tinamus major, Crypturellus soui, C. boucardi, Harpia harpya* y *Patagioenas nigrirostris* entre otros. La Planicie Costera del Golfo de México (*sensu* Morrone, 2019) forma parte del puente centroamericano, lo que ha favorecido el establecimiento de un gran número de taxones provenientes del sur, y en menor medida, del norte (Vuilleumier, 1985). Esto determina los altos valores de riqueza de especies y el bajos número de especies endémicas. En esta área se ubica la porción norte del Istmo de Tehuantepec, accidente geográfico que constituye un corredor biológico y área de contacto entre las cuencas del Atlántico y el Pacífico (Wendt, 1992; Pérez-García y Meave, 2006), lo que podría explicar la agrupación de un pequeño número de celdas de la Provincia Veracruzana con la de las Tierras bajas del Pacífico y viceversa. Estudios genéticos enfocados en taxones como *Lepidocolaptes affinis* (Arbeláez-Cortés *et al*., 2010), *Icterus pustulatus* (Cortés-Rodríguez *et al*., 2008), *Melanerpes santacruzi* (Llanes-Quevedo *et al*., 2022) y *Podocarpus matudae* (Ornelas *et al*., 2010), no han encontrado diferenciación genética entre poblaciones separadas por el Istmo, lo que sugiere que este accidente ha permitido la dispersión de individuos y el flujo de genes entre

poblaciones distribuidas en las vertientes Atlántica y Pacífica de México (Navarro-Sigüenza *et al*., 2008; Arbeláez-Cortés *et al.,* 2010; Llanes-Quevedo *et al*., 2022).

CONSIDERACIONES FINALES

Los patrones de distribución identificados y la regionalización obtenida con el PAE en este trabajo, nos permiten tener una aproximación a la complejidad de la historia evolutiva de la avifauna de los BTM. Las condiciones ambientales actuales y las variaciones Pleistocénicas en el clima y la vegetación posiblemente han repercutido de manera diferente en las selvas secas y húmedas, generando diversos escenarios que han conducido a una diferenciación desigual de la avifauna de la región. Los patrones biogeográficos que presentamos aquí sugieren la interacción de procesos de vicarianza y dispersión, que sólo pueden resolverse mediante el estudio de las filogenias moleculares que permitan desentrañar las múltiples historias evolutivas (compartidas o idiosincráticas) que han conformado los patrones de distribución de la biodiversidad.

Además, nuestro trabajo aporta indicios relevantes de la drástica variación de los patrones de esta distribución en el corto periodo de unos cientos de años. Aun cuando las fuentes de datos empleadas presentan sesgos importantes, nuestros resultados tienen al menos dos grandes implicaciones para los estudios de la biodiversidad en los BTM: (1) indican áreas poco muestreadas que deben ser atendidas para registrar y proteger su biodiversidad y (2) indican áreas que ya no son adecuadas para las especies por cambios de uso de suelo y vegetación, y que con poca probabilidad volverán a serlo en un futuro (e.g., asentamientos humanos o tierras de cultivo). Esto último ha de ser considerado en estudios de modelado de distribución potencial y del impacto de presiones selectivas sobre la diversidad genétics de las especies (*e.g*., *genomic offset;* Capblancq *et al*., 2020), para predecir correctamente las áreas de idoneidad ambiental y genómica hacia el futuro, así como para la evaluación del estado de conservación de las especies por organismos nacionales e internacionales.

Declaración de contribución de autoría

AL-Q: Conceptualización, Metodología, Análisis, Curación de los datos, Escritura: Manuscrito original, revisiones y edición, Visualización. **LES-G**: Metodología, Análisis, Escritura: revisiones y edición, Visualización. **AGN-S**: Conceptualización, Metodología, Escritura: Manuscrito original, revisiones y edición, Supervisión, Adquisición de fondos.

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TABLAS

Tabla 1. Valores de cambio en la composición de especies de aves en los bosques tropicales de México (BTM) y en cada una de las provincias biogeográficas donde estos se distribuyen, estimados mediante el cálculo del índice β_{SOR}. β_{SIM} indica el recambio de especies medido con el índice de Simpson y β_{NES}, el anidamiento o diferencias en riqueza de acuerdo con Baselga (2010).

	Distribución Histórica					Distribución GBIF 2013-2023				
Provincia Biogeográfica	**n Especies**	**n Celdas**	**β_{SIM}**	**β_{NES}**	**β_{SOR}**	**n Especies**	**n Celdas**	**β_{SIM}**	**β_{NES}**	**β_{SOR}**
Balsas	149	122	0.8391	0.0949	0.9340	102	98	0.8368	0.1009	0.9377
Península de Yucatán	206	150	0.7736	0.1298	0.9035	208	149	0.8320	0.0928	0.9248
Tierras Bajas del Pacífico	222	285	0.9366	0.0465	0.9831	213	203	0.9117	0.0712	0.9829
Veracruzana	236	254	0.8755	0.0412	0.9767	233	235	0.8440	0.1002	0.9442
BTM	260	811	0.9356	0.0641	0.9997	260	685	0.9353	0.0603	0.9956

FIGURAS

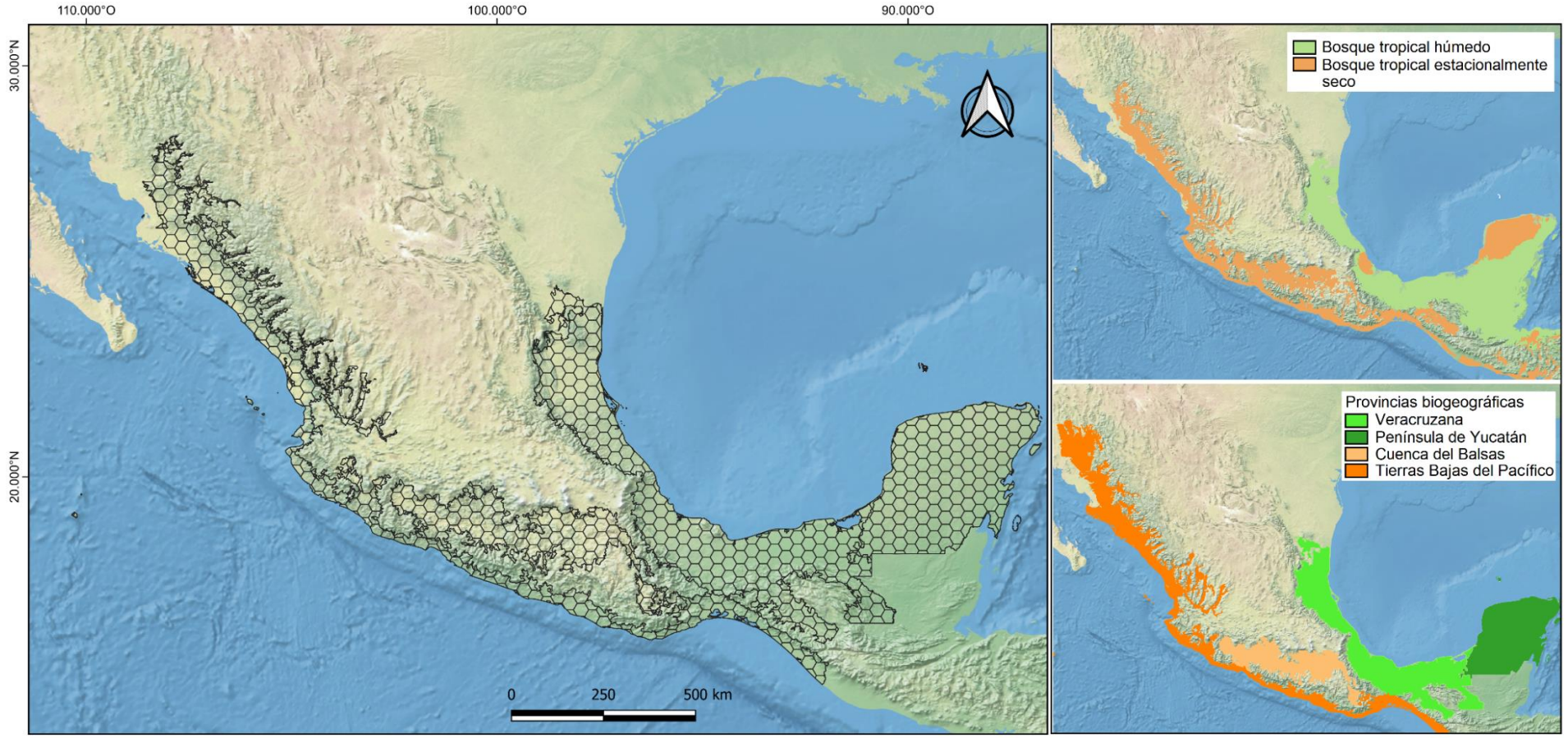


Figura 1- Área de estudio dividida en celdas de 0.33º para los análisis de diversidad y de Parsimonia de Endemismos. En los mapas de la derecha, se representan: arriba, las áreas correspondientes a bosques tropicales húmedos y los estacionalmente secos de acuerdo a Olson et al. (2001), y debajo, las provincias biogeográficas propuestas por Morrone (2009).

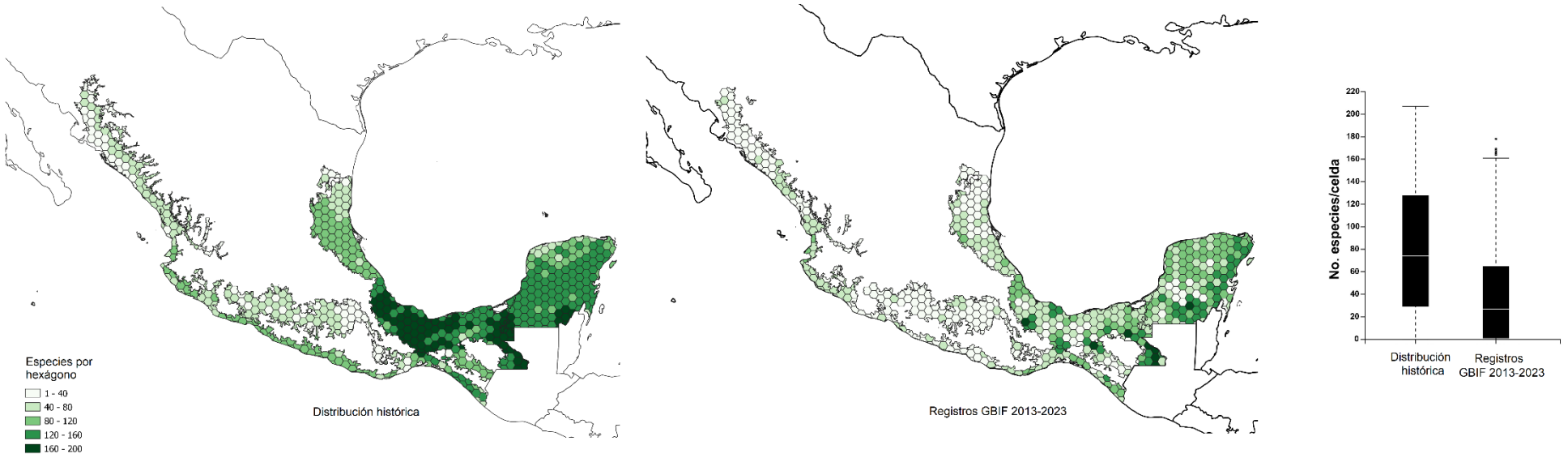
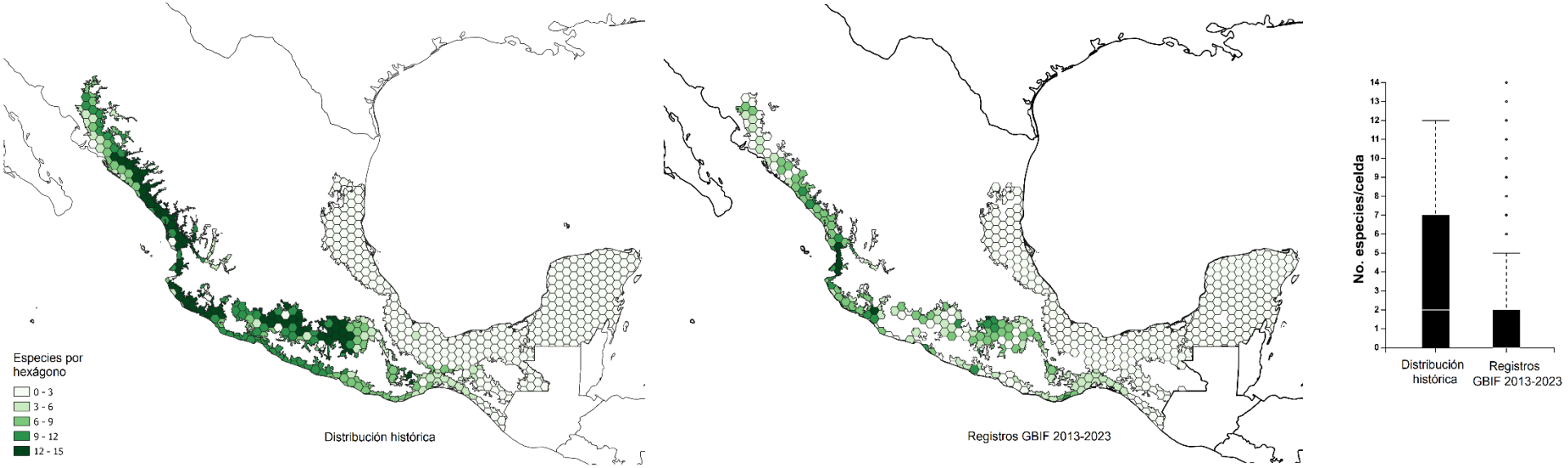
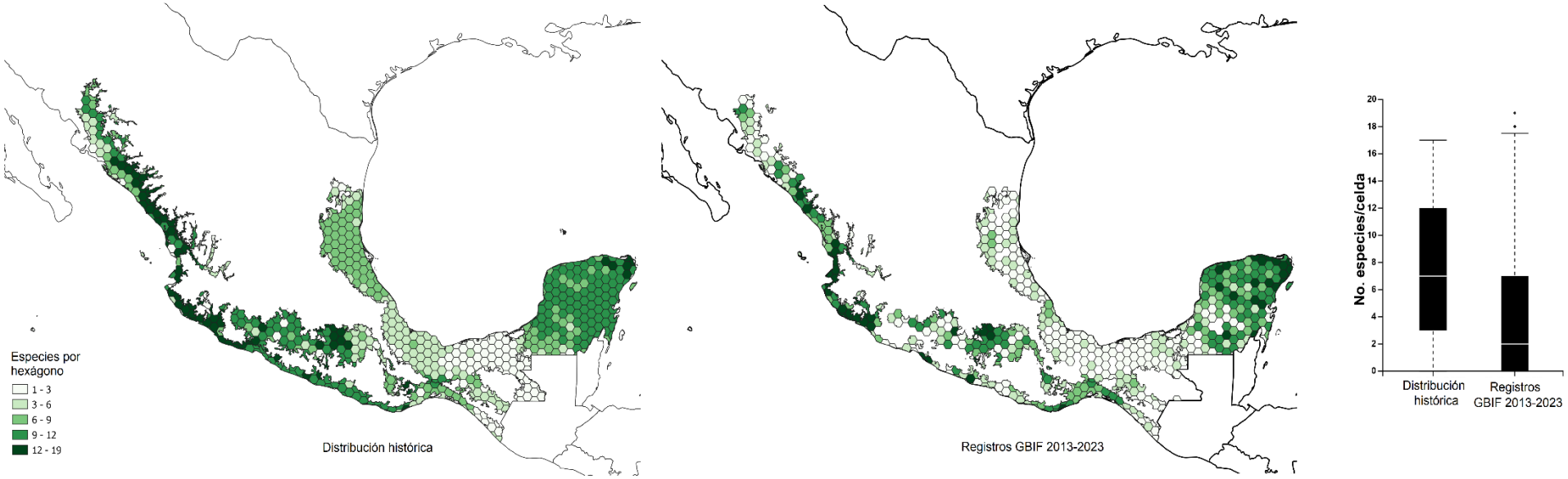


Figura 2- Patrones de distribución de la riqueza de aves residentes permanentes de los bosques tropicales de México de acuerdo a los mapas de distribución generados a partir de datos históricos (de especímenes colectados entre el siglo XVIII y 2007; a la izquierda) y a partir de registros de ocurrencia de GBIF de la última década para 260 especies (mapa de la derecha). Los gráficos de la derecha indican la comparación entre las medianas y las varianzas de las matrices de datos usados (en todos los casos, $p \leq 0.0001$ Mann-Whitney). (a) Riqueza total de especies.



Continuación Figura 2- (b) Riqueza de especies de aves endémicas.



Continuación Figura 2- (c) Distribución de la riqueza de especies con alguna categoría de endemismo de acuerdo con Berlanga *et al*. (2015).

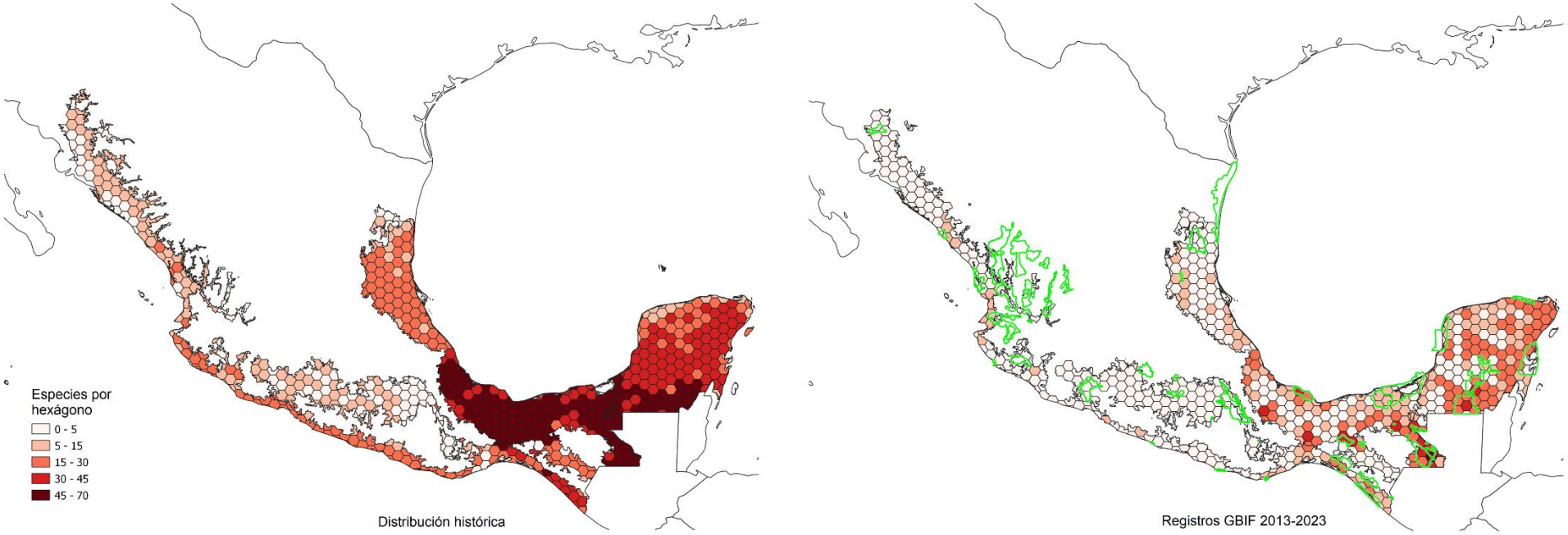
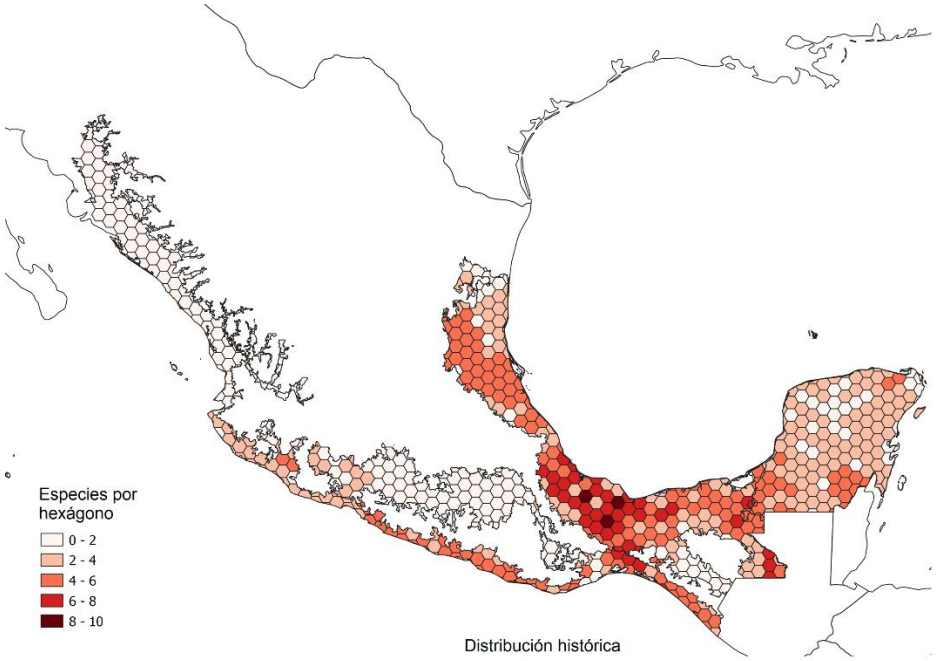
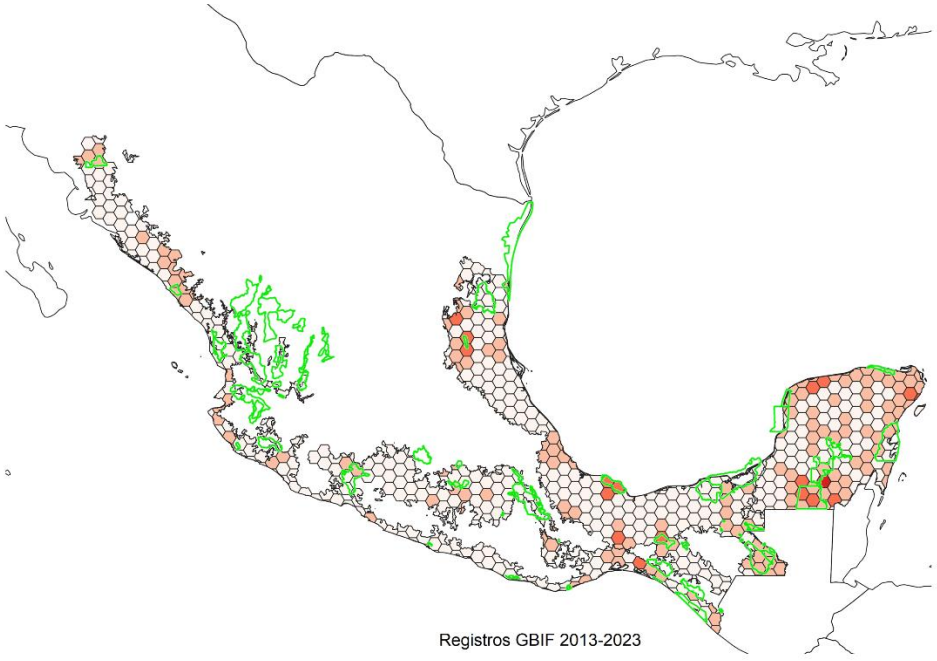


Figura 3a- Distribución de la riqueza de especies de aves residentes permanentes de los bosques tropicales de México con algún grado de amenaza según la Norma Oficial Mexicana (NOM-059, SEMARNAT) y la Lista Roja de la UICN. A la izquierda se muestra el patrón de riqueza esperado de acuerdo con mapas de distribución generados a partir de datos históricos (de especímenes colectados entre el siglo XVIII y 2007) y a la derecha, a partir de registros de ocurrencia de GBIF de la última década para 260 especies. Con líneas verdes se han marcado los límites de las Áreas Naturales Protegidas que incluyen bosques tropicales en alguna medida. (a) Riqueza de especies con algún grado de amenaza según la NOM-059.





Continuación Figura 3- (b) Riqueza de especies con algún grado de amenaza según la Lista Roja de la UICN.

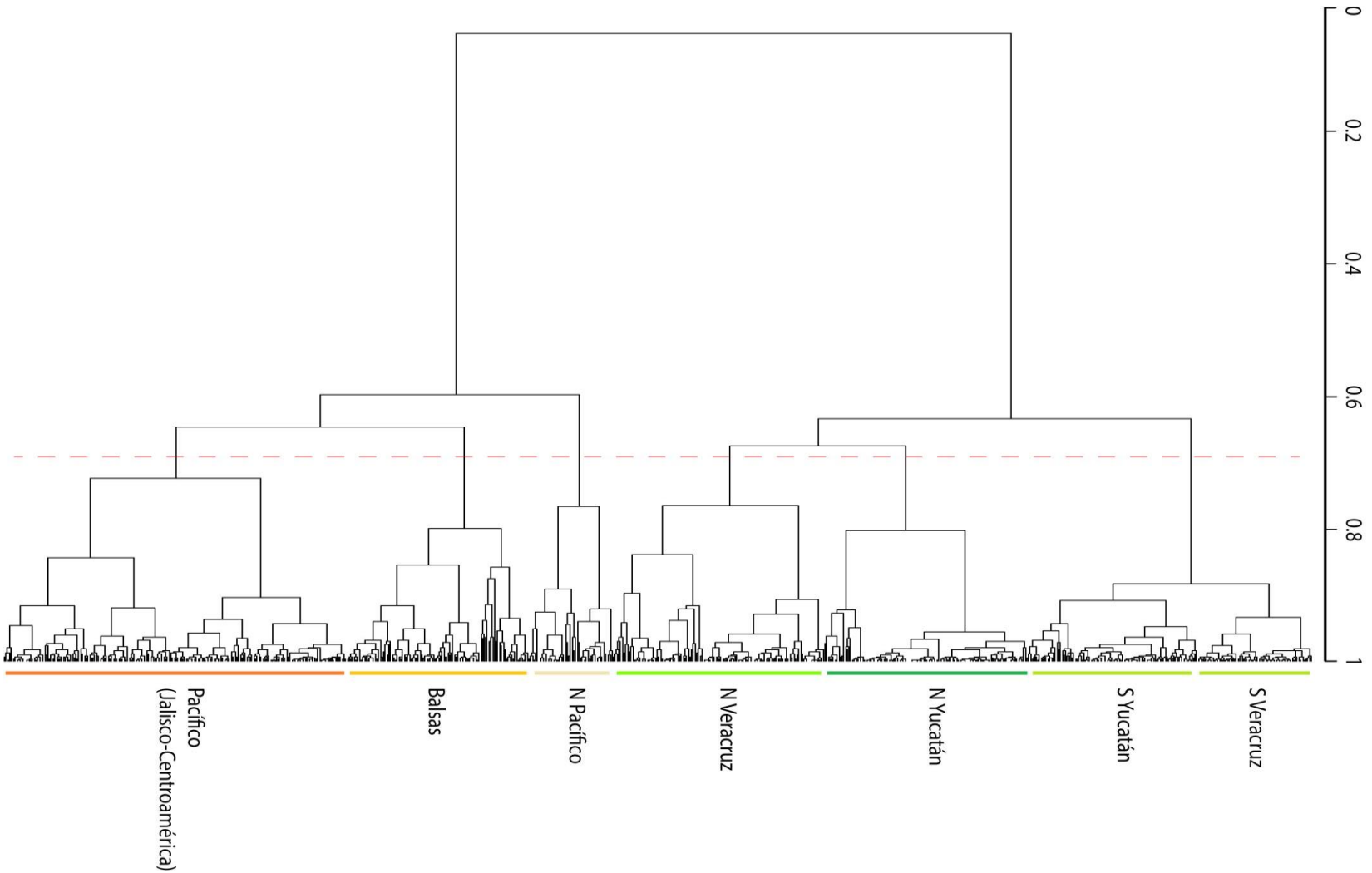
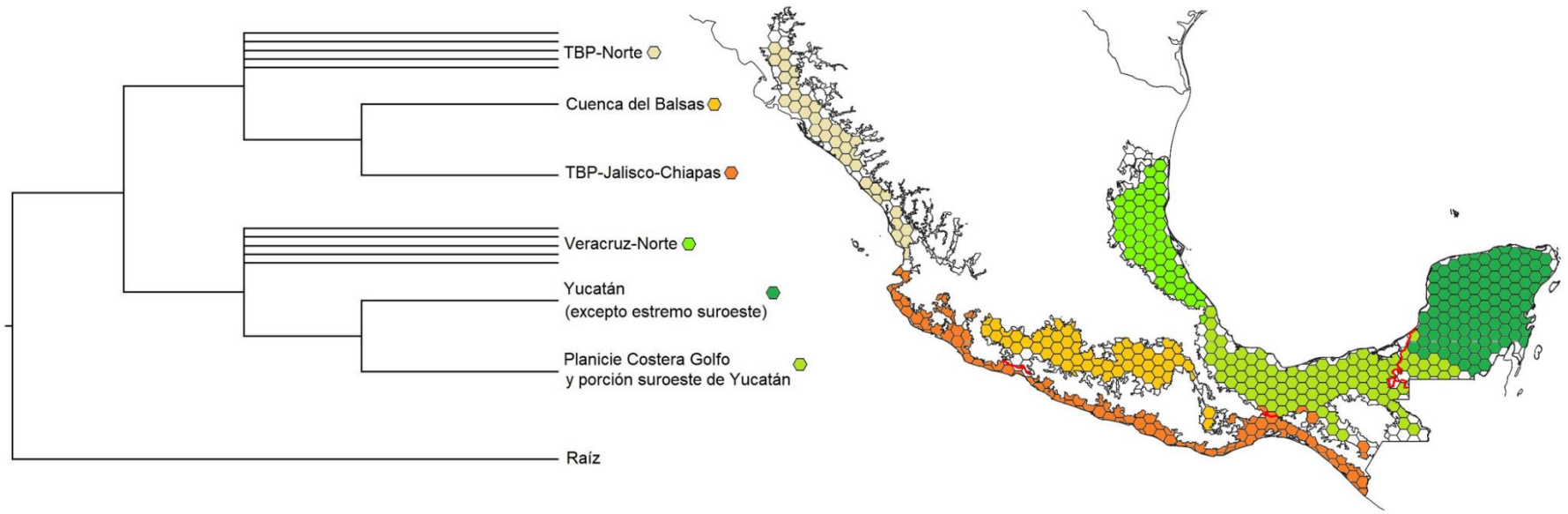


Figura 4- Dendrograma obtenido a partir del análisis de clasificación basado en disimilitud por recambio taxonómico general de la avifauna residente permanente de los bosques tropicales de México.



Clados del dendrograma consenso estricto y sinapomorfías encontradas	
Clado	Especies
	Crotophaga sulcirostris
TBP Jalisco-Chiapas + Cuenca del Balsas	*Calocitta formosa*
Planicie Costera Golfo	*Tinamus major, Crypturellus boucardi*
Yucatán	*Amazona xantholora*

Figura 5- Esquema simplificado de relaciones jerárquicas entre las áreas inferidas por el Análisis de Parsimonia de Endemismos de aves residentes permanentes de los bosques tropicales de México y sinapomorfías encontradas para los nodos del dendrograma. Las celdas se han coloreado en correspondencia con los colores indicados para los grupos del cladograma. Los límites entre provincias biogeográficas (de acuerdo con Morrone, 2019) se han representado con líneas rojas sobre el mapa.

MATERIALES SUPLEMENTARIOS

Patrones históricos y actuales de diversidad y relaciones biogeográficas de la avifauna residente de los bosques tropicales de México

Alexander Llanes-Quevedo, Luis E. Sánchez-Ramos y Adolfo G. Navarro-Sigüenza

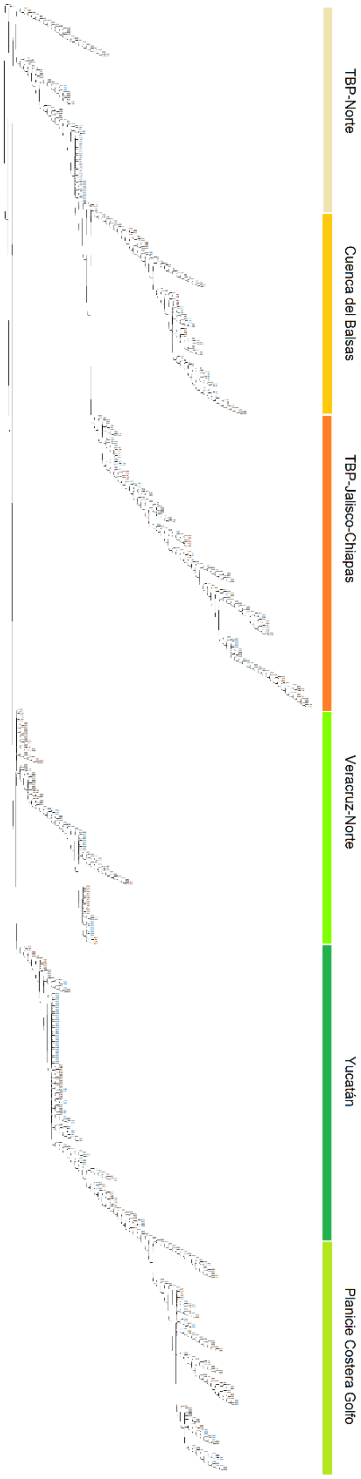


Figura S1- Árbol consenso generado a partir de los tres árboles más parsimoniosos obtenidos por el Análisis de Parsimonia de Endemismos de aves residentes permanentes de los bosques tropicales de México

Tabla S1. Especies de aves incluidas en el presente estudio, categorías de endemismo y riesgo de acuerdo con la Norma Oficial Mexicana NOM59 y la Lista Roja de la UICN. Categorías de Endemismo = END: endémica, CUA: cuasiendémica, Niveles de riesgo según la NOM-059 = sc: sin clasificación, Pr: sujeta a protección especial, A: amenazada, P: en peligro de extinción. IUCN (Birdlife International, 2018) = LC: least concern (preocupación menor), NT: near threatened (casi amenazada), VU: vulnerable (vulnerable).

Especie	Categoría de endemismo	Nivel de riesgo según NOM-059	Nivel de riesgo según UICN
Accipiter bicolor		A	LC
Amazilia candida			LC
Amazilia cyanocephala			LC
Amazilia rutila			LC
Amazilia tzacatl			LC
Amazilia yucatanensis	CE		LC
Amazona albifrons		PR	LC
Amazona auropalliata		PE	VU
Amazona autumnalis			LC
Amazona farinosa		PE	LC
Amazona finschi	EN	PE	VU
Amazona oratrix	CE	PE	EN
Amazona viridigenalis	CE	PE	EN
Amazona xantholora	CE	A	LC
Antrostomus badius	SE		LC
Antrostomus salvini	EN		LC
Ara macao		PE	LC
Ara militaris		PE	VU
Arremonops chloronotus			LC
Arremonops rufivirgatus	CE		LC
Asio clamator		A	LC
Attila spadiceus			LC
Automolus ochrolaemus		PR	LC
Basileuterus culicivorus			LC
Basileuterus lachrymosus			LC
Basileuterus rufifrons	CE		LC
Brotogeris jugularis		A	LC
Burhinus bistriatus			LC
Busarellus nigricollis		PR	LC
Buteo brachyurus			LC
Buteo plagiatus			LC
Buteogallus urubitinga		PR	LC
Callipepla douglasii	EN		LC

Calocitta colliei	EN		LC
Calocitta formosa			LC
Calothorax pulcher	EN		LC
Campephilus guatemalensis		PR	LC
Campylopterus curvipennis			LC
Campylopterus excellens	EN	PR	NT
Campylorhynchus chiapensis	EN	PR	LC
Campylorhynchus jocosus	EN		LC
Campylorhynchus rufinucha			LC
Campylorhynchus yucatanicus	EN	PE	NT
Campylorhynchus zonatus			LC
Caryothraustes poliogaster			LC
Cassiculus melanicterus	CE		LC
Cathartes burrovianus		PR	LC
Celeus castaneus		PR	LC
Ceratopipra mentalis			LC
Cercomacroides tyrannina			LC
Chiroxiphia linearis		PR	LC
Chloroceryle aenea			LC
Chloroceryle amazona			LC
Chloroceryle americana			LC
Chlorostilbon canivetii			LC
Chondrohierax uncinatus		PR	LC
Claravis pretiosa			LC
Coccyzus minor			LC
Coereba flaveola			LC
Colaptes rubiginosus			LC
Colinus nigrogularis	CE		LC
Columbina minuta			LC
Columbina talpacoti			LC
Corvus sinaloae	EN		LC
Cotinga amabilis		A	LC
Crax rubra		A	VU
Crotophaga sulcirostris			LC
Crypturellus boucardi		A	LC
Crypturellus cinnamomeus		PR	LC
Crypturellus soui		A	LC
Cyanerpes cyaneus			LC
Cyanocompsa cyanoides			LC
Cyanocompsa parellina			LC
Cyanocorax sanblasianus	EN		LC
Cyanocorax yncas			LC
Cyanocorax yucatanicus	CE		LC
Cyclarhis gujanensis			LC

Cynanthus sordidus	EN		LC
Dendrocincla anabatina		PR	LC
Dendrocincla homochroa			LC
Dendrocolaptes sanctithomae		PR	LC
Dives dives			LC
Doricha eliza	EN	PE	NT
Dromococcyx phasianellus			LC
Dryobates fumigatus			LC
Dryocopus lineatus			LC
Elaenia flavogaster			LC
Elaenia martinica	CE		LC
Electron carinatum		PE	VU
Eucometis penicillata		PR	LC
Eumomota superciliosa			LC
Eupherusa ridgwayi			
Euphonia affinis			LC
Euphonia gouldi		PR	LC
Euphonia hirundinacea			LC
Eupsittula nana		PR	LC
Falco femoralis		A	LC
Falco rufigularis			LC
Florisuga mellivora			LC
Formicarius analis			LC
Forpus cyanopygius	EN	PR	LC
Galbula ruficauda		A	LC
Geothlypis flavovelata	EN	PE	VU
Geothlypis poliocephala			LC
Geotrygon montana			LC
Geranoaetus albicaudatus		PR	LC
Geranospiza caerulescens		A	LC
Glaucidium brasilianum			LC
Glyphorynchus spirurus		A	LC
Granatellus sallaei			LC
Granatellus venustus	EN		LC
Habia fuscicauda			LC
Habia rubica			LC
Harpagus bidentatus		PR	LC
Harpia harpyja		PE	NT
Heliomaster longirostris		PR	LC
Henicorhina leucosticta			LC
Herpetotheres cachinnans			LC
Hydropsalis maculicaudus			LC
Hylocharis eliciae			LC
Hylomanes momotula		A	LC

Hylophilus decurtatus		PR	LC
Hylophilus ochraceiceps		PR	LC
Ibycter americanus		E	LC
Icterus auratus	CE		LC
Icterus chrysater			LC
Icterus graduacauda	CE		LC
Icterus gularis			LC
Icterus mesomelas			LC
Icterus pectoralis			LC
Lanio aurantius		PR	LC
Legatus leucophaius			LC
Lepidocolaptes souleyetii			LC
Leptodon cayanensis		PR	LC
Leptopogon amaurocephalus			LC
Leptotila jamaicensis	CE		LC
Leptotila verreauxi			LC
Lipaugus unirufus			LC
Lophornis helenae		A	LC
Lophostrix cristata		A	LC
Manacus candei		PR	LC
Megaceryle torquata			LC
Megarynchus pitangua			LC
Megascops cooperi	CE	PR	LC
Megascops guatemalae			LC
Megascops seductus	EN	A	NT
Melanerpes aurifrons			LC
Melanerpes chrysogenys	EN		LC
Melanerpes hypopolius	EN		LC
Melanerpes pucherani			LC
Melanerpes pygmaeus	CE		LC
Melanoptila glabrirostris	CE	PR	NT
Melozone albicollis	EN		LC
Micrastur ruficollis		PR	LC
Micrastur semitorquatus		PR	LC
Microrhopias quixensis		PR	LC
Mimus gilvus			LC
Mionectes oleagineus			LC
Molothrus oryzivorus			LC
Momotus mexicanus	CE		LC
Morococcyx erythropygus			LC
Myiarchus nuttingi			LC
Myiarchus tyrannulus			LC
Myiarchus yucatanensis	CE		LC
Myiobius sulphureipygius			LC

Myiopagis viridicata			LC
Myiozetetes similis			LC
Nyctibius jamaicensis			LC
Nyctidromus albicollis			LC
Nyctiphrynus yucatanicus	CE		LC
Odontophorus guttatus		PR	LC
Oncostoma cinereigulare			LC
Onychorhynchus coronatus		PE	LC
Ortalis leucogastra		PR	LC
Ortalis vetula			LC
Ortalis wagleri	EN		LC
Pachyramphus aglaiae			LC
Pachyramphus cinnamomeus			LC
Panyptila cayennensis		PR	LC
Passerina leclancherii	EN		LC
Passerina rositae	EN	A	NT
Patagioenas cayennensis			LC
Patagioenas flavirostris			LC
Patagioenas nigrirostris		PR	LC
Patagioenas speciosa		PR	LC
Penelope purpurascens		A	LC
Peucaea humeralis	EN		LC
Peucaea sumichrasti	EN	PE	NT
Phaethornis longirostris			LC
Phaethornis striigularis		PR	LC
Pheugopedius felix	EN		LC
Pheugopedius maculipectus			LC
Piaya cayana			LC
Piranga roseogularis	CE		LC
Pitangus sulphuratus			LC
Platyrinchus cancrominus		PR	LC
Poecilotriccus sylvia			LC
Polioptila albiloris			LC
Psarocolius montezuma		PR	LC
Psarocolius wagleri		PR	LC
Pseudastur albicollis		PR	LC
Psilorhinus morio			LC
Psittacara holochlorus		A	LC
Psittacara strenuus		A	NR
Pteroglossus torquatus		PR	LC
Pulsatrix perspicillata		A	LC
Pyrilia haematotis		PE	LC
Quiscalus mexicanus			LC
Ramphastos sulfuratus		A	LC

Ramphocaenus melanurus			LC
Ramphocelus passerinii			LC
Ramphocelus sanguinolentus			LC
Ramphotrigon flammulatum			
Rhodinocichla rosea			LC
Rhynchocyclus brevirostris			LC
Rhytipterna holerythra			LC
Rostrhamus sociabilis		PR	LC
Rupornis magnirostris			LC
Saltator atriceps			LC
Saltator maximus			LC
Sarcoramphus papa		PE	LC
Setophaga pitiayumi			LC
Sittasomus griseicapillus			LC
Spizaetus melanoleucus		PE	LC
Spizaetus ornatus		PE	NT
Spizaetus tyrannus		PE	LC
Sporophila funerea			LC
Sporophila minuta			LC
Sporophila torqueola			LC
Streptoprocne zonaris			LC
Strix nigrolineata			LC
Strix virgata		A	LC
Synallaxis erythrothorax			LC
Tapera naevia			LC
Taraba major		PR	LC
Thamnophilus doliatus			LC
Thraupis abbas			LC
Thraupis episcopus			LC
Tiaris olivaceus			LC
Tinamus major		A	NT
Tityra inquisitor			LC
Tityra semifasciata			LC
Todirostrum cinereum			LC
Tolmomyias sulphurescens			LC
Trogon citreolus	EN		LC
Trogon collaris		PR	LC
Trogon massena		A	LC
Trogon melanocephalus			LC
Turdus assimilis			LC
Turdus grayi			LC
Tyrannus couchii			LC
Tyrannus dominicensis			LC
Tyrannus melancholicus			LC

Tyrannus savana			LC
Vireo hypochryseus	EN		LC
Vireo magister	CE		LC
Vireo nelsoni	EN	PR	LC
Vireo pallens		PR	LC
Vireolanius pulchellus		A	LC
Volatinia jacarina			LC
Xenops minutus		PR	LC
Xenotriccus mexicanus	EN	PR	LC
Xiphorhynchus flavigaster			LC
Zenaida aurita		PR	LC

CAPÍTULO 2. The biogeography of Neotropical birds in the genomics era

Alexander Llanes-Quevedo[1,2]

[1] Posgrado en Ciencias Biológicas, Universidad Nacional Autónoma de México

[2] Museo de Zoología “Alfonso L. Herrera”, Facultad de Ciencias, Universidad Nacional Autónoma de México

The biogeography of Neotropical birds in the genomics era

ABSTRACT.- The recent and accelerated development of genomics have provided tools to analyze biogeographic patterns and processes, pushing our understanding of evolution of areas and taxa to upper levels. The Neotropics harbor the most diverse avifauna compared to other biogeographic regions, but our understanding of general patterns of divergence and its underlying process is still incipient. Distributional and divergence patterns are intricate, suggesting that the high diversity of Neotropical birds has been fueled by a complex interplay of geological and paleoclimatic events along with idiosyncratic responses from each taxon. Herein I reviewed the biogeographic literature focused on the Neotropical ornithology using genomic approaches and discuss how it have been used to approach some critical questions in biogeography. I found an increasing tendency to incorporate genomic methods to ornithological research, although in most cases without a comparative approach and important biases towards the study of rainforests, the Brazilian subregion, and Passeriformes species. Most genomic studies have focused on the historical drivers of spatial and temporal patterns in avian diversity, the role of gene flow in the diversification and persistence of species, and on the influence of ecology in processes of local adaptation and differentiation. Finally, I offer some considerations on the potentialities and limitations of the use of methods of population genomics and phylogenomics in the study of Neotropical birds' biogeography and evolution.

Key words: avifauna, diversification, gene flow, historical drivers, ornithological research, spatial and temporal patterns

INTRODUCTION

Biogeography is a discipline that analyzes the distribution patterns of life and its causes (Sanmartín, 2012). Traditionally, biogeography has advanced based on two approaches that are apparently contrasting: geographical ecology, that study contemporary ecological processes acting on current patterns of species distribution; and historical biogeography, that study the origin and evolutionary history of species and the long-term changes in the distribution of organisms in conjunction with past processes (Lexer et al., 2013). However, due to the complex evolution of biotas, it has become evident the need of integrating and reconciling both approaches (Morrone, 2008). Indeed, to fully understand the geography of speciation, dispersal, and extinction, the biotas should be studied through different methods, this is, integrating distributional, phylogenetic, molecular, and paleontological data, an approach currently known as “integrative and evolutionary biogeography” (Morrone, 2008; Riddle et al., 2008).

The rise of methods for studying biodiversity at molecular level has helped biogeography integration of ecological and historical factors. Nowadays, with the High Throughput Sequencing (HTS) or massive sequencing strategies we have the ability of analyzing the genomic information of thousands of species. Such methods have become popular rapidly, allowing to explore the composition of entire genomes (or reduced representations of these) at unprecedented scales, leading to an annual increase in the annual production of DNA sequences of approximately 70 times (McCormack et al., 2013).

There are five main approaches of HTS methods: (1) whole genome sequencing, (2) transcriptome sequencing, (3) sequencing of fragments generated with restriction enzymes (e.g., Restriction sites associated DNA markers, RADseq), (4) PCR amplicons, and (5) target enrichment (McCormack et al., 2013). Most of these approaches can generate hundreds of millions of sequence reads per run (with variable lengths depending on the employed platform) that could be directly analyzed for the discovery and assessment of molecular markers as the Single Nucleotide Polymorphisms (SNPs) or assembled for reconstructing nuclear or mitochondrial genomes (Metzker, 2010; Lexer et al., 2013). Such genomic data may be relevant in biogeographic studies by helping to identify and pinpoint the evolutionary drivers of species divergence, expansion, and persistence (Lexer et al., 2013).

Neotropical birds constitute attractive models for approaching biogeographic and evolutionary processes. Neotropics has been proposed as the center of origin for most of modern birds and it is suggested that the world's bird diversity evolved from repeated dispersal events out of then-isolated South America (Claramunt and Cracraft, 2015). That

high diversity at all taxonomic levels is thought to be promoted by the complex ecology and physiography of this biogeographic region. Specifically, paleoclimatic and geological events producing the uplifting of the Andean range (e.g., Chapman, 1917) and the Panama Isthmus (Coates et al., 2004), and the habitat fragmentation associated with Pliocene and Pleistocene glacial cycles (e.g., Haffer, 1969) have been suggested to be some of the major drivers of the avian diversification in the region. However, many questions remain regarding the time and form of the processes driving diversification, resulting in the lack of consensus on mechanisms underlying species richness patterns (Ribas et al., 2012).

Genomic approaches provide some interesting tools to tackle these issues, bringing light to the ecological and historical processes that have driven the evolution of the Neotropical avifauna. In this review, I aim to analyze how genomic data have been incorporated into the study of processes and patterns in Neotropical birds' biogeography. First, I present some of the milestones in the application of genomics to the study of birds. Then, I analyze the biogeographic literature focused on the Neotropical ornithology using genomic approaches and discuss how it has been used to explore critical questions related to the drivers of spatial and temporal distributional patterns in avian diversity, the role of gene exchange in the diversification and persistence of species, and the influence of ecology in processes of local adaptation and differentiation. Finally, I offer some considerations on the potential and limitations of the use of methods of population genomics and phylogenomics in the study of Neotropical birds' biogeography and evolution.

LITERATURE SEARCH

For this review, I examined biogeographic literature of Neotropical avian research using genomic tools available in Google Scholar, and Web of Science, including all its databases (i.e., Biological Abstracts, Biosis, Current Contents Connect, SciELO Citation Index, Russian Science Citation Index, and Zoological Records). The combination of keywords used in searches was: (Aves or bird*) + (Neotropic*, Caribbean, Mexico, Central America, or South America) + (Biogeograph* or phylogeograph*) + (*genomic*, NGS, next-generation sequencing, high-throughput sequencing, massive parallel sequencing, SNP or exon capture). In addition, we examined the literature cited of the found articles to expand and verify the compilation made. The publications were analyzed to extract the information regarding the geographic area (based on the biogeographic regionalization of Morrone 2014) and the species studied, the type of genomic markers used, and the objectives and biogeographic problems addressed.

INCORPORATION OF GENOMICS TO ORNITHOLOGY

The first complete sequence of a bacterial genome (*Haemophilus influenzae*) was obtained in 1995, starting the genomic era in Biology. Since then, the genomes of a variety of organisms from yeast to humans have been sequenced. (e.g., *Saccharomyces cerevisiae* in 1996, *Caenorhabditis elegans* in 1998, and *Homo sapiens* in 2003; Kraus and Wink, 2015). Early genomic works on birds were motivated by agricultural interests in poultry, resulting in the whole genome sequencing of *Gallus gallus* (Hillier et al., 2004), *Meleagris gallopavo* (Dalloul et al., 2010), and *Anas platyrhynchos* (Huang et al., 2013). However, it took some time to become a regular tool in ornithological studies despite, the availability of the required technology for some years (Edwards, 2007). The first phylogenomic avian tree was published in 2014, and besides the reconstruction of phylogenetic relationships among major bird groups, genomic data were used to comparatively address the evolution of birds and their genomes (Jarvis et al., 2014). Some genomic studies appeared shortly after, focusing in the evolution of sex chromosomes and the development of complex traits, such as flight, loss of teeth, and vocal learning (Kraus and Wink, 2015).

Nowadays, many projects for sequencing complete genomes from most avian groups are advancing. As for 2021, GenBank had entries for information of genomes from 812 bird species and information of reduced genomic representation approaches for 3692 species. Although these numbers of records might seem surprising considering how relatively recent HTS technologies are, much more ambitious research projects are in progress such as the Bird 10,000 Genomes (B10K, https://b10k.genomics.cn/), which attempts to eventually sequence genomes of all species of birds.

GENOMIC APPROACHES TO BIOGEOGRAPHIC PROCESSES AND PATTERNS IN NEOTROPICAL BIRDS

The implementation of HTS in biogeography (not only in ornithology) have been slow when compared to other fields, such as metagenomics and disease genetics (Mardis, 2008). This delay was caused, among other reasons by a predominant focus on non-model organisms, a lack of consensus regarding the protocols for solving particular questions, and the transitional state of technology in its early stages (McCormack at al., 2013). Although the progressive reduction in costs and standardization of HTS methods has encouraged a more frequent incorporation of genomic data in biogeographic research, some important limitations remain. For example, current genomic studies pose competing demands on the fraction of

the genome to include versus the sample size of individuals by population or species, which is critical in the biogeographical framework (see for instance Aguirre-Liguori et al., 2020). Also, these studies have shifted the burden of data acquisition to its analysis, being this a challenging transition for many researchers, especially since bioinformatics tools for molecular phylogenomics and population genomics are in constant development, and still far to reach maturity (Cutter, 2013).

Nevertheless, since the sequencing of the first avian genome in 2004 (Hillier et al., 2004) and early attempts for reconstructing the bird life tree based on genomic information (e.g., Hackett et al., 2008; McCormack et al., 2013; Jarvis et al., 2014), there has been an increasing tendency to incorporate genomics to ornithological research. Particularly, in Neotropical biogeography I found a sustained increase in the number of publications on the subject. Since 2014, the number of papers ranges from one to seven by year, yielding to date almost 30 publications focused on more than 370 bird species. Interestingly, less than half of these publications were carried out as comparative biogeographic studies, while the vast majority are dedicated to analyze the evolutionary history of single taxa (Fig. 1). Most of these studies used reduced genomic representation, while only two are based on whole genome sequencing methods (Fig. 2a). The taxonomic representation in such studies has been wide, so that genomic information has been generated for around 15 orders, although Passeriformes represent almost three-quarters of the species studied (Fig. 2b).

There is also an uneven distribution of the research work along the Neotropics, with a higher interest in the Boreal, South Brazilian, Pacific, and Mesoamerican dominions within the Brazilian Subregion, as well as the Chacoan Subregion. On the other hand, the Antillean Subregion and the South American Transition Zones are the worst represented in both number of publications and number of species (Fig. 3). There is a bias to study moist forests and savanna biomes, while most of the other habitats remain largely unexplored. Tropical rainforests are, by far, the most commonly investigated biome (more than half of papers), followed by tropical dry forests and tropical-subtropical grasslands, savannas and shrublands (near 30%). Conversely, there is a lack of publications addressing biogeographic patterns in tropical and subtropical coniferous forests, flooded grasslands and savannas, montane grasslands and shrublands, deserts, xeric shrublands and mangroves.

Most genomic studies on Neotropical birds have been focused on groups with taxonomic uncertainties (e.g., *Sporophila*, Mason et al., 2019; *Scytalopus*, Cadena et al., 2020) or for improving the resolution at population level of previous studies at much finer temporal and spatial scales (e.g., Llanes-Quevedo et al., 2022). In general, it is possible to

distinguish three main biogeographic questions frequently addressed in Neotropical ornithology research: (1) historical drivers of spatial and temporal patterns in avian diversity, (2) the role of gene exchange in the diversification and persistence of species, and (3) the influence of ecology in processes of local adaptation and differentiation.

Historical drivers for spatial and temporal patterns in avian diversity.- The origin and distribution patterns of Neotropical avifaunas have been one of the most active fields in recent ornithological research, especially emphasizing the role of geologic and paleoclimatic events in the processes of dispersal and vicariance. These are often contrasted as the main processes shaping spatial and temporal patterns of biological diversity. Although recent biogeographic reconstruction methods recognize that both processes have an important role in the formation of distributional areas and patterns, the emerging question is to discover their relative frequencies (Zink et al., 2000).

For elucidating these patterns (Box 1), genes from organelles (and often from nuclear DNA) and microsatellites have been traditionally used. These markers have been widely used for reconstructing phylogenetic hypotheses and characterize the genetic diversity of taxa, respectively (e.g., Avise, 2004; Selkoe and Toonen, 2006). However, the proportion of use of these markers has gradually decreased, as compared to those generated by HTS. These methods provide a wide range of markers, from SNPs to whole genomes, to test biogeographic hypotheses at various timeframes and taxonomic scales. At the population level, the most widely used markers are those produced by RADseq and parallel tagged sequencing, while at higher taxonomic scales and to resolve deeper phylogenies the data obtained target enrichment sequencing has been mainly used (McCormack et al., 2013). Since the information produced is composed of millions of sequences, it allows phylogenetic inferences to be made from nucleotide substitution patterns, although the length of each individual locus is relatively short. They can also be used to quantify the number of alleles and heterozygosity, allowing the making of phylogenetic inferences and those related to the population structure with the same data set (Ree and Hipp, 2015; Pante et al., 2015).

Avian diversity in the Neotropics has been traditionally attributed to the effect of vicariant forces promoting speciation in allopatry (e.g., Brumfield, 2012). Nevertheless, genomic studies have shown that phylogeographical patterns shared among co-distributed species cannot be explained by a single vicariant event, as species responses to a common barrier are different, depending on their biological attributes (Lavinia et al., 2019). The Amazon, Andes, Tehuantepec Isthmus, and forested areas (for savanna birds) have been

recognized as major barriers for dispersal; however, they are differentially permeable, depending on the bird species' habits, e.g., canopy versus understory birds (Weir et al., 2015; Ferreira et al., 2018). Also, most of the biogeographic genomic studies in birds support the Refuge Hypothesis, which suggests that the historical alternation of wet (interglacial periods) and dry (glacial periods) cycles has generated the contraction and expansion of available habitats, creating unconnected patches that can promote allopatric speciation (Haffer, 1969; Brown et al., 1974).

Moreover, many studies have focused on the detection of cryptic species and the historical factors that have produced it (e.g., Raposo et al., 2018; Imfeld et al., 2020; Cadena et al., 2020; Buainain et al., 2021). Such works leaded to reanalyze the processes and patterns that have generated the biodiversity of the Neotropics due to the limitations of previous studies based in few genetic markers in assessing recent historical events and population structure (e.g., Smith et al., 2014; Raposo Amaral et al., 2018). Analyses based on genomic data result in narrower confidence intervals in estimates of important parameters as effective population sizes, and migration rates between populations allowing more robust approaches to cryptic species evolutionary history (Smith et al., 2014).

Role of gene flow in avian diversification and species persistence.- Although allopatric speciation is typically considered as the primary mode of speciation in vertebrates (Coyne and Orr, 2004; Provine, 2004), there is growing evidence that gene-flow often occurs during clade diversification (Burbrink and Gehara, 2018; Gompert et al., 2014; Thom et al., 2018). Introgression is generally thought to prevent differentiation by homogenizing allele frequencies among populations (Slatkin, 1987), but it can also promote speciation, via adaptive introgression or hybrid speciation (Delmore et al., 2015; Barrera-Guzmán et al., 2018; Marques et al., 2019). Nevertheless, identifying gene-flow events may be problematic, due to the similarity of patterns produced by these or by common ancestry, being a pending task to resolve for population genetics and phylogenetics theory and methodologies.

Due to most of the genomic studies on Neotropical birds are based on reduced representations of the genomes, the “global ancestry” family of methods (see Box 2) is the most commonly used for inferring genetic structure and gene flow. Such methods have allowed the identification of admixture in a variety of taxa, including Mesoamerican (Musher et al., 2019), Amazonian (Weir et al., 2015; Harvey et al., 2017), Andean (Cabane et al., 2019), and Atlantic Forest species (Berv et al., 2021). These findings fit a general pattern of widespread occurrence of hybridization (Ottenburghs et al., 2015) and the slow evolution of

postzygotic isolation in birds (Ottenburghs et al., 2017). They also revitalized the biogeographic controversy about the role of geographic barriers in the evolution of avifauna. Some of these works have incorporated coalescence-based demographic models (e.g., those implemented in momi2 [Kamm et al., 2020] and G-PhoCS [Gronau et al., 2011]), that allow to test explicit historical models, and have more realistic assumptions (Malinski et al., 2021), which constitutes a step forward in the understanding of the evolution of taxa.

The use of genomic data has also revealed inconsistencies with previous findings based on traditional phylogeographic studies (e.g., *Vireo olivaceus* [Battey and Klicka, 2017], *Galbula leucogastra/chalcothorax* [Ferreira et al., 2018], and *Tunchiornis ochraceiceps* [Buainain et al., 2021]). The inconsistency of genealogies obtained from a large number of markers, such as those generated by reduced representation of genomes, or form nuclear versus mitochondrial markers, allows to detect not only the existence of introgression or the signatures of shared ancestry (as incomplete lineage sorting), but some other biological attributes or processes of taxa, such as philopatry, natural selection and dissimilar dispersal capacities between sexes, as well as indicate incomplete sampling or improper taxonomy (Ferreira et al., 2018).

Genome-phenotype variation and their relationship with ecology.- Genetic and phenotypic variation within species determines how they respond to environmental change (Willi et al., 2006), the propensity to form new species (Riginos et al., 2014; Harvey et al., 2017), and their susceptibility to extinction (Keller and Waller, 2002; Harvey et al., 2017). Thus, the understanding of relationship between genomes, phenotypes, and environment is essential for elucidate the patterns of biodiversity.

Near a third of the publications analyzed herein incorporated phenotypic and environmental data, including habitat parameters, climate niche modelling, among others to explain phenotypic and genomic variation. Even so, the diversity of approaches (Box 3) was not fully exploited to detect evidence of local selection or adaptation. Most of the studies analyzed outlier loci and its correlations with environmental variables. For some taxa, genomic variation is mainly explained by historical barriers to gene flow and paleoclimatic changes, while phenotypic diversity is better predicted by contemporary environmental heterogeneity (e.g., *Icterus gularis*, Moreira et al., 2020). Species ecology, in the form of habitat association, is an important predictor of genetic diversity and population divergence. For example, in the Amazon basin, species of upland forest have greater genetic diversity

and divergence across the landscape as well as signatures of older histories and less gene flow than floodplain species (Harvey et al., 2017).

Ecological niche modelling is often employed as complementary to genomic methods for reconstructing paleodistribution of taxa, and consequently explain geographical patterns of species and speciation process. Thus, it has been hypothesized that although changes in physical barriers to dispersal, current habitat heterogeneity, and geographic distance are relevant, the evolutionary history of the Neotropical bird species is mainly related to past climatic changes (Buainain et al., 2019; Corbett et al., 2019).

OPPORTUNITIES AND CHALLENGES

Although to date the incorporation of genomics to Neotropical birds' biogeography has been limited, there are tremendous opportunities in incorporating these new genomic tools to investigate patterns and processes of emergence and maintenance of biodiversity. The technical advances and new analytical tools from molecular biology and bioinformatics offer great possibilities to solve obstacles that hindered research studies in the past and provide new avenues to improve our understanding of the evolutionary history of the birds and its environment.

Genomics from non-invasive methods of sampling.- The sampling of elusive, rare, or endangered species constitutes a major challenge to the study of biodiversity. For such species, non-invasive sampling methods (e.g., feathers, eggshells, and feces) constitute an important alternative. Although some extraction protocols from non-invasively collected starting materials typically yield low concentrations of DNA and may also contain PCR inhibitors (Beja-Pereira et al., 2019; Smith and Wang, 2014), it has been demonstrated that genomic data collection from non-invasively collected starting materials is efficient (Rusello et al., 2015). This is especially true for modern genotyping-by-sequencing (GBS) approaches, which typically call for less than 10 µg of DNA for library construction (Baird et al., 2008; Etter et al., 2011).

Metagenomic and metabarcoding applications. – In biogeography and phylogeography it is essential to have a proper knowledge of the distributional range of organisms and, ideally, to obtain a genetic sampling that adequately represents the geographic distribution. Metagenomic and metabarcoding methods allow to extract DNA from environmental samples, and then use bioinformatic pipelines to find and match sequenced DNA of species

with reference to online database. This way, the presence or absence of species can be detected in the environment and in some cases abundance estimates might be obtained (Olah et al., 2021).

However, some limitations persist linked to the short-read sequencing, because the fragmentation and incompleteness of genomes, limiting the accuracy of some downstream uses of genomic data. The ongoing further development and popularization of third-generation sequencing technologies, which can produce reads greater than 10 Kb in length and allow for the assembly of chromosomal-level genomes, will pave the way for more accurate and reliable studies (Olah et al., 2021).

Genomics on ancient DNA.- Modern molecular biology methods enable the use of ancient DNA obtained from specimens from museums and other ornithological collections (e.g., Tsai et al., 2019). Such material offers the opportunity to sample very rare or extinct taxa or populations, allowing a more precise reconstruction of their evolutionary history and that of their closest contemporary taxa/ populations and postulate hypotheses about the physical and paleo-environmental causes that have shaped their distributional patterns (Posadas et al., 2006). From a population standpoint, the use of ancient DNA would allow better estimates of the influence of events such as bottlenecks, population expansions, gene flow, and isolation on the genetic diversity of species. From a phylogenetic point of view, these samples could also provide valuable information for a more realistic estimation of morphological and molecular evolution rates and their fit to the predictions of ecological and evolutionary theory, which are essential to critically test speciation models within a historical biogeography setting (Riddle et al., 2008).

However, there are some issues related to the use of ancient DNA including difficulties in the collection of samples (Tsai et al., 2019), the higher error rates, missing data, and lower diversity of SNPs (Olah et al., 2021). Therefore, studies using low-quality museum samples to generate phylogenomic data must be careful and follow best practices for assembling, processing, and analyzing such data to avoid misinterpretations (Olah et al., 2021).

Prediction of species response to future environmental changes.- Genomic offset methods (Capblancq et al., 2020) combine genomic and environmental data from different time points and/or locations to assess the degree of possible maladaptation to new environmental conditions (Rellstab et al., 2021). This is done by calculating statistical relationship between environmental factors and allele frequencies of populations or

individuals through genotype-environment association analysis, and then predicting the necessary genetic composition for the new or future (modeled) environment. These analyzes, aim to evaluate the degree of possible maladaptation of these populations to their new environmental conditions and to quantify the potential lack of adaptation of a population to its current environment (Borrell et al., 2019). To date, most genomic offset studies have been carried out with forest trees (e.g., Jia et al., 2020; Sang et al., 2022) and cultivated plants (e.g., Morales-Cruz et al., 2021; Aguirre-Liguori et al., 2019); however, their application to Neotropical birds may contribute to most effective planning of their management and conservation.

Comparative studies using genomic data.- Comparative studies are essential given the relevance of testing spatial and temporal congruence between different co-distributed taxa for the inference of processes in biogeography. These analyses will allow to delve into the processes of allopatric, parapatric, and sympatric speciation, providing information on the causes of diversification (Bowen et al., 2014). However, addressing these analytical challenges requires the development of computationally powerful and flexible bioinformatics tools that will allow inferring large-scale patterns of spatial and temporal congruence between co-distributed taxa. To date ther is some software to analyze these patterns including the R package Multidice (Xue and Hickerson, 2017), msBayes (Hickerson et al., 2007) and Ecoevolity (Oaks, 2019). Nevertheless, in most cases, some improvements are required for optimizing time and computational resources needed, as well as for the calculation of parameters such as population sizes and divergence times. Furthermore, it is necessary to improve the reticulation events analysis tools during the evolutionary process, both within genomes in the form of genetic recombination and between populations and species in the gene flow and introgression. In the case of population genomics approaches, it is also not feasible to perform genomic-scale comparisons on hundreds or thousands of samples given the high costs, wasting efforts in sequencing non-informative parts or with redundant information in DNA, among other factors.

CONCLUDING REMARKS

The use of genomic markers and methods is still not common but constitute very valuable tools for analyzing processes and patterns in biogeography, bringing historical and ecological approaches closer. Although each genetic marker and molecular method have its

limitations, their integration can help us unravel the different phenomena that have marked the evolutionary history of populations and species.

The development of genomics has provided access to a large amount of information that allows us to analyze, like never before, patterns of biodiversity and the processes that generated them; however, it is far from being the ideal solution to all problems and questions in biogeography. Given the high costs of these technologies for most of the world's biodiversity hotspots that are concentrated in underdeveloped countries, such as the Neotropics (e.g., Navarro et al., 2003), studies with traditional markers (such as those generated by Sanger sequencing) remain effective in addressing key issues of evolution and conservation. However, it is highly recommended to promote capacities that allow the exploitation of the most advanced technologies to better understanding and conservation of the Neotropical biota. Likewise, it is important to reorient efforts in the study of areas or little studied environments such as islands, xeric areas, and some others that have remained in the background, even when they are very relevant from the biogeographical point of view.

Finally, it is necessary to bear in mind that genomic methods may also contribute to improve the contributions of biogeography to conservation. DNA of species not only contains evidence of their history but is the basis of their future. Understanding the contribution of past environmental change, vicariance, and dispersion in the connectivity and genetic diversity of populations and species, together with their adaptive capacity, is essential to make predictions about the future of their persistence and consequently of their evolution. The incorporation of genomic information will allow the generation of more solid evidence with application to the conservation and management of biodiversity.

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COMPLEMENTARY BOXES

Box 1. How to recognize dispersal and vicariance with genetic information?

Two main approaches involving the use of calibrated molecular phylogenies and the comparison of genetic characteristics of taxa are available. Molecular phylogenies allow estimating molecular clocks which are used to date cladogenetic events on a large time scale (millions of years) and relate them to ecological and/or geological events that might be responsible of the divergence.

For example, if the dating of the divergence between the disjunctly distributed lineages is more recent than the barrier formation, a long-distance dispersal hypothesis might be supported. On the contrary, if the divergence is after the emergence of the barrier, it may be due to vicariant events, although long-distance dispersal cannot be excluded (Posadas et al., 2006).

At smaller timescales (thousands of years), other surrogates are often used, which are based either on the different lineage-area relationships produced by vicariance and dispersal or in the different patterns of genetic diversity resulting from these processes. In the first approach, the reciprocal monophyly of lineages from disjunct areas may support the vicariant hypothesis (except for insufficient sampling), while under a long-distance dispersal hypothesis, it is expected that lineages from a disjunct area will nest within those of another area.

The second approach suggests that the genetic diversity should be comparable between disjunct distribution areas resulting from vicariance, while a reduced genetic diversity could be expected in a disjunct area, when it was colonized by dispersed organisms from another area, especially if dispersal is produced by a few individuals (Kropft et al., 2006).

Box 2. Investigating genetic admixture and their causes

Approaches to identify genetic admixture are grouped within the so-called global and local ancestry analyses and may include several species or individuals (Twyford and Ennos, 2012).

Under the local ancestry model, it is assumed that the genome of each individual or species is divided into chromosomal segments with a defined ancestral origin. The objective is, then, to find the limits of the segments and assign the origin of each. Several methods have been proposed for such estimations, which generally match the genomes of interest with representative panels from the putative source populations to find the patterns of continuous ancestry (Gravel, 2012).

In the case of global ancestry, the main objective is to estimate the proportion of ancestry of each contributing population, considered as an average over the entire genome of the individual (Alexander et al., 2009). There are two main methods, those based on models which estimate the ancestry coefficients as parameters of a statistical model (as implemented in Structure, Admixture, among others); and those of "algorithmic estimation" that use multivariate analysis techniques, primarily Cluster Analysis and Principal Component Analysis, to explore the structure within the data (Gravel, 2012).

Admixture in individuals and populations may be caused by different processes such as Incomplete Lineage Sorting (ILS) and Introgression. In practice it is possible to distinguish among them, although it is not always clear. The main difference between these processes is that in an ILS hypothesis it is not expected to find any biogeographic pattern, while in introgression it is possible to find it (Toews and Brelsford 2012). Also, bioinformatic pipelines have been developed recently to differentiate and to test statistically the sites that support introgression or ILS hypotheses, considering the possible topologies of a tree (e.g., ABBA-BABA test, Malisnki et al., 2021).

Box 3. Genomic methods for environmental association studies

Before the popularization of HTS methods, empirical studies used quantitative trait loci (QTL) mapping in crosses made between divergent lines, to find loci of large phenotypic effect. However, these studies often lack the power to detect loci with less effect on phenotype and therefore cannot fully test the theory on the genetic basis of differences between divergent phenotypes or identify all genes involved (Orr, 2005).

At present genomic methods opened new research pathways to genome-wide association studies (GWAS) by analyzing thousands of polymorphic sites scattered throughout the coding- and non-coding parts of the genome. They allow uncover regions that show signatures of selection and local adaptation on the genome (Olah et al., 2021) by three main approaches: sequencing and comparison of whole genomes, identification of loci through association studies and sequencing of candidate genes (De Villemereuil and Gaggiotti, 2015).

From whole genomes sequencing, comparisons can be made with phylogenetically close species whose genomes are annotated. In this way, genetic variants can be analyzed considering ecological characteristics and evolutionary history of the reference genome.

On the other hand, loci identification approaches may be based on: (1) genomic analysis of populations where genotypic data of individuals from divergent populations are used to identify outlier loci; (2) genome-wide association studies (GWAS), that seek to find an association between certain genetic traits and markers in large population samples based on a linkage map or reference genome; (3) analysis of coverage depth where the depth between loci and populations of transcriptome sequences is quantified to find different expression patterns and splicing variants.

Finally, using the candidate gene sequencing method, specific regions based on a priori knowledge of the function of genes are sequenced by complete genome sequencing or by GWAS (Stapley et al., 2010).

FIGURES

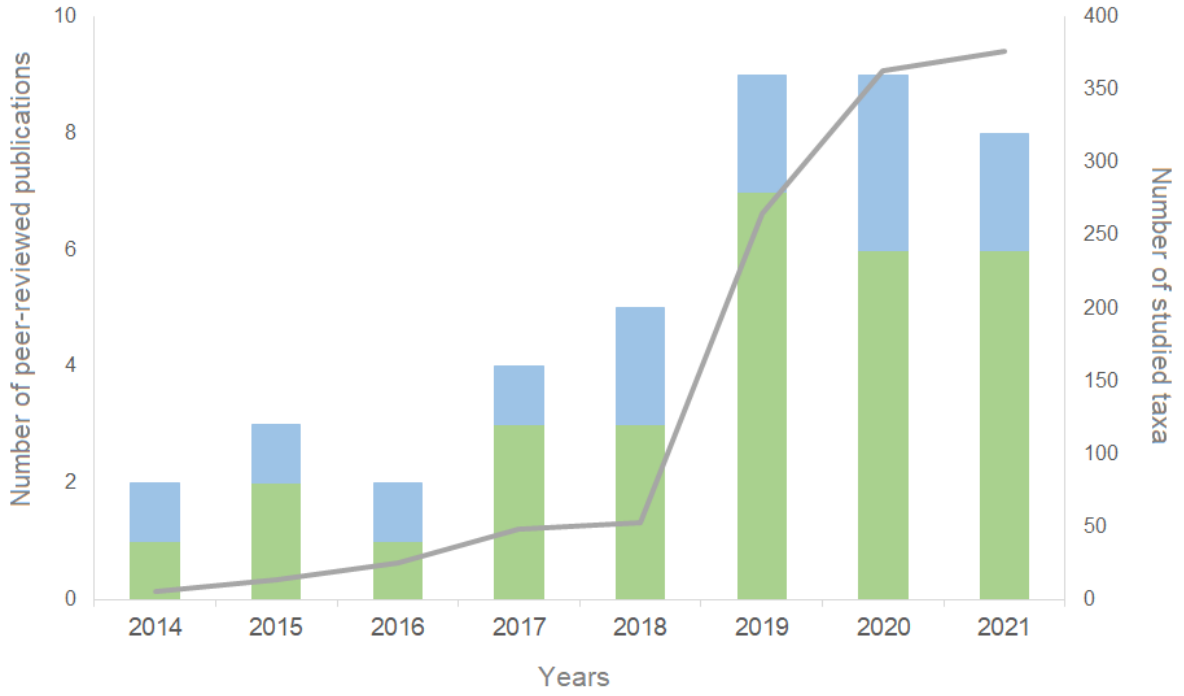

Figure 1- Genomic studies on Neotropical birds since 2014. Bars and primary axes show the number of publications by year, in blue are represented the fraction of works analyzing comparatively two or more species. Continuous gray line and secondary axis show the accumulative number of species which genomic data has been generated within these publications.

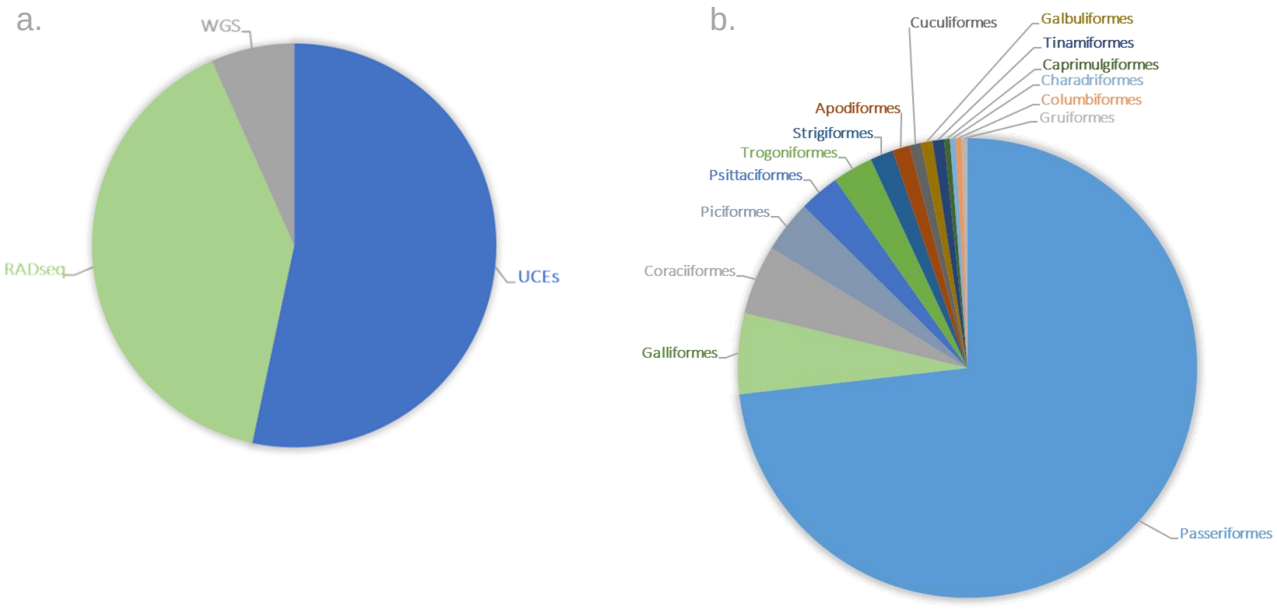

Figure 2a- Genomic markers used in publications on the Neotropical bird's biogeography since 2014: RADseq (Restriction site associated DNA markers), UCEs (Ultra-Conserved Elements), WGS (whole genome sequencing). (b) Number of species by order represented in publications on the Neotropical bird's biogeography since 2014.

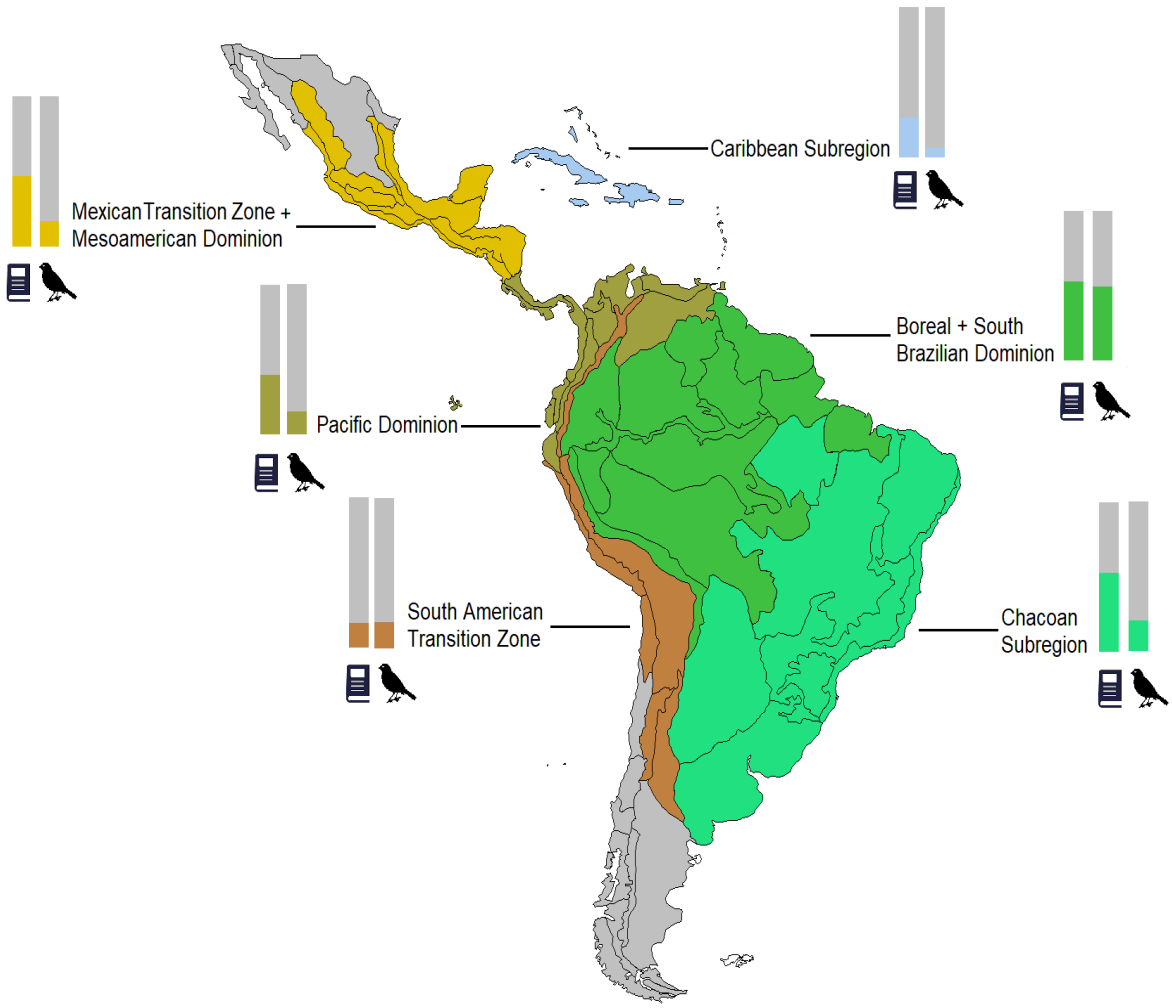

Fig. 3- Representativeness of biogeographic regions and their species in publications on the Neotropical bird's biogeography since 2014. Biogeographic regionalization according to Morrone (2013). Left bars (identified with a book icon) represent the number of publications referring each area and ranges from 1 to 29. Bars on the right (identified with a bird icon) represent the number of bird species of the area analyzed in each publication, and ranges from 1 to 377.

CAPÍTULO 3. The tangled evolutionary history of a long-debated Mesoamerican taxon: the Velazquez Woodpecker (*Melanerpes santacruzi*, Aves: Picidae)

Alexander Llanes-Quevedo [1, 2], Alicia Mastretta-Yanes [3, 4], Luis A. Sanchez-Gonzalez[1], Vicente J. Castillo-Chora [1,2], Adolfo G. Navarro-Sigüenza[1]

[1]Museo de Zoología "Alfonso L. Herrera", Facultad de Ciencias, Universidad Nacional Autónoma de México, México

[2]Posgrado en Ciencias Biológicas, Universidad Nacional Autónoma de México, México

[3]Comisión Nacional para el Conocimiento y Uso de la Biodiversidad, México

[4]Consejo Nacional de Ciencia y Tecnología, México

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The tangled evolutionary history of a long-debated Mesoamerican taxon: The Velazquez Woodpecker (*Melanerpes santacruzi*, Aves: Picidae)

Alexander Llanes-Quevedo [a,b,*], Alicia Mastretta-Yanes [c,d], Luis A. Sánchez-González [a], Vicente J. Castillo-Chora [a,b], Adolfo G. Navarro-Sigüenza [a]

[a] Museo de Zoología "Alfonso L. Herrera", Facultad de Ciencias, Universidad Nacional Autónoma de México, Mexico
[b] Posgrado en Ciencias Biológicas, Universidad Nacional Autónoma de México, Mexico
[c] Comisión Nacional para el Conocimiento y Uso de la Biodiversidad, Mexico
[d] Consejo Nacional de Ciencia y Tecnología, Mexico

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ABSTRACT

The Velazquez Woodpecker *Melanerpes santacruzi* is a highly polytypic species distributed from east-central Mexico to northern Nicaragua. The ample variation in body size, barring of the plumage, and the coloration of nasal tufts, neck, and belly have fueled debates about the taxonomy and evolutionary history of the species; however, the processes generating these patterns of variation and the underlying population dynamics throughout the species' distribution remain poorly understood. Here, we employed reduced representation genome sequencing (NextRAD) and Ecological Niche Modeling methods to test the distinctiveness of the Velazquez Woodpecker based on this new set of genomic data and analyze the correspondence of the genetic structure and ecological differentiation with phenotypic variation and geographic distribution. From phylogenetic and demographic analyses including the Golden-Fronted (*M. aurifrons*) and Red-bellied Woodpecker (*M. carolinus*), we obtained results congruent with previous molecular phylogenies. The clades of *M. santacruzi* and *M. carolinus-M. aurifrons* are reciprocally monophyletic, although the sister group relationship of *M. aurifrons* is ambiguous. Using genetic and ecological analyses, we found that the species is structured into three genetically and ecologically differentiated groups comprising the subspecies (1) *M. s. santacruzi*, (2) *M. s. dubius* and (3) *M. s. grateloupensis-polygrammus-veraecrucis*. These groups diverged recently, with two splits between 250,000 and 150,000 years ago, and show a significant genetic admixture among them, especially in their current contact zones. Ecological and demographic models suggest the existence of intermittent areas of sympatry and connectivity among populations of *M. santacruzi* since the Last Interglacial period. We also found evidence of bi-directional gene flow between the species *M. aurifrons* and the nearby populations of *M. santacruzi* (*M. s. grateloupensis*), along the Sierra Madre Oriental in northeastern Mexico. Gene flow seems to be uneven, with prevalence of movement in the direction from *M. aurifrons* to *M. s. grateloupensis*.

1. Introduction

Understanding the relationship between phenotypic and genetic divergence is an important goal in the study of speciation. However, connecting these elements to elucidate the evolutionary history of species is often challenging because phenotypic and genotypic variation may be uncoupled (Raposo do Amaral et al. 2018). Phenotypic variation in genetically determined traits is generally attributed to adaptation of different genotypes to local conditions (Antoniazza et al., 2010); however, it can also arise from processes like mutations and genetic drift in populations connected by spatially limited gene flow (Endler, 1977), admixture of populations in secondary contact (Slatkin, 1973, Barton and Hewitt, 1985), or spatial population expansions (Klopfstein et al., 2006, Excoffier and Ray, 2008). Analyzing the influence of these processes constitute the next step for understanding the patterns of variation and the underlying population dynamics throughout the species' distribution.

The Velazquez Woodpecker *Melanerpes santacruzi* is a polytypic taxon distributed from eastern Mexico to northern Nicaragua. Due to the ample phenotypic variation of the species and the morphological

* Corresponding author.
E-mail address: alex.llanesquevedo@gmail.com (A. Llanes-Quevedo).

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Highlights

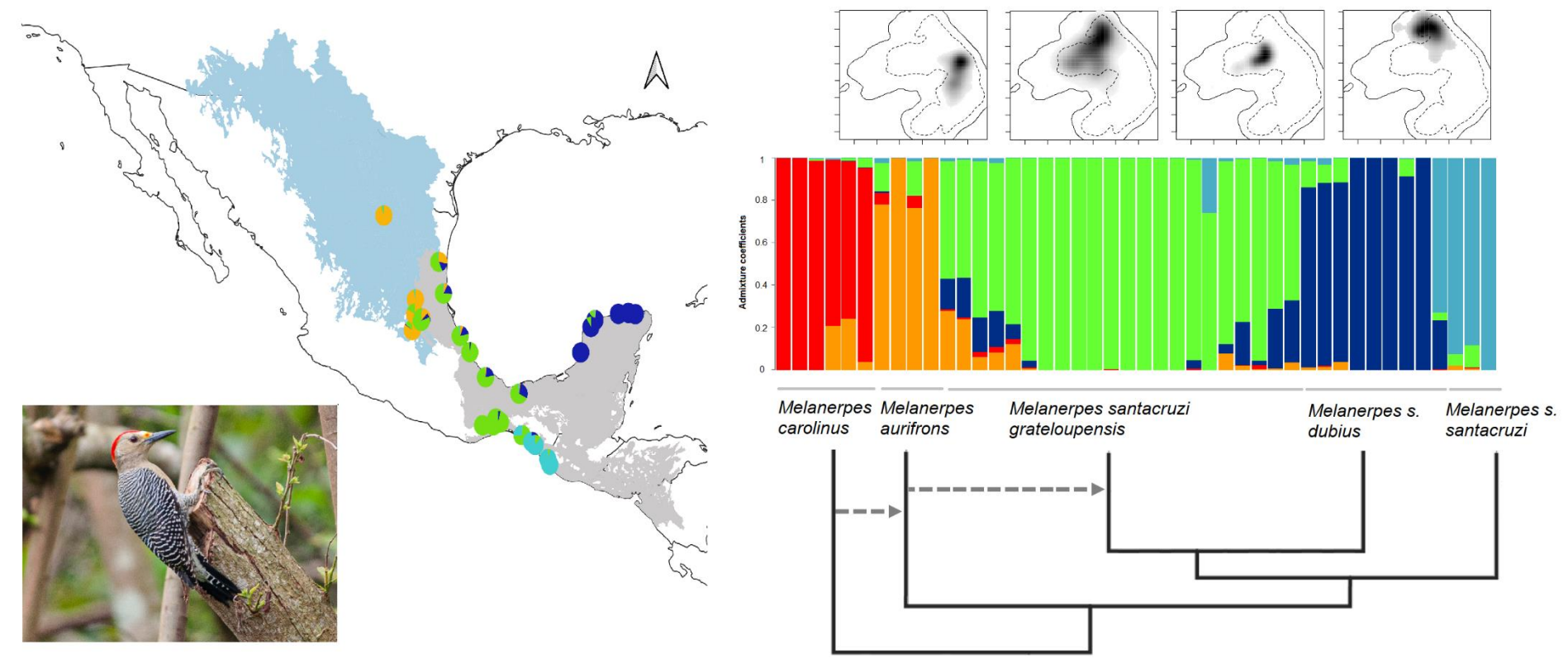
- The Velazquez Woodpecker (*Melanerpes santacruzi*) is a polytypic species whose taxonomy and evolutionary history have been historically controversial, due to complex distribution patterns of morphologically differentiated populations, and the lack of phylogeographic structure among them.

- Genomic data provided good resolution for detecting population structure within the Velazquez Woodpecker, which is consistent with some major geographical features in southeastern Mexico, and only partially congruent with previous grouping hypotheses.

- Populations of *M. santacruzi* diverged recently and showed genetic admixture among them, but also with the Golden-Fronted Woodpecker (*M. aurifrons*), although each one maintained its own morphological and evolutionary identity.

- Ecological niche models supported the genetic differentiation of *M. santacruzi* populations and suggested intermittent areas of sympatry since Last Interglacial.

Graphical Abstract



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ABSTRACT

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Key words: contact areas, gene flow, genomics, ecological niche modeling

INTRODUCTION

Understanding the relationship between phenotypic and genetic divergence is an important goal in the study of speciation. However, connecting these elements to elucidate the evolutionary history of species is often challenging because phenotypic and genotypic variation may be uncoupled (Raposo do Amaral et al., 2018). Phenotypic variation in genetically determined traits is generally attributed to adaptation of different genotypes to local conditions (Antoniazza et al., 2010). However, it can also arise from processes like mutations and genetic drift in populations connected by spatially limited gene flow (Endler, 1977), admixture of populations in secondary contact (Slatkin, 1973; Barton and Hewitt, 1985), or spatial population expansions (Klopfstein et al., 2006; Excoffier and Ray, 2008). Analyzing the influence of these processes constitute the next step for understanding the patterns of variation and the underlying population dynamics throughout the species' distribution.

The Velazquez Woodpecker *Melanerpes santacruzi* is a polytypic taxon distributed from eastern Mexico to northern Nicaragua. Due to the phenotypic variation of the species throughout its distribution range and the morphological similarity of some of these forms with other species of the genus, its taxonomy and evolutionary history have been historically controversial and is still under debate (Selander and Giller, 1963; García-Trejo et al., 2009). This taxon has been traditionally included within a complex formed by the Golden-fronted Woodpecker (*M. aurifrons*) and the Red-bellied woodpecker (*M. carolinus*) (Ridgway, 1914; Peters, 1948; AOU 1957, 1983, 1998). However, it was recognized as a distinct species by García-Trejo et al. (2009), Navarro-Sigüenza et al. (2017), and Gill et al. (2021) based in mitochondrial markers (ND2, ND3, COIII, and tRNA-Met). These authors found that *M. santacruzi* is a monophyletic group, sister to a clade composed by *M. aurifrons* from northern Mexico and the southern United States, and the North American Red Bellied Woodpecker (García-Trejo et al., 2009; Dufort, 2016; Navarro-Sigüenza et al., 2017). It also has been suggested that *M. santacruzi* diverged from *M. aurifrons-M. carolinus* between two and five million years ago (Dufort, 2016; Navarro-Sigüenza et al., 2017). Nonetheless, the phylogenetic trees based on four nuclear genes obtained by Navarro-Sigüenza et al., (2017) failed to support the monophyly of *M. santacruzi* regarding to *M. aurirons* and *M. carolinus.*

Throughout its distribution, the Velazquez Woodpecker shows clear morphological differentiation in terms of body size, the coloration of the nasal tufts, neck, and ventral plumage, and in the patterning of the bars on the dorsal feathers and rectrices (Selander and Giller, 1963). This variation led to the recognition of up to 11 subspecies (Gill et al., 2021),

five distributed in mainland: v*eraecrucis, dubius, polygrammus grateloupensis*, and *santacruzi* (Fig. 1a) and another six in islands nearby. *M. s. grateloupensis* ranges from central San Luis Potosí and southern Tamaulipas to eastern Puebla and central Veracruz, where it overlaps with *M. s. veraecrucis*, which ranges along the Atlantic slope from southern Veracruz to northeastern Guatemala. *M. s. dubius* inhabits the Yucatan Peninsula, Belize, and northeastern Guatemala, and *M. s. santacruzi* is distributed from southeastern Chiapas to northern Nicaragua. Finally, *M. s. polygrammus* is found on the Pacific slope of Oaxaca, along the southern portion of the Isthmus of Tehuantepec, and in the interior valley of Chiapas.

Based on color patches and the barring of the plumage, Benites et al. (2020) suggested that these subspecies could be grouped into three basic morphs (Fig. 1b): (1) red nape/red belly with higher barring frequency and lower barring ratio; (2) red nape/yellow belly with intermediate barring frequency and intermediate barring ratio; and (3) yellow nape/yellow belly with lower barring frequency and higher barring ratio. Some of these morphs are associated to well-defined regions such as the Yucatan Peninsula (red nape/red belly, high barring frequency), and the Pacific coast of the Tehuantepec Isthmus (yellow belly/yellow nape, low barring frequency); however, to the northern and southern extremes of the species range, morphs are more variable, showing orange-to-red nape/yellow belly, and intermediate barring frequency (Benites et al., 2020). Distributional patterns of these groups are complex, with several zones of sympatry or parapatry, and although some level of variation and intergradation is present in these contact zones (Benites et al., 2020), they presumably have limited interbreeding (García-Trejo et al., 2009).

Despite several studies using morphological differentiation, genetics, and distribution patterns, the phylogeographic structure within *M. santacruzi* populations is still not well-resolved, fueling debates on the systematics and evolution of this taxon. After more than a century of debate, Benites et al. (2020) noticed that the subspecies traditionally proposed for this taxon do not properly reflect the patterns of plumage variation. Patterns found by these authors are hypothesized to be the consequence of local adaptation to environmental variation, which after range expansion to humid lowland forests in the Late Pliocene-early Pleistocene met different selection pressures and conditions for interspecific competition (Navarro- Sigüenza et al., 2017).

Genomic approaches are enlightening the complexity of species' evolutionary histories. High-throughput sequencing (HTS) methods have overcome some of the most important limitations of traditional markers by expanding the number of sampled loci by

several orders of magnitude, covering the whole genome or at least a representative part of it (Goodwin et al., 2016). Genetic data retrieved from some HTS methods, like the reduced representation genome sequencing, have proven useful for population structure analyses (Ree and Hipp, 2015; Pante et al., 2015), phylogenetic inferences, and the identification of loci or genes related to adaptations of populations to local environmental conditions (De la Torre at al., 2019). It also allows inferences about the extent of hybridization and events of lineage sorting in many taxa, offering new opportunities to understand the complexity of phylogenetic relationships among taxa (Twyford and Ennos, 2012).

To complement genomic analyses, Ecological Niche Modelling (ENM) constitutes a powerful set of tools for investigating evolutionary patterns and processes (Peterson et al., 2011; Rodriguez-Rodriguez et al., 2020) since they are linked to the variation in the abiotic environment (e.g., temperature, precipitation, and topography; Hozak et al., 2008). These methods allow the inference of environmental suitability of species (Guisan and Thuiller, 2005), the testing of ecological niches similarity between species, and the reconstruction of paleodistributions, among other applications (Van Tran et al., 2020; Raxworthy et al., 2007). Its combination with genetic studies may provide a better understanding on the evolutionary biology and geographical patterns of species and speciation (Rodriguez-Rodriguez et al., 2020), by allowing to infer connectivity among suitability areas of taxa over time, especially in those of recent divergence, as well as to test hypothesis of speciation modes (Hozak et al., 2008).

In this study, we address the evolutionary history of the Velazquez Woodpecker *M. santacruzi* by integrating information retrieved from genomic methods and ENM analyses. We analyzed the role of isolation and gene flow events in the differentiation dynamics within the Velazquez Woodpecker populations and with the closely related Golden-fronted and Red-bellied Woodpeckers. We tested the hypothesis that morphological variation in this taxon is a consequence of geographic isolation, which led to genomic and ecological differentiation despite current patterns of sympatry and parapatry in some of its populations. Our main goals were: 1) to test for the distinctness of Velazquez Woodpecker based on a new set of genomic data; 2) to characterize genetic diversity and population structure within the taxon; 3) to evaluate the correspondence of the genetic structure with the phenotypic geographic variation (recognized subspecies and morphological and coloration-based grouping hypotheses), 4) to infer scenarios of isolation and gene flow explaining current patterns of genetic variation through phylogenetic analyses and demographic modelling, 5) to characterize the ecological niche and evaluate the correspondence between genomic and

ecological differentiation, and 6) to reconstruct the geographic distribution of populations in the past, for analyzing the role of past climatic changes in the connectivity and isolation among *M. santacruzi* populations.

MATERIAL AND METHODS

Taxonomic Sampling.- We collected frozen or ethanol-preserved tissue samples (muscle, heart or liver), or pieces of toepads from study skins of *M. santacruzi* (n = 54), covering most of the mainland distribution in Mexico and belonging to the subspecies *veraecrucis, santacruzi, dubius, polygrammus*, and *grateloupensis* (Table S1). We also included samples of *M. aurifrons* (n = 5) and *M. carolinus* (n = 6), the species in the sister clade of the Velazquez Woodpecker (García-Trejo et al., 2009; Dufort, 2016; Navarro-Sigüenza et al., 2017). As outgroups for phylogenetic analyses, we sampled individuals of *M. chrysogenys* (n = 2) and *M. pucherani* (n = 1).

Most of the samples were obtained from specimens and tissues in the Scientific Ornithological Collection of the Museum of Zoology “Alfonso L. Herrera” of the Faculty of Sciences of the National Autonomous University of Mexico, Mexico. Samples of *M. carolinus* were obtained from loans from scientific collections in the USA (Supplementary Materials, Table S1).

DNA Extraction and NextRAD Sequencing.- For preserved tissue samples, total genomic DNA was extracted using Proteinase K digestion, followed by isolation with the DNeasy Blood & Tissue Kit (Quiagen) according to manufacturer instructions. For toepad samples, we followed McCormack et al. (2016), which includes incubation and EtOH and STE Buffer washing steps prior to the Quiagen extraction protocol. We estimated the quality and amount of DNA by electrophoresis in 1 % agarose gels and spectrophotometry at 650nm with an Epoch microplate spectrometer (BioTek Instruments). All samples yielded high molecular weight DNA at concentrations above 50 ng/µl.

Genomic sampling and sequencing were performed using the NextRAD (Nextera-tagmented, Reductively Amplified DNA) method. It yields short sequences (~ 150 base pairs) flanked by engineered transposon cut sites, which results in thousands of loci distributed throughout the genome (Russello et al., 2015). This method requires small amounts of DNA (less than 50 ng; Russello et al., 2015), allowing the recovery of sequence data even from museum specimens with low DNA concentration.

NextRAD genotyping-by-sequencing libraries were made from genomic DNA by SNPsaurus LLC (Oregon, USA) following Russello et al. (2015). To do this, genomic DNA (~10 ng) was fragmented with Nextera Flex reagent (Illumina, Inc), which also ligated short adapter sequences to the ends of the fragments. Fragmented DNA was amplified with one of the primers matching the adapter and extending 10 nucleotides into the genomic DNA with the selective sequence GTGTAGAGCC. The constructs were amplified by PCR using Phusion® Hot Start Flex DNA Polymerase (New England Biolabs; Massachusetts, USA). PCR program consisted of an initial step of 3min at 72°C, followed by 30s at 98°C, and five denaturation cycles at 98°C for 10s, annealing at 63°C for 30s, and 3min extension at 72°C. Amplicons were pooled and the resulting libraries were purified using AMPure XP beads (Agencourt Bioscience Corporation; Massachusetts, USA) at 0.7×. Then, purified libraries were size-selected to 350-800 base pairs and sequenced on a HiSeq 4000 with one lane of 150 bp reads (Genomics Core Facility, University of Oregon).

Processing of raw data.- The quality and adapter content of NextRAD sequences were evaluated with FastQC (Andrews, 2010). Then, sequences were quality-filtered (Phred quality score > 33) and adapter-trimmed in Trimmomatic using the sliding-window function (Bolguer et al., 2014). Subsequent filtering and clustering were performed in ipyrad (Eaton and Overcast, 2020). We assembled the DNA matrix using as reference the genome of *M. aurifrons* (Wiley and Miller, 2020) available in GenBank under the accession number: PRJNA598863.

We produced several datasets with different parameter combinations to evaluate their performance in genetic analyses, following recommendations in the ipyrad documentation (Eaton and Overcast, 2020). Evaluated parameters were: denovo vs. assembly with reference genome; maximum number of low-quality base calls (0, 5, 10, 15); phred Q score offset (20, 33, 43); clustering threshold (0.75, 0.80, 0.85, 0.90, 0.95, 1); minimum depth for statistical base calling (7 to 10); number of heterozygotes and uncalled bases in consensus (5%, 10%, 20%, 30%); minimum number of samples per locus for output (20%, 30%, 40%, 50%); and numbers of SNPs, heterozygous sites, and indels per locus (5%, 10%, 20%, 30%).

We then choose the dataset produced with the parameter settings that maximized the number of phylogenetically informative sites while minimizing missing data and eliminating potential paralogous loci. This parameter combination included a minimum coverage value of 9, grouping threshold of 0.85, a minimum value of groups (taxa) included of 30%, and

maximum number of polymorphic sites shared within a locus of 10%. All other parameters were set at default values.

The loci were concatenated into combined matrices of NextRAD. Output data was filtered to remove alleles with a frequency of less than 5% and loci that were heterozygous in all samples. We applied additional filters to eliminate individuals and loci with more than 30% of missing data using VCFtools v1.15 (Auton and Marcketta, 2015).

The final analyses were performed using two data matrices: one including *M. santacruzi, M. aurifrons, M. carolinus,* and two outgroups for phylogenetic analyses, and the second containing only *M. santacruzi*, *M. aurifrons*, and *M. carolinus* for population genetics analyses. From the Variant Call Format (VCF) files, we made the conversion to the required input formats for subsequent analyses with bash script vcf2phylip (Ortiz, 2019).

Data availability.- Raw data is available at SRA (BioProject ID: PRJNA802310). VCF file, metadata and usage notes are available at Dryad repository (doi:10.5061/dryad.crjdfn35z)**.** Additional documentation is also available at Github repository of the Museum of Zoology "Alfonso L. Herrera" (https://github.com/MZFCgenomics/santacruzi_project).

Phylogenetic Analyses.- We explored the phylogenetic relationships with our SNPs dataset in a coalescent framework with SVDQuartets (Chifman and Kubatko, 2014; Chifman and Kubatko, 2015) as implemented in PAUP4.0a (Swofford, 2003). Quartet evaluation comprised all possible quartets, which were assembled into a species tree using a variant of Quartet FM, as recommended by the SVDquartets developers. Support for nodes was assessed using nonparametric bootstrapping with 1000 replicates. Additionally, we performed a phylogenetic analysis using Maximum Likelihood (ML) approach with IQ-Tree2 (Minh et al., 2020). For IQ-TREE, the FreeRate and ascertainment bias correction (+ASC) model were used as suggested for SNP data. Branch support was assessed using the ultrafast bootstrap approximation (Minh et al., 2013; Hoang et al., 2018) with 1,000 replicates. Trees were visualized and edited in Figtree v1.4.3 (Rambaut, 2016).

We constructed a phylogenetic network with unlinked SNPs file by implementing the Neighbor-Net algorithm in SplitsTree (Huson and Bryant, 2006). The Neighbor-Net method consists of the agglomeration of weighted collection of splits or partitions of the set of taxa which constitute the building blocks of a phylogenetic tree and provides the visualization of the space of feasible trees. This method allows the representation of relationships among taxa in which the underlying evolutionary history may not be treelike due to processes such

as recombination, hybridization, gene conversion and gene transfer (Hernández-Langford et al., 2020).

Population genetics.- To explore spatial patterns of genetic structure, we initially performed algorithmic estimation methods to infer clusters based on genetic similarity of each multilocus genotype: Discriminant analysis of principal components (DAPC) and Principal Component Analysis (PCA) as implemented in the packages *adegenet* (Jombart and Ahmed, 2011) and *SNPRelate* v1.6.4 (Zheng et al., 2012) respectively. We used the function *find.clusters* in DAPC to find the best number of genetic clusters (K) in unlinked SNP datasets for each clade. We set the maximum number of clusters (K) to ten. The optimal number of clusters was determined by the lowest value of the Bayesian Information Criterion (BIC) statistic. Based on the number of groupings and the identity of individuals included in each one, we displayed the conglomerates in 3D PCA graphics.

Next, we inferred population structure and estimated individual admixture coefficients from the genotype matrix with the model-based method implemented in the function *snmf* of the LEA package v2.3.4 (Frichot and François, 2015). We evaluated *K* values from 1 to 6 with 20 repetitions of each one. The best number of populations (*K*) was inferred from the entropy criterion, which evaluates the goodness of fit of the statistical model to the data using a cross-validation technique (Frichot and François, 2015).

From the groupings obtained by PCA and LEA, we calculated pairwise weighted F_{ST} according to Weir and Cockerman (1984) using Arlequin (Excoffier and Lischer, 2010). Calculations were performed with a Markov chain length of 100,000 steps and 10,000 dememorization steps. The allowed level of missing data was set to 0.05. Genetic diversity within groupings was estimated using the nucleotide diversity (Pi) proposed by Nei (1987) as implemented in DnaSP v6 (Librado and Rozas, 2009). The estimation was based on the sequence variation information stored in the VCF file retrieved from the ipyrad alignment.

Based on the clusters obtained from phylogenetic and population genetics analyses for *M. santacruzi*, we estimated divergence times using *momi2* (Kamm et al., 2020) (https://momi2.readthedocs.io/en/latest/tutorial.html#Inference). This method calculates the Site Frequency Spectrum and its linear functions for demographies described by general directed acyclic graphs, and it can be used to infer the history of population size changes, migrations, split times, and other demographic events that may affect a set of populations. We set the mutation rate as $1.5x10^{-9}$ per site per year (Ellegreen, 2007) and generation time

as 1 year (Husak and Maxwell, 2020). For each model, we ran 40 optimizations, selecting the one with the highest maximum likelihood value.

Gene flow estimation.- To evaluate the amount of shared genetic diversity explained by ancestry and/or processes involving gene flow among clusters/lineages, we used two different approaches: TreeMix analyses (Pickrell and Pritchard, 2012) and Patterson's D tests as implemented in DSuite (Malisnski et al., 2021).

TreeMix employs the allele frequency data from populations or species to infer the structure of the graphs that connect ancestral populations to modern-day ones (i.e., population splits and admixture events; Pickrell and Pritchard, 2012). We evaluated a number of admixtures edges (m) from zero to the number of populations +1, and retained the m-value for which the likelihood of trees reached an initial plateau. For this value, we ran ten iterations over different subsamples of SNPs as suggested in the ipyrad analysis toolkit: treemix (https://ipyrad.readthedocs.io/en/latest/API-analysis/cookbook-treemix.html) and selected the replicate with the highest likelihood.

D-statistics (also called ABBA BABA statistics) provide a simple and powerful test for deviation from a strictly bifurcating evolutionary history. Patterson's D tests calculate the proportion of ABBA and BABA site patterns, and an excess of either is indicative of admixture rather than incomplete lineage sorting (Durand et al., 2011). ABBA-BABA calculations were performed following authors´ scripts (https://github.com/millanek/Dsuite). The significance of each test was determined by performing 1,000 bootstrap replicates in which loci were resampled with replacement.

After identifying the pairs of lineages with genetic admixture resulting from gene flow events, we estimated demographic parameters and tested the support for competing demographic scenarios using *momi2* (Kamm et al., 2020). The demographic scenarios we tested were pure isolation, isolation with migration (bidirectional and unidirectional), and isolation with secondary contact. We set the mutation rate and generation time as in the previous section. Divergence time for *M. santacruzi* and *M. carolinus-M. aurifrons* was set to two million years ago (YA) following Navarro-Sigüenza et al. (2017). For each model, we ran 20 optimizations, selecting the one with the largest maximum likelihood value.

Contemporary and paleo-distribution modeling.- To complement our genetic analyses with an ecological approach, we developed niche models and climatic niche similarity tests

to evaluate ecological differentiation and the past potential distribution of lineages within *M. santacruzi*.

We assessed ecological niche using two different approaches: ENMs and an ordination technique. For the ENM approach, we used occurrence data from our genetic sample sites, as well as occurrence records throughout the species distribution extracted from the Atlas of the Birds of Mexico (Navarro-Sigüenza et al., 2003), records from museums and information downloaded from GBIF (http://www.gbif.org DOI: 10.15468/dl.97qx8h). We dealt with spatial autocorrelation by filtering occurrences at < 5 Km using the *spThin* R package (Aiello-Lammens et al., 2015). The final thinned datasets per lineage and populations (see Results) comprised the following number of occurrences: *Melanerpes santacruzi+M. aurifrons*=424, *M. aurifrons*=157, *M. s. dubius*=93, *M. s. veraecrucis+polygrammus+grateloupensis*=129, and *M. s. santacruzi*=45. We used 80% of the data for calibration and the remaining 20% for testing. We used the 19 bioclimatic variables for current conditions from WorldClim version 1 (worldclim.org; Hijmans et al., 2005) at 2.5 arc-minute resolution. We removed highly correlated variables (based on a Pearson's coefficient correlation > 0.75), which resulted in a set of nine climatic variables for model building: mean diurnal range, isothermality, maximum temperature of the warmest month, minimum temperature of the coldest month, mean temperature of the driest quarter, precipitation seasonality, precipitation during the wettest quarter, precipitation during the driest quarter, and precipitation during the coldest quarter. We built a calibration area M (Soberón and Peterson, 2005) for each lineage (Supplementary Materials, Fig. S1) in ArcMap 10.2.2 (ESRI, 2010) according to the genetic clusters, and unequivocal occurrence records and their intersection with ecoregions (Dinerstein et al., 2017).

To generate the suitability maps, we used MaxEnt v 3.4.4 (Phillips et al., 2021), whose algorithm fits models by optimizing the Regularization Multiplayer (RM) and Feature Types (L = linear, Q = quadratic, H = hinge and P = product) parameters. We selected the best values for these two parameters using *ENMeval* for R (Muscarella et al., 2014). We used eight values of RM from 0.5 to 4.0 (increasing by 0.5) and 15 Feature Types combination thereof: L, P, Q, H, LP, LQ, LH, PQ, PH, QH, LPQ, LPH, LQH, PQH, and LPQH. This resulted in 120 preliminary models, from which we the selected the best settings based on the corrected Akaike Information Criterion (AICc). We evaluated the model's performance by using the area under the receiver operating characteristic curve (AUC), and partial receiver operating characteristic (PROC). We selected the 10^{th} percentile training presence threshold to determine suitable/unsuitable area for each lineage.

After ENM building, we conducted a Mobility Oriented Parity (MOP) analysis to compare sets of environmental conditions between the calibration area and multiple areas or scenarios to which models are transferred (Owens et al., 2013) as implemented in the R package *KUENM* (Cobos et al., 2019). We used MOP to detect areas of strict extrapolation (i.e., novel environmental values in comparison to those represented in the calibration area) and to estimate environmental similarity between the calibration and transference areas to three past periods: Mid-Holocene (MH; 6,000 YA), Last Glacial Maximum (LGM; 22,000 YA) and Last Inter-glacial (LIG; 120,000-140,000 YA). The analysis restricted the calculation of environmental average multivariate distances between transference region and the nearest portion (50 % of points sampled) from the calibration area.

To transfer the ecological niche to past conditions, we used climatic models for MH and LGM from three different paleoclimatic environmental reconstructions: CCSM4 (Gent et al., 2011), MIROC-ESM (K-1 model developers, 2004) and MPI-ESM-P (Stevens et al., 2013); for LIG we used the NCAR-CCSM model (Otto-Bliesner et al., 2006). The last set of bioclimatic layers is only available in 0.5 arc-minute resolution, so we downsampled it to 2.5 arc-minutes. Procedures for niche transferences were performed in R using *ENMwizard* package (Heming et al., 2018).

The ordination approach was based on a Principal Component Analysis (PCA) of uncorrelated climatic variables (Guisan et al., 2014) to perform niche overlap analyses within *M. santacruzi* populations. For these analyses, we performed niche equivalency tests (Are the two niches identical?) and niche similarity tests (Are two niches more- or less-similar than expected by chance?).

To assess niche overlap, we used Schoener's D (Schoener, 1968), which ranges from 0 (no overlap) to 1 (total overlap). For both the niche equivalency and similarity tests, we tested the conservatism hypothesis (overlap greater than expected by chance) and the divergence hypothesis (overlap lower than expected by chance) as implemented in the R package *Ecospat* (Di Cola et al., 2017). The null hypothesis is rejected when the empirical overlap is significantly lower or higher than the null distribution (Warren et al., 2008). The null distribution for both analyses were drawn by performing 100 iterations. All maps were generated and visualized in ArcGIS 9.0 and ArcView 3.2a.

RESULTS

NextRAD sequencing, filtering and SNPs calling.- We obtained 200 Megabases of sequence data on average per sample, equivalent to ~ 2.3 million of 150 base-pair reads,

from each NextRAD sample library. From these libraries, we obtained, on average 2,280,924 reads per sample that mapped to the *M. aurifrons* genome, yielding 225,423 clusters and 17,740 “loci” after final assembly. After subsequent filtering of the ipyrad SNP dataset, we obtained a matrix of 3,846 SNPs for 48 individuals for phylogenetic analyses (3 outgroups, 5 *M. carolinus,* 5 *M. aurifrons,* and 35 *M. santacruzi*), with an average of 6.2% of missing data by sample, and another for population genetics analyses with 45 individuals (5 *M. carolinus,* 5 *M. aurifrons* and 35 *M. santacruzi*), 4,004 SNPs and 3.8% of missing data.

Phylogenetic Analyses.- Phylogenetic trees obtained from SNPs with the multispecies coalescent model in SVDquartets (Fig. 2b) showed *M. carolinus* as monophyletic and sister to one clade comprising most of *M. aurifrons* samples. All individuals of *M. santacruzi* constitute a clade characterized by a lack of structure congruent with subspecies designations or color morphs, and low support at inner nodes. On the other hand, Maximum Likelihood analysis (Fig. 2a) yielded a different topology with *M. carolinus* constituting a clade sister to *M. aurifrons* + *M. santacruzi*. The latter two species are resolved as reciprocally monophyletic. As in SVDQuartets tree, most of the internal nodes of *M. santacruzi* showed low support values and lack of structure.

In the phylogenetic network reconstruction (Fig. 3), *M. carolinus* was sister to *M. aurifrons*. *M. santacruzi* formed a group with reticulations that roughly correspond to subspecies. Reticulation patterns at the base of the network indicated incomplete lineage sorting and/or events of gene flow.

Population genetics.- DAPC analysis yielded a nested pattern when samples of *M. carolinus, M. aurifrons* and *M. santacruzi* were analyzed together. The *find.clusters* function from DAPC analysis suggested that samples were differentiated into two main genetic groupings (lowest BIC score, 135): one containing *M. carolinus* individuals (subdivided according to their origin: Florida vs. New York-Minnesota) and the other containing samples from *M. aurifrons* and *M. santacruzi.* Removing the *M. carolinus* individuals, left two main clusters (lowest BIC score, 208): one containing *M. s. santacruzi* and the other containing *M. aurifrons* + *M. s. polygrammus-veraecrucis-grateloupensis-dubius.* Within this last cluster, we obtained a subdivision (lowest BIC score: 178) between *M. aurifrons, M. s. dubius,* and a group containing the subspecies *M. s. veraecrucis+polygrammus+grateloupensis* that is consistent in all the remaining analyses (hereafter referred as *Melanerpes s. grateloupensis* due to nomenclatural priority).

The PCA suggested a similar separation of three groups comprising (1) *M. carolinus*, (2) *M. s. santacruzi* and (3) *M. aurifrons* + *M. s. dubius* + *M. s. grateloupensis* (Supplementary Materials, Fig. S2a). A closer look at this last grouping suggests that *M. aurifrons, M. s. dubius,* and *M. s. grateloupensis* clustered in three separated groups (Supplementary Materials, Fig. S2b).

The structure-like analyses indicated that the number of populations was most likely two or five populations (K = 2 and K = 5), both of which received similar support based on the entropy criterion (cross-entropy = 0.12475; Fig. 4a). Plotting the admixture coefficient by individuals using K = 2, showed one group containing *M. carolinus* and the other containing *M. aurifrons* + *M. santacruzi* (Fig. 4b). Using K = 5, the samples contained in each cluster were as follows: (1) *M. carolinus*, (2) *M. aurifrons*, (3) *M. s. santacruzi* and (4) *M. s. dubius,* and (5) *M. s. grateloupensis* (Fig. 4c). Although these groups seem to be genetically differentiated, most individuals have a substantial amount of genetic information from other groups (up to 40 % in the most admixed individuals).

When plotting admixture coefficients by sampling location (Fig. 4d) we found a pattern of increased mixing in contact zones of *M. aurifrons-M. s. grateloupensis* and *M. s. grateloupensis-M. s. santacruzi.* Also, *M. s. grateloupensis* from the northern Gulf slope presents a higher proportion of shared genetic information with *M. aurifrons* and *M. s. dubius* than the individuals of *M. s. grateloupensis* from the Pacific slope, which share genetic information with *M. s. santacruzi* and a minimum quantity with *M. s. dubius.*

The F_{ST} values and their associated probabilities (Table 1) indicated significant genetic differentiation between *M. santacruzi- M. aurifrons, M. santacruzi- M. carolinus, and M. aurifrons- M. carolinus*. When calculating the genetic differentiation considering separately the genetic groups within *M. santacruzi*, we found significant values for the following pairs: *M. aurifrons- M. s. dubius*, *M. aurifrons- M. s. santacruzi*, and *M. s. santacruzi* with the rest of lineages. Genetic differentiation was not significant in *M. aurifrons* vs. *M. s. grateloupensis* and *M. s. dubius* vs. *M. s. grateloupensis.* The overall nucleotide diversity (Pi) for *M. santacruzi* was 0.0247. For groups detected by population genetics analyses, Pi values were: *grateloupensis*: 0.0242, *dubius*: 0.0249, and *santacruzi*: 0.0323. For *M. carolinus* and *M. aurifrons*, Pi values were 0.0258 and 0.0177 respectively.

In the analyses for divergence times estimation among *M. santacruzi* populations, the best model (relative likelihood = -13,014.81) yielded an initial split around 250,000 years between *M. s. santacruzi* and the rest. *M. s. dubius-M. s. grateloupensis* diverged more recently, around 150,000 YA.

Gene flow inference.- In line with what was suggested by the phylogenetic and population genetics analyses, gene flow events were detected between some groups with both the Treemix and Patterson's *D* tests. Treemix test showed that the most likely number of admixture edges was two (Fig. 5a): one between *M. aurifrons* and *M. s. grateloupensis*, and another between *M. carolinus* and *M. aurifrons* (Fig. 5b).

The *D*-statistics supported the hypothesis of gene flow between *M. aurifrons* and *M. s. grateloupensis,* yielding positive and significant values of *D* as well as the highest Z-scores (Table 2). These statistics also suggested introgression between *M. s. dubius* and *M. s. grateloupensis,* although this was never obtained with the method implemented in Treemix. For the rest of the paired comparisons, the D values were positive, but not significant, or showed low Z-scores, indicating that the ABBA and BABA site patterns arose purely through incomplete lineage sorting, and the difference between them is purely random (Table 2).

To explain the pattern of genetic admixture between *M. aurifrons*-*M. s. grateloupensis* yielded by several of our analyses, we produced four different demographic models depending on the directionality and timing of gene flow. The best-fit demographic model was isolation with bidirectional migration (relative likelihood = -4,117.98, Fig. 6). The second-best model was isolation with unidirectional migration from *M. aurifrons* to *M. s. grateloupensis* (-4,977.33).

Contemporary and paleo-distribution modelling.- Final model parameters selected based on AICc values were as follows: *M. santacruzi*+*M. aurifrons*: FT = QPH, RM = 1 (QPH-1); *M. s. dubius*: QPH-2; *M. aurifrons*: LPH-1.5; VPG: QP-0.5 and *M. s. santacruzi*: PH-2.5. Model diagnostics showed that all of the models were superior to random models: *M. santacruzi*+ *M. aurifrons*: AUC = 0.89, PROC = 1.50, $P < 0.01$; *M. s. dubius*: AUC = 0.97, PROC = 1.94, $P < 0.01$; *M. aurifrons*: AUC = 0.95, PROC = 1.74, $P < 0.01$; VPG: AUC = 0.97, PROC = 1.74, $P < 0.01$, and *M. s. santacruzi*: AUC = 0.98, PROC = 1.83, $P < 0.01$.

The potential distribution of the populations for current climatic conditions (Fig. 7, detailed projections in Supplementary Materials, Fig. S3), showed the geographic distribution for *M. dubius* mainly centered in the Yucatan Peninsula, while *M. aurifrons* is widely distributed in north-central Mexico, separated from the Gulf slope *M. s. grateloupensis* by the Sierra Madre Oriental and the Transvolcanic Mexican Belt. *M. s. grateloupensis* is distributed along the Gulf slope and the Isthmus of Tehuantepec, although suitable potential areas are also located on the Central Pacific slope and in the lowlands of Chiapas and Guatemala. Highest suitability areas for *M. s. santacruzi* were located east of the Isthmus of Tehuantepec

on the Pacific slope of Central America, but some suitable areas west of the Isthmus of Tehuantepec (Pacific slope in Guerrero and Oaxaca) were also highlighted. The relative percentages of variables' contribution in the Maxent models of potential distribution are shown in Supplementary Table 2.

MOP analysis (Supplementary Materials, Fig. S4) showed that, for *M. santacruzi* and *M. aurifrons*, most of the past climatic conditions were analogous to current climatic conditions, except for the northernmost part of the distribution during LGM and LIG. For the genetic groups roughly coincident with the distribution of the subspecies *M. s. dubius* and *M. s. santacruzi*, analogous climatic conditions in the past were restricted to the Yucatan Peninsula and most of Central America, respectively. For *M. s. grateloupensis*, central and northern Mexico were areas of strict extrapolation in all four time periods. Finally, for *M. aurifrons*, areas of strict extrapolation were in Central America and the northernmost part of the range.

Transference maps to the three different time periods (LIG, LGM, MH) were based on areas with analogous climatic conditions to the Present (Fig. 7). For *M. aurifrons,* the areas of suitability were the Mexican plateau during MH, the Yucatan Peninsula and western Mexico during LGM, and northwest and central Mexico during LIG. Considering the genetic groups within *M. santacruzi*, for *M. s. dubius* most areas of high suitability in all three time periods were located in Central America, with smaller areas in the Yucatan Peninsula. The areas of suitability for *M. s. grateloupensis* were the central Pacific slope of Mexico, the Isthmus of Tehuantepec, and Central America during MH and LIG; during the LGM, areas of suitability were restricted to the Pacific slope. For *M. s. santacruzi*, areas of high suitability during the three past time periods were located along the Pacific slope of Mexico and Central America. When areas of ecological suitability by lineage were superimposed (Fig. 7), our analyses suggest that *M. santacruzi* lineages were sympatric over a wide and continuous distribution range from southern Mexico to Central America during LIG. Such a range is mapped as fragmented in the LGM, separating populations from Central America, the Yucatan Peninsula, and the Pacific and Gulf slopes of Mexico. In the MH, ranges of populations from Pacific and Gulf slopes of Mexico are reconnected, although those from Yucatan remained isolated. Those areas are roughly coincident with the present distribution of the genetically differentiated groups found herein.

According to the PCA analyses of the climatic data, the first two components explained 73.61% of the variation (Fig. 8). PC1 and PC2 were used to represent the environmental conditions and the environmental niche for each lineage. There was low to medium niche

overlap (Schoener's *D*) depending on the pair of lineages compared (Table 3). The highest values for Schoener's *D* were between the pair *M. s. dubius* x *M. s. grateloupensis* (Schoener's *D* = 0.37) and *M. s. grateloupensis* x *M. s. santacruzi* (*D* = 0.45).

For the equivalency tests (Supplementary Materials, Fig. S5), all pairwise comparisons rejected the null hypothesis of niche conservatism (p-value = 1, for all comparisons), therefore niche divergence hypothesis in the equivalency test was supported (p-value = 0.009, for all comparisons). For the similarity tests (Supplementary Materials, Fig. S6), most of the comparisons showed non-significant p-values for the niche conservatism hypothesis (p-value > 0.05), except for *M. s. dubius* x *M. s. grateloupensis*, and *M. s. grateloupensis* x *M. s. santacruzi* (p-value = 0.019 in both cases), although the opposite comparison for both pairs showed non-significant p-values (p-value > 0.05). The divergence hypothesis for the similarity test also was rejected (p-value > 0.05, for all comparisons).

DISCUSSION

Phylogenetic distinctiveness of *Melanerpes santacruzi* and relationships with *M. carolinus* and *M. aurifrons*.- Based on its morphological similarities, *M. santacruzi* has been included in the Red-bellied Woodpecker superspecies (*sensu* Short, 1982) along with *M. carolinus, M. uropygialis, M. superciliaris M. hoffmannii,* and *M. aurifrons,* and historically classified as subspecies of the latter (Peters, 1948; AOU 1957, 1983, 1998). At the same time, its phenotypic variation led to description of several subspecies (Peters, 1948; Selander and Giller, 1963; Short, 1982), some of which resemble other forms from the *carolinus* group (e.g., *M. s. dubius* of the Yucatan Peninsula is morphologically highly similar to *M. carolinus*), while others resemble species outside the group (e.g., *M. s. santacruzi* is similar to *M. pygmaeus*; García-Trejo et al., 2009), resulting in confusing classifications.

Recent studies on the molecular systematics of the group (García-Trejo et al., 2009; Navarro-Sigüenza et al., 2017) have already addressed these issues. Based on mitochondrial information, García-Trejo et al. (2009) suggested species rank for the Velazquez Woodpecker, sister to the clade *M. carolinus* + *M. auriforns*. However, such relationships were not obtained by Navarro-Sigüenza et al. (2017) in a ML phylogeny inferred from concatenated sequences of four nDNA genes, given that the three taxa are mixed together in a polytomy, and are not resolved as reciprocally monophyletic. Our genomic data, which added many more specimens and increase the representation of loci across the genome, recovered a topology congruent with that in García-Trejo et al. (2009) and Navarro-Sigüenza et al. (2017), based on mitochondrial genes. Our phylogenetic analyses using both

ML and coalescence approaches, strongly support the monophyly of *M. santacruzi,* although the sister taxa relationships between *M. aurifrons* and *M. carolinus* are not identical following the methods employed herein.

Additionally, population genetics analyses showed high and significant F_{ST} values in *M. santacruzi* (as a whole)*,* as compared to *M. carolinus* and *M. aurifrons*, and two of the three genetic groups within it were significantly differentiated from *M. aurifrons*; and all were clearly differentiated from *M. carolinus*. The phylogenetic network obtained in SplitsTree also supported this differentiation, although the reticulation pattern observed suggests that the evolutionary processes underlying this differentiation have been complex and in the presence of gene flow (Huson and Bryant, 2006).

Our genomic and ecological evidence, coupled with the previously documented morphological variation (size and coloration, Benites et al., 2020) and mtDNA differentiation (García-Trejo et al., 2009; Navarro-Sigüenza et al., 2017), renders *M. santacruzi* as a diagnosable and evolutionarily independent taxon, fulfilling the criteria of species status under different species delimitation approaches (e.g., deQueiroz, 1998; Gill and Donsker, 2021, Sangster, 2014).

Genetic diversity, population structure and ecological differentiation in the Velazquez Woodpecker.- The study of the Velazquez Woodpecker (*Melanerpes santacruzi)* evolution and systematics has relied largely on morphological characters and coloration analyses (e.g., Selander and Giller, 1963; Short, 1982; Benites et al., 2020). However, such sources of information may confound the underlying evolutionary relationships as they are influenced by many additional factors, such as population demographic histories, phenotypic plasticity, and local adaptation. Consequently, although several hypotheses of geographic structure in morphology and coloration within the species have been proposed, these remain controversial.

Molecular data derived from HTS methods and ENM analyses presented in this study provide evidence on the genetic structure and ecological differentiation within the taxon. Our population structure analyses revealed three main genetic groups: *M. s. santacruzi*, *M. s. dubius* and *M. s. grateloupensis* [including forms *grateloupensis* (Lesson 1839)*; polygrammus* (Cabanis, 1862)*,* and *veraecrucis* Nelson, 1900]. This is partially consistent with the clustering hypothesis proposed by Benites et al. (2020) and corroborates the finding of these authors that the currently recognized subspecies and their main morphological groups (e.g., Selander and Giller, 1963) do not accurately reflect intraspecific variation.

Similar to Benites et al. (2020), our findings on the geographic distribution of genetic groups suggests complex relationships between environmental/climatic, phenotypic, and genetic variation across the range of the species. Groups within *M. santacruzi* are consistent with some major geographical features in southeastern Mexico, as *M. s. santacruzi* is distributed in the Soconusco ecoregion, while *M. s. dubius* inhabits the Yucatan Peninsula. The range of *M. s. grateloupensis* spans along the ecoregions of the Coastal plain and hills of the Gulf of Mexico and the southern Isthmus of Tehuantepec, which is unexpected given the marked ecological differences between these areas. However, highly polymorphic lineages, such as *M. s. grateloupensis*, may tolerate a wider range of ecological conditions (Fig. 8) because variation in phenotypic and morphological traits could be associated with adaptation to different habitat conditions (Gray and McKinnon, 2007; Hugall and Stuart-Fox, 2012; Campagna et al., 2017).

Our niche similarity and equivalence tests revealed that each group occupies non-identical environmental niches, suggesting a scenario in which niches for each group are less equivalent (identical) than expected by chance. This could indicate that the observed niche differentiation between species depends on the habitat selection or suitability, rather than due to environmental differences between available habitats (Warren et al., 2008).

On the other hand, non-significant divergence hypotheses in the niche similarity tests (except for *M. s. dubius* x *M. s. grateloupensis*, and *M. s. grateloupensis* x *M. s. santacruzi*) suggests that groups have differentiated recently, therefore sharing similar climatic requirements (Rodríguez-Rodríguez et al., 2020). Alternatively, the niches of these groups could evolve in a complex scenario, promoting a wide diversity of adaptations (Rodríguez-Rodríguez et al., 2020).

We found evidence of genetic admixture among all genetic groups within the Velazquez Woodpecker, which may reinforce recent differentiation among populations. Although genetic differentiation was significant among all pairs of groups (except *M. s. grateloupensis* vs *M. s. dubius*), nearly all individuals exhibited some probability of being assigned to other groups, especially those distributed in contact areas. Recent ecological differentiation, along with the recent divergence time estimations for these populations suggests that *M. s. santacruzi, M. s. grateloupensis,* and *M. s. dubius* constitute groups that are in the process of differentiation but have not diverged completely, probably due to the partial sympatry (parapatry), which has been maintained since at least since the LIG.

Melanerpes s. polygrammus (described from the southern Isthmus of Tehuantepec), is a phenotypically well-differentiated group within *M. santacruzi*, being highly similar to *M.*

aurifrons. Consequently, it has been considered as a single morphological group according to Ridgway (1914), Selander and Giller (1963), Short (1982), and Benites et al. (2020). However, our genomic data do not support this hypothesis, but rather it groups *M. s. polygrammus* with populations of the Gulf of Mexico slope (*veraecrucis* and *grateloupensis*). Such a finding indicates that coloration and morphological differentiation occur despite gene flow, suggesting that color variation is most likely the result of local adaptation and selection may be the primary force maintaining color variation among these populations (Antoniazza et al., 2010). Although for the other groups obtained here, we cannot rule out that the phenotypic geographic variation reflects the underlying genetic structure, for *M. s. polygrammus* this variation could be explained by the association of plumage color variation to a few adaptative loci, which apparently do not introgress, in spite of high genetic flow among different morphs (e.g., Poelstra et al., 2014; Uy et al., 2016; Campagna et al., 2017).

Gene flow in the *Melanerpes carolinus- aurifrons- santacruzi* complex.- Our data support the existence of bi-directional gene flow between *M. aurifrons* and adjacent populations of *M. santacruzi*, (*M. s. grateloupensis*) along the Sierra Madre Oriental in northeastern Mexico. Gene flow, however, seems to be uneven, with prevalence of movement from *M. aurifrons* to *M. s. grateloupensis*. Based on the geographic representation of admixture coefficients, we suggest that gene flow has occurred mainly in contact areas with both mixed and seasonal conditions (i.e., wet and dry) in Tamaulipas, Querétaro, and southwestern San Luis Potosí. This region has been identified as one of the main contact zones for birds in North America (Swenson and Howard, 2005).

Contact areas in woodpeckers may vary widely from extensive areas with frequent hybridization events, to small ones with nearly no interbreeding (Manthey et al., 2019). Different levels of gene flow and hybridization have been reported in contact areas of several North American groups like *Melanerpes* (between *M. carolinus* and *M. aurifrons*; Smith, 1987) *Colaptes* (Grudzien et al., 1987), *Dryobates* (Manthey et al,. 2019), *Sphyrapicus* (Billerman et al., 2019), *Campephilus* (Fuchs et al., 2013) as well as in Old World genera like *Dendrocopos* (Figarski et al., 2018). This evidence suggests that secondary contact and hybridization may play an important role in the ecology and evolutionary history of the woodpeckers of the World.

CONCLUSIONS.- The historical and ecological complexity of Mesoamerica apparently have driven equally complex evolutionary processes that may have resulted in the large

biodiversity of the area. Differentiation in the Velazquez Woodpecker seems to be a consequence of the effects of alternate episodes of geographic isolation- that have resulted in a high phenotypic and ecological divergence- and the maintenance of an evolutionary identity in spite of gene flow across areas of secondary contact.

Credit authorship contribution statement

AL-Q: Conceptualization, Methodology, Formal analysis, Data curation, Writing: original draft, review & editing, Visualization. **AM-Y**: Methodology, Formal analysis, Writing: review & editing, Visualization, Supervision. **LAS-G**: Conceptualization, Methodology, Formal analysis, Writing: review & editing, Supervision. **VJC-C**: Formal analysis, Data curation, Writing: original draft, review & editing, Visualization. **AGN-S**: Conceptualization, Methodology, Formal analysis, Writing- original draft, review & editing, Visualization, Supervision, Funding Acquisition

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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TABLES

Table 1. Pairwise F_{ST} (above the diagonal) and p-value (below the diagonal) for clusters obtained in structure-like analyses for *Melanerpes carolinus*, *M. aurifrons*, and *M santacruzi*. Significant values of genetic differentiation are in bold.

	M. carolinus	*M. aurifrons*	*M. santacruzi*	*M. s. santacruzi*	*M. s. dubius*	*M. s. grateloupensis*
M. carolinus	*	0.223	0.138	0.210	0.156	0.157
M. aurifrons	**0.006 +-0.001**	*	0.068	0.236	0.176	0.173
M. santacruzi	**0.000 +-0.000**	**0.000 +-0.000**	*	-	-	-
M. s. santacruzi	**0.006 +-0.001**	**0.029 +-0.002**	-	*	0.171	0.186
M. s. dubius	**0.000 +-0.000**	**0.021 +-0.001**	-	**0.001 +-0.000**	*	0.011
M. s. grateloupensis	**0.000 +-0.000**	0.074 +-0.002	-	**0.000 +-0.000**	0.194 +-0.004	*

Table 2. ABBA-BABA statistics calculated among genetic groups of *Melanerpes santacruzi* and *M. aurifrons*. *Melanerpes carolinus* was used as outgroup. Positive D-statistics represent an excess of loci supporting ABBA versus BABA topologies, thus indicating potential introgression between taxa P2 and P3. Significant Z-scores are in bold, as are the species involved with introgression.

P1	P2	P3	D-statistic	Z-score	p-value	f4-ratio	ABBA	BABA
dubius	*grateloupensis*	*aurifrons*	0.30	**3.92**	**0.00**	0.02	81.71	43.75
santacruzi	*grateloupensis*	*aurifrons*	0.50	**4.64**	**0.00**	0.02	80.17	27.02
santacruzi	*dubius*	*aurifrons*	0.23	1.20	0.12	0.01	39.93	24.74
dubius	*santacruzi*	*grateloupensis*	0.13	2.02	0.02	0.24	142.38	108.92
aurifrons	*santacruzi*	*grateloupensis*	0.20	2.94	0.00	0.24	121.44	81.71
aurifrons	*dubius*	*grateloupensis*	0.31	**3.15**	**0.00**	0.44	153.20	80.17
santacruzi	*aurifrons*	*dubius*	0.23	1.20	0.12	0.01	39.93	24.74
grateloupensis	*santacruzi*	*dubius*	0.04	0.64	0.26	0.01	154.14	142.38

Table 3. Schoener's *D* and p-values (equivalency and similarity for niche conservatism (C) and niche divergence (D) hypotheses of intraspecific lineages within *Melanerpes santacruzi* and *M. aurifrons*. Significant p-values are in bold.

	M. s. dubius x M. aurifrons	*M. s. dubius x M. s. grateloupensis*	*M. s. dubius x M. s. santacruzi*
Schoener's *D*	0.003	0.37	0.1
Equivalency *C/D*	1/**0.009**	1/**0.009**	1/**0.009**
Identity *C/D* 1->2	0.28/0.46	**0.019**/0.91	0.31/0.87
Identity *C/D* 1<-2	0.58/0.46	0.13/0.91	0.13/0.87
	M. aurifrons x M. s. grateloupensis	*M. aurifrons x M. s. santacruzi*	*M. s. grateloupensis x M. s. santacruzi*
Schoener's D	0.019	< 0.001	0.45
Equivalency C/D	1/**0.009**	1/**0.009**	1/**0.009**
Identity C/D 1->2	0.47/0.44	1/0.25	0.29/1
Identity C/D 1<-2	0.49/0.44	1/0.73	**0.019**/1

FIGURES

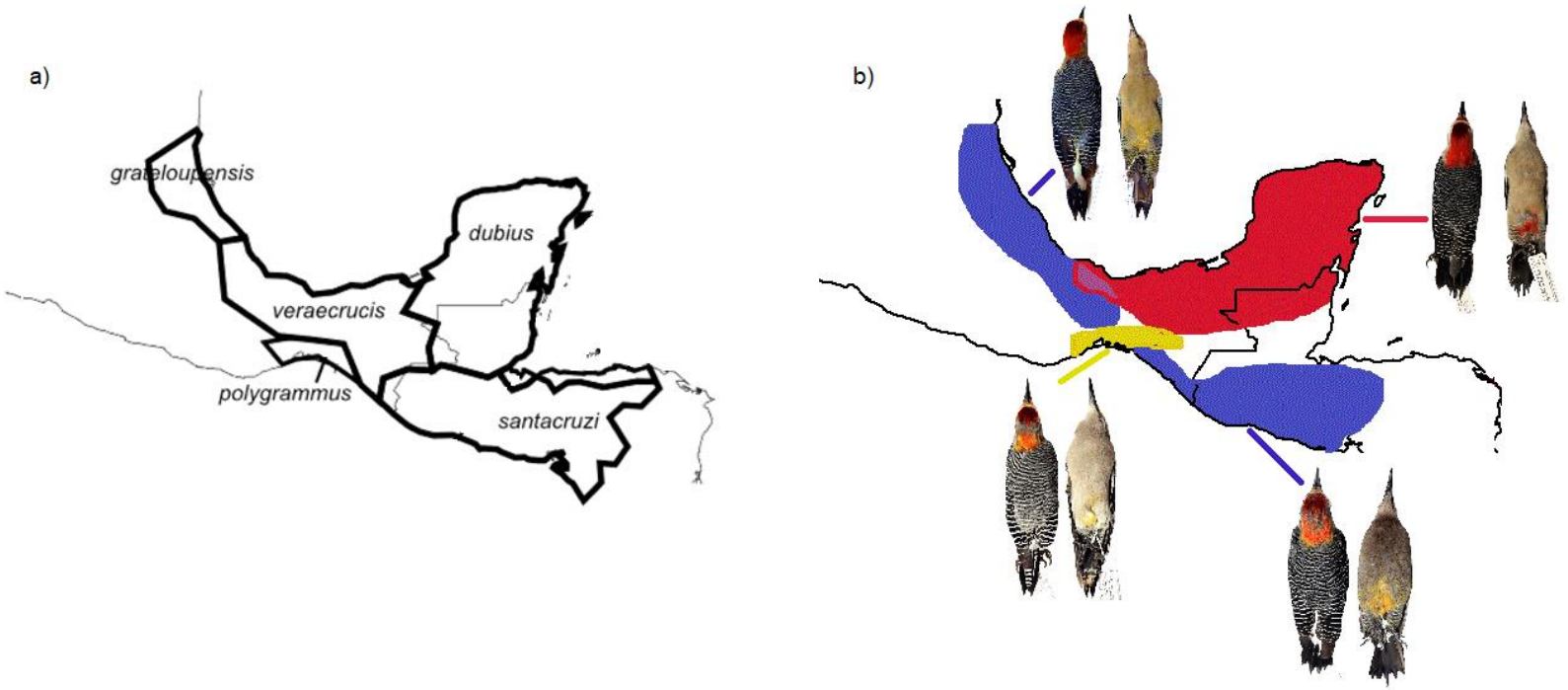

Figure 1. Geographic distribution of the Velazquez Woodpecker, *Melanerpes santacruzi*: a) distribution of mainland subspecies, modified from Benites et al. (2020); b) distribution and examples of morphotypes according to Benites et al. (2020). In red, we depict morphotype associated to Yucatan Peninsula and Tabasco with red nape/red belly with higher barring frequency and lower barring ratio (photo MZFC specimen, subspecies *dubius*); in yellow, the morphotype associated to the southern Tehuantepec Isthmus with red nape/yellow belly with intermediate barring frequency and intermediate barring ratio (photo MZFC specimen subspecies *polygrammus*); and in blue the morphotype present in the northern (photo MZFC specimen subspecies *veraecrucis*) and southern range (photo MZFC specimen subspecies *santacruzi*) with yellow nape/yellow belly with lower barring frequency and higher barring ratio.

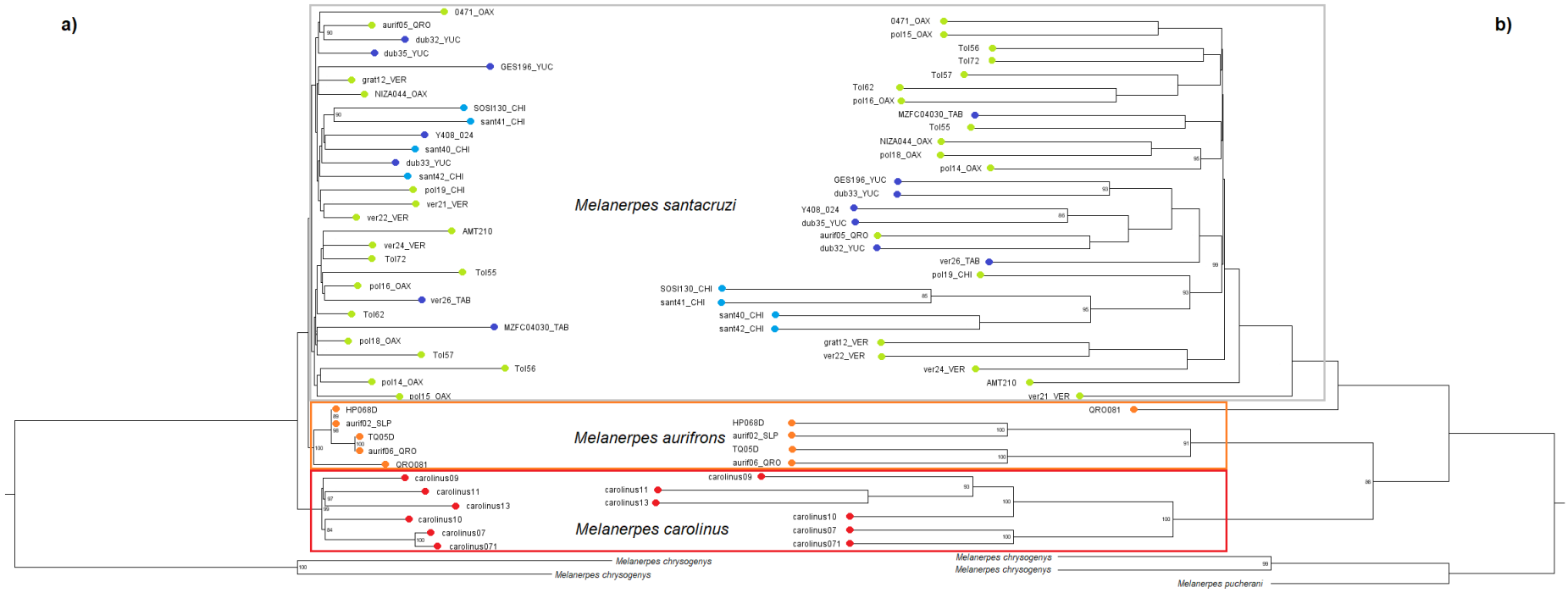

Figure 2. Phylogenetic trees estimated by Maximum Likelihood method in IQ-Tree (a) and coalescence framework as implemented in SVDQuartets (b). Bootstrap values are depicted for nodes with values greater than 85%. *M. carolinus*: red dots; *M. s. santacruzi*: light blue dots; *M. s. grateloupensis*: green dots; *M. aurifrons*: orange; and *M. s. dubius*: dark blue.

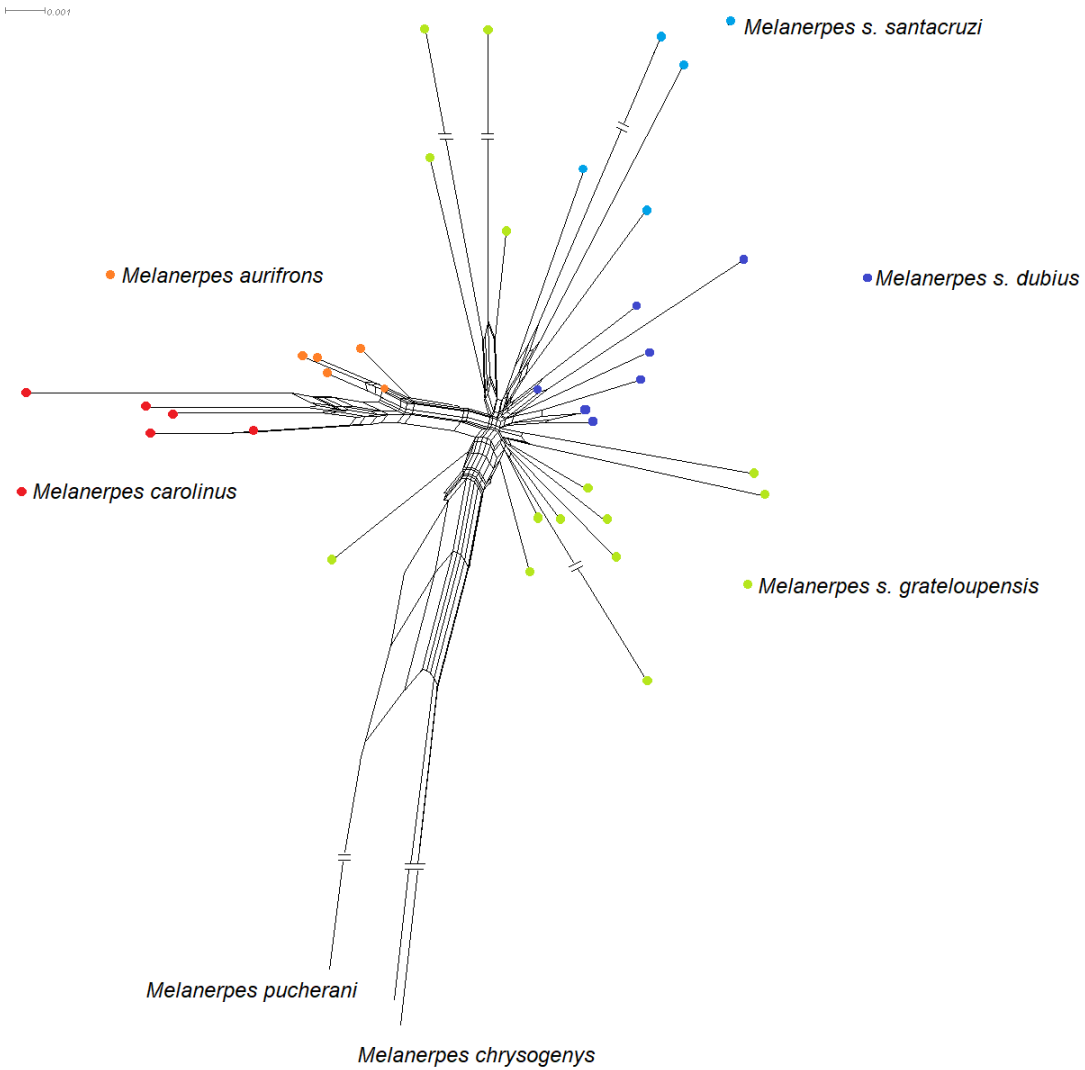

Figure 3. Phylogenetic network (Neighbor-Net) constructed using SplitsTree based on unlinked SNPs. *M. carolinus*: red dots; *M. s. santacruzi:* light blue dots; *M. s. grateloupensis*: green dots; *M. aurifrons*: orange; and *M. dubius*: dark blue.

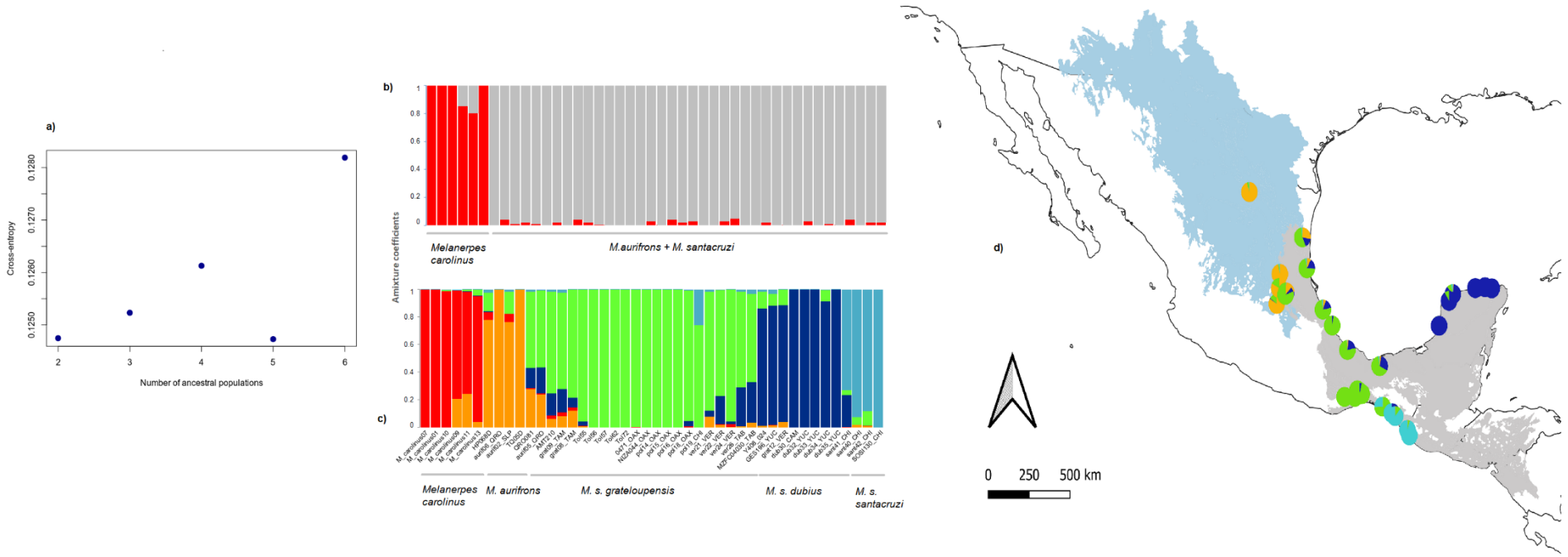

Figure 4. Genetic and geographical structure of *Melanerpes santacruzi:* a) Values of the cross-entropy criterion as a function of the number of populations obtained when analyzing the genetic structure of *Melanerpes carolinus-M. aurifrons-M. santacruzi* individuals included in this study. The lower values for K=2 and K=5 suggest that both two and five clusters best explain of genetic structure in sample. **b**) Ancestry coefficients of *M. carolinus-M. aurifrons-M. santacruzi* individuals included in this study when K=2 is assumed (admixture coefficients of *M. carolinus* membership is indicated with red color; *M. aurifrons-M. santacruzi:* gray). **c**) Ancestry coefficients of *M. carolinus-M. aurifrons-M. santacruzi* individuals included in this study, when K=5 is assumed (admixture coefficients of *M. carolinus* membership is indicated with red color; *M. s. santacruzi:* light blue; *M. s. grateloupensis:* green; *M. aurifrons*: orange; and *M. dubius*: dark blue). **d)** Geographic origin of samples included in this study and its admixture coefficients (color code of Fig. 4c is maintained in scatter pies). Distribution range of *M. aurifrons* and *M. santacruzi* are depicted in light blue and gray respectively.

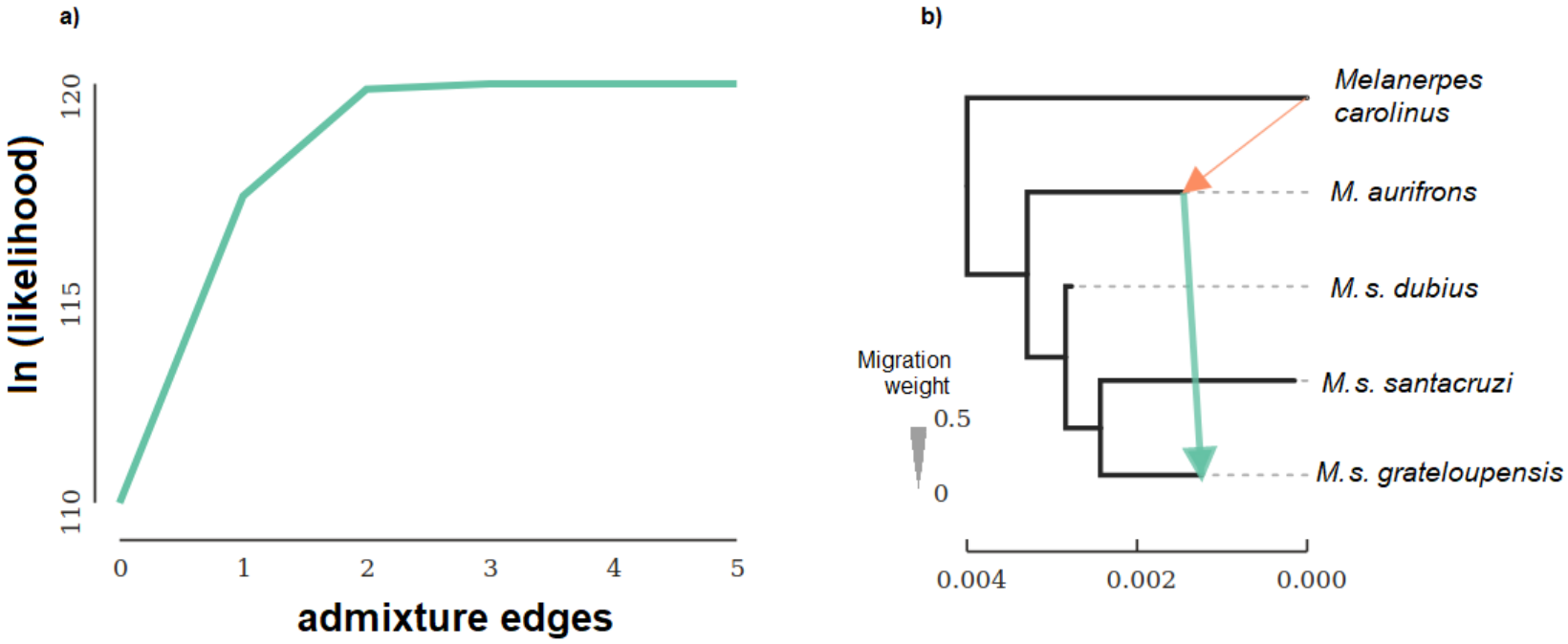

Figure 5. Events of gene flow and directionality between lineages of *Melanerpes santacruzi*, *M. aurifrons*, and *M. carolinus* as inferred in the dataset by Treemix: a) plot of values of ln(L) associated with each number of admixture events; b) tree inferred based on the most likely number of admixture events and the groupings obtained by population genetic analyses.

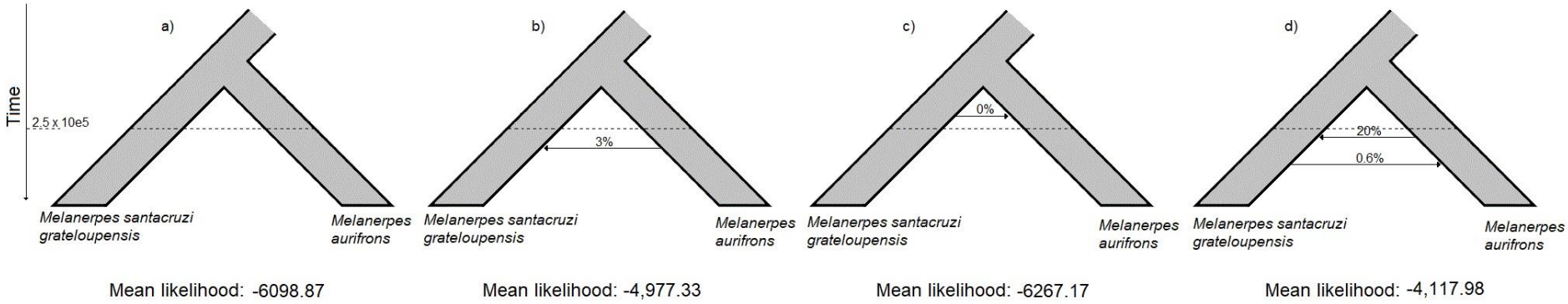

Figure 6. Demographic scenarios modeled with momi2 for explaining current patterns of admixture between *M. aurifrons* and *M. s. grateloupensis* and the associated mean likelihood: **a**) pure isolation; **b)** isolation with unidirectional gene flow from *M. aurifrons* to *M. s. grateloupensis* assuming no barrier to flow since the time of divergence; **c)** isolation with unidirectional gene flow from *M. s. grateloupensis* to *M. aurifrons* assuming no barrier to flow since the time of divergence; **d**) bidirectional gene flow between lineages assuming no barrier to flow since the time of divergence.

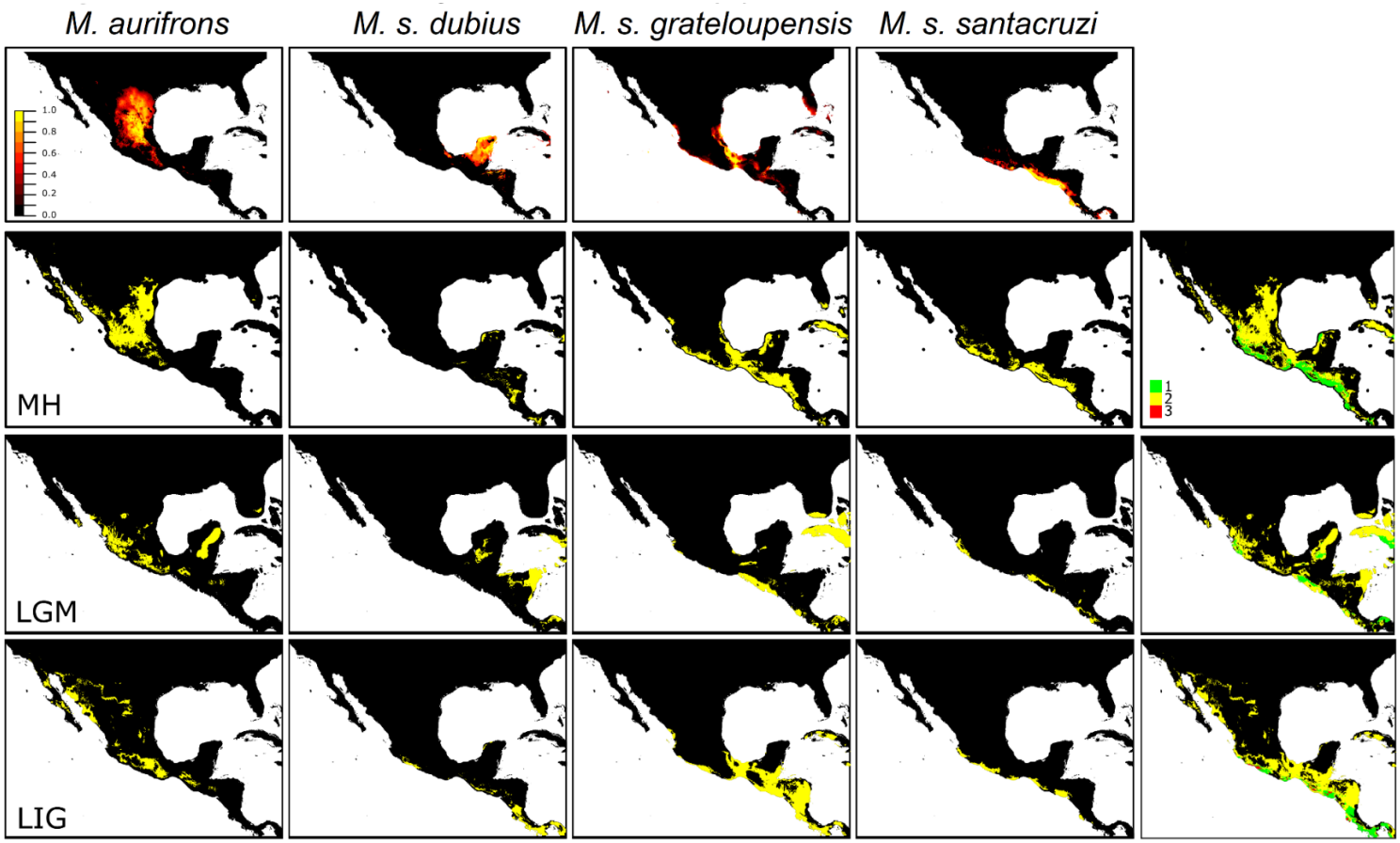

Figure 7. Maxent results for present and past climatic conditions. Maps in rows showed areas of high suitability conditions for *Melanerpes aurifrons* and genetics groups within *M. santacruzi*. Binary maps represent the suitability areas (Yellow) for three time periods: Middle Holocene (MH, 6,000 YA), Last Glacial Maximum (LGM, 22,000 YA) and Last Inter Glacial (LIG, 120,000-140,000 YA). For MH and LGM three models of paleoclimatic environmental reconstructions were used: CCSM4, MIROC-ESM and MPI-ESM-P. For LIG NCAR-CCSM model was used. Fifth row indicates overlapping areas for *M. aurifrons* and genetics groups within *M. santacruzi*: green color represents areas of co-occurrence of two groups, red color represents areas of co-occurrence of three groups.

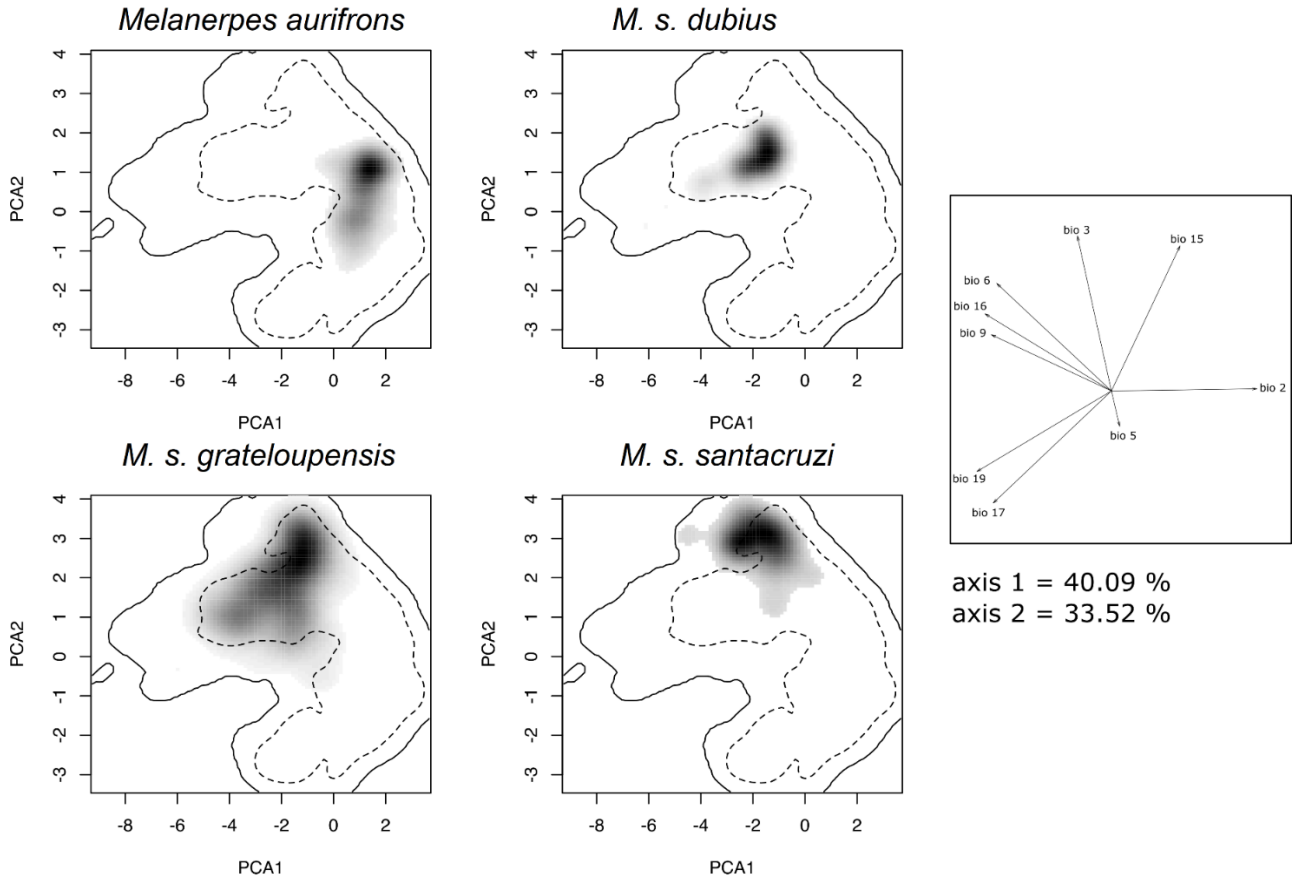

Figure 8. Niches of the genetic lineages within *Melanerpes santacruzi* and *M. aurifrons* in the environmental space of the study area represented along the two first principal components (PC). The contribution of the environmental variables to the two axes of the PC analysis and the percentage of variation explained by the two axes. Variables: mean diurnal range (bio 2), isothermality (bio 3), max temperature of warmest month (bio 5), min temperature of coldest month (bio 6), mean temperature of driest quarter (bio 9), precipitation seasonality (bio 15), precipitation of wettest quarter (bio 16), precipitation of driest quarter (bio 17) and precipitation of coldest quarter (bio 19).

SUPPLEMENTARY MATERIALS

The tangled evolutionary history of a long-debated Mesoamerican taxon: the Velazquez Woodpecker (*Melanerpes santacruzi*, Aves: Picidae)

Alexander Llanes-Quevedo, Alicia Mastretta-Yanes, Luis A. Sánchez-Gonzalez, Vicente J. Castillo-Chora, Adolfo G. Navarro-Sigüenza

SUPPLEMENTARY TABLES

Table S1. Samples used for genomic analyses in this study. Acronyms used in the Collection Catalog Number field correspond to the following institutions: MZFC: Museo de Zoología "Alfonso L. Herrera" of the Faculty of Sciences at the UNAM, Mexico; AMNH: American Museum of Natural History, USA; and FMNH: Field Museum of Natural History, USA.

Species	Subspecies	Sample identificator	Collection Catalog Number	Locality	Longitude	Latitude
Melanerpes santacruzi	*veraecrucis*	0471	MZFC 16890	Oaxaca, Mexico	-95.83	16.45
	grateloupensis	AMT210	MZFC 17560	Veracruz, Mexico	-97.03	20.48
	veraecrucis	MZFC04030	MZFC 18385	Tabasco, Mexico	-93.88	17.87
	veraecrucis	NIZA044	MZFC 17254	Oaxaca, Mexico	-95.12	16.75
	veraecrucis	Tol55	MZFC uncatalogued	Oaxaca, Mexico	-94.89	16.58
	veraecrucis	Tol56	MZFC uncatalogued	Oaxaca, Mexico	-94.87	16.58
	veraecrucis	Tol57	MZFC uncatalogued	Oaxaca, Mexico	-94.89	16.58
	veraecrucis	Tol62	MZFC uncatalogued	Oaxaca, Mexico	-94.87	16.58
	veraecrucis	Tol72	MZFC uncatalogued	Oaxaca, Mexico	-94.89	16.58
	grateloupensis	aurif05	MZFC 13315	Queretaro, Mexico	-99.07	21.27
	grateloupensis	grat08	MZFC 16742	Tamaulipas, Mexico	-98.17	23.81
	grateloupensis	grat12	MZFC 17559	Veracruz, Mexico	-87.66	21.46
	grateloupensis	grat09	MZFC 17556	Tamaulipas, Mexico	-97.92	22.38
	polygrammus	pol14	MZFC 17259	Oaxaca, Mexico	-95.02	16.68
	polygrammus	pol15	MZFC 18683	Oaxaca, Mexico	-95.26	16.45
	polygrammus	pol16	MZFC 16890	Oaxaca, Mexico	-95.01	16.67
	polygrammus	pol18	MZFC 16891	Oaxaca, Mexico	-94.99	16.63
	polygrammus	pol19	MZFC 20347	Chiapas, Mexico	-93.72	16
	veraecrucis	ver21	MZFC 23882	Veracruz, Mexico	-93.88	17.87
	veraecrucis	ver22	MZFC 15656	Veracruz, Mexico	-95.67	18.61
	veraecrucis	ver24	MZFC 23593	Veracruz, Mexico	-96.51	19.74
	veraecrucis	ver26	MZFC 24536	Tabasco, Mexico	-93.88	17.87

	dubius	Y408_024	MZFC 25741	Campeche, Mexico	-90.58	19.73
	dubius	GES196	MZFC 13518	Yucatan, Mexico	-89.82	21.19
	dubius	dub30	MZFC 21794	Campeche, Mexico	-87.66	21.46
	dubius	dub32	MZFC 22348	Yucatan, Mexico	-88.03	21.51
	dubius	dub33	MZFC 23064	Yucatan, Mexico	-90.03	20.88
	dubius	dub34	MZFC 27186	Yucatan, Mexico	-88.57	21.47
	dubius	dub35	MZFC 21797	Yucatan, Mexico	-90.58	19.73
	santacruzi	sant41	MZFC 20410	Chiapas, Mexico	-90.58	19.73
	santacruzi	sant40	MZFC 17637	Chiapas, Mexico	-93.2	15.7
	santacruzi	sant42	MZFC 17638	Chiapas, Mexico	-93.02	15.56
	santacruzi	SOSI130	MZFC 25867	Chiapas, Mexico	-92.34	14.92
Melanerpes aurifrons	-	aurif06	MZFC 15130	Queretaro, Mexico	-99.59	20.74
	-	HP068D	MZFC 15129	San Luis Potosi, Mexico	-101.11	25.9
	-	aurif02	MZFC 15007	San Luis Potosi, Mexico	-99.42	22.12
	-	TQ05D	MZFC 15287	Queretaro, Mexico	-99.46	21.47
	-	QRO081	MZFC 13091	Queretaro, Mexico	-99.11	21.16
Melanerpes carolinus	-	M_carolinus07	AMNH PAC 774	New Jersey, USA	-74.15	40.79
	-	M_carolinus71	AMNH PRS 613	New York, USA	-73.34	40.90
	-	M_carolinus09	AMNH PRS 134	New York, USA	-73.42	40.87
	-	M_carolinus10	FMNH 387666	Florida, USA	-	-
	-	M_carolinus11	FMNH 393573	Florida, USA	-81.41	27.24
	-	M_carolinus13	FMNH 437259	Minnesota, USA	-93.713	46.5
Melanerpes crhysogenys	-	URRA81	MZFC 25261	Jalisco, Mexico	-103.85	19.58
	-	URRA70	MZFC 25264	Jalisco, Mexico	-103.94	19.59
Melanerpes pucherani	-	CHIMA409	MZFC 11394	Oaxaca, Mexico	-94.05	17.06

Table S2. Relative percentages and permutation importance of variables' contribution in the Maxent models of potential distribution for *Melanerpes aurifrons-M. santacruzi*, *M. aurifrons,* and genetic groups within *M. santacruzi.*

	Melanerpes		*M. aurifrons*		*M. s. dubius*		*M. s. grateloupensis*		*M. s. santacruzi*	
	% contribution	Permutation importance	% contribution	Permutation importance	% contribution	Permutation importance	% contribution	Permutation importance	% contribution	Permutation importance
bio2	0.9	0.8	0.4	2.4	0.1	1.2	12.3	3.1	1.1	3.3
bio3	22.1	5.6	1.3	1	11.7	0.5	10.3	4.7	54	46.3
bio5	1.7	1.8	0.6	0.1	0.4	0	5.6	0.3	0	0
bio6	7.6	6.6	31.4	38.3	56.2	39.9	15.1	44.1	1.3	0.1
bio9	3.7	4.8	27.7	31.4	11	49.1	3.6	1.6	0.1	0
bio15	24.9	23.4	3.9	0.1	0	0	23	30.9	6.5	9.2
bio16	7.6	6.6	5.8	5.9	9.8	4.5	22.1	2	21.6	9.2
bio17	6.5	6.7	4.3	1.8	10.7	4.8	4	10.9	12.7	24.8
bio19	3.5	2.3	24.7	18.9	0.1	0	4	2.5	2.7	7

SUPPLEMENTARY FIGURES

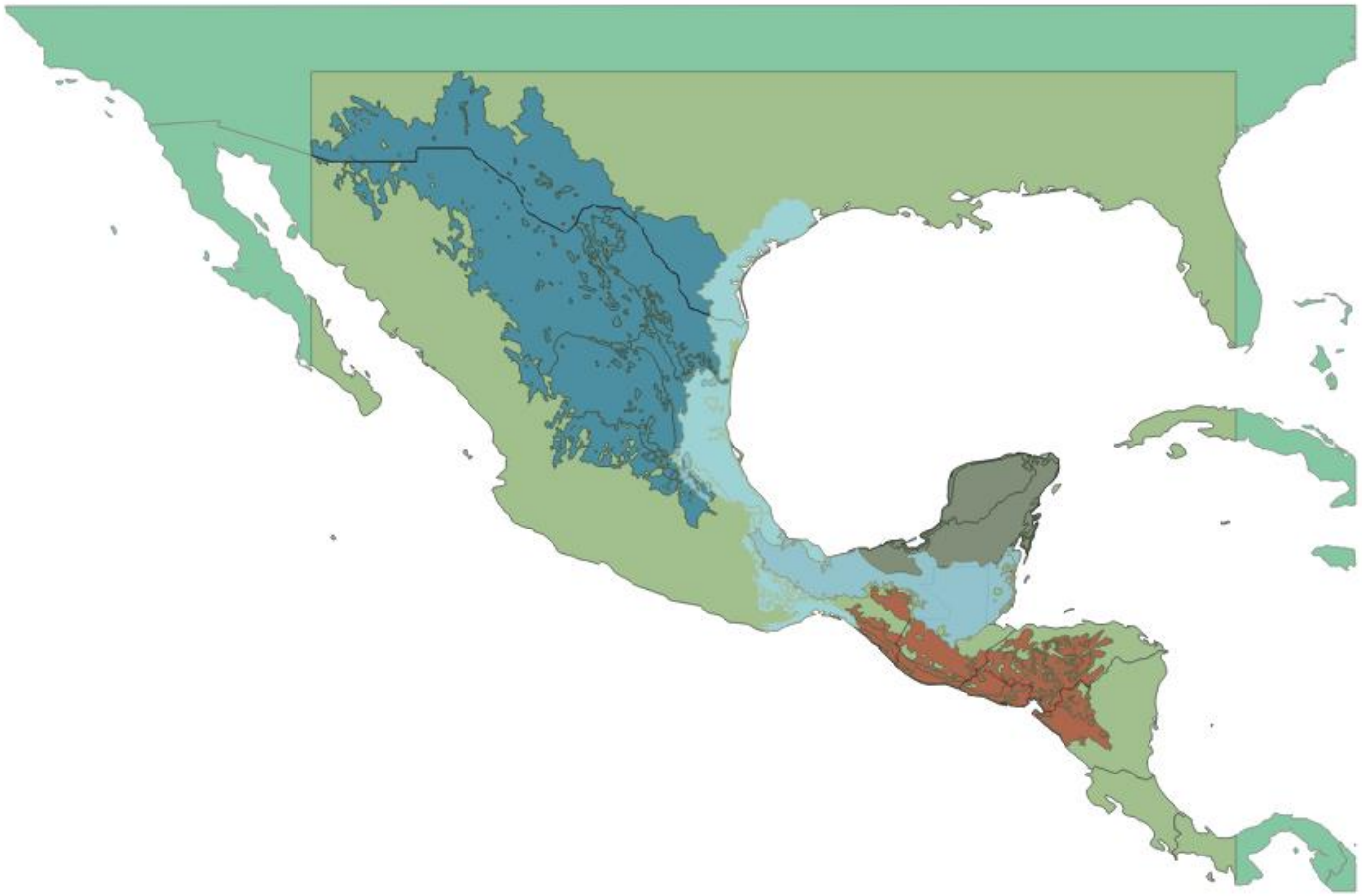

Figure S1- Calibration areas used for ecological niche models. Large extent (green) is the area used for ecological niche models analysis and the small extent (olive) is the area used for the ordination technique. The calibration area for lineages area as follows: blue: *Melanerpes aurifrons;* light-blue: *M. s. grateloupensis*; gray: *M. s. dubius*; and red: *M. s. santacruzi*.

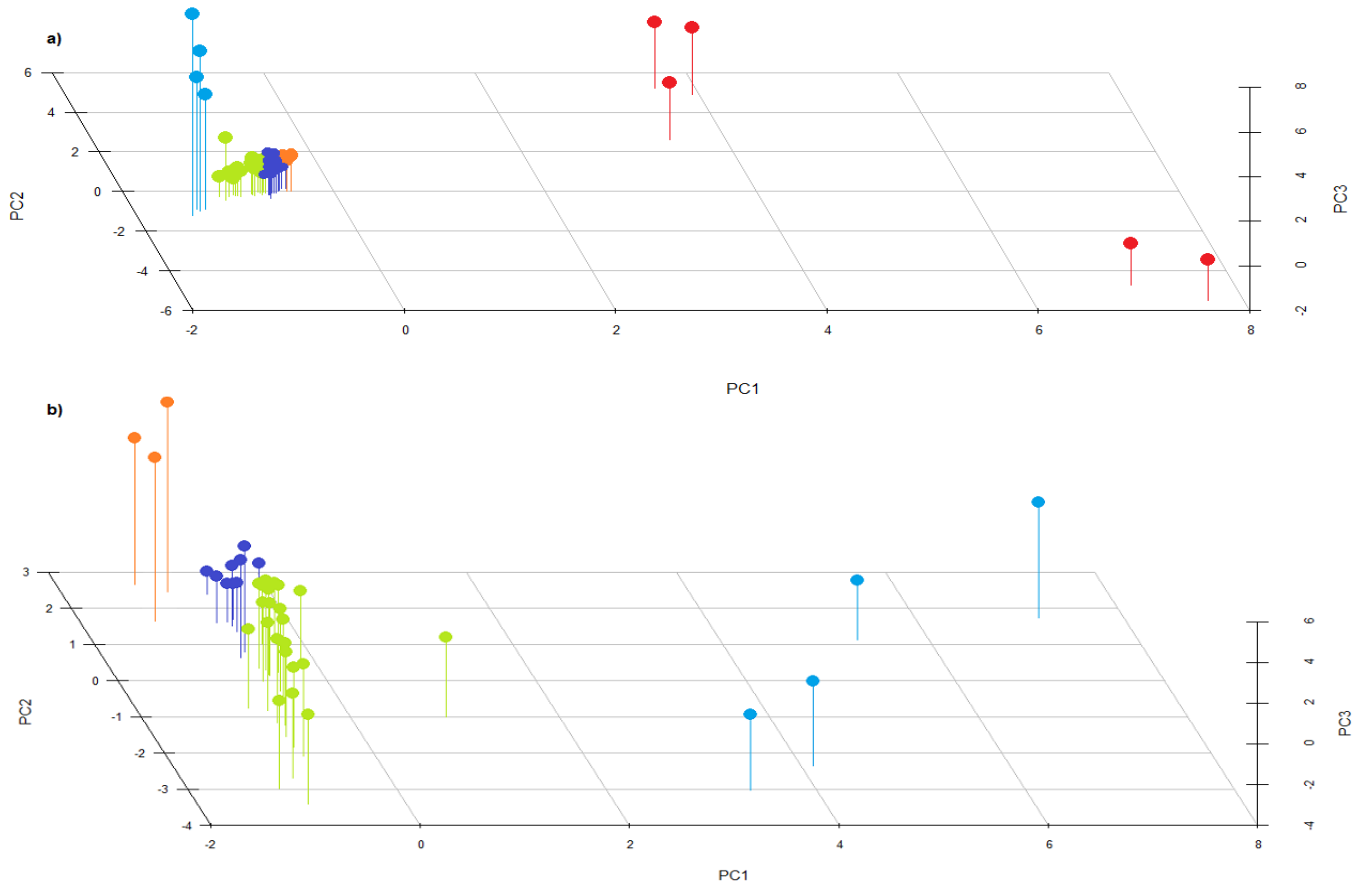

Figure S2- a) Three-dimensional PCA of the *Melanerpes carolinus-M. aurifrons-M. santacruzi* complex: *M. carolinus*: red dots; *M. s. santacruzi:* light blue dots; *M. s. grateloupensis:* green dots; *M. aurifrons*: orange; and *M. s. dubius*: dark blue. **b**) Three-dimensional PCA of the samples from *M. aurifrons, M. s. grateloupensis,* and *M. s. dubius,* samples *M. carolinus* and *M. s. santacruzi* were excluded (*M. s. grateloupensis*: green dots; *M. aurifrons*: orange; and *M. s. dubius*: dark blue).

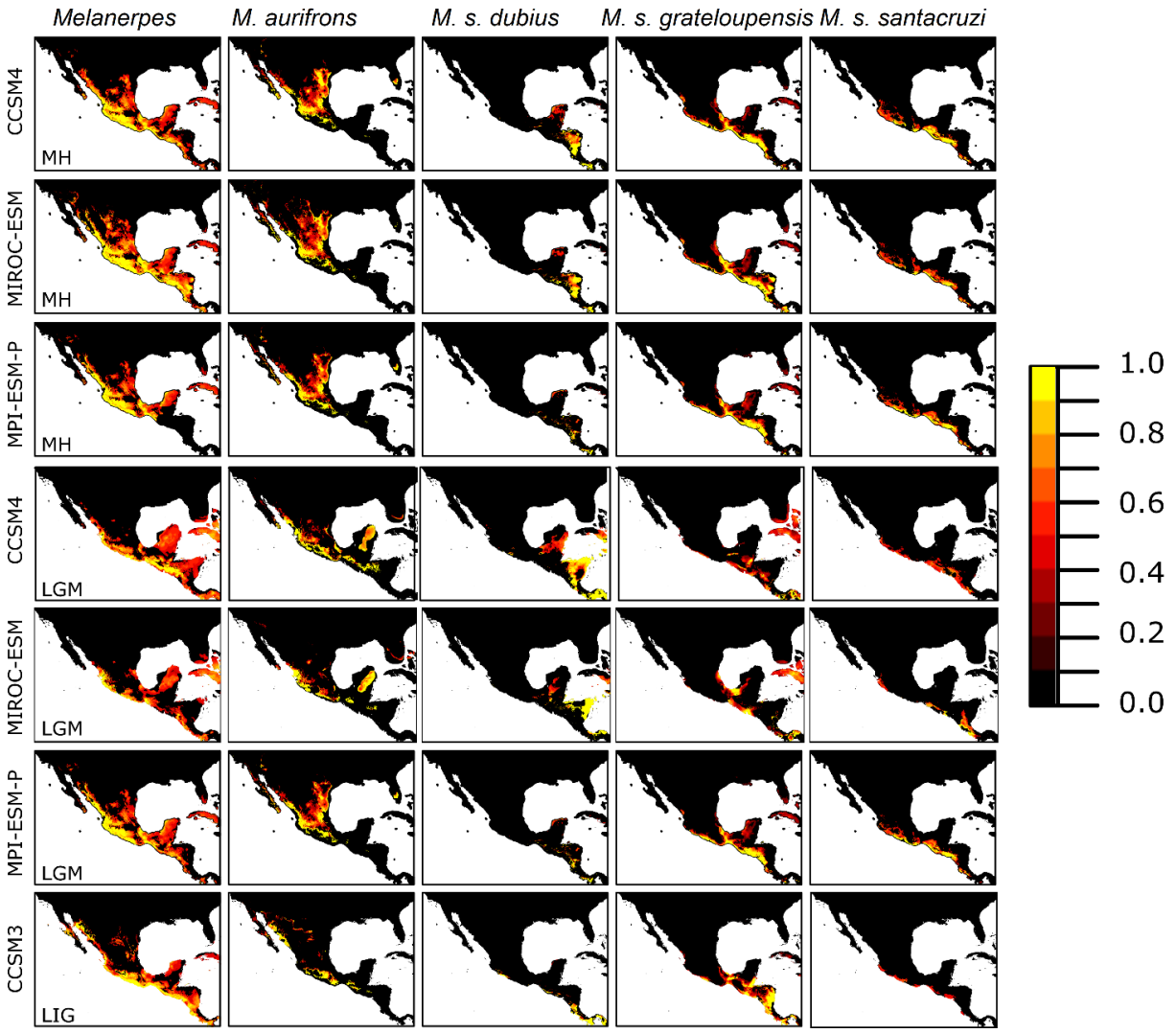

Figure S3-. Maxent results for past climatic conditions suitability for the *Melanerpes aurifrons* and *M. santacruzi* lineages. Continuous maps showed areas of high suitability conditions for species and lineages in three past time periods: MH (6,000 YA), LGM (22,000 YA) and LIG (120,000-140,000 YA).

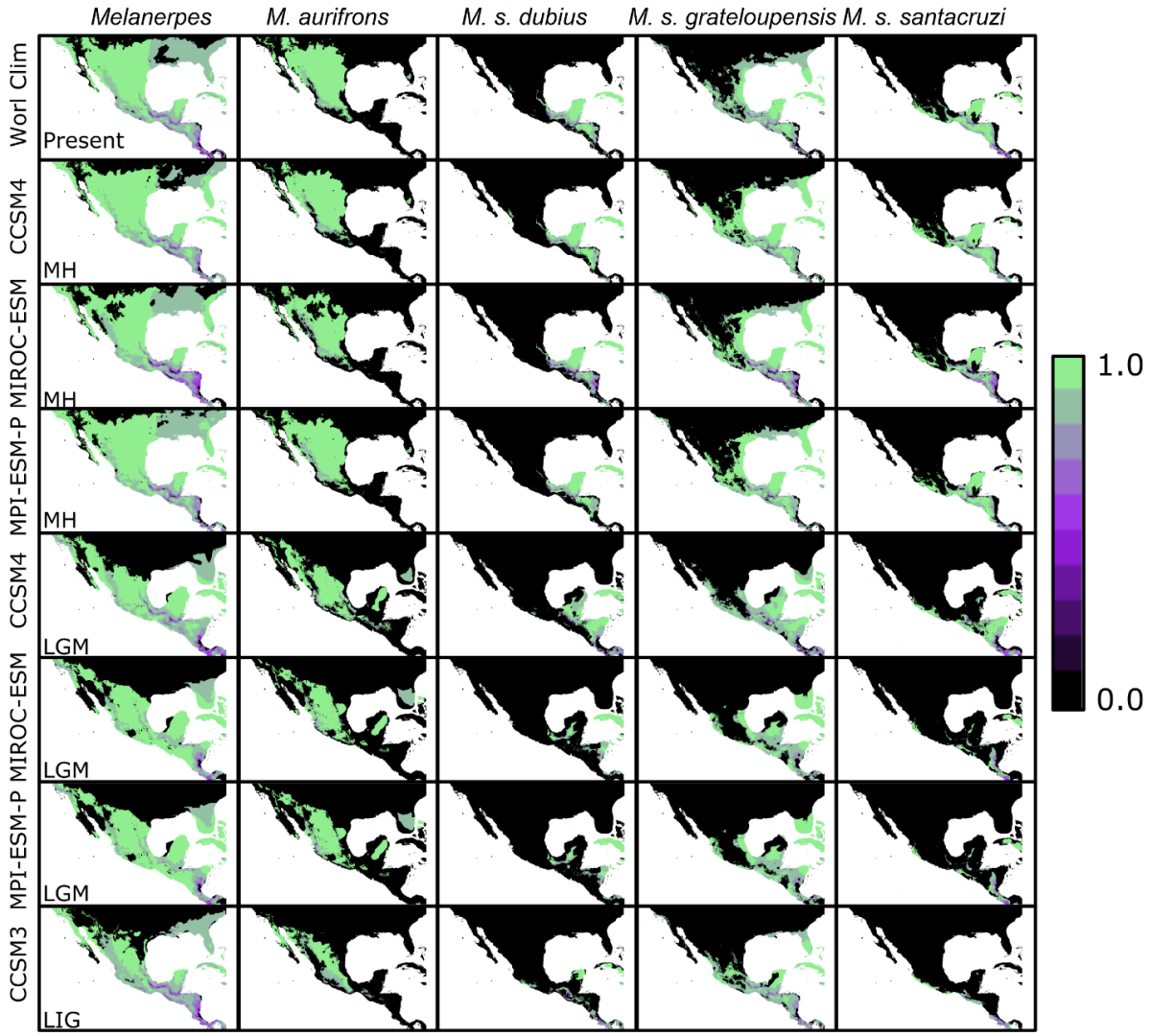

Figure S4- Extrapolation risk for the present and past model transferences (MOP results) for the *Melanerpes aurifrons* and *M. santacruzi* lineages. Three past time periods were analyzed: MH (6,000 YA), LGM (22,000 YA) and LIG (120,000-140,000 YA).

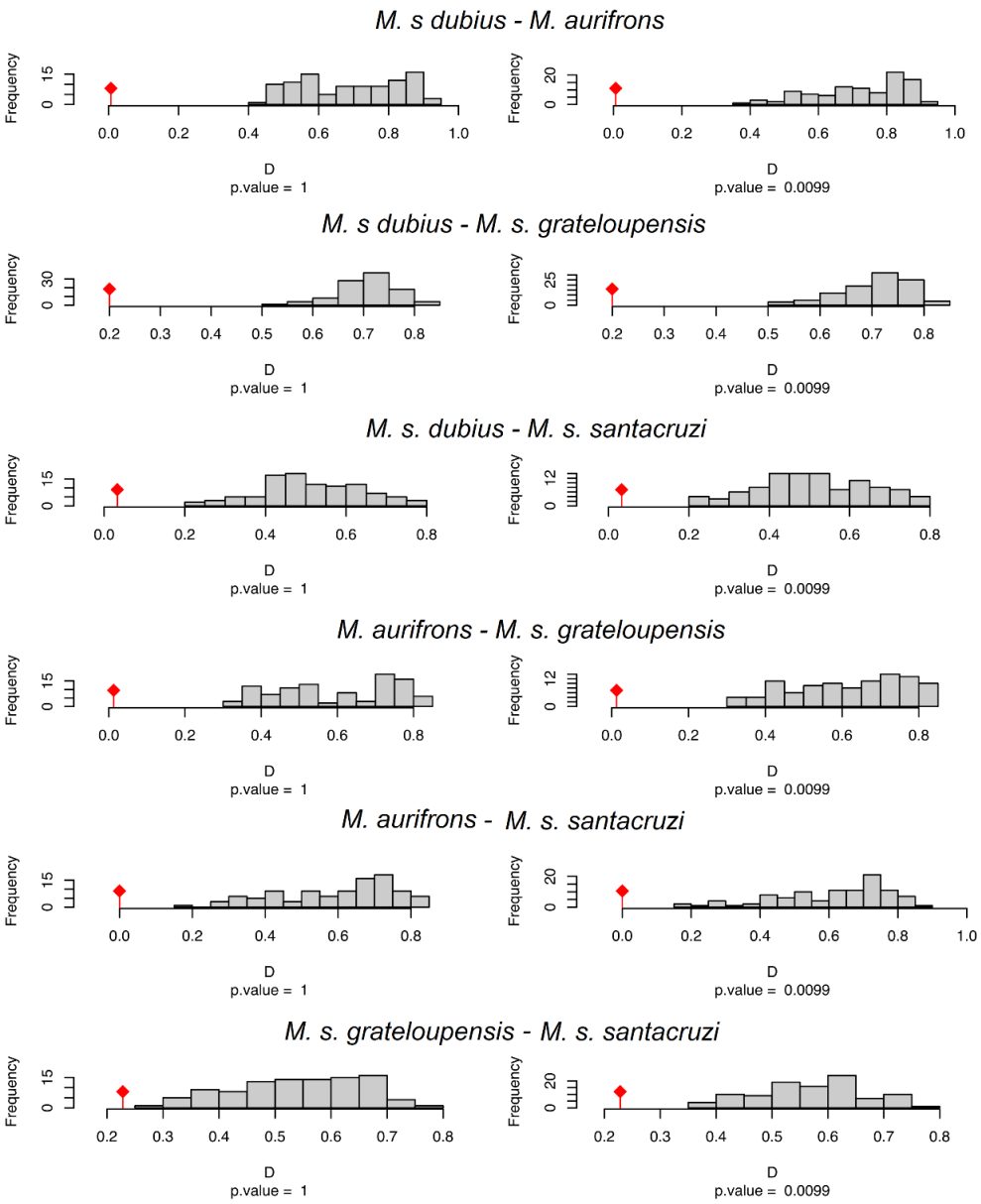

Figure S5- Equivalency test for all lineage pairs. Red diamonds indicate the results of niche overlap representing the true calculated niche overlap. Columns show the result of similarity test with 100 replicates using Ecospat. Conservatism hypothesis (left): the true calculated overlap Schoener's D values were below the bottom 5% of null distribution, so the ecological conservatism hypothesis was rejected (p-value = 1). Divergence hypothesis (right): the true calculated overlap Schoener's D values were below the bottom 5% of the null distribution, so the divergence hypothesis was not rejected (p-value = 0.0099).

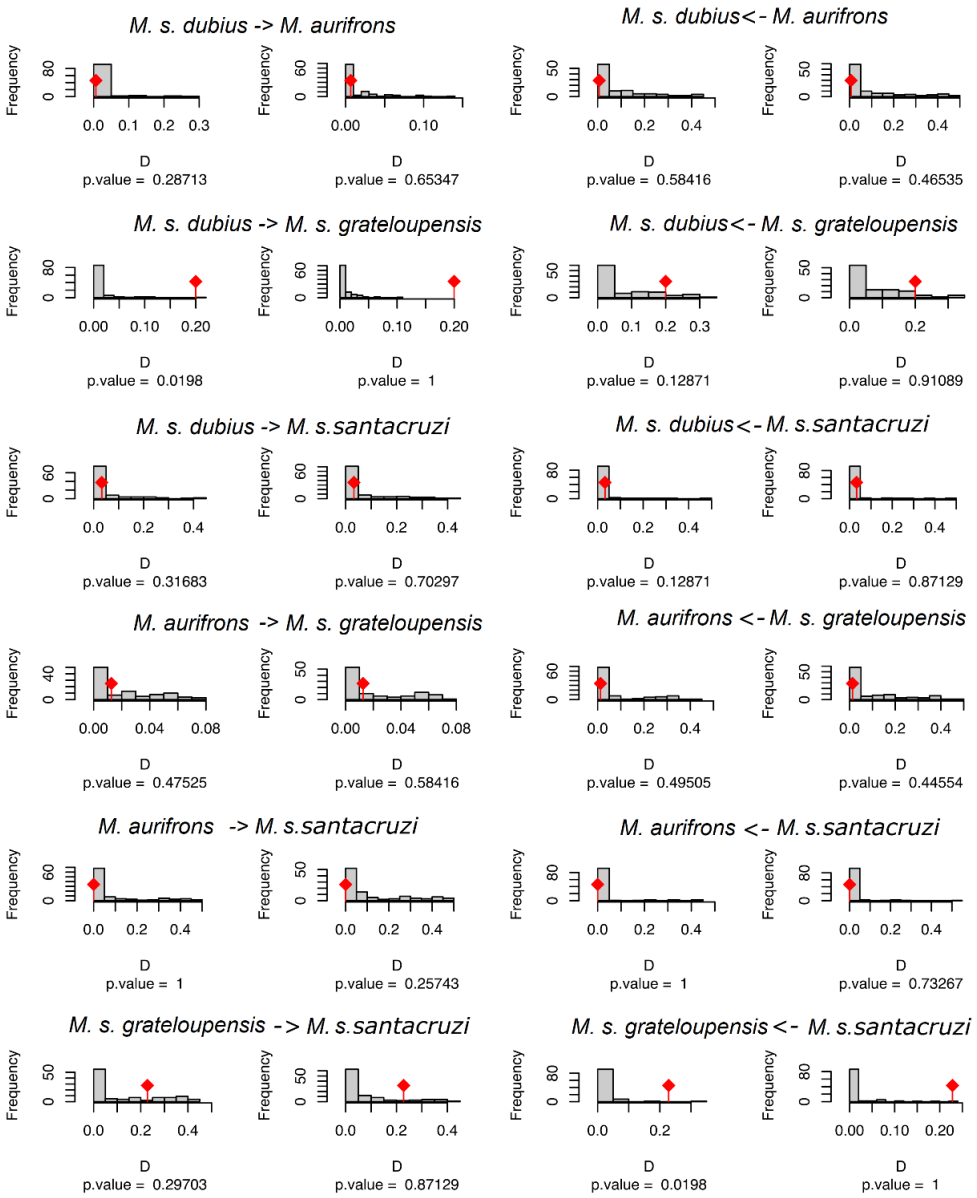

Figure S6- Identity test for all lineage pairs. Red diamonds indicate the results of niche overlap representing the true calculated niche overlap. Columns show the result of identity test with 100 replicates using Ecospat. Arrows point in the direction of analysis (focal lineage -> background lineage). **Column 1:** conservatism hypothesis: the true calculated overlap Schoener's D value was higher than 5% of the null distribution, so the ecological conservatism hypothesis was not rejected for *C. s. dubius* -> VPG (p-value = 0.0198). In all other cases, the conservatism hypothesis was rejected (p-value > 0.05). **Column 2:** divergence hypothesis: the true calculated overlap Schoener's D values were not below the bottom of the null distribution, so the divergence hypothesis was rejected (p-value > 0.05). **Column 3:** conservatism hypothesis: the true calculated overlap Schoener's D value was higher than 5% of the null distribution, so the ecological conservatism hypothesis was not rejected for VPG -> *C. s. santacruzi* (p-value = 0.0198). In all other cases, the conservatism hypothesis was rejected (p-value > 0.05). **Column 4:** divergence hypothesis: the true calculated overlap Schoener's D values were not below the bottom 5% of the null distribution, so the divergence hypothesis was rejected (p-value > 0.05).

CAPÍTULO 4. Distinctive evolutionary processes explain the distribution of genomic variation among co-distributed avian species in the Mexican tropical forests

Alexander Llanes-Quevedo[1, 2], Luis A. Sánchez-González[1], Alicia Mastretta-Yanes[3, 4], Alberto Rocha-Méndez, María Recuerda[5], Borja Milá[5], and Adolfo G. Navarro-Sigüenza[1]

[1] Museo de Zoología “Alfonso L. Herrera”, Facultad de Ciencias, Universidad Nacional Autónoma de México, México

[2] Posgrado en Ciencias Biológicas, Universidad Nacional Autónoma de México, México

[3] Comisión Nacional para el Conocimiento y Uso de la Biodiversidad, México

[4] Consejo Nacional de Humanidades, Ciencia y Tecnología, México

[5] Museo Nacional de Ciencias Naturales, Consejo Superior de Investigaciones Científicas, España

Distinctive evolutionary processes explain the distribution of genomic variation among co-distributed avian species in the Mexican tropical forests

ABSTRACT.- Avian diversity of Mexican tropical forests (MTF) has been strongly influenced by the presence of physical barriers to dispersal -such as the mountain ranges constituting the Mexican Transition Zone- and the climatic cycles of the Pleistocene. While some studies suggest that differentiation patterns are associated to common geological and climate shifts, others propose more complex and species-specific responses to these changes. Nevertheless, the understanding of processes generating these patterns are limited, especially those related to the role of local adaptation of populations in species diversification. Here, we analyzed genomic and ecological data for approaching spatial and temporal divergence patterns of four bird species co-distributed in the MTF: *Saltator atriceps* (Passeriformes: Thraupidae), *Xiphorhynchus flavigaster* (Passeriformes: Furnariidae), *Melanerpes santacruzi* (Piciformes: Picidae) and *Icterus gularis* (Passeriformes: Icteridae). We performed population genomic analyses based on SNP data (*S. atriceps:* n=24, SNP= +3,300; *X. flavigaster:* n=53, SNP= +8,900; *M. santacruzi:* n=35, SNP= +4,000; *I. gularis*: n=60, SNP=+ 11,000) to test if the demographic dynamics and distribution of genetic diversity of species obey to the influence of synchronic historical events or common physical barriers, as well as the influence of climatic variables in such differentiation. We found that there is some spatial congruence in the population structure of the species analyzed in the Tehuantepec Isthmus and wetlands of Tabasco. However, the divergence times and populations' dynamics are distinctive for each species (idiosyncratic). We also found evidence of the differential effects of distance and environment in the genetic diversity of each species. For *M. santacruzi* and *S. atriceps,* we obtained a significant correlation between genomic and environmental variation, while for *X. flavigaster* and *I. gularis* we inferred that genetic differentiation must be a consequence of the geographic distance and limited gene flow among populations. Our work provides new evidence on the historical processes that have driven the evolution of the birds of the MTF, and of the influence of the processes of local adaptation of the populations to different environmental conditions, which together have promoted the outstanding diversification of the Neotropical avifauna.

Key words: asynchronous divergence, genomic-environmental association, *Icterus gularis*, *Melanerpes santacruzi*, population dynamics, *Saltator atriceps, Xiphorhynchus flavigaster*

INTRODUCTION

One of the major objectives in biogeography and evolutionary biology is the understanding of the historical processes affecting the contemporary geographic distribution of biodiversity. Neotropical avifauna, the most diverse of any biogeographic unit of the world (Stotz et al., 1996), presents interesting patterns of distribution of morphological and genetic differentiation that reveal clues for approaching the processes that shaped the complex biotic evolution of the region. Different studies addressing these patterns have documented the existence of a clear phylogeographic structure of bird taxa, with discontinuous ranges due to physical barriers to gene flow (e.g., Harvey et al., 2015; Berv et al., 2021; Buainain et al., 2022). These studies highlighted the importance of barriers and of the geological processes creating them -such as the Amazon River or the Andes- in reducing gene flow between populations while increasing genetic differentiation and speciation process. On the other hand, species distributed along areas with no apparent barriers to gene flow -such as the Brazilian Cerrado (Bates et al., 2003) or the Mesoamerican seasonally dry forests (e.g., Arbeláez-Cortés et al., 2014; Sánchez-González et al., 2021)- exhibit less congruent patterns. These poorly congruent genetic differentiation patterns have commonly been explained as consequence of species-specific responses (idiosyncratic) of taxa to past climate changes and current ecological conditions (Arbeláez-Cortés et al., 2014; Castillo-Chora et al., 2021). Differences in food requirements, habitat needs, physiological tolerances, and behavioral flexibilities among species (Cady et al., 2019), may enforce also diverse demographic and evolutionary responses in their co-distributed populations.

Northernmost Neotropical forests are found in Mexico, where they come in contact with the Nearctic region (Morrone, 2019). These forests are distributed along narrow and continuous strips of land on the slopes of the Pacific Ocean and the Gulf of Mexico, and on the Yucatan Peninsula (Morrone, 2019). These areas present drastic climatic differentiation which has allowed the establishment of seasonally dry forests in the Pacific coast, while in the Atlantic slope wet forests predominate. Both types of forests are in contact at the Tehuantepec Isthmus. The biodiversity of the Mexican tropical forests (hereafter MTF) has been strongly influenced by the presence of physical barriers to dispersal in various taxa of plants and animals (e.g., mountain ranges [Sierras Madres Occidental, Oriental, del Sur, and Chiapas]) and Pleistocene climatic cycles (Prance 1973, Toledo 1982), that oscillated from dry and cold climate

during glacial to wet, and warm interglacial periods. During the glacial periods, populations of biotas inhabiting the MTF were isolated by the expansion of non-forest vegetation adapted to xeric conditions. Later, during interglacials, contracted populations were reconnected again by the expansion of their distributional areas (Haffer, 1969; Prance, 1973; Ramírez-Barahona and Eguiarte, 2013; Mastretta-Yanes et al., 2015).

Responses of MTF biota to the joint effect of the Pleistocene climate changes and physiographic barriers have been diverse. While differentiation patterns of some taxa, such as *Bursera* trees and some species of fishes and snakes, seem to be associated with geological activity of the Trans Mexican Volcanic Belt (Becerra, 2005; Mateos, 2005; Devitt et al., 2008; De-Nova et al., 2012), some other plant species and birds show low geographic structuring of genetic variation (e.g., Chávez-Pesqueira and Núñez-Farfán, 2016; Licona-Vera et al., 2018), suggesting complex and species-specific responses to geological and climate shifts. However, most of these studies have been performed using few molecular markers, mostly mitochondrial genes, which has prevented further inferences about the processes that have generated the differentiation patterns obtained.

Recent advances in DNA massive parallel sequencing techniques, and in the analysis methods of genomic information have brought a revolution in the study of evolutionary processes at different spatial and temporal scales. Massive genomic data have improved the robustness of population genetics and phylogeographic analyses and opened a door to the exploration of questions such as the role of adaptive variation and of hybridization in the species' evolution (e.g., Taylor and Larson, 2019; Chaves et al, 2016).

One of the most promising and fastest growing fields associated to these advances is what can be called "comparative population genomics", which attempts to compare populations and species for drawing inferences about the evolutionary forces affecting genetic variation (Edwards et al., 2021). The integration of population genomics methods along with phylogeographic approaches, for instance by seeking to understand biotic history by finding landscape features that create vicariant breaks in the genetic variation of species (see Avise et al., 1986; 2016), offers a more complete vision of the evolutionary processes that have occurred in populations than pre-genomic population genetics analyses (Edwards et al., 2021).

Herein, we aimed to integrate methods from phylogeography and population genomics in a comparative framework to investigate patterns of genomic variation of co-distributed birds in the MTF. For this, we analyzed the concordance between phylogeographic structure (genetic differentiation, based on genomic data) and distributional data of each species, and explored the association of environmental variables with the structure. We attempted to discover not only the influence of the Mexican geography in the spatial arrange of the avifauna's genetic diversity, but also in the neutral and adaptive processes acting on populations.

Specifically, we used a genomic reduced representation dataset based in SNP data produced by NextRAD sequencing (Rusello et al., 2015) in a comparative phylogeographic and population genomics framework to test whether: (1) there is geographically structured genetic variation in each species; (2) genetic structure is spatially concordant among species; (3) population dynamics and differentiation are temporally congruent; and (4) populations' differentiation is related to neutral processes acting on isolated populations or to local adaptation to diverse environmental conditions. Our work provides new evidence on the historical processes that have driven the evolution of the birds of the TMF, as well as on the influence of processes of local adaptation of populations to different environmental conditions, which together have promoted the outstanding diversification of Neotropical avifauna.

MATERIALS AND METHODS

***Taxon Selection*.-** We selected a set of four distantly related co-distributed bird species from the MTF. All species show important morphological variation, such that they are considered polytypic taxa, each with between five and ten described subspecies. In addition to evolutionary (taxonomic) diversity, these species reflect ecological diversity in terms of habitat use and feeding habits, and also exhibit differences in the geographic connectivity of their populations, some showing allopatric while other parapatric distributions (Fig 1a-d).

The selected species were:

1) Black-headed Saltator (*Saltator atriceps,* Passeriformes: Thraupidae). The geographic variation of this species has led to the recognition of six subspecies (Supplementary File 1). The variation consists of differences in size and coloration of the throat (which is white in most populations but pale cinnamon in subspecies from southeastern Mexico) and the size of the pectoral band located below the throat patch

(being broad and black, but in some subspecies, it is reduced or absent; Howell and Webb, 1995; Deshwal et al., 2020). No molecular studies have been carried out at the population level in the species, so it has not been possible to establish any relationship between genetic diversity and morphological variation.

2) Ivory-billed woodcreeper (*Xiphorhynchus flavigaster,* Passeriformes: Furnariidae). Eight subspecies have been recognized (Supplementary File 2). Geographic variation in the species consists mainly of differences in overall and bill sizes; differences in coloration on both the dorsal and ventral regions (which can be from pale brown and greyish to very dark brown with black crown and nape); the striped pattern (in terms of width and shape), and the striped or spotted pattern on the crown (Howell and Webb, 1995; Marantz et al., 2020). A molecular study based on mitochondrial sequences of the ND2 gene showed some agreement with the morphological groups (Castillo-Chora et al., 2021). However, conflicts were found with the distribution of the subspecies distributed in the coastal plain of the Gulf of Mexico, Yucatan, and Central America (Castillo-Chora et al., 2021).

3) Altamira Oriole (*Icterus gularis,* Passeriformes: Icteridae). Six subspecies have been recognized because of differences in overall size, wing, covert feathers, culmen, and tarsus lengths, the extant of white color in the secondary feathers, and the intensity of yellow coloration (Fig. 1, Supplementary File 3). In general, subspecies on Caribbean slope of Mexico are smaller and more intensely colored than those on Pacific slope, although it has been recognized that the diagnosable differences between some races are doubtful and require further study (Howell and Webb, 1995; Brush and Pleasants, 2020). Population analyzes based on genomic data yielded a discordant structure in the number and limits of the proposed subspecies. Although the differentiation in the north-south axis of the Isthmus of Tehuantepec is recovered, this is not obtained for the limits of the subspecies distributed on the Pacific and Atlantic slopes (Rocha-Moreira et al., 2020).

4) Velazquez Woodpecker (*Melanerpes santacruzi,* Piciformes: Picidae). Eleven subspecies have been recognized according to the morphological differentiation in the coloration of the nasal brushes and the plumage of the neck and ventral regions, the patterns of the dorsal region bars and the central rectal feathers, and size (Selander and Giller, 1963; IOC, 2019) (Fig. 1, Supplementary File 4). The patterns of occurrence of their populations are complex, with several zones of sympatry or parapatry of the forms of the species that also have a limited cross between them (García-Trejo et al.,

2009). Several genetic studies have been conducted to elucidate the population structure of the species and the phylogenetic relationships with their closest relatives *M. aurifrons* and *M. carolinus* (García-Trejo et al., 2009; Navarro-Siguenza et al., 2017; Llanes-Quevedo et al., 2022). Mitochondrial studies (García-Trejo et al., 2010; Navarro-Siguenza et al., 2017) could not resolve the structure within the species, and genomic data analyses (Llanes-Quevedo et al., 2022) did not support the number and limits of proposed subspecies from Veracruz and the southern portion of the Tehuantepec Isthmus.

***Project workflow*.-** A general overview of the methods flow is depicted in Supplementary Fig. 1. First, we present new genomic data for two taxa *Xiphorhynchus flavigaster* and *Saltator atriceps* and analyzed their genetic structure algorithmic and model-based methods of clustering and the calculation of diversity and fixation indexes (detailed methods below).

Then, we incorporated published data of other two taxa that has been generated by similar techniques: *Melanerpes santacruzi* (NextRAD sequencing; Llanes-Quevedo et al., 2022) and *Icterus gularis* (ddRAD sequencing; Rocha-Moreira et al., 2020) to test for congruent phylogeographic and adaptive patterns across four co-distributed species in the MTF. From raw sequence data of *M. santacruzi* and *I. gularis*, we performed bioinformatic processing for SNP data assamblage using same parameters to those employed for *X. flavigaster* and *S. atriceps.* With the full species dataset, we investigated the congruence of evolutionary history of the four taxa by evaluating: 1) Spatial congruence of the genetic structure among species; 2) Past demographic changes experienced by populations, throughout Bayesian Skyline plot calculations; 3) Existence of synchronic events of population differentiation, by using a full-likelihood Bayesian model choice framework; and 4) Correlation of genomic and environmental variation along the species' ranges with two complementary methods: differentiation outliers and genetic-environment association methods (detailed methodology below).

***Tissue Sampling*.-** We collected frozen or ethanol-preserved tissue samples (muscle, heart, or liver), or pieces of toepads from specimens of *Saltator atriceps* (n=31) and *Xiphorhynchus flavigaster* (n=64), covering most of the distribution range of taxa in Mexico. As outgroups for phylogenetic analyses, we sampled *Saltator vigorsii* (n=2),

S. grandis (n=2), and *S. maximus* (n=1) for *S. atriceps*. For *X. flavigaster*, we selected a closely related taxon of Dendrocolaptine for which we had available tissues [*Lepidocolaptes affinis* (n=2)*, L. souleyetti* (n=2)*,* and *L. leucogaster* (n=2)]. All tissues and specimens were obtained from the Scientific Ornithological Collection housed at the Museum of Zoology, Facultad de Ciencias, UNAM, Mexico (Supplementary Table S1).

For *Icterus gularis,* and *Melanerpes santacruzi,* we downloaded available raw sequence data in fastq format (http://www.ncbi.nlm.nih.gov/sra, BioProjects PRJNA636585 and PRJNA802310). The total number of samples included were 60 for *Icterus gularis,* and 35 for *Melanerpes santacruzi.*

DNA Extraction and NextRAD Sequencing.- Total genomic DNA was extracted using Proteinase K digestion, followed by isolation with the DNeasy Blood & Tissue Kit (Quiagen) following manufacturer instructions. We estimated the quality and amount of DNA by electrophoresis in 1% agarose gels. All samples yielded high molecular weight DNA of more than 50 ng/µl.

Genomic DNA was converted into NextRAD genotyping-by-sequencing libraries in SNPsaurus LLC (Oregon, USA), as in Russello et al. (2015). Genomic DNA (~10 ng) was fragmented with Nextera Flex reagent (Illumina, Inc), which also ligated short adapter sequences to the ends of the fragments. Fragmented DNA was amplified with one of the primers matching the adapter and extending 10 nucleotides into the genomic DNA with the selective sequence GTGTAGAGCC. The constructs were amplificated by PCR using Phusion® Hot Start Flex DNA Polymerase (New England Biolabs; Massachusetts, USA). The PCR program used includes an initial step of 3 min at 72 °C, followed by 30 s at 98 °C, and five cycles of denaturation at 98 °C for 10 s, annealing at 63 °C for 30 s, and 3 min extension at 72 °C. Amplicons were pooled and the resulting libraries were purified using AMPure XP beads (Agencourt Bioscience Corporation; Massachusetts, USA) at 0.7 ×. Then, the purified libraries were size selected to 350-800 base pairs and sequenced on a HiSeq 4000 with one lane of 150 bp reads (Genomics Core Facility, University of Oregon).

Processing of raw data.- The quality and adapter content of NextRAD sequences were evaluated with FastQC (Andrews, 2010). Then, sequences were quality-filtered (Phred quality score > 33) and adapter-trimmed in Trimmomatic, using the sliding-

window function (Bolguer et al., 2014). We assembled our DNA matrices in ipyrad (Eaton and Overcast, 2020) using the following parameters: *de novo* assembly, minimum coverage value of 9, grouping threshold of 0.85, minimum value of groups (taxa) included of 33 %, and maximum number of polymorphic sites shared within a locus of 15 %. All other parameters were set at default values. Loci were concatenated into combined matrices of NextRAD. We applied additional filters to eliminate individuals and loci with more than 30 % of missing data using VCFtools v1.15 (Auton and Marcketta, 2015). All the required file format conversions were conducted using PGDSpider version 2.1 (Lischer and Excoffier, 2012), using VCF files as input.

Genetic structure in* Saltator atriceps *and* Xiphorhynchus flavigaster*.- We explored spatial patterns of genetic structure among populations using both non-parametric algorithmic estimation and model-based methods. We performed Discriminant Analyses of Principal Components (DAPC), as implemented in *adegenet* package (Jombart and Collins, 2015) in R (R Core Team, 2022). We used the function *find.clusters* to calculate best number of genetic clusters (*k*), setting the maximum number of clusters (*max.n.clust*) = 10. The lowest value of the Bayesian Information Criterion (BIC) statistics was used to detect the optimal number of *k* clusters.

We also conducted a genetic assignation analysis with STRUCTURE (version 2.3; Pritchard et al., 2000), considering an admixture model with correlated allele frequencies and no prior information regarding sampling locations. We evaluated number of clusters (*k*) from 1 to 6 (given the outputs of DAPC, see Results), with 20 replicates for each value of *k*, and a run length of 1,000,000 MCMC (Markov chain Monte Carlo) replicates after a burn-in period of 500,000. The most likely number of clusters was determined by plotting the log probability of data (ln Pr(*X*|*k*) across the range of *k* values tested and selecting *k* where the value of ln Pr(*X*|*k*) plateau as suggested in STRUCTURE's manual (Pritchard et al., 2000).

In addition, we explored the genetic relationship among individuals, with a maximum likelihood tree from the concatenated SNP data set using IQTREE with statistical support from 1,000 ultra-fast bootstrap (UFBoot) replicates (Hoang et al., 2018). For tree inference, we applied the option for correcting for ascertainment bias (GTR + ASC) recommended for reducing distortions of branch lengths and topologies in SNP-based phylogeny estimations (Leaché et al., 2015).

From the groupings obtained by DAPC and Structure, we calculated pairwise weighted F_{ST} (Weir and Cockerham, 1984), and summary statistics of genetic diversity such as heterozygosity, expected heterozygosity, and the inbreeding coefficient (F_{IS}) in the R package *hierfstat* (Goudet, 2005).

Demographic dynamics inference.- Historical signatures of demographic fluctuations in the species were examined using a coalescent-based estimation of population size changes over time with Bayesian Skyline Plot (Drummond et al., 2005), as implemented in BEAST v2.6. (Bouckaert et al., 2019). We ran analyses for 100,000,000 generations, sampling every 1,000 generations, discarding the first 20% as burn-in. The verification of adequate effective sample size for each parameter (ESS > 200) and Bayesian Skyline reconstruction were performed in Tracer v1.6.0 (Rambaut and Drummond, 2013). We performed independent runs for each genetic group obtained for *S. atriceps* and *X. flavigaster,* and for those reported for *Melanerpes santacruzi* and *Icterus gularis* by Llanes-Quevedo et al. (2022) and Rocha-Moreira et al. (2020), respectively.

***Test for synchronic divergence of populations*.-** Based on the grouping hypotheses obtained by population structure and phylogenetic analyses, we tested for synchronic events of differentiation among the populations of *S. atriceps*, *X. flavigaster, M. santacruzi,* and *I. gularis*. Due to sampling limitations in some sections of the species' ranges, we considered the belonging of samples to different biogeographic provinces for paired comparisons among populations. In this way, we identified pairs of differentiated populations spatially concordant (distributed in the same biogeographic unit) among different species.

After grouping pairs of differentiated populations, we tested for synchronicity of population differentiation events in a full-likelihood Bayesian model choice framework by using *ecoevolity* (Oaks, 2019). This approach estimates the number and timing of divergence events across pairs of populations directly from SNPs (Bryant et al., 2012; Oaks, 2019), and has proved to be less sensitive to prior assumptions and less biased toward favoring shared divergences than other methods, such as Approximate Bayesian Calculation methods (Oaks, 2019; Leaché et al., 2020).

Following the author recommendations, we set the concentration parameter, which characterizes the Dirichlet process used by program to determine prior

probability for shared divergence events, to a value that puts +50% of prior probability on maximum number of divergence events. Thus, if results support shared divergences, it is more likely that data are driving the result (http://phyletica.org/ecoevolity/ecoevolity/tutorials/). We set the average mutation rate across species to a mean μ rate of $1.5x10^{-9}$ substitutions per site per generation (Ellegren, 2007). We performed ten independent runs with 10,000,000 generations, with a 10% burn-in and sampling every 1,000 iterations. Runs were concatenated with pyco-sumchains, implemented in *ecoevolity* (Oaks, 2019). MCMC convergence parameters and effective sample size were accessed through pyco-sumchains (Oaks, 2019) and Tracer v1.6.0 (Rambaut and Drummond, 2015). Next, we used sumcoevolity, a tool to calculate posterior probabilities and Bayes factors for divergence models and plotted posterior probabilities of the number of events and marginal divergence time with the pyco-sumtimes of the *pycoevolity* package (Oaks 2019).

Genetic-environment association analyses.- The final step of our analyses comprised the exploration of relationship between genomic and environmental (climatic) variation found for each species along the geographical area sampled. First, we performed a Mantel test for accounting on the effect of isolation by distance in our study systems, the null hypothesis in landscape genomic analyses (Garrido-Garduño and Vazquez-Dominguez, 2013). We first converted the information of geographic coordinates into a distances matrix by using the function *geodist* from the namesake R package (Padgham et al., 2021). Then, we performed Mantel test with 9,999 free permutations, and the use of the Spearman correlation in *vegan* R package (Okansen et al., 2020).

Then, we investigated association of genomic information with environmental variables through two complementary methods: a differentiation outliers method (Bayescan; Foll and Gaggiotti, 2008) and a genetic-environment association method (GEA; RDA: Redundancy Analyses; Forester et al., 2015). Combining methods is important to reduce error rates, particularly in a species with hierarchical genetic structure, because this can lower statistical power (De Villemereuil et al., 2014) and may allow to differentiate between processes leading to differential fixation of alleles (historical differentiation vs. environmental adaptation).

RDA multivariate ordination method allows analyzing many loci and environmental predictors simultaneously, determining how groups of loci covary in response to the multivariate environment (Rellstab et al., 2015; Forester et al., 2018) and has emerged as a powerful GEA method with a relatively low rate of false positive discovery (Capblancq and Forester, 2021; Contreras-Moreira et al., 2019; Forester et al., 2018). RDA approach was implemented in the R-package vegan (Okansen et al., 2020). Significant RDA axes were selected using 1,000 permutations with the *anova.cca* function. Candidate outlier SNPs were identified as those with loadings outside the 95% quantiles for any significant RDA axis (following Forester et al., 2018). In GEA studies, it is common to account for population structure to control the effect of neutral loci with significant allele frequency variance among populations. However, correcting for population structure could artificially increase false negatives and result in a failure to detect true positive loci under selection (Capblanq et al., 2023). To overcome this issue, the RDA was performed either using the averaged locality scores on the first two axes of the genetic PCA as conditioning variables and with no correction for population structure. Ecological data were obtained from variables available in the BioClim database (Hijmans et al., 2005) and specifically chosen in accordance to their independence, weight in models and relevance to each species' ecology.

As a second approach, we tried to identify loci that present population coefficients of differentiation (F_{ST}) that are 'distinct' from those under neutral expectations by using Bayescan (Foll and Gaggiotti, 2008). The method implemented in this software has been widely used to detect recent episodes of selection in non-model species, where the absence of detailed genomic information does not allow other alternatives emerging as a highly efficient method for detecting outliers, yielding low values of false positives (Perez-Figueroa et al., 2010). The analyses consisted of 25 pilot runs with a length of 10,000 and using the default chain parameters given in the program: sample size was set to 5,000 and thinning interval to 20.

RESULTS

NextRAD sequencing, filtering, and SNPs calling.- For *Saltator* samples, we obtained on average ~ 2.6 million of 150 base-pair reads per sample from each NextRAD sample library. From these libraries, we found, on average, 114,959 clusters and 7,039 "loci", after final assembly. After subsequent filtering of the ipyrad SNP

dataset, we obtained a matrix of 28 individuals (including outgroups) and 3,366 SNPs, with an average of 24 and 28 % of missing data by sample and by “locus”, respectively.

In the case of *Xiphorhynchus* samples, we got an average of 3,508,096 reads per sample, yielding 130,142 clusters and 14,251 “loci” in ipyrad assembly. With the bioinformatic processing, we obtained a dataset with 55 individuals (including outgroups) and 8,929 SNPs containing 13 % of missing data by sample and 7% by “locus”.

***Genetic structure in* Saltator atriceps *and* Xiphorhynchus flavigaster.-** DAPC indicated the existence of three and four main groupings in *Saltator* and *Xiphorhynchus* samples respectively. For *Saltator atriceps*, *find.clusters* function yielded *K* = 3 as the best supported value (lowest BIC score, 112.91), slightly better than *K* = 4 (BIC= 113.20) (Supplementary Fig. S2a). The plot of the two first DC, which explained 19.71 and 9.92 % of variation respectively, revealed the presence of three genetic clusters composed by individuals of (1) the Pacific coast of Mexico, (2) Veracruz, and (3) Yucatan Peninsula (Supplementary Fig. S2b).

The DAPC analysis for *Xiphorhynchus flavigaster* indicated the existence of four genetic groups (BIC= 162.81), being the other best supported value *K* = 3 (BIC= 163.78) (Supplementary Fig. S3a). These groups were composed by individuals from (1) the northeastern portion of Pacific coast of Mexico, (2) the Yucatan Peninsula + southern extreme of Pacific coast of Mexico + Los Tuxtlas, (3) remaining individuals from Los Tuxtlas, and (4) northern Veracruz (Supplementary Fig. S3b). Variance explained by the two first DC, were 22.47 and 10.52%.

As suggested in the DAPC, STRUCTURE analyses indicated *K* = 3 or 4 as the best clustering hypotheses for partitioning the genomic diversity in the samples for both, *S. atriceps* and *X. flavigaster*, according to the values of ln Pr(*X*|*K*) and Δ*K.* Bar plots of STRUCTURE results indicate similar hypotheses of groupings and individuals belonging to these, although with varying levels of genetic admixture between clusters (Figs. 2a and 3a). The genetic admixture levels were proportionally higher in *S. atriceps*, which reached an average of 28%, whereas in *X. flavigaster* this average was 21 %. The display of admixture plots by geographic origin of samples (Figs. 2b and 3b) was consistent with the structure obtained by DAPC, although the visualization of the analysis of the samples of *S. atriceps* for *K* = 4 (Figs. 2c) showed a subdivision of the group obtained for the Pacific coast. One of those subgroups consisted of

samples from northern Oaxaca and Guerrero and another from southern Oaxaca to Chiapas.

The ML phylogenetic trees obtained for each species widely agree with the genetic relationship among individuals inferred by DAPC and STRUCTURE (Figs. 2 and 3), although the support of inner nodes was generally low. For *S. atriceps*, the four clusters recovered in STRUCTURE constituted monophyletic groups, being the populations from the Pacific coast (northern Oaxaca + Guerrero and southern Oaxaca + Chiapas) sister clades. The localities from the Gulf of Mexico slope (Veracruz and Yucatan) were similarly recovered as sister and reciprocally monophyletic, constituting the sister clade of Pacific slope populations. For *X. flavigaster,* individuals sampled in the Pacific coast west of Tehuantepec Isthmus, clustered together in a clade sister to the remaining samples. Individuals from Yucatan Peninsula and eastern Chiapas also constituted a clade, closely related with those from Los Tuxtlas and northern Veracruz, these latter constituting a paraphyletic grouping.

The genetic clusters obtained in STRUCTURE (K = 4) for *S. atriceps* showed high values of pairwise weighted F_{ST} (Weir and Cockerham, 1984; Table 1) for all evaluated pairs, except for samples from the northern portion of Oaxaca and Guerrero, as compared to those from southern Oaxaca to Chiapas. The genetic diversity indexes calculated (allelic richness, heterozygosity, expected heterozygosity, and inbreeding coefficient (Table 1)) indicated that populations from Yucatan and north Pacific slope are the most diverse, while samples from Veracruz exhibited the lowest genetic diversity.

Clusters obtained for *X. flavigaster* (Structure, K = 4) also had high F_{ST} values for all pairs (Table 2), especially for the population from the Pacific regarding the rest. The samples from North Veracruz showed lower values in paired comparison with samples from Los Tuxtlas and Yucatan. This latter group showed highest values of genetic diversity, while the Pacific and Northern Veracruz presented the lowest ones (Table 2).

Demographic dynamics of the four co-distributed species in the MTF.- Our Bayesian estimation for demographic dynamics indicated different population trends for the co-distributed species in the MTF in both Pacific and Atlantic slopes (Fig. 4a and b). For most populations of the dry forests of the Pacific coast of Mexico, we inferred expansions of about one to six times the previous sizes of those populations,

starting approximately between 140,000 and 110,000 years ago (YA). The population of *S. atriceps* presented opposite dynamics, exhibiting a recent sharp decrease (from $4.0e^6$ to less than $1.0e^6$) (Fig. 4a).

On the other hand, for populations of the Gulf slope, we obtained a more variable and species-specific range of times for demographic growth (Fig. 4b). Populations of *X. flavigaster* experienced expansions around the 160,000 YA, while for those of *I. gularis* we estimated expansions starting approximately since the 140,000 YA. For *M. santacruzi*, we estimated the more recent expansions specially the population from the Yucatan Peninsula, which last pulse of growth began nearly 10,000 YA. As in the Pacific slope, the populations of *S. atriceps* from the Gulf of Mexico exhibited contrasting trends with those from Yucatan, decreasing from the 60,000 YA and those from Veracruz growing since 150,000 YA approximately (Fig. 4).

***Synchronic population divergence test for co-distributed species in the MTF*. –** By comparing the genetic structure of the four co-distributed bird taxa analyzed here, we identified two areas related to population genetic differentiation of two or more species: the Tehuantepec Isthmus, and Tabasco wetlands. The first is associated to the differentiation in *S. atriceps, X. flavigaster,* and *Icterus gularis*, while the second, in *M. aurifrons* and *S. atriceps* (Fig. 5a).

Our Bayesian analysis for synchronic evolutionary events strongly rejected hypothesis of shared divergence in the two scenarios evaluated. Instead, based on the 95% credible set of models, three independent divergence events were supported for populations of *I. gularis, X. flavigaster,* and *S. atriceps* distributed in the Pacific slope *versus* those in the Gulf of Mexico slope (PP>0.99, Fig. 5a; Table S3) and two for *M. santacruzi* and *S. atriceps* when comparing populations from Yucatan *versus* Veracruz (PP>0.99, Fig. 5b; Supplementary Table S3).

Estimated times of divergence for populations of *I. gularis, X. flavigaster,* and *S. atriceps* in both sides of the Tehuantepec Isthmus were 10,000 YA (CI 95%: 8,000-11,000 YA); 125,000 YA (CI 95%: 120,000-131,000 YA); and 350,000 YA (CI 95%: 310,000-385,000 YA), respectively. On the other hand, divergences between the Yucatan Peninsula and Veracruz, were estimated around the 95,000 YA (CI 95%: 85,000-105,000 YA) for *M. santacruzi* and 225,000 YA (CI 95%: 155,000-320,000 YA) for *S. atriceps*.

Environmental association analyses.- Analyses based on Mantel tests for isolation by distance in the studied species yielded low values of correlation between genetic and geographical distances. For *S. atriceps* (r: 0.2177, p = 0.0838; Supplementary Fig. S4) and *M. santacruzi* (r: -0.1618, p = 0.958; Supplementary Fig. S6) the correlations were not statistically significant, while for *X. flavigaster* (r: 0.235, p = 1e-04; Supplementary Fig. S5) and *I. gularis* (r: 0.1522, p = 0.0064; Supplementary Fig. S7) were statistically significant.

Out of 19 potentially relevant ecological variables for the species, we retained a range from six to eight after the forward selection method intended for excluding non-significant effects. Retained variables selected for more than one RDA model among all species included: Temperature Annual Range (TAR), Precipitation Seasonality (PrS), Annual Precipitation (Apr), Annual Mean Temperature (AMT), Isothermality (Ist), Mean Diurnal Range (MDR), Mean Temperature of Warmest Quarter (PWQm), and Precipitation of Driest Quarter (PDQ). Other variables important for single models were Temperature Seasonality (TmS), Precipitation of Coldest Quarter (PCQ), Precipitation of Wettest Quarter (PWQ), and Precipitation of Driest Month (PDM).

We found significant association of the genomic variation with climatic variables in *S. atriceps* and *M. santacruzi* for RDA, with and without correction by population structure. On the other hand, for *I. gularis* and *X. flavigaster* we obtained non-significant p-values for the tested models, although partial RDA for *X. flavigaster* yielded a significant association (Table 3). In all cases, we obtained low percentages of variance explained by the models tested ranging from 0.9% in *Icterus gularis,* to 8% in *Saltator atriceps* (Table 3). The number of outliers detected were: 129 for *S. atriceps*, 350 for *X. flavigaster*, 427 for *M. santacruzi*, and 462 for *I. gularis,* being the variables APr, TAR, PrS, and PDM those with higher number of candidates SNPs associated (Fig. 6a-d).

RDA plots for species with significant values of correlation indicate a correspondence of clustering with those found with population analyses (Figs. 6a and 6c). For *S. atriceps*, individuals from the Pacific coast clustered together, despite belonging to any of the populations detected, while those from Veracruz and Yucatan constituted different groupings separated on the axis 1 of RDA, mainly by the effect of variables PWQ, AMT, TAR, and PrS. For *M. santacruzi*, axis 1 of RDA discriminates individuals from southeastern Mexico from the remaining samples being the most

contributing variables Apr and Ist. The second axis allow to separate these latter samples in three groups, which corresponds with individuals from Yucatan Peninsula, Veracruz, and the southern portion of the Tehuantepec Isthmus (most contributing variables: PDQ, TAR, and PrS).

Finally, Bayescan analyses resulted in a low number of loci showing higher population coefficients of differentiation than those expected under neutral model. Considering an FDR=0.05, we obtained 28 outliers for *S. atriceps*, 46 for *X. flavigaster*, 51 for *M. santacruzi*, and 52 for *I. gularis.* The overlapping of these "loci" with those recovered by RDA was low, never exceeding the 30% of the Bayescan outliers (Supplementary Fig. S8).

DISCUSSION

Concordance between biogeographical processes and genetic structure of multispecies assemblages is expected in comparative phylogeographic analyses. Common barriers are expected to have similar impact on different lineages (e.g., those produced by shared vicariance; Brumfield 2012), causing shared divergence times across such barriers. Herein, we studied the congruence in the evolutionary history of four co-distributed species of birds in the MTF using methods from phylogeography and population genomics. These fields aim to study genetic variation over space and time through somewhat different conceptual and methodological approaches, but with the same type of data and may be integrated for a more solid approach for the understanding of species' evolution.

We analyzed the responses of tropical forest bird species to historical and ecological processes affecting the areas where they co-occur by testing four lines of evidence: the congruence of phylogeographic structure, the congruence of demographic dynamics of populations, the synchronicity of divergence events among such populations, and lastly, the signatures of local adaptations in these diversification processes. Our analysis revealed a partially congruent phylogeographic structure, and differences in demographic dynamics, population divergence times, and the effect of geographic distance and environmental variables on the genetic diversity of all evaluated taxa.

Comparative analyses of species co-distributed in the MTF.- Comparative analyses of genetic structure in the four co-distributed studied species indicate the

existence of some common phylogeographic barriers, although most limits of differentiated populations are idiosyncratic, (i.e., different for each species; Fig 5a), reflecting differences in biological attributes of each that affect divergence time of their populations.

Some of these geographical/ecological barriers that seem to be influencing the genetic structure of single species are the Soconusco mountains in southern Chiapas for *M. santacruzi*, and the southern extensions of Sierra Madre del Sur to the adjacent lowlands for *S. atriceps*. On the other hand, the Tehuantepec Isthmus and the wetlands of Tabasco appear to act as common barriers for some of the species analyzed herein, agreeing with previous studies which suggested that these features have strongly influenced the biodiversity of the Mesoamerican region (e.g., Barber and Klicka, 2010, Sánchez-González et al., 2023).

The Tehuantepec Isthmus is a lowland region located in southeastern Mexico bounded by the mountain ranges Sierra Madre Oriental, Sierra Madre del Sur, and the Chiapas-Guatemala Highlands. This region is characterized by a climatic contrast in a north-south direction, with an average of annual rainfall on the Atlantic slope of 3,960 mm and annual temperatures between 22-26 °C, while the Pacific slope has a drier climate, with averages of 800-1,200mm per year and temperatures above 26°C (Sánchez and Oropeza, 2003). This climatic variation is accompanied by important hydrological differences in the number and characteristic of water bodies (Sánchez and Oropeza, 2003), which together have caused the establishment of wet forest in the Atlantic slope and seasonally dry forest towards the Pacific. The effect of the Tehuantepec Isthmus on the diversification of lowland birds, and other taxa, have been diverse depending on attributes exhibited by each of the studied species. We found genetic connectivity in *M. santacruzi* (Llanes-Quevedo et al., 2021), while phylogeographic breaks have been found in wrens, toads, and snakes, among others (e.g., Vázquez-Miranda et al., 2009; Mulcahy et al., 2006; Daza et al., 2010). These patterns are also found for taxa inhabiting nearby highlands, which comparatively have received more attention. Genetic breaks have been found in some plants (Gutiérrez-Rodríguez et al., 2011; Ornelas and Rodríguez-Gómez, 2015), amphibians and reptiles (Mulcahy et al., 2006; Castoe et al., 2009), mammals (Sullivan et al., 2000; León-Paniagua et al., 2007) and birds (Cortés-Rodríguez et al., 2013; Barrera-Guzmán et al., 2012; Ornelas et al., 2015); whereas genetic connectivity has been

detected in in other plants (Ornelas et al., 2010) and in some birds species (Arbeláez-Cortés et al., 2010; Cortés-Rodríguez et al., 2008; Navarro-Sigüenza et al., 2008).

Our tests of differentiation events synchronicity among populations sharing barriers, highlighted the ecologically unique Isthmus of Tehuantepec and Pantanos de Centla regions (Fig. 5a). Our Bayesian analyses detected several pulses of divergence among MTF bird taxa, indicating that the genetic divergence observed across spatial boundaries identified herein are asynchronous among lineages. These results are consistent with previous findings in the Tehuantepec Isthmus for lowland snakes (Daza et al., 2010) and birds from the nearby highlands (Barber and Klicka, 2010; Ornelas et al., 2013), and suggest a complex evolutionary history of the area, where successive oscillation of climate from Miocene-Pleistocene have differentially affected the taxa occurring in it (Ornelas et al., 2013).

Phylogeographic incongruences among co-distributed species inhabiting same areas may result not only from the "true" differences in the temporal framework of diversification events, but also from the species' biology. In consequence, the life-history traits need to be considered to shed light onto the processes that generate evolutionary diversification (Ditchfield, 2000; Sullivan et al., 2000; Gutiérrez-García and Vázquez-Domínguez, 2011; Paz et al., 2015). Therefore, discrepancies in phylogeographic patterns among co-distributed species could result from differences among individual phylogeographic processes, such as colonization time of the areas and differential responses to geographic barriers, but also from the existence of selection gradients and gene flow, as well as diversity in rates of molecular evolution, effective population sizes, and generation time and differences in the physiology and ecology (Zink, 1996).

Our demographic analyses found mostly idiosyncratic responses of co-distributed bird species to changes in the MTF. We found a more variable dynamics in wet forests from the Gulf of Mexico than in the dryer forest of the Pacific coast, where the pulses of population expansions are more concentrated into a temporal range roughly coincident with the Riss-Wurm interglacial, 140,000 and 110,000 YA (Dahl-Jensen et al., 2013), that apparently triggered population growth in both MTFs, Atlantic and Pacific. Similar patterns of rapid and sharp growth of lowland tropical birds' species in interglacial periods have been found for *Cacicus melanicterus* and *Melanerpes chrysogenys* (Castillo-Chora et al., 2021). In these species low values of nucleotide but high haplotype diversity were reported, suggesting population

bottlenecks followed by rapid population growth and accumulation of mutations (Castillo-Chora et al., 2021).

Icterus gularis and *X. flavigaster* seem to have parallel demographic histories, but *M. santacruzi* the expantions are more recent. In contrast, *S. atriceps* from both slopes exhibist a decrease of effective population sizes in the last 50,000 YA. These results are apparently congruent with previous studies modelling paleoclimatic suitability, showing contractions of suitable climatic areas in the LGM nearly in in Yucatan, Veracruz, and the Pacific coast (Llanes-Quevedo et al., 2022; Castillo-Chora et al., 2021; Rocha-Moreira et al., 2020). Nevertheless, the expansion of suitable areas in interglacial periods is dissimilar among the species, which may produce the differences in genetic structure and population dynamics as found herein.

The genomic-environment association analyses yielded a dissimilar pattern of association of genetic variation with the climatic variables tested. *Saltator atriceps* and *M. santacruzi* genetic structure seems to be correlated with AMT, TAR, PrS, and PWQ climatic variables (among others). Main variables contributing the differentiation of RDA clusters in *S. atriceps* and *M. santacruzi* are related with temperature and precipitation (i.e., annual average and range of variation) suggesting processes of local adaptation to changing environments. In contrast, in *X. flavigaster* and *I. gularis* genetic structure seems to be more dependent on geographic distance among populations, although there is a higher number of loci associated with climatic variables.

Large estimates of effective sizes over time may support this hypothesis of local adaptation, as it would be difficult to find fixation of alleles correlated with environmental variation due to genetic drift, but this fixation may be consequence of purifying selection or “allele surfing” in their history of expansion. Purifying selection reduces the allele frequency of variants that are generated by mutation and have low fitness (Cvijović et al., 2018). According the “allele surfing” model, the loss of genetic diversity is due to expansion of populations and sequential founder events causing some neutral or even deleterious alleles of founder individuals to rapidly propagate through space (Braga et al., 2018).

***Genetic structure and taxonomic congruence in* Saltator atriceps *and* Xiphorhynchus flavigaster.-** Our results also allowed to generate inferences about taxonomic and genomic correspondence in the designation of evolutionarily significant

units (ESUs; Moritz, 1994), and the conservation importance of populations within *S. atriceps* and *X. flavigaster*. Besides being common and widespread, these species have received comparatively less attention in previous genetic studies, being included only as a few mitochondrial sequences analyzed in more ample phylogenetic works of the taxonomic groups they belong (Chaves et al., 2013; Klicka et al., 2007; Aleixo, 2002). Recently, the population structure of *X. flavigaster* was investigated within a comparative phylogeographic studie of the birds of seasonally dry forest of Mexico (Castillo-Chora et al., 2021).

For *S. atriceps* we detected a population structure partially congruent with the distribution of the subspecies as described based on morphological and coloration data (Supplementary File 1, Miller et al., 1957). Individuals from Yucatan, Guerrero + western Oaxaca, and eastern Oaxaca + Chiapas form genetically differentiated groups, which overall correspond with the ranges of subspecies *raptor, peeti,* and *flavicrissus*, respectively. On the contrary, we did not found any hint of separation among samples from Veracruz, where two subspecies (*suffuscus* and the nominate *atriceps*) are supposed to occur. The few individuals analyzed herein always clustered together, therefore a higher number of samples from these areas would be needed for making more robust inferences on the genetic structure.

For *X. flavigaster* we found a geographic structure similar to that previously found with mitochondrial ND2 gene using a larger sample size (n= 53 in this work vs. 69 individuals in Castillo-Chora et al., 2021). However, we found a different level of structuring with samples from Los Tuxtlas, which were clustered apart from those of Yucatan, and all samples from the Pacific Coast in a single clade. Another important difference with the mitochondrial DNA results (Castillo-Chora et al., 2021) is the paraphyly of the Veracruzan population.

As for *S. atriceps*, the structure found herein only matches partially the subspecies described based on morphological characters (Miller et al., 1957). Along the Atlantic slope, the three genetic groupings detected are congruent with the distribution of subspecies *saltarius, ascensor*, and *yucatanensis.* On the contrary, in the Pacific coast we only detected two clusters, one that would group subspecies *flavigaster* and *mentalis* and another with the two individuals from southern Chiapas, which clustered with those from Yucatan, this latter also detected by Castillo-Chora et al. (2021) mitochondrial study.

CONCLUSIONS AND PERSPECTIVES

There is some spatial congruence in the population structure of four tropical forest bird species; nevertheless, the structure seems to be consequence of diverse and idiosyncratic responses of each taxa to common processes that have affected the evolution of the MTF biota. Although physiographic features as the Tehuantepec Isthmus and wetlands of Tabasco appear to be relevant for the genetic structuring of birds and other species of plants and animals, the divergence times and trends of populations distributed around it obey to particular biological attributes of each species.

We presented evidence for the four species of the differential effect of isolation by distance and environment differences in the micro-evolutionary dynamics of each species. While for two species (*X. flavigaster* and *I. gularis*) the geographic distance and limited gene flow among populations seem to generate genetic differentiation, for the other two (*S. atriceps* and *M. santacruzi*) there is a significant correlation between genomic and environmental variation, suggesting that local adaption has had a relevant role in the differentiation of the populations under diverse environmental pressures.

Looking towards the future, next studies should focus on improving the sampling of the species, especially in contact areas between morphologically differentiated forms; as well as incorporate a greater number of co-distributed taxa with high phylogenetic and ecological diversity. In addition to improving geographic and taxonomic sampling, it is highly recommendable the sequencing of complete genomes of the species of interest (Thorburn et al., 2023). This information can provide a better framework for the assemblage of SNP matrices, can function as a reference to map the SNPs obtained from genomic-environmental correlation analyzes such as those carried out in this work, and also open the door to other population and evolutionary studies using medium coverage genomes or complete exomes.

CRediT authorship contribution statement

Alexander Llanes-Quevedo: Conceptualization, Methodology, Formal analysis, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Luis A. Sánchez-González:** Conceptualization, Methodology, Writing – review & editing, Supervision. **Alicia Mastretta-Yanes:** Conceptualization, Methodology, Writing – review & editing, Supervision. **Alberto Rocha-Méndez:** Formal analysis, Data curation, Writing – review & editing. **María Recuerda:** Formal analysis, Data curation, Writing – review & editing**. Borja Milá:** Conceptualization, Methodology, Writing – review & editing, Supervision. **Adolfo G. Navarro-Sigüenza:** Conceptualization, Methodology, Writing – original draft, Visualization, Supervision, Funding acquisition.

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TABLES

Table 1. Summary statistics of genetic diversity and weighted Pairwise F_{ST} (Weir and Cockerman, 1984) for genetic groups of *Saltator atriceps* in the MTF. Ar: Allelic richness; Ho: Observed heterozygosity; Hs: expected heterozygosity; F_{IS}: inbreeding coefficient.

	Summary statistics of genetic diversity				Pairwise F_{ST}			
Genetic Groups	Ar	Ho	Hs	F_{IS}	N. Pacific	S. Pacific	Veracruz	Yucatan
N. Pacific (n=8)	1.114	0.178	0.126	0.159	-	0.051	0.162	0.344
S. Pacific (n=6)	1.112	0.172	0.123	0.179		-	0.243	0.284
Veracruz (n=5)	1.075	0.163	0.118	0.333			-	0.289
Yucatan (n=5)	1.132	0.116	0.170	0.553				-

Table 2. Summary statistics of genetic diversity and weighted Pairwise F_{ST} (Weir and Cockerman, 1984) for genetic groups of *Xiphorhynchus flavigaster* in the MTF. Ar: Allelic richness; Ho: Observed heterozygosity; Hs: expected heterozygosity; F_{IS}: inbreeding coefficient.

	Summary statistics of genetic diversity				Pairwise F_{ST}			
Genetic Groups	Ar	Ho	Hs	F_{IS}	Pacific	N. Veracruz	Tuxtlas	Yucatan
Pacific (n=14)	1.039	0.096	0.143	0.393	-	0.265	0.324	0.267
N. Veracruz (n=9)	1.046	0.091	0.181	0.466		-	0.080	0.073
Tuxtlas (n=7)	1.042	0.125	0.157	0.262			-	0.110
Yucatan (n=15)	1.049	0.157	0.175	0.216				-

Table 3. Redundancy analyses (RDA) on four species of birds co-distributed in Mexican Tropical Forests. For models of partial RDA, eigenvalues of the first two axes (EV1 and EV2) of the genetic PCA are the conditioning variables. Climatic variables included in the analyses are: Temperature Annual Range (TAR), Precipitation Seasonality (PrS), Annual Precipitation (Apr), Annual Mean Temperature (AMT), Isothermality (Ist), Mean Diurnal Range (MDR), Mean Temperature of Warmest Quarter (PWQm), Precipitation of Driest Quarter (PDQ), Temperature Seasonality (TmS), Precipitation of Coldest Quarter (PCQ), Precipitation of Wettest Quarter (PWQ), and Precipitation of Driest Month (PDM).

Species	Model	Pr(>F)	Signif.	Variance explained
Saltator atriceps	RDA (Condition, Y = EV1, Z = EV2, variables: TAR + TmS + PCQ + PWQ + PrS + APr + AMT)	0.001	***	0.0876
	RDA (variables: TAR + TmS + PCQ + PWQ + PrS + APr + AMT)	0.001	***	0.0840
Xiphorhynchus flavigaster	RDA (Condition, Y = EV1, Z = EV2, variables: AMT + Apr + Ist + MDR + PrS + TAR)	0.01	**	0.0719
	RDA (variables: AMT + Apr + Ist + MDR + PrS + TAR)	0.321	ns	0.0248
Melanerpes santacruzi	RDA (Condition, Y = EV1, Z = EV2, variables: TAR + Ist + MDR + PWQm + PDQ + PrS + Apr)	0.001	***	0.0263
	RDA (variables: TAR + Ist + MDR + PWQm + PDQ + PrS + Apr)	0.023	**	0.0170
Icterus gularis	RDA (Condition, Y = EV1, Z = EV2, variables: Ist + MDR + PrS + PDM + Apr + TWQm + AMT)	0.102	ns	0.0098
	RDA (variables: Ist + MDR + PrS + PDM + Apr + TWQm + AMT)	0.679	ns	0.0125

FIGURES

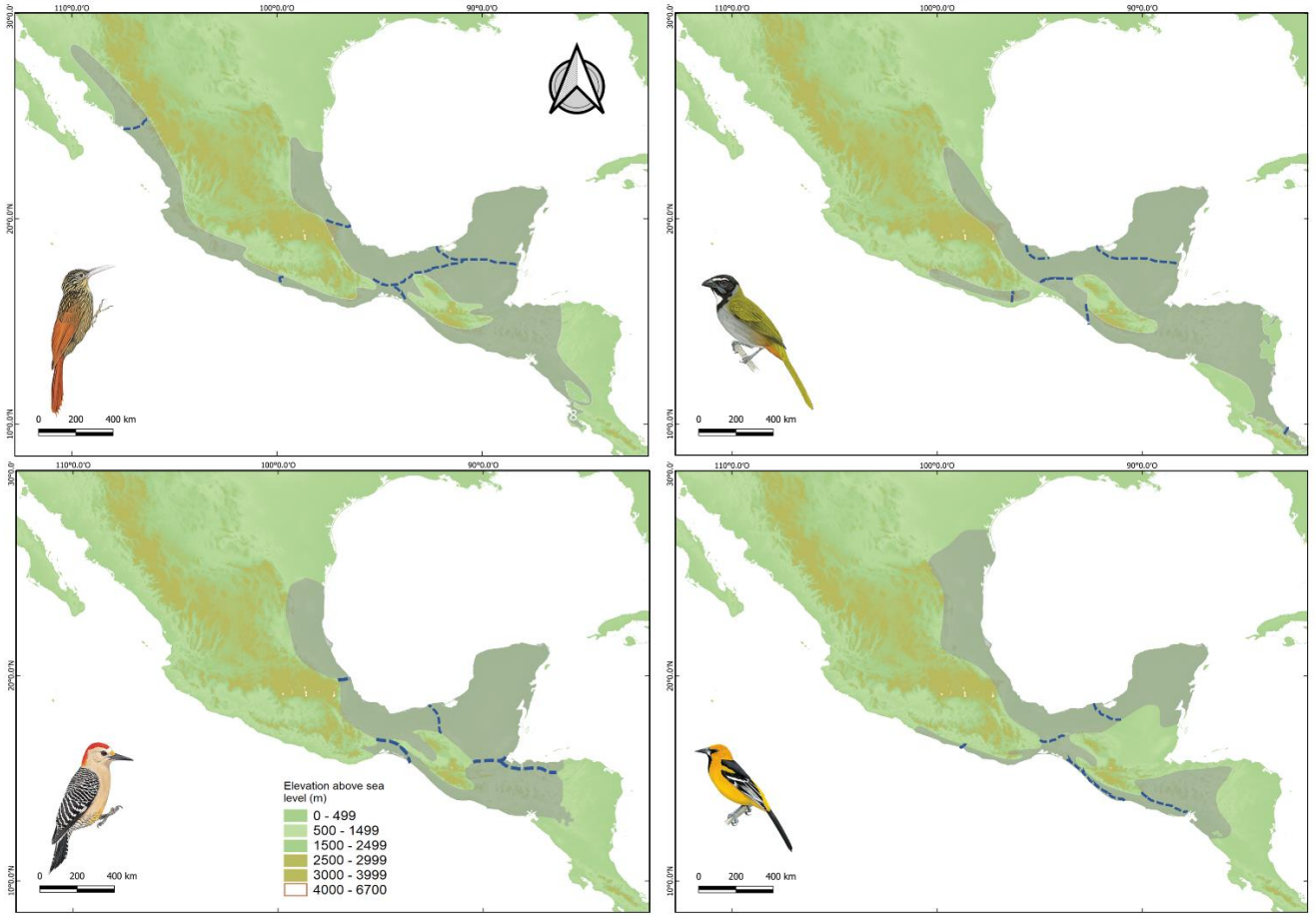


Figure 1- Ocurrence areas of four co-distributed bird species in Mexican Tropical Forests. From left to right and from top to bottom are represented: *Xiphorhynchus flavigaster, Saltator atriceps, Melanerpes santacruzi and Icterus gularis*. Distribution ranges of species are depicted in gray and subspecies limits in dotted blue line. Maps generated from the digital elevation model from Hydro1k (https://www.usgs.gov/centers/eros/science/usgs-eros-archive-digital-elevation-hydro1k).

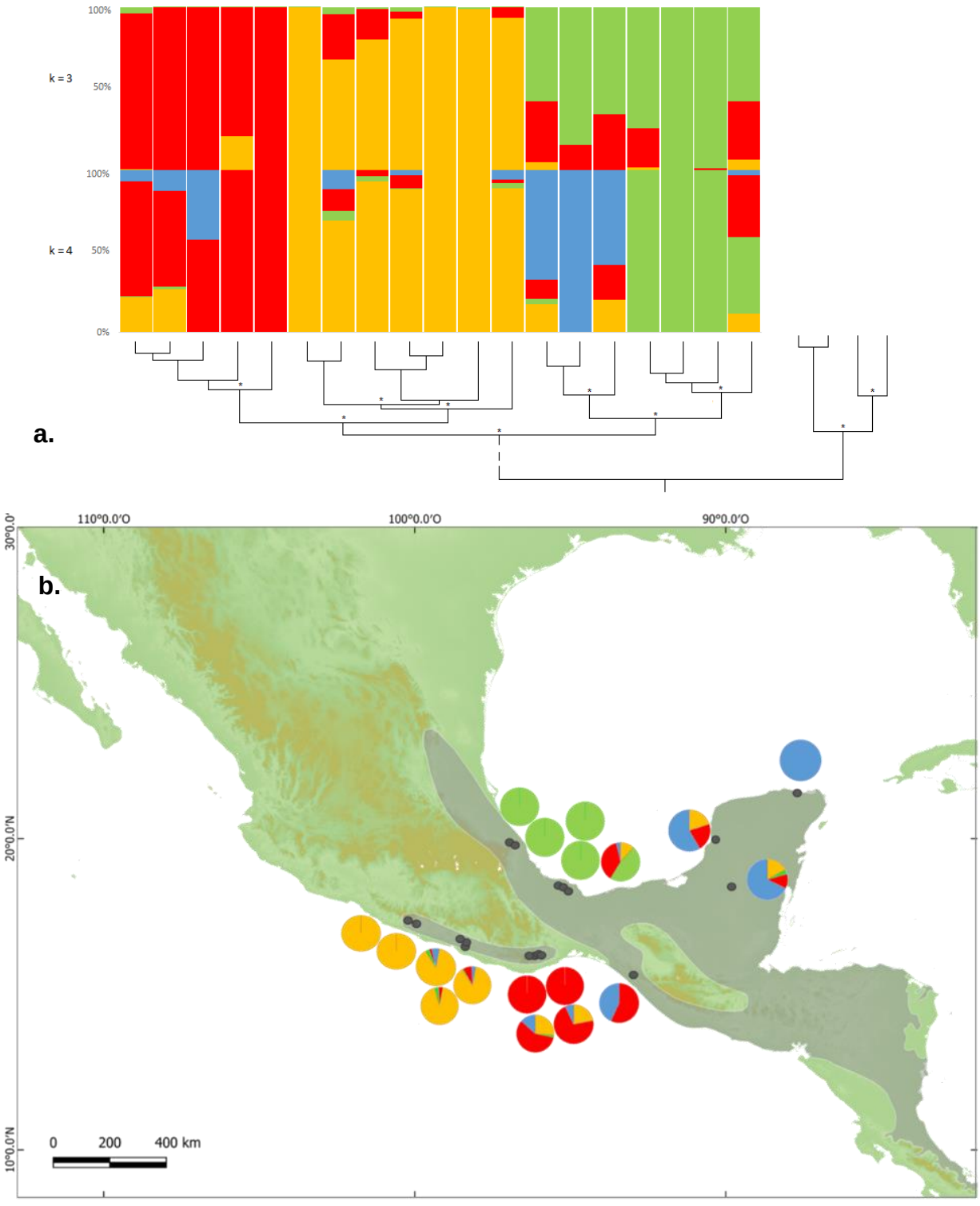


Figure 2- Genetic structure in *Saltator atriceps;* (a) Structure bar plots and Maximum Likelihood tree, nodes with Bootstrap values greater than 80% are depicted with asterisk; (b) Geographic distribution of individuals and its probabilities of assignment for K=4. Distribution ranges of species are depicted in gray and sampling localities in dark gray.

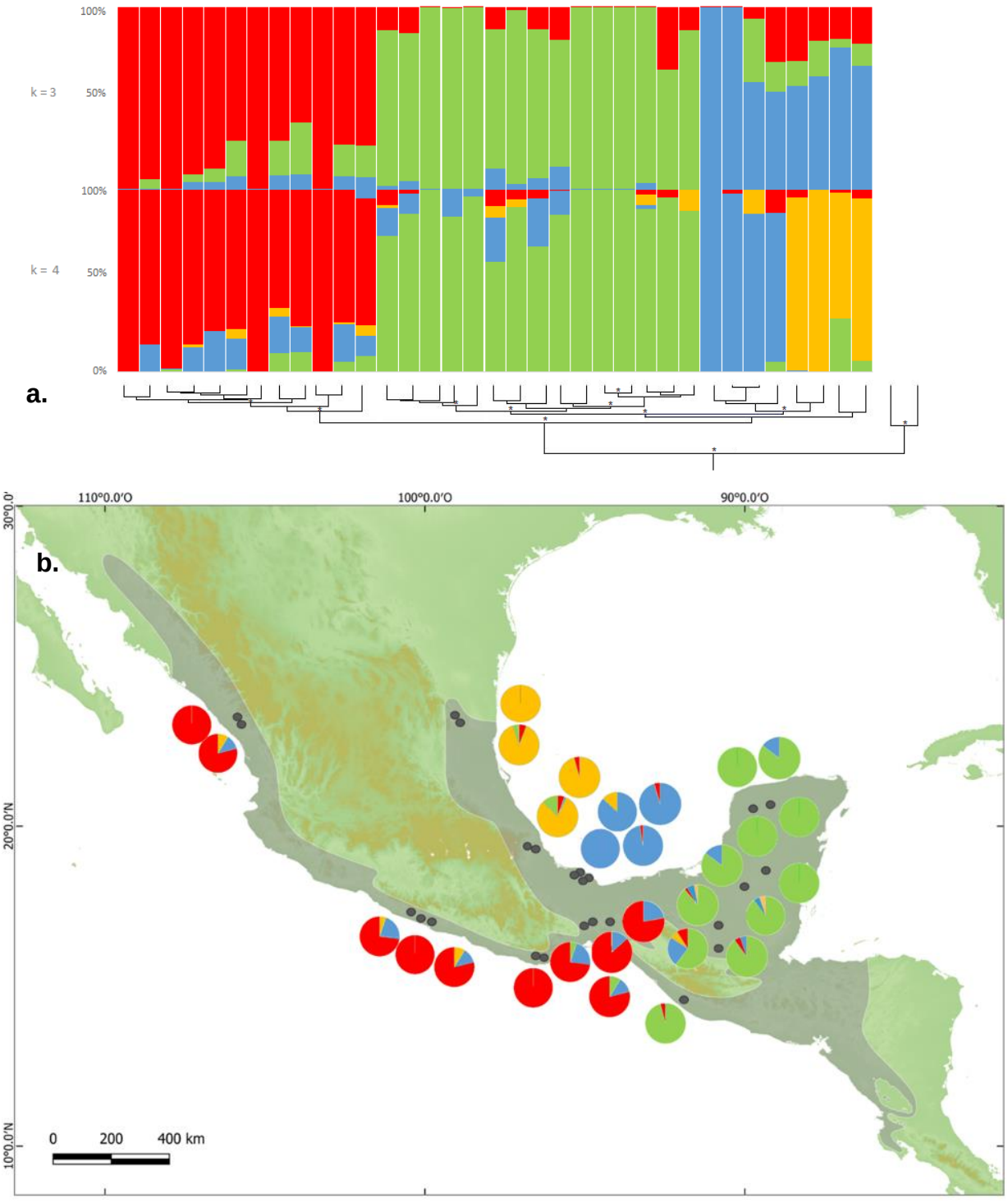


Figure 3- Genetic structure in *Xiphorhynchus flavigaster*; (a) Structure bar plots and Maximum Likelihood tree, nodes with Bootstrap values greater than 80% are depicted with asterisk; (b) Geographic distribution of individuals and its probabilities of assignment for K=4. Distribution ranges of species are depicted in gray and sampling localities in dark gray.

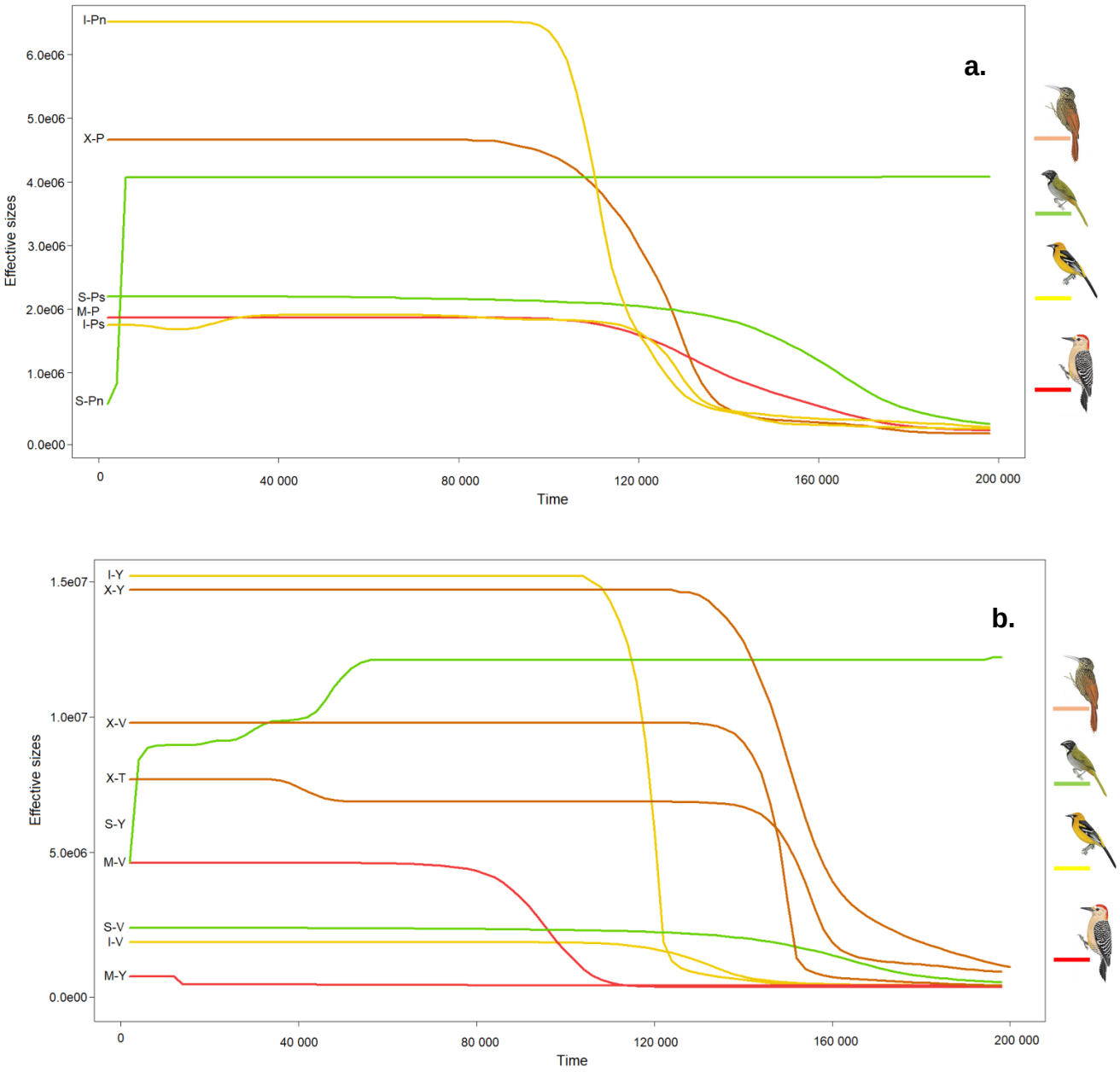


Figure 4- Effective population size through time for populations of *Saltator atriceps*, *Xiphorhynchus flavigaster*, *Melanerpes santacruzi*, and *Icterus gularis* as estimated by the Bayesian Skyline Plot method. The *x*-axis shows times from ~200,000 years to the present, assuming a substitution rate of $1.5x10^{-9}$ per site per year and a generation time of two years. (a) Populations distributed in the Pacific coast, (b) populations distributed in Veracruz, and Yucatan Peninsula. Acronyms are interpreted as follows, first letter depicts taxon (M: *Melanerpes;* S: *Saltator*; X: *Xiphorhynchus*; I: *Icterus*) and second letters corresponds with geographic origin of populations (Y: Yucatan, V: Veracruz, T: Tuxtlas, P: Pacific coast, Pn: North Pacific coast, Ps: South Pacific coast).

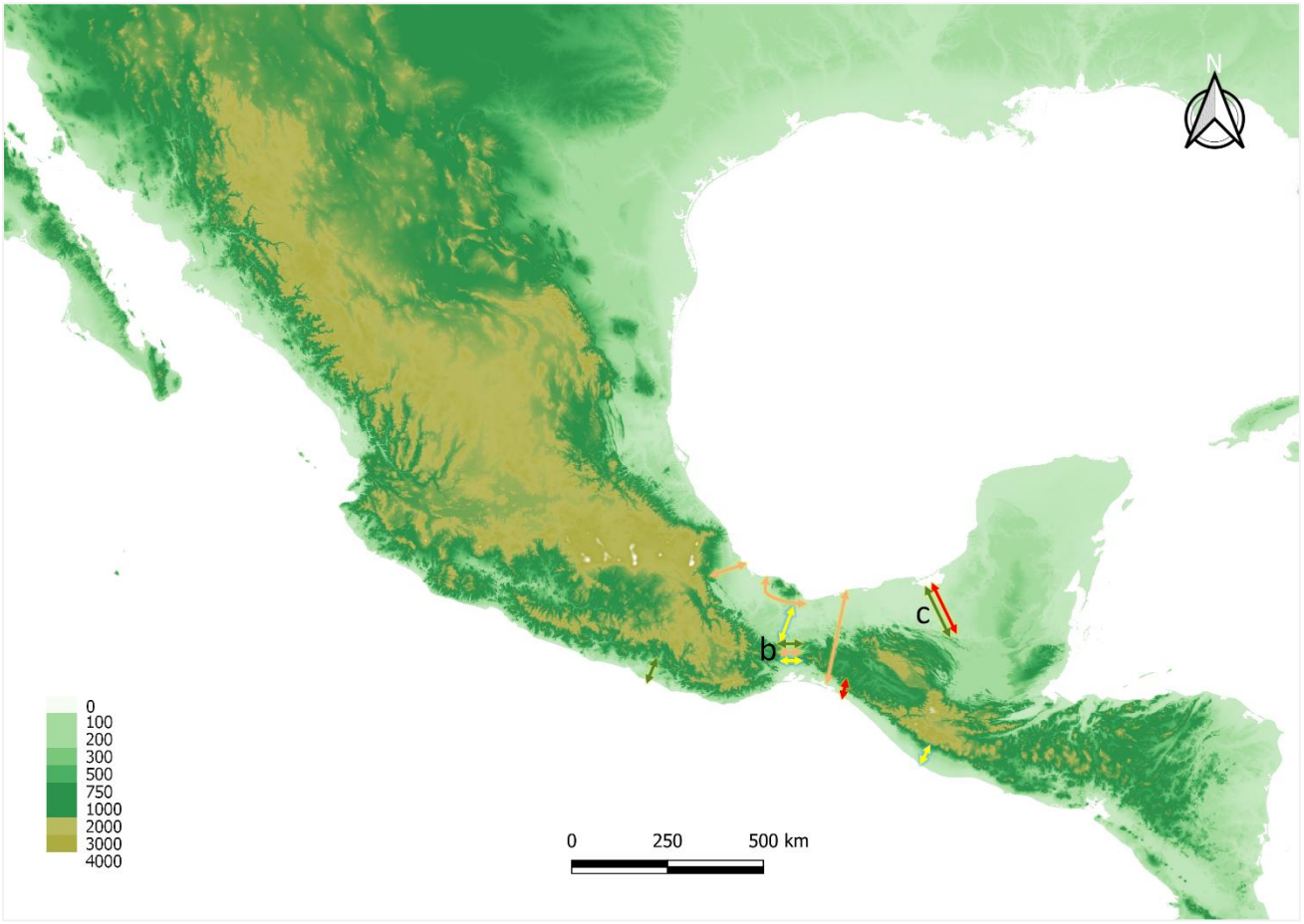
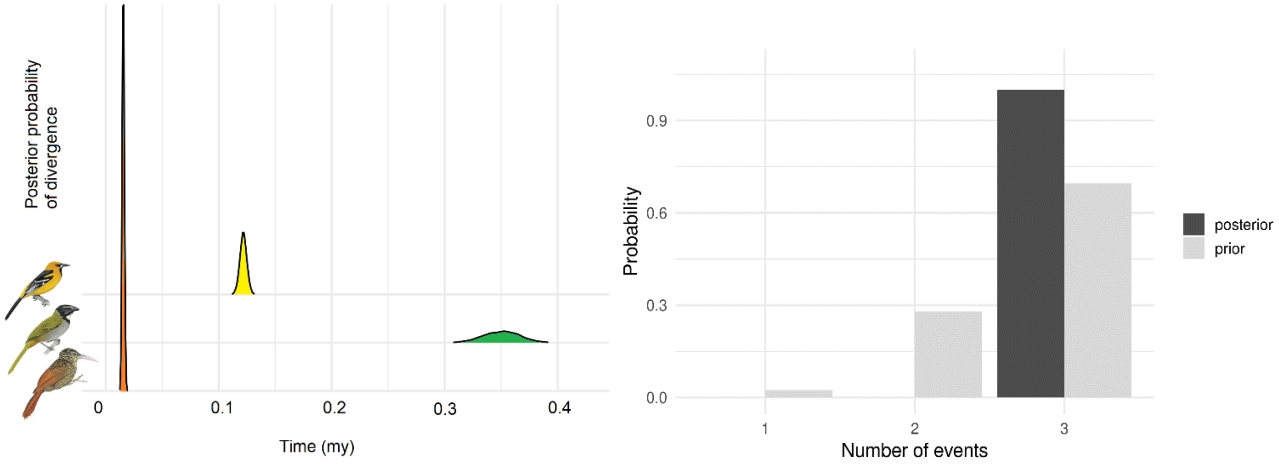


Figure 5- Synchronic divergence test among four species of birds co-distributed in Mexican Tropical Forests. (a) Spatial pattern of genetic structure, approximate population limits are depicted with arrows: *Saltator atriceps* (green), *Xiphorhynchus flavigaster* (light brown), *Melanerpes santacruzi* (red), and *Icterus gularis* (yellow). Shared barriers, Tehuantepec Isthmus and Centla Wetlands, are depicted with letters (b) and (c) respectively. Base map constructed from digital elevation model Hydro1k (https://www.usgs.gov/centers/eros/science/usgs-eros-archive-digital-elevation-hydro1k).

b.



c.

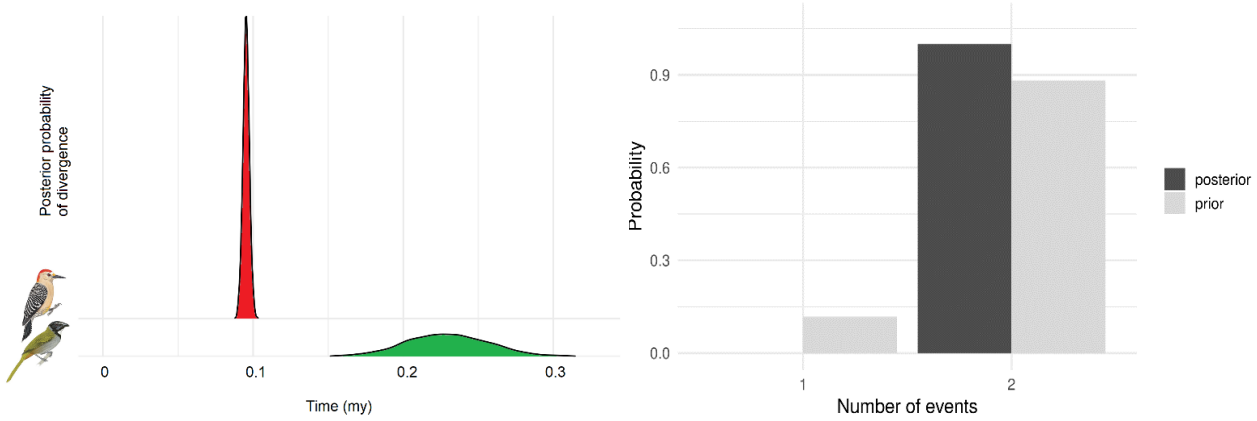
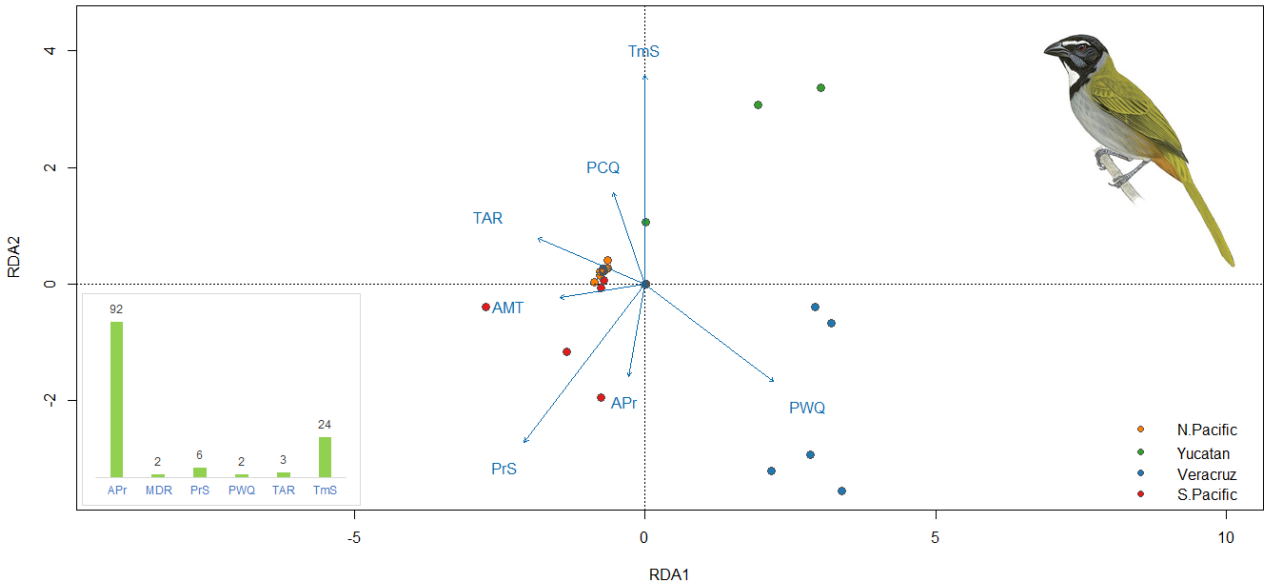


Figure 5 (cont.). (b) Distribution of prior (light bars) and posterior (dark bars) probabilities of the divergence events between pairs of populations distributed in the Pacific and Gulf of Mexico slopes (separated by Tehuantepec Isthmus), and approximate posterior densities of divergence times (in millions of years) for each pair of populations. (c) Distribution of prior (light bars) and posterior (dark bars) probabilities of the divergence events between pairs of populations distributed in the Yucatan Peninsula and Veracruz (separated by Centla Wetlands), and approximate posterior densities of divergence times (in millions of years) for each pair of populations.

a.



b.

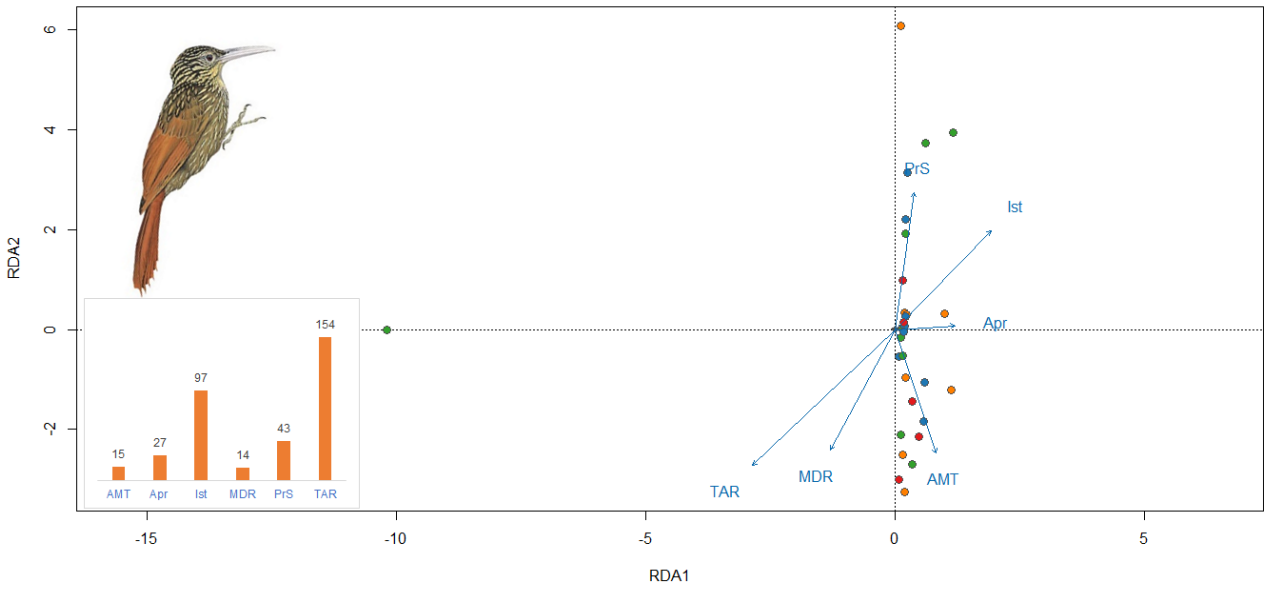
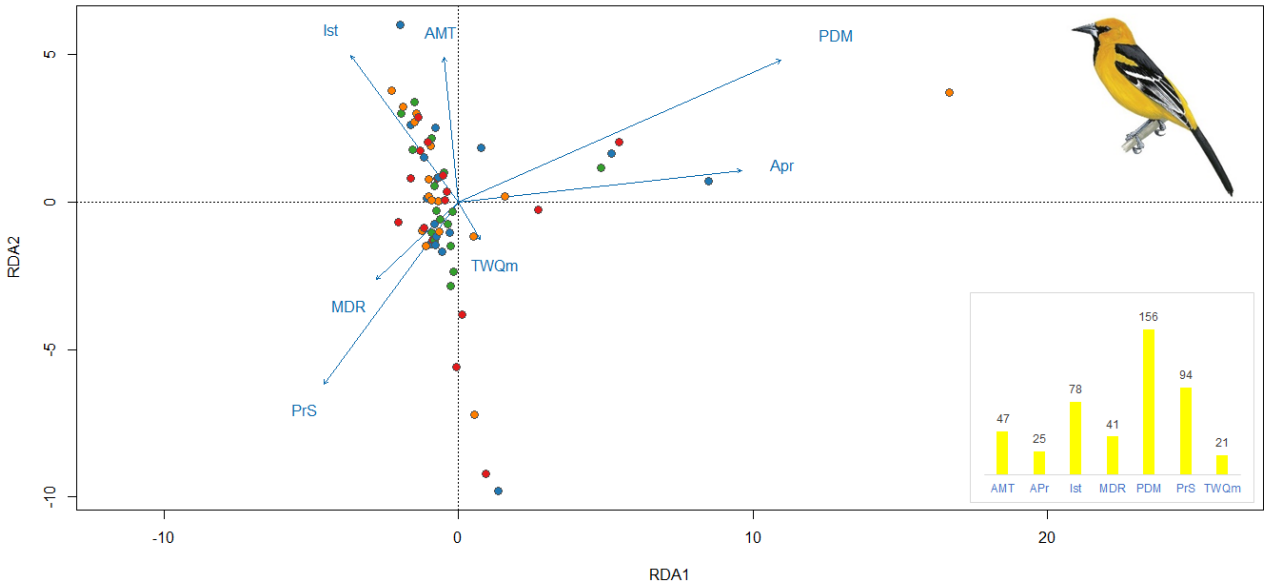


Figure 6- Genetic-environment association analyses for four species of birds co-distributed in Mexican Tropical Forests. Points represent the projection of individual genotypes on the first two RDA axes. The explanatory variables are shown within the space defined by RDA1 and RDA2 by labeled vectors. Their contribution to each axis is represented by the length of their orthogonal projections over the scale bars along the top and right sides of the graphs. Arrows indicate the direction of the gradient of variation for the corresponding environmental parameter. (a) Projection

for *Saltator atriceps*; (b) projection for *Xiphorhynchus flavigaster*; Environmental variables: Temperature Annual Range (TAR), Precipitation Seasonality (PrS), Annual Precipitation (Apr), Annual Mean Temperature (AMT), Isothermality (Ist), Mean Diurnal Range (MDR), Mean Temperature of Warmest Quarter (PWQm), Precipitation of Driest Quarter (PDQ), Temperature Seasonality (TmS), Precipitation of Coldest Quarter (PCQ), Precipitation of Wettest Quarter (PWQ), and Precipitation of Driest Month (PDM).

c.



d.

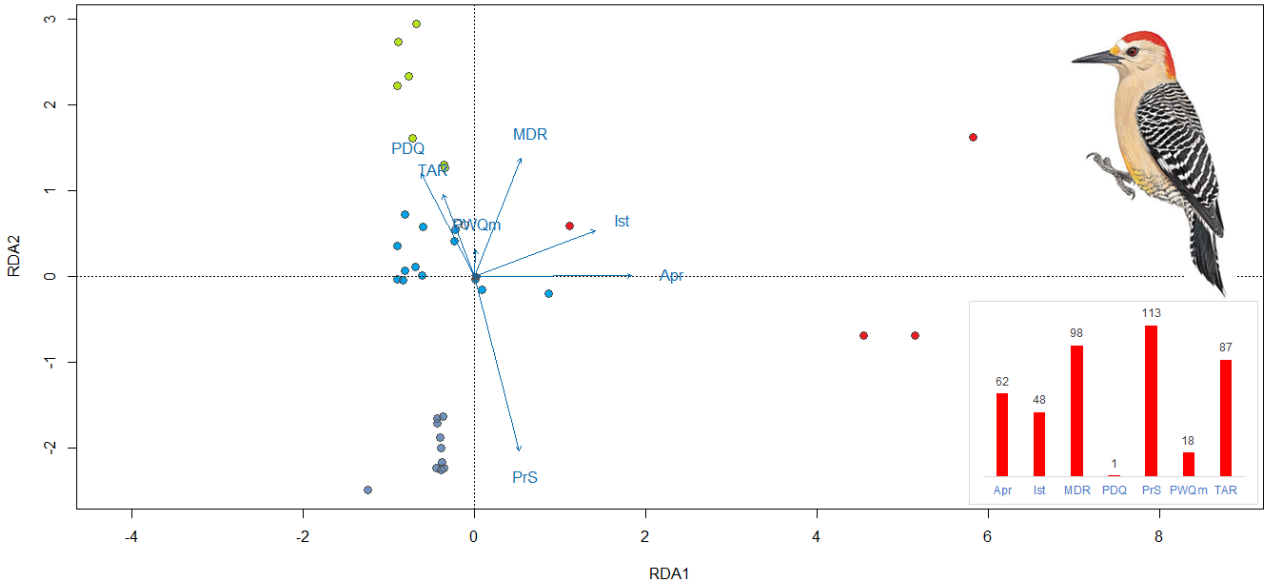


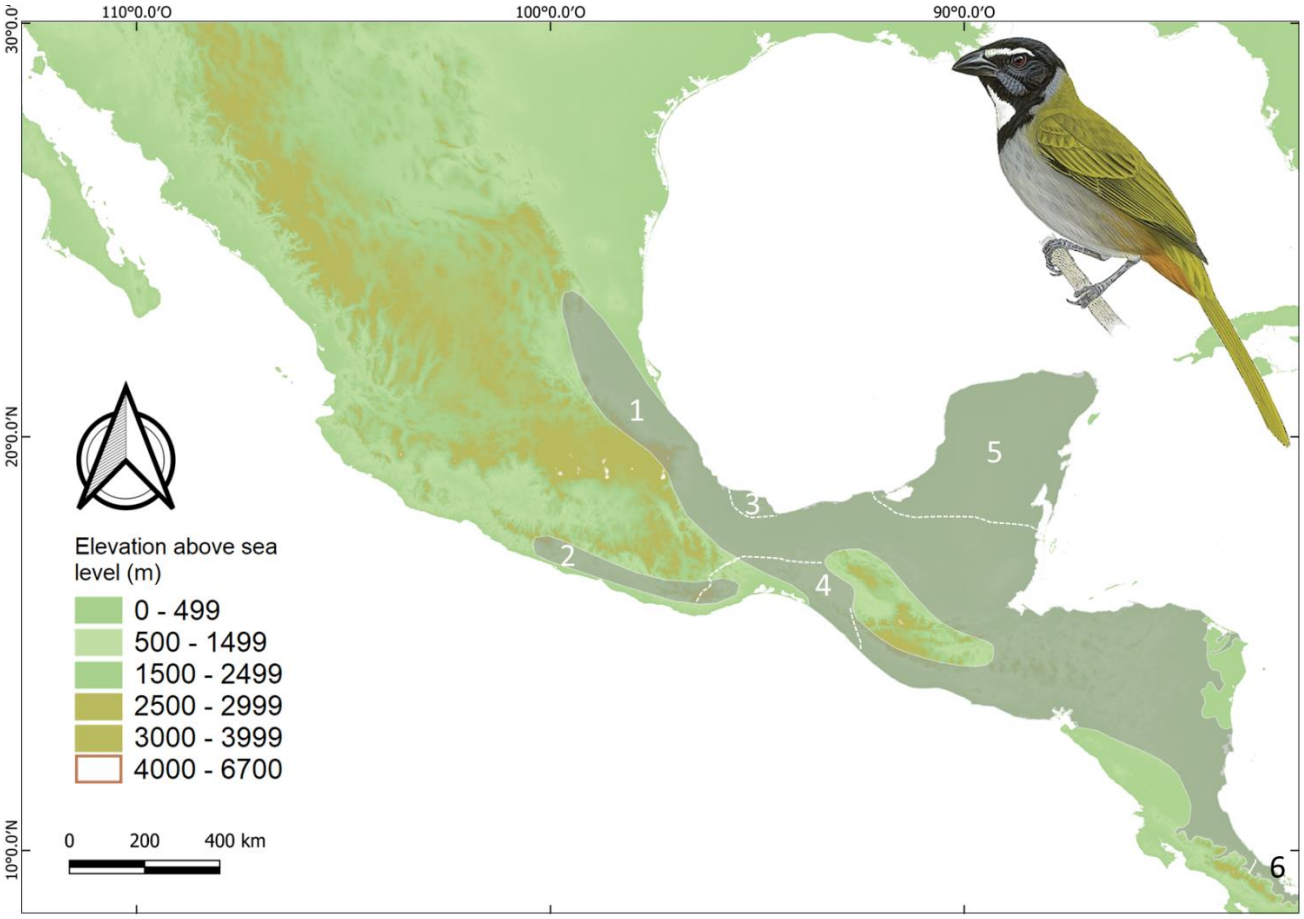
Figure 6 (cont.)- Genetic-environment association analyses for four species of birds co-distributed in Mexican Tropical Forests. (c) projection for *Melanerpes santacruzi*; (d) projection for *Icterus gularis*.

SUPPLEMENTARY MATERIALS

Comparative population genomics provide evidence of independent evolutionary history of co-distributed avifauna of Mexican tropical forests

Alexander Llanes-Quevedo, Luis A. Sánchez-González, Alicia Mastretta-Yanes, Alberto Rocha-Méndez, Borja Milá, María Recuerda and Adolfo G. Navarro-Sigüenza

Supplementary File 1. Geographic variation of the Black Headed Saltator (*Saltator atriceps:* Passeriformes: Thraupidae).



Subspecies *atriceps* occurs along the Caribbean slope from eastern Mexico (except for the southeast coast of Veracruz and the Yucatán Peninsula) south to eastern Costa Rica, also on the Pacific slope from Guatemala to Costa Rica (1).

Subspecies *suffuscus* is restricted to the Sierra de Los Tuxtlas, in the southeastern coast of Veracruz, Mexico (3).

Subspecies *flavicrissus* occurs on the Pacific slope of southern Mexico, from central Guerrero to east-central Oaxaca (west of the Isthmus of Tehuantepec (3);

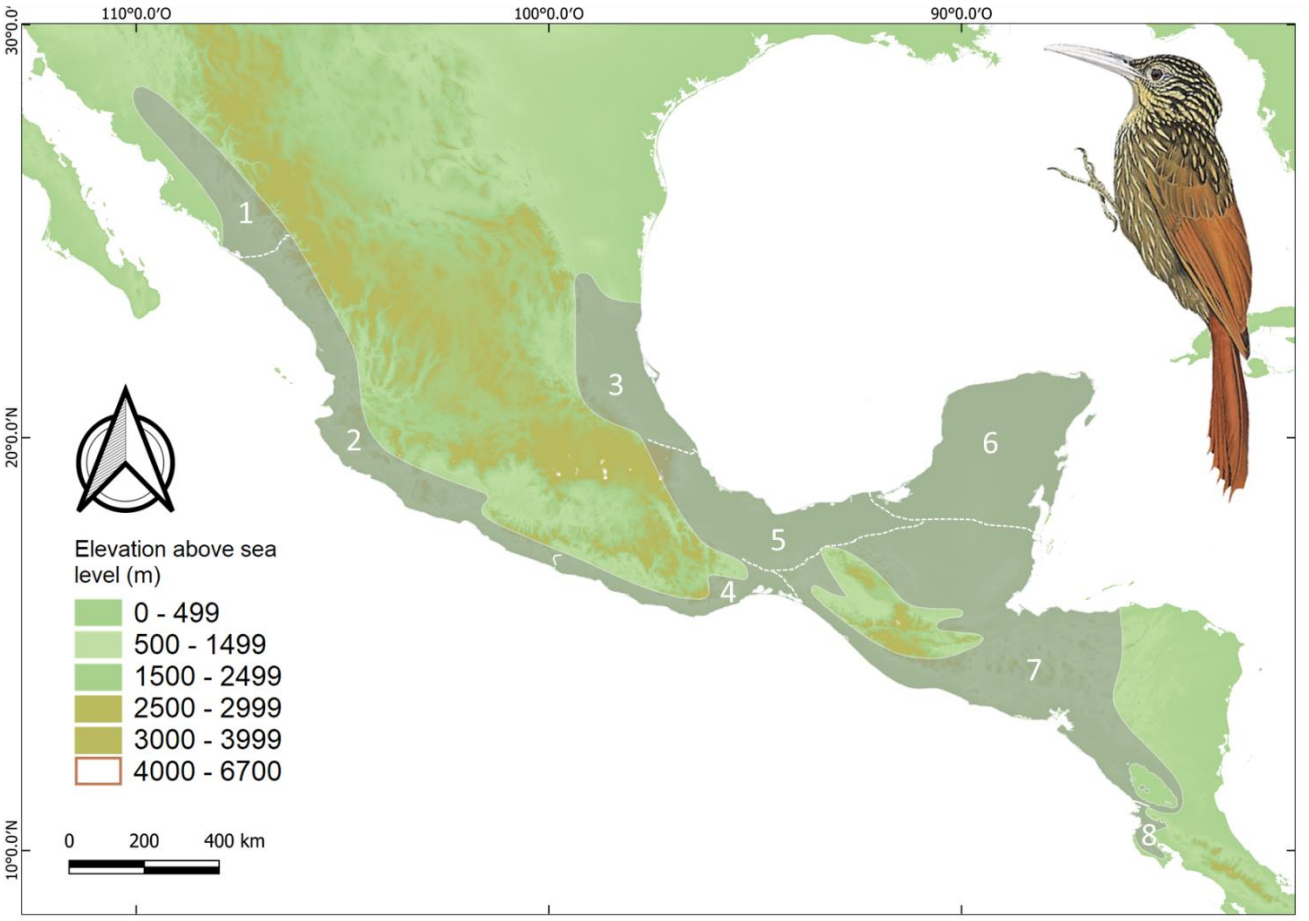
Subspecies *peeti* occurs on the Pacific slope of southern Mexico, in eastern Oaxaca (east of the Isthmus of Tehuantepec) and Chiapas (4);

Subspecies*raptor* occurs in southeastern Mexico on the Yucatan peninsula, in Yucatan, Quintana Roo, and Campeche (5);

Subspecies *lacertosus* occurs in Panama, east of the Canal Zone; possibly also in Costa Rica (Ridgway, 1901) (6).

For subspecies *peeti* and *raptor*, intergradation with *atriceps* has been described (Paynter 1955; Binford 1989).

Supplementary File 2. Geographic variation of the Ivory-billed woodcreeper (*Xiphorhynchus flavigaster:* Passeriformes: Furnariidae).



Subspecies *flavigaster* is distributed in the southwestern Mexico, including the states of Guerrero and South Oaxaca (4).

Subspecies *tardus* occurs in northwestern Mexico in southeastern Sonora, north Sinaloa, and west Durango (1).

Subspecies *mentalis* in the coasts of western Mexico (from center and south Sinaloa and west Durango to Michoacán and west Guerrero)(2);

Subspecies *saltarius*, in northeastern Mexico (south Tamaulipas and southeastern San Luis Potosí to north Veracruz) (3); *ascensor*, in the Caribbean slope of Mexico from south Veracruz and north Oaxaca to Tabasco, possibly also in North Chiapas and Guatemala (5)

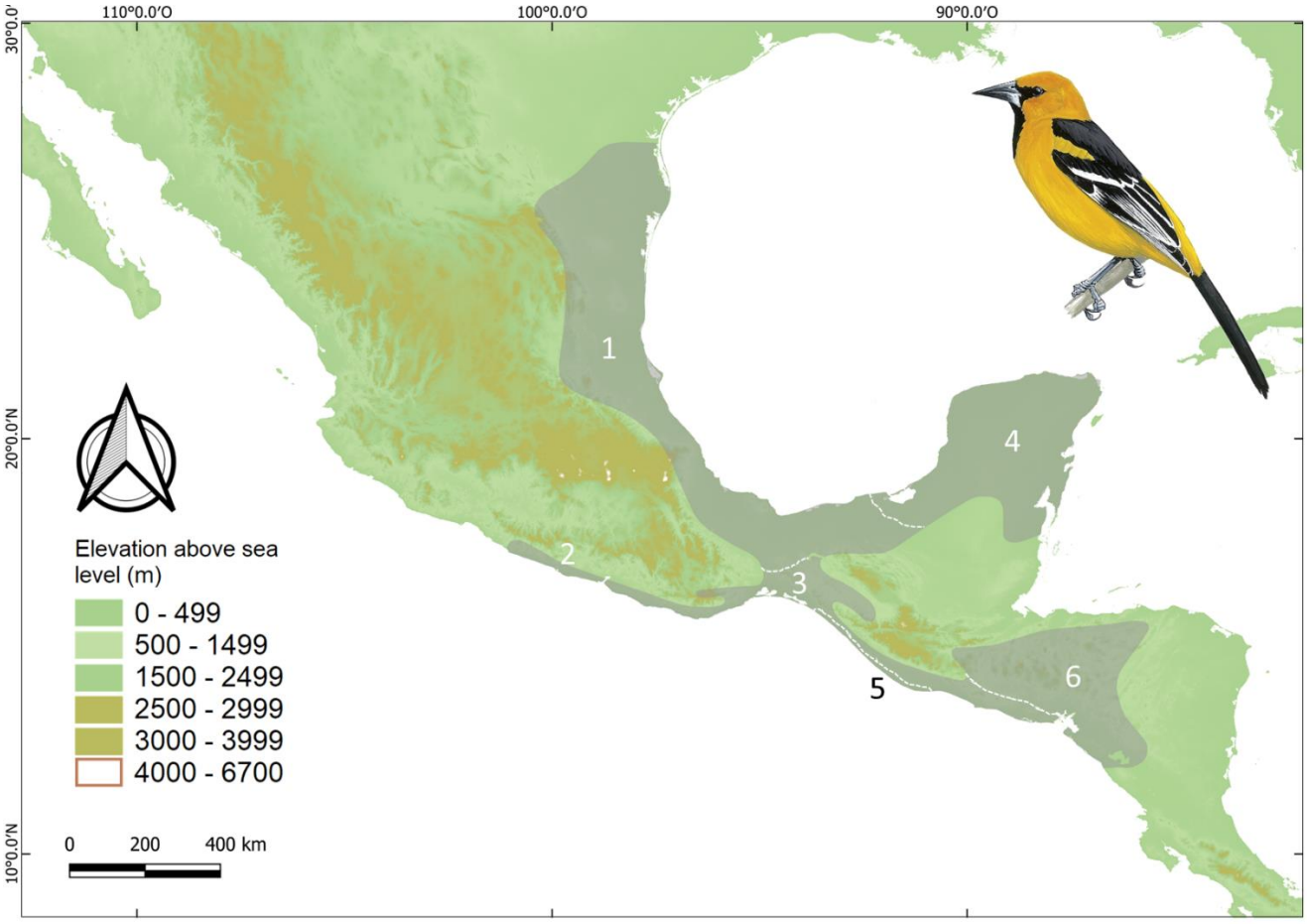
Subspecies *yucatanensis* is distributed along the Yucatán Peninsula to Belize and Guatemala (6);

Subspecies *eburneirostris*, ranges in the Caribbean slope of Belize, Guatemala and Honduras and the Pacific slope from south Mexico (southeastern Oaxaca and Chiapas) to northwestern Costa Rica (7).

Subspecies *ultimus* occurs in hills and adjacent lowlands of Nicoya Peninsula, in northwestern Costa Rica (8).

For subspecies *mentalis* has been described intergradation with the nominal one in western Guerrero and with *tardus* in western Durango and *yucatanensis* populations at the base of the Yucatan Peninsula show signs of intergradation with *eburneirostris*, as perhaps *ultimus* does in the lowlands of northeastern Costa Rica.

Supplementary File 3. Geographic variation of the Altamira Oriole (*Icterus gularis:* Passeriformes: Icteridae).



Subspecies *gularis* occupy the arid tropical zone of Pacific coastal slope from southwestern Mexico (Oaxaca and Chiapas) south to interior Guatemala and El Salvador (3).

Subspecies *tamaulipensis* occurs from south Texas (lower Rio Grande Valley) through eastern Mexico to Puebla, and Campeche (1).

Subespecie *yucatanensis* is distributed in Yucatan Peninsula, Cozumel Islands, and extreme north Belize (4).

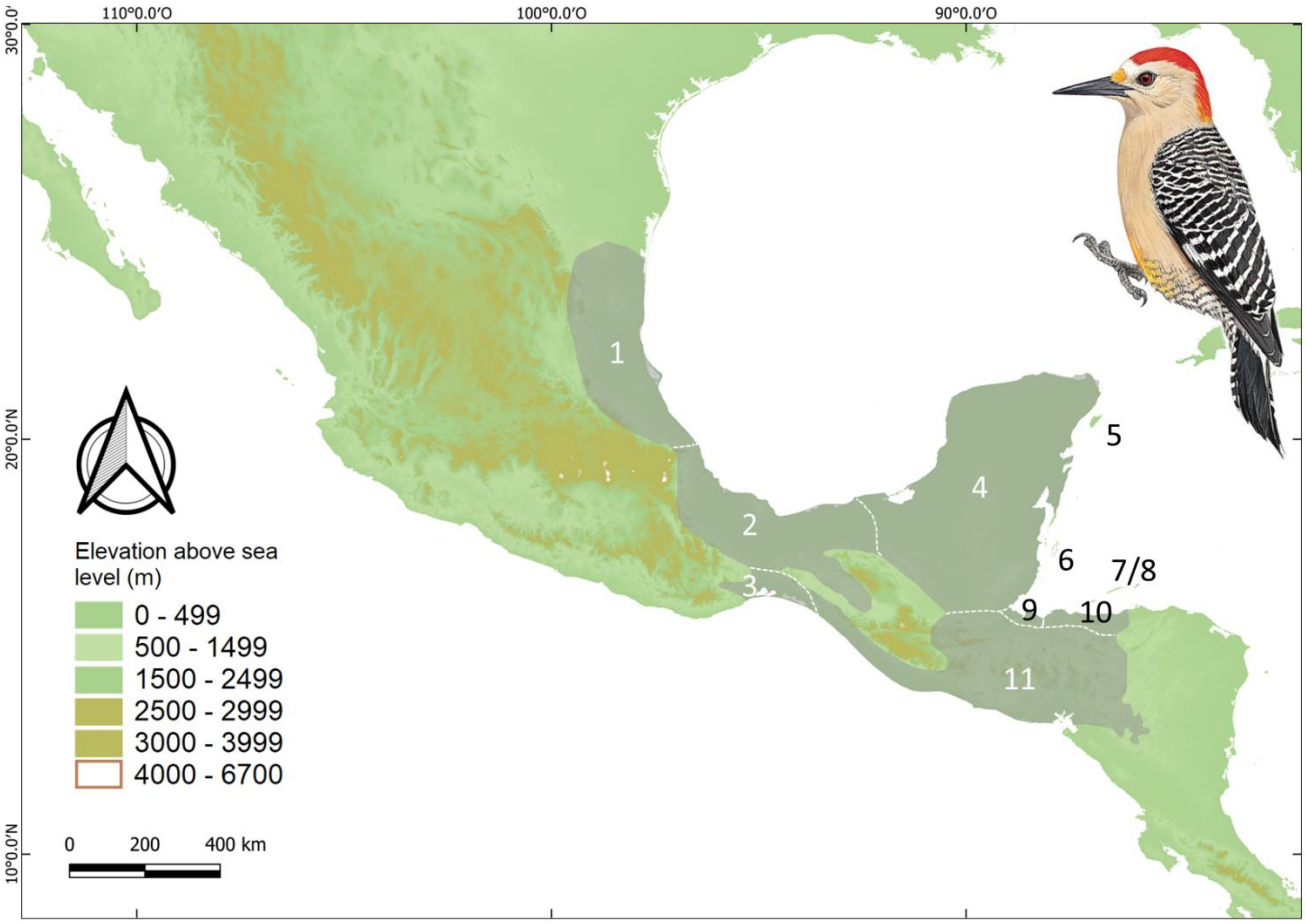
Subespecie *flavescens* in the coast of Guerrero state (2).

Subespecie *troglodytes* in eastern Chiapas, and Pacific slope of Guatemala (5).

Subespecie *gigas,* in interior Guatemala (dryer portions of Río Negro and Río Motagua valleys) to Honduras and Nicaragua (6).

The subspecies *troglodytes* has been described to intergrade with *gularis* (Brush and Pleasants, 2020).

Supplementary File 4. Geographic variation of the Velazquez Woodpecker (*Melanerpes santacruzi:* Piciformes: Picidae).



The subspecies of *M. santacruzi* have been classified in three morphological groups:

1) Group *santacruzi*: Orange-red nape continuous with red crown-patch; belly yellow to yellow orange; nasal tufts yellow to orange; white bars relatively narrow; central rectrices sometimes have white at base; white wing-patch reduced or absent. The *santacruzi* group includes subspecies *santacruzi* (11)*, grateloupensis* (1)*, hughlandi* (9)*, pauper* (10), and *insulanus* (7), and ranges from San Luis Potosí and southwestern Tamaulipas to north Nicaragua.

2) Group *dubius:* red nape, nasal tufts, and belly; narrow white bars dorsally; and *polygrammus* group: white barring on central rectrices, yellow nape merges with red crown-patch, and white barring slightly narrower. The *dubius* group occurs Atlantic slope of south Veracruz to northeastern Guatemala, the Yucatan Peninsula and nearby islands, and includes subspecies *dubius* (4)*, veraerucis* (2), *leei* (5)*, turneffensis* (6)*,* and *canescens* (8)*.*

3) Group *polygrammus* (3) is monotypic group and is distributed on the Pacific slope of Oaxaca, across southern portion Isthmus of Tehuantepec and arid interior valley of Chiapas.

Supplementary Figures

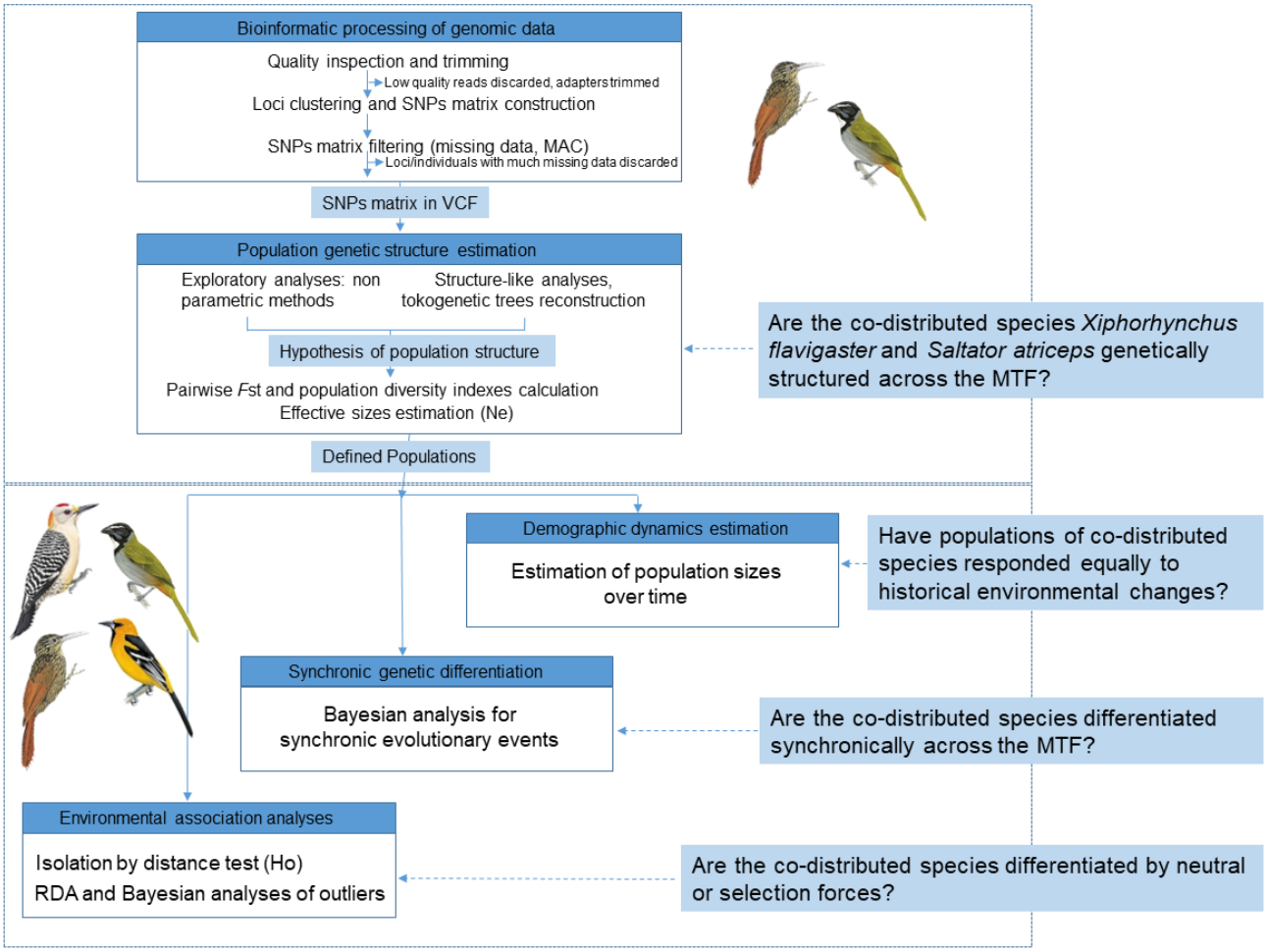


Figure S1. General overview of the project.

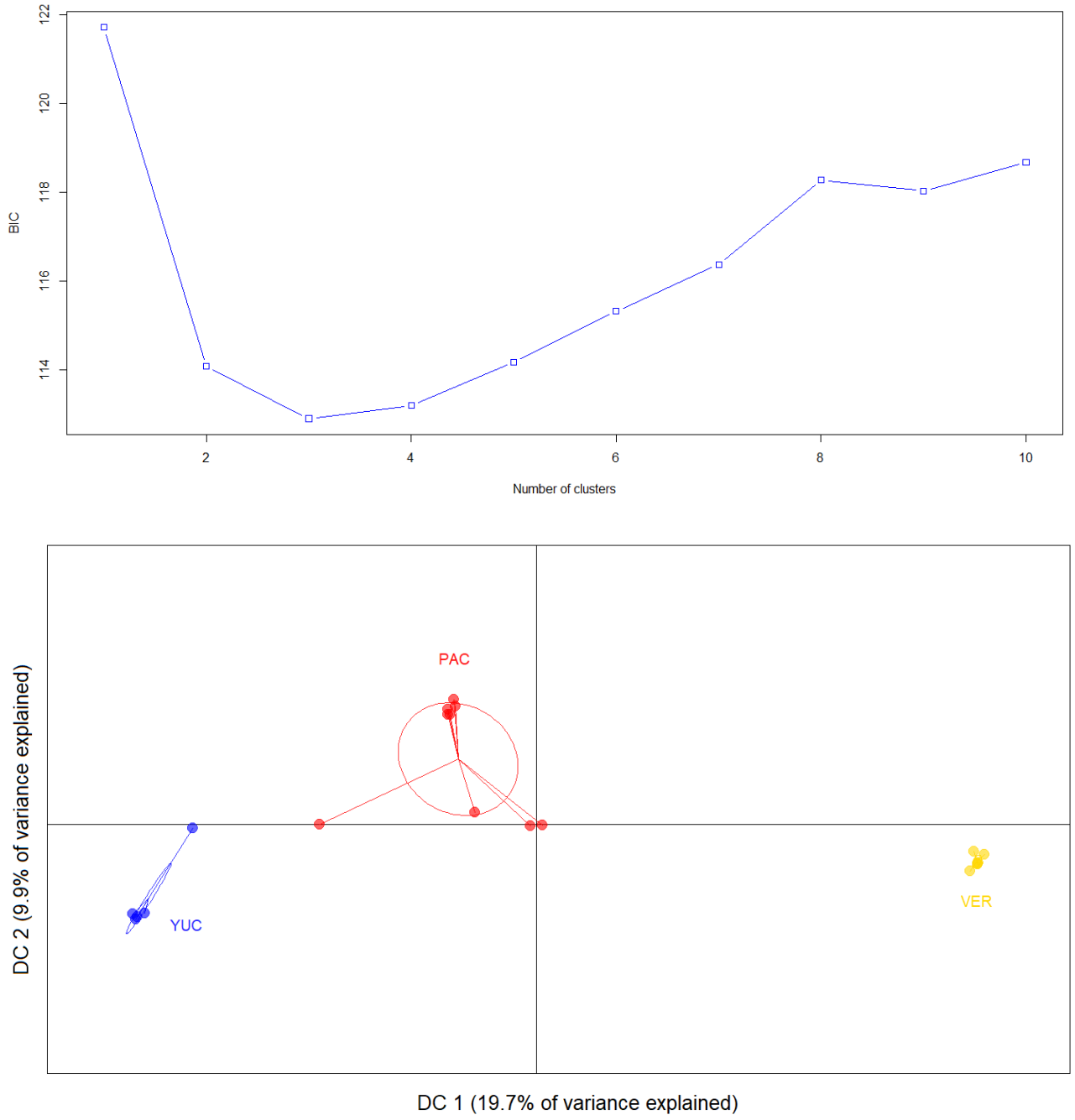


Figure S2. Genomic variation by non-parametric DAPC on *Saltator atriceps* from Mexican Tropical Forests. (a) Values of BIC for tested numbers of genetic clusters (k); (b). DAPC plot for optimal value of k=3, samples from the Pacific coast of Mexico are depicted in red, from Veracruz in yellow, and from Yucatan Peninsula, in blue.

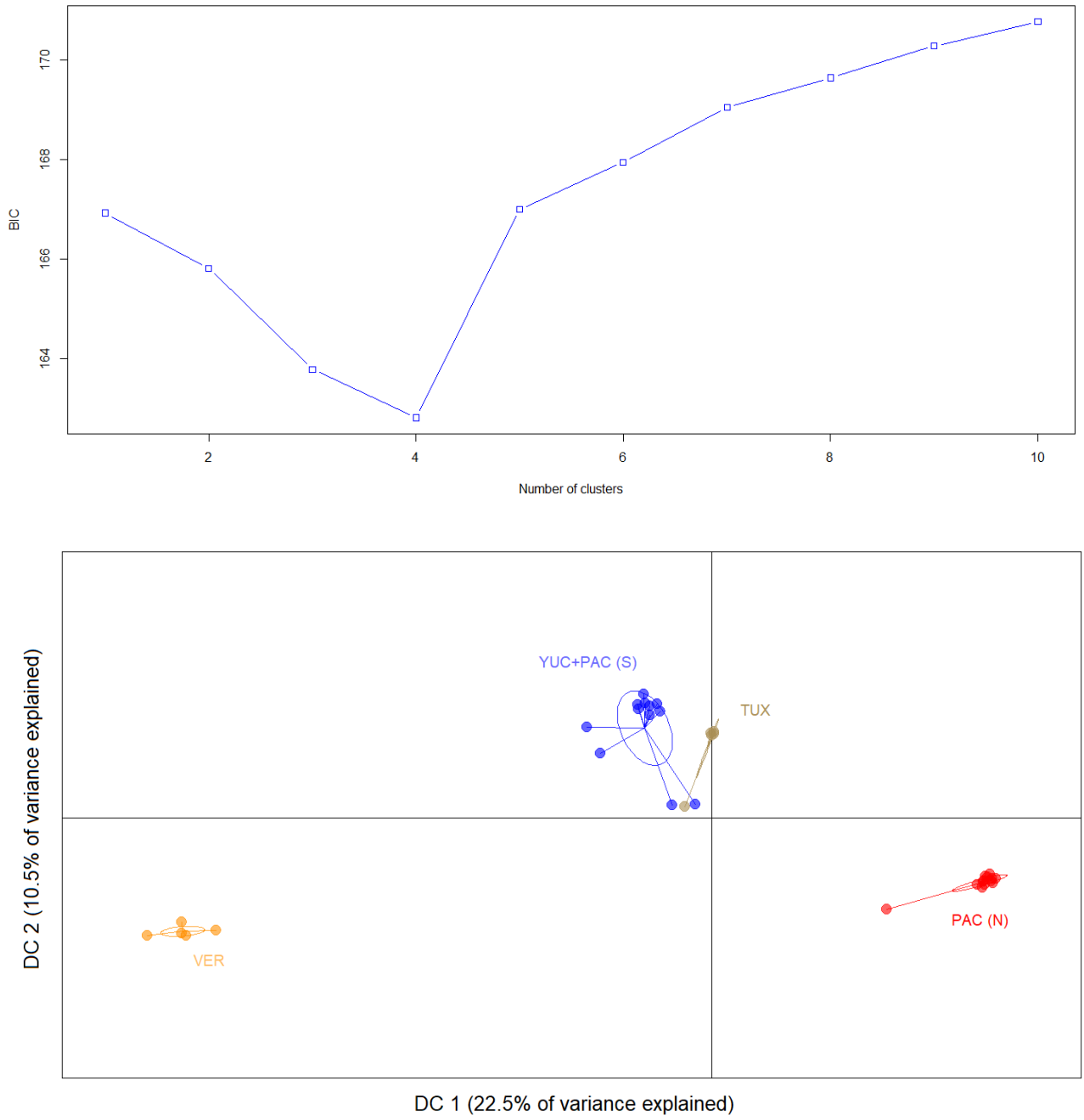


Figure S3. Genomic variation by non-parametric DAPC on *Xiphorhynchus flavigaster* from Mexican Tropical Forests. (a) Values of BIC for tested numbers of genetic clusters (k); (b). DAPC plot for optimal value k=4, samples from the northeastern portion of Pacific coast of Mexico are depicted in red, from the Yucatan Peninsula + southern extreme of Pacific coast of Mexico + Los Tuxtlas, in blue, another group of individuals from Los Tuxtlas in gray, and samples from northern Veracruz, in yellow.

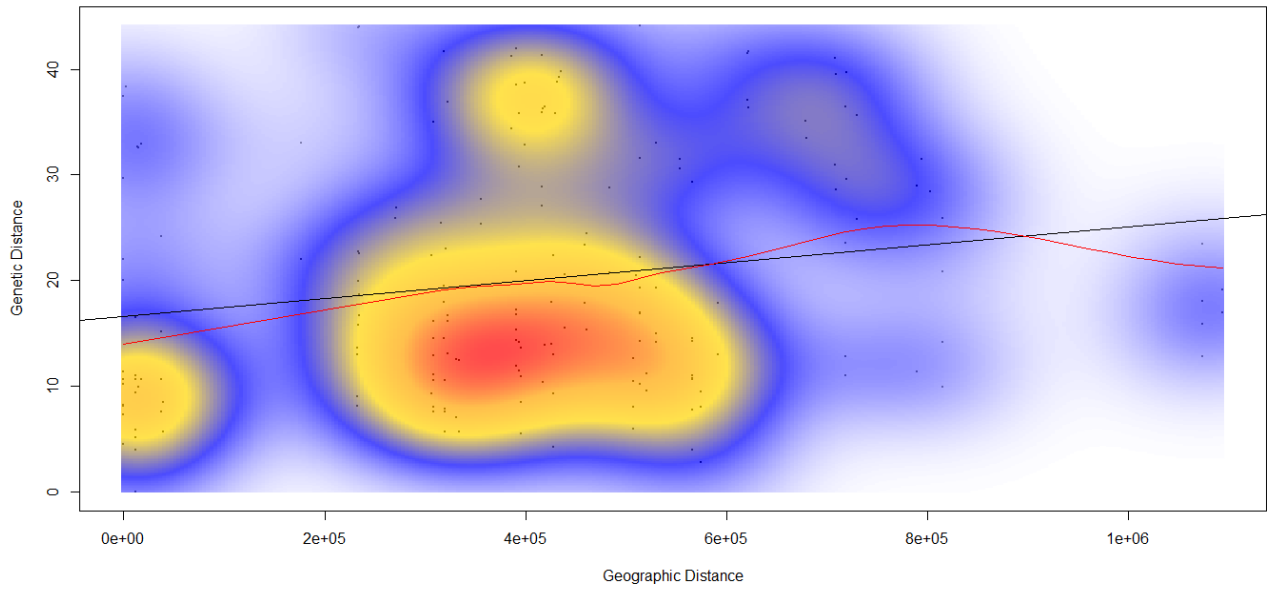


Figure S4. Mantel Test for *Saltator atriceps* (R^2= 0.2177; p: 0.1838).

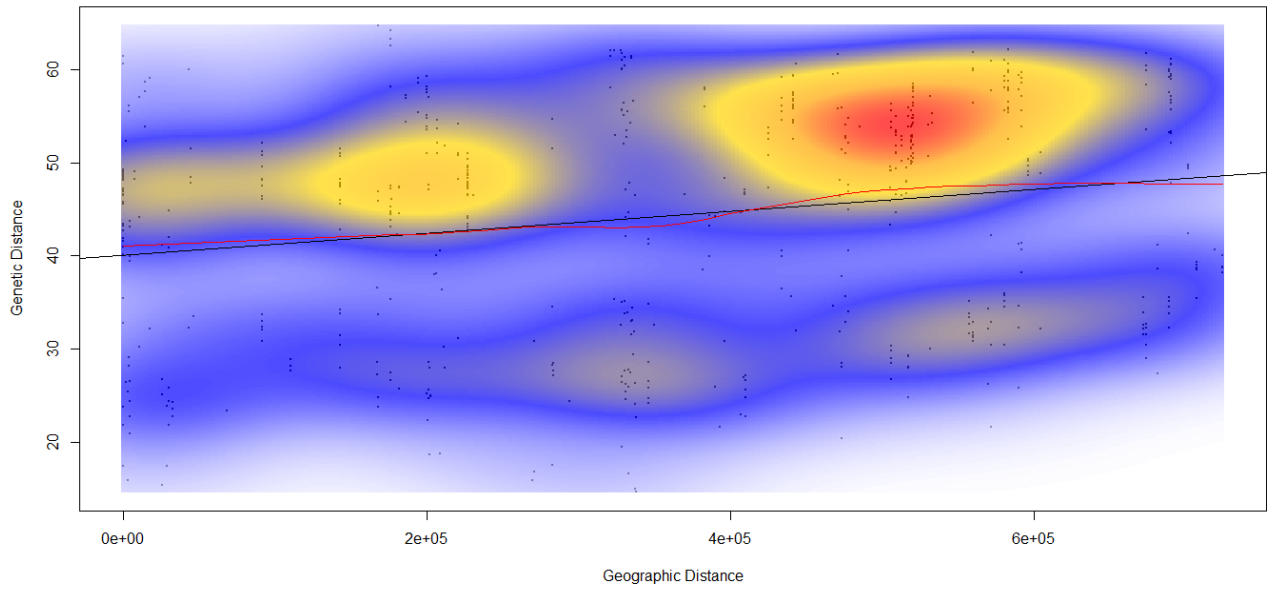


Figure S5. Mantel Test for *Xiphorhynchus flavigaster* (R^2= 0.235; p: 1e-04).

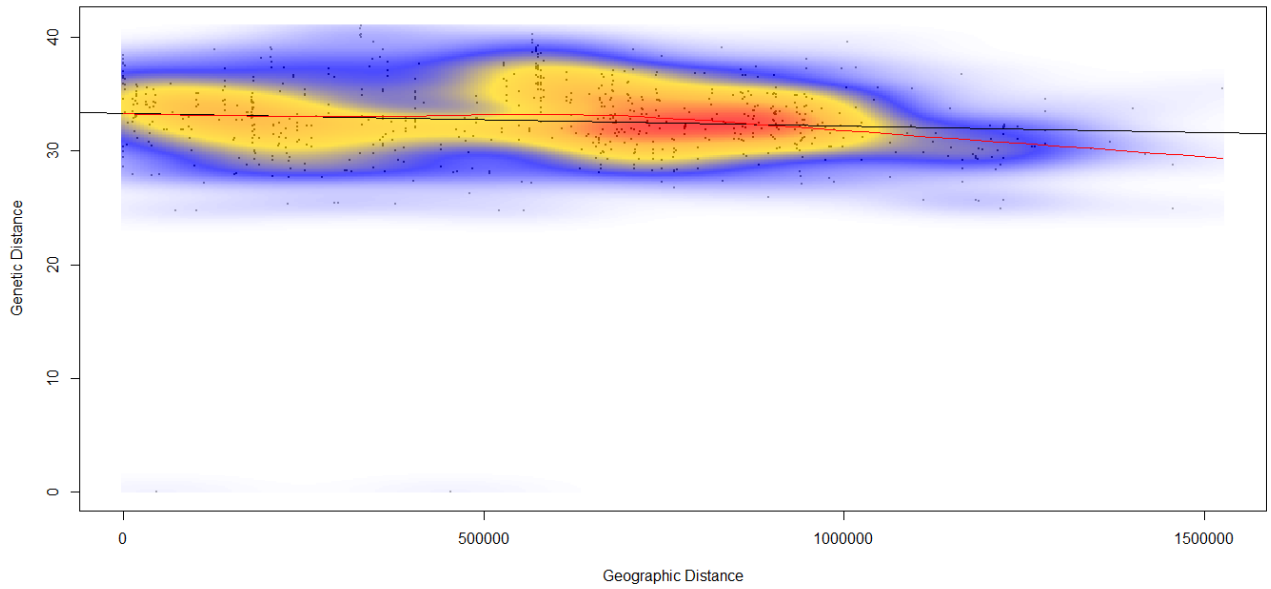


Figure S6. Mantel Test for *Melanerpes santacruzi* (R^2= -0.1618; p: 0.958).

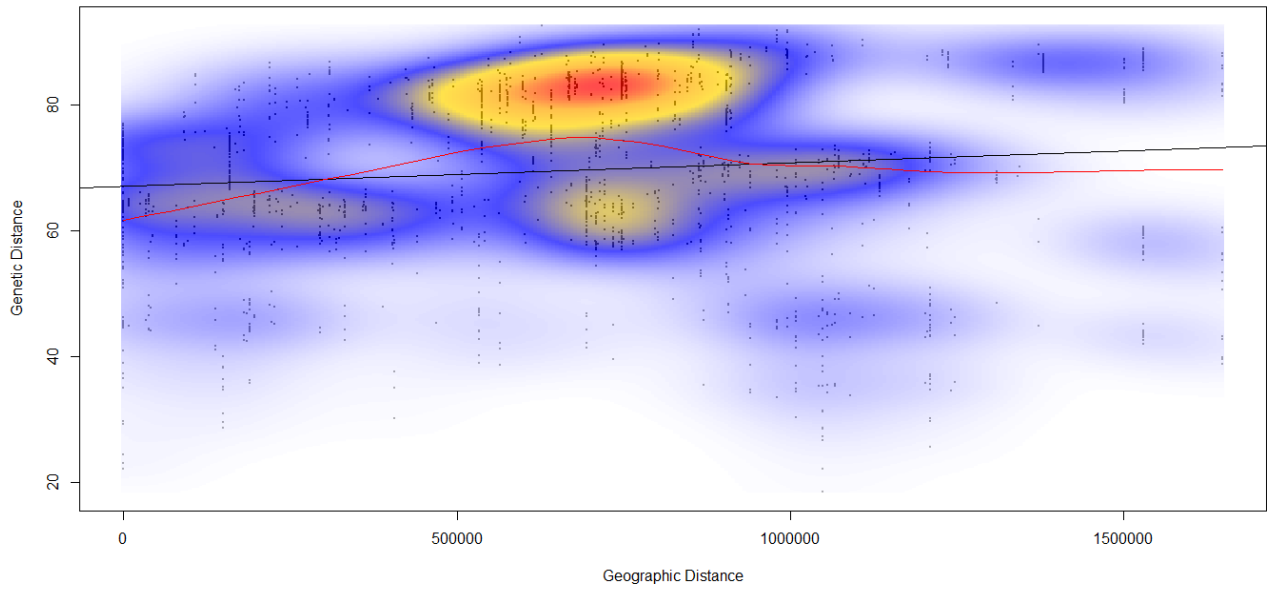


Figure S7. Mantel Test for *Icterus gularis* (R^2= 0.1522; p: 0.0064).

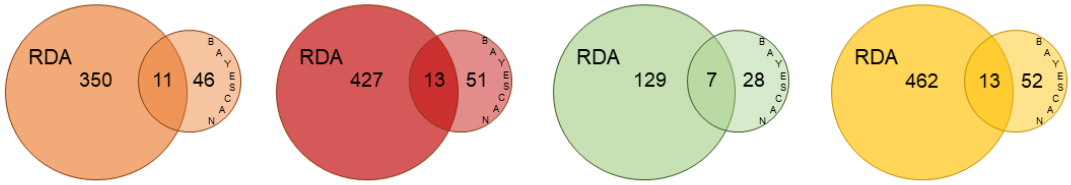


Figure S.8. Venn diagram showing the overlap of the outlier loci identified with Redundancy Analyses (RDA) and Bayescan methods for *Xiphorhynchus flavigaster* (depicted in brown), *Melanerpes santacruzi* (depicted in red), *Saltator atriceps* (depicted in green), and *Icterus gularis* (depicted in yellow).

Table S1. Samples of *Saltator atriceps* and close relatives, used for genomic analyses in this study. Acronyms used in the Collection Catalog Number field correspond to the specimens in the Museo de Zoología "Alfonso L. Herrera" of the Faculty of Sciences at the UNAM, México.

Species	NUMCOLEC	Sample	Longitude	Latitude
Saltator atriceps	YUC13 031	Skin	-87.66008	21.4646
Saltator atriceps	PEP 0711	Skin	-100.25	17.3333333
Saltator atriceps	PEP 0096	Skin	-95.0666667	18.6
Saltator atriceps	PEP 0071	Skin	-95.0666667	18.6
Saltator atriceps	PEP 0058	Skin	-95.0666667	18.6
Saltator atriceps	PEP 0016	Skin	-95.0666667	18.6
Saltator atriceps	ORSDON 133	Skin	-96.9435111	19.5139306
Saltator atriceps	OMVP 0707	Tissue/Skin	-97.8883333	16.82
Saltator atriceps	OMVP 0704	Tissue	-97.88	16.8283333
Saltator atriceps	OMVP 0186	Tissue	-97.9066667	16.9633333
Saltator atriceps	MZFC04 141	Tissue	-93.8833333	17.8708333
Saltator atriceps	MOL15 90	Skin	-96.36522	15.86905
Saltator atriceps	MOL15 85	Skin	-96.36522	15.86905
Saltator atriceps	MOL14 080	Skin	-96.5469	15.9531
Saltator atriceps	MISA 072	Tissue	-96.8795278	19.79625
Saltator atriceps	MISA 051	Tissue	-96.8706111	19.8161667
Saltator atriceps	MEX 118	Skin	-94.8319444	18.3211111
Saltator atriceps	MEX 115	Skin	-94.8319444	18.3211111
Saltator atriceps	KABS 012	Skin	-96.3116667	18.1
Saltator atriceps	KAB 044	Skin	-96.2966667	18.22
Saltator atriceps	GES 289	Skin	-90.2561111	18.5927778
Saltator atriceps	GAL 307	Skin	-95.0666667	18.6
Saltator atriceps	GAL 201	Skin	-95.0666667	18.6
Saltator atriceps	CHISTR 139	Skin	-92.8219722	15.7715556
Saltator atriceps	CONACYT99 105	Skin	-90.395	20.1761111
Saltator atriceps	CONACYT04 253	Skin	-96.4844444	16.0691667
Saltator atriceps	CJL 059	Skin	-96.2616667	18.0166667
Saltator atriceps	AMT 385	Tissue	-96.42	15.925
Saltator atriceps	AGNS 0430	Skin	-100.3	17.25
Saltator atriceps	AGNS 0276	Skin	-100.3	17.25
Saltator atriceps	AV 502	Skin		
Saltator atriceps	AV 571	Skin		
Saltator atriceps	AV 799	Skin		
Saltator vigorsii	CPM 210	Tissue		
Saltator vigorsii	CPM 065	Tissue		
Saltator grandis	HGO-SLP 418	Tissue		
Saltator grandis	CHIS 2007	Tissue		
Saltator maximus	MZFC04 017	Tissue		

Table S2. Samples of *Xiphorhynchus flavigaster* and close relatives, used for genomic analyses in this study. Acronyms used in the Collection Catalog Number field correspond to the specimens in the Museo de Zoología “Alfonso L. Herrera” of the Faculty of Sciences at the UNAM, México.

Species	NUMCOLEC	Sample	Longitude	Latitude
Xiphorhynchus flavigaster	YACH 174	Skin	-90.9766667	16.0841667
Xiphorhynchus flavigaster	YACH 139	Tissue	-90.9766667	16.0841667
Xiphorhynchus flavigaster	YACH 109	Skin	-90.9766667	16.0841667
Xiphorhynchus flavigaster	YACH 082	Tissue	-90.9766667	16.0841667
Xiphorhynchus flavigaster	YACH 071	Tissue	-90.9733333	16.9016667
Xiphorhynchus flavigaster	YACH 069	Tissue	-90.9733333	16.9016667
Xiphorhynchus flavigaster	YACH 025	Tissue	-90.9733333	16.9016667
Xiphorhynchus flavigaster	Y408 180	Tissue	-90.3200333	18.0200667
Xiphorhynchus flavigaster	Y408 179	Tissue	-90.3200333	18.0200667
Xiphorhynchus flavigaster	Y408 165	Tissue	-90.3200333	18.0200667
Xiphorhynchus flavigaster	Y408 164	Tissue	-90.3200333	18.0200667
Xiphorhynchus flavigaster	Y408 139	Tissue	-90.3200333	18.0200667
Xiphorhynchus flavigaster	Y408 122	Tissue	-90.3200333	18.0200667
Xiphorhynchus flavigaster	TXT 42	Tissue	-95.13797	18.53537
Xiphorhynchus flavigaster	TUXFPO 19	Tissue	-95.0592222	18.5871111
Xiphorhynchus flavigaster	TUXFPO 09	Tissue	-95.0979944	18.5887583
Xiphorhynchus flavigaster	TUXFPO 04	Skin	-95.0979944	18.5887583
Xiphorhynchus flavigaster	SOSI 143	Skin	-92.23057	14.67249
Xiphorhynchus flavigaster	SLAW 084	Skin	-95.0666667	18.6
Xiphorhynchus flavigaster	PLU 283	Tissue	-96.3965611	15.8941694
Xiphorhynchus flavigaster	PLU 282	Tissue	-96.3965611	15.8941694
Xiphorhynchus flavigaster	PLU 197	Tissue	-96.3965611	15.8941694
Xiphorhynchus flavigaster	PLU 019	Skin	-96.3965611	15.8941694
Xiphorhynchus flavigaster	PGD 163	Skin	-93.405	16.6933333
Xiphorhynchus flavigaster	OMVP 0587	Tissue	-94.8852778	16.8302778
Xiphorhynchus flavigaster	MT 338	Tissue	-96.3166667	17.7566667
Xiphorhynchus flavigaster	MT 291	Tissue	-96.8716667	19.3666667
Xiphorhynchus flavigaster	MOL15 71	Skin	-96.17075	15.75595
Xiphorhynchus flavigaster	MOL14 081	Skin	-96.5469	15.9531
Xiphorhynchus flavigaster	MEX 015	Skin	-94.8319444	18.3211111
Xiphorhynchus flavigaster	JEMP 748	Skin	-93.78351	17.022226
Xiphorhynchus flavigaster	GES 256	Skin	-90.2561111	18.5927778
Xiphorhynchus flavigaster	GES 245	Skin	-90.2561111	18.5927778
Xiphorhynchus flavigaster	GAL 045	Skin	-95.0666667	18.6
Xiphorhynchus flavigaster	CHIMA 519	Skin	-94.6894444	17.0066667
Xiphorhynchus flavigaster	CHIMA 415	Tissue	-94.05	17.066819
Xiphorhynchus flavigaster	CHIMA 123	Tissue	-94.1183333	17.066819

Xiphorhynchus flavigaster	CPM 302	Tissue	-96.1945	15.76953
Xiphorhynchus flavigaster	CPM 290	Tissue	-96.1945	15.76953
Xiphorhynchus flavigaster	CONACYT99 133	Skin	-90.395	20.1761111
Xiphorhynchus flavigaster	CONACYT04 252	Tissue	-96.4844444	16.0691667
Xiphorhynchus flavigaster	CONACYT04 233	Tissue	-96.4273611	15.9340028
Xiphorhynchus flavigaster	CONACYT 1291	Tissue	-95.0425	16.7924999
Xiphorhynchus flavigaster	CONACYT 1290	Skin	-95.0425	16.7924999
Xiphorhynchus flavigaster	BMM 306	Skin	-97.0769444	16.1555556
Xiphorhynchus flavigaster	BMM 277	Skin	-97.1833333	16.1
Xiphorhynchus flavigaster	BMM 250	Skin	-97.1833333	16.1
Xiphorhynchus flavigaster	AMT 442	Skin	-96.42	15.925
Xiphorhynchus flavigaster	AMT 404	Skin	-96.42	15.925
Xiphorhynchus flavigaster	AMT 400	Skin	-96.42	15.925
Xiphorhynchus flavigaster	AMT 395	Tissue	-96.42	15.925
Xiphorhynchus flavigaster	AMT 386	Skin	-96.42	15.925
Xiphorhynchus flavigaster	AGNS S/N	Skin	-96.55	17.5316667
Xiphorhynchus flavigaster	AM 057	Skin		
Xiphorhynchus flavigaster	AM 056	Skin		
Xiphorhynchus flavigaster	YUC13 153	Skin		
Xiphorhynchus flavigaster	YUC13 152	Skin		
Xiphorhynchus flavigaster	TEPE 39	Skin		
Xiphorhynchus flavigaster	SIN 009	Tissue		
Xiphorhynchus flavigaster	SIN 005	Tissue		
Xiphorhynchus flavigaster	SIN 001	Tissue		
Xiphorhynchus flavigaster	ORT11 039	Skin		
Xiphorhynchus flavigaster	ORT11 038	Skin		
Xiphorhynchus flavigaster	ORRS-X-15	Skin		
Xiphorhynchus flavigaster	ORRS-X-11	Skin		
Xiphorhynchus flavigaster	MOLGRO 453	Tissue		
Xiphorhynchus flavigaster	MOL11 11	Tissue		
Xiphorhynchus flavigaster	JGCC 06	Tissue		
Xiphorhynchus flavigaster	CONACYT 1245	Tissue		
Xiphorhynchus flavigaster	CONACYT 1213	Tissue		
Lepidocolaptes affinis	QRO 283	Tissue		
Lepidocolpates affinis	BMM 925	Tissue		
Lepidocolaptes souleyetii	Chima473	Tissue		
Lepidocolaptes souleyetii	Yach155	Tissue		
Lepidocolaptes leucogaster	BMM 470	Tissue		
Lepidocolaptes leucogaster	MT407	Tissue		

Table S3. Model comparisons for synchronic divergence tests of four species of birds co-distributed in Mexican Tropical Forests.

Divergence test for Pacific slope versus Gulf of Mexico slope populations (*Saltator atriceps, Xiphorhynchus flavigaster,* and *Icterus gularis*)				
Number of events	Posterior prob.	Cumulative post. prob.	Prior prob.	BF
3	>0.99959	1	0.696375	>1063.86
1	<0.000409668	1	0.024629	<0.0162305
2	<0.000409668	1	0.278996	<0.00105913
Divergence test for Yucatan Peninsula vs Gulf of Mexico slope populations (*Saltator atriceps* and *Melanerpes santacruzi*)				
Number of events	Posterior prob.	Cumulative post. prob.	Prior prob.	BF
2	>0.999643	1	0.882317	>373.329
1	<0.000357143	1	0.117683	<0.0026786

DISCUSIÓN Y CONCLUSIONES GENERALES

En esta tesis presento diversos análisis de información biogeográfica, ecológica y genómica, los cuales proveen información novedosa y relevante para un mejor entendimiento de los procesos subyacentes en los patrones actuales de distribución de las especies de aves de los Bosques Tropicales de México (BTM) y su variación genética. La integración de múltiples líneas de evidencia es crucial para tratar de desentrañar la complejidad de las historias evolutivas de los taxones que se co-distribuyen en esos ecosistemas, dado que permite obtener aproximaciones a diferentes escalas espaciales y temporales. De esta forma, los análisis biogeográficos y ecológicos permiten inferir procesos históricos y ecológicos a escala regional, incluyendo grandes números de especies y amplias áreas geográficas; mientras que los estudios filogeográficos, de genética de poblaciones y de modelado de distribución potencial permiten estudiar a mayor detalle ciertas especies focales y sus procesos evolutivos (como los cuellos de botella, procesos de introgresión y de adaptación local, entre otros).

Los BTM cubren actualmente poco más del 15% de la superficie nacional (cerca de 318.000 km^2; CONABIO, https://www.biodiversidad.gob.mx/ecosistemas/), sin embargo concentran los mayores valores de riqueza de especies, gran parte de las cuales son endémicas (*e.g.*, Flores-Villela y Gerez, 1994). En lo que respecta a las aves, estos bosques albergan cerca de la mitad de todas las especies descritas para México, las cuales ocupan estos ecosistemas como residentes permanentes, temporales o de tránsito durante sus periodos migratorios (Navarro-Sigüenza *et al*., 2014; Berlanga *et al*., 2015). Este carismático grupo de organismos constituye una parte esencial del funcionamiento de los BTM, dado que constituyen enlaces móviles que son cruciales para mantener la función, la memoria y la resiliencia de los ecosistemas, por sus múltiples papeles ecológicos en la polinización, dispersión de semillas, deposición de nutrientes, depredación y descomposición (Sekercioglu, 2006).

Las aves, además de su importancia ecológica, desde el punto de vista científico constituyen modelos interesantes y útiles para estudiar los procesos históricos y ecológicos que han modelado no solo su evolución, sino la de las áreas que estas ocupan (Mayr, 1986). Por tanto, los estudios encaminados a esclarecer la historia evolutiva de la avifauna pueden aportar conocimientos relevantes para la comprensión de los patrones contemporáneos de la biodiversidad y, por tanto, para la planeación eficiente de su manejo y conservación (*e.g.*, Escalante *et al*., 2020).

En el primer capítulo de esta tesis, mediante el análisis de los cambios en las áreas de distribución de las aves de los BTM, pudimos detectar diferencias temporales en sus patrones de ocurrencia. Históricamente, los mayores valores de diversidad total de especies se encontraban en los bosques húmedos distribuidos hacia la vertiente del Golfo de México y el Mar Caribe, como consecuencia del corredor de selvas tropicales que se extienden hasta Centroamérica y que ha permitido la colonización de estas áreas por taxones neotropicales (Vuilleumier, 1985). Sin embargo, en la actualidad esas áreas presentan valores inferiores de biodiversidad, probablemente debido a los drásticos cambios de uso de suelo y vegetación que han tenido lugar, sobre todo en Veracruz y Tabasco, como consecuencia de los planes de desmonte implementados durante la década de 1970 para impulsar el desarrollo agrícola y ganadero de la región (SEMARNAT, 2023). Este patrón de alta diversidad de los bosques tropicales húmedos también ha sido encontrado en otros grupos taxonómicos como anfibios y mamíferos (Koleff *et al*., 2008). Para estos, y de manera semejante a lo encontrado en este trabajo, se han identificado en el área importantes riesgos a la persistencia de las especies dadas las crecientes presiones antropogénicas sobre los bosques tropicales (*e.g*., Challenger, 1998).

Por otra parte, los bosques estacionalmente secos de la costa del Pacífico, aunque con un número comparativamente menor de especies, presentan la mayor diversidad de taxones endémicos. Esta riqueza ha sido explicada como resultado de procesos de aislamiento y reconexión de las poblaciones, generados por las oscilaciones climáticas del Pleistoceno y potenciados por el efecto de barrera de los sistemas montañosos del oeste de México (*e.g*., Arbelaez-Cortés *et al*., 2014; Prieto-Torres *et al*., 2019). Sin embargo, al igual que en el caso de las aves de los bosques húmedos, la distribución actual de la avifauna del oeste mexicano es menor a la esperable de acuerdo con los registros históricos, posiblemente debido a causas similares a las antes mencionadas, principalmente en Michoacán y Jalisco donde se han deforestado más de 3,000,000 ha de bosque en los últimos 30 años (SEMARNAT, 2023).

Todo lo anterior es especialmente preocupante si se considera que la cobertura actual del Sistema de Áreas Naturales Protegidas de los BTM es baja (alrededor de 49.100 km^2, lo que correspondería con aproximadamente el 15% de la superficie ocupada actualmente por los BTM; CONANP http://sig.conanp.gob.mx), lo que podría hacer que las reducciones de las poblaciones llegaran a un punto crítico de no retorno, a partir del cual su extinción sería inevitable. Algunos trabajos previos han señalado que las Áreas Protegidas en la región son ineficientes e insuficientes, y sugieren que las amenazas antropogénicas disminuirán aún

más su efectividad hacia el futuro (*e.g*., Prieto-Torres *et al*., 2021; Ramírez-Albores *et al.*, 2021). Así, por ejemplo, la superficie terrestre cubierta por el sistema de áreas protegidas actuales apenas representa un promedio de ~19% de la distribución potencial total de las especies de aves endémicas de Mesoamérica. Esto que indica una necesidad imperativa de re-enfocar los futuros esfuerzos de conservación para lograr una red regional de áreas representativa y bien conectada, en función de los valores actuales de biodiversidad y las proyecciones de distribución potencial hacia el futuro (Ramírez-Albores *et al.*, 2021).

A partir de los datos distribucionales recabados en este trabajo, pudimos generar un análisis de regionalización biogeográfica y estimar los valores de recambio taxonómico entre las distintas áreas que componen los BTM. Nuestros resultados sugieren complejos patrones de ocurrencia de las aves neotropicales en México, los cuales han debido formarse como consecuencia de la combinación de eventos de vicarianza y dispersión entre diferentes taxones. Esto es consistente con lo encontrado en varios estudios filogeográficos previos (*e.g*., Becerra, 2005; Arbeláez-Cortés *et al*., 2014; Ortiz-Ramírez *et al*., 2020; Castillo-Chora *et al.,* 2021) que han hipotetizado que a lo largo de su historia evolutiva reciente las poblaciones de bosques de tierras bajas han quedado aisladas como consecuencia de la expansión de vegetación no forestal adaptada a condiciones xéricas durante los secos y fríos del Pleistoceno (*e.g*.,Toledo, 1982; Prance, 1973). Es probable que bajo estas condiciones de aislamiento, se hayan dado procesos de diferenciación, por la ocurrencia de mutaciones en ausencia (o reducción) de flujo genético, por adaptación local, o por eventos de cuello de botella y deriva genética sobre las poblaciones contraídas. Posteriormente, durante los períodos climáticos húmedos, esas poblaciones ampliaron sus áreas de distribución, entrando en contacto cuando los parches de bosques aislados se conectaron nuevamente (Haffer, 1969). Sin embargo, aunque esta hipótesis explica gran parte de los patrones de diferenciación encontrados en varios taxones distribuidos en los BTM, queda por esclarecer la importancia relativa de los procesos históricos y las condiciones ambientales contemporáneas de heterogeneidad topográfica y climática en la conformación del complejo mosaico de diferenciación morfológica (*e.g*., Benites *et al*., 2019) y genética (Arbeláez-Cortés *et al*., 2014; Castillo-Chora *et al*., 2021) de la avifauna actual.

Para obtener acercamientos más detallados a los procesos de diversificación, adaptación local y especiación, es necesario profundizar en el estudio de las filogenias moleculares y realizar análisis detallados de genética de poblaciones. Estos permiten desentrañar las múltiples historias evolutivas (que pueden ser tanto compartidas entre varios grupos o idiosincráticas de cada taxón en particular) que han conformado los patrones de

distribución y variación de la biodiversidad. La incorporación de información molecular y en especial la derivada de la secuenciación de genomas completos o en su defecto, representaciones reducidas de estos, es esencial para complementar el estudio los patrones biogeográficos en aves y en todo tipo de organismos (*e.g*., Toews *et al*. 2016; Llanes-Quevedo *et al*. 2022).

El empleo de las herramientas genómicas en la investigación ornitológica y biogeográfica ha experimentado un auge notable en el Neotrópico, de acuerdo a los datos recopilados para la revisión realizada en esta tesis. La utilización de información proveniente de representaciones reducidas de genomas en el estudio de las aves neotropicales ha permitido el reconocimiento de nuevas especies (crípticas; *e.g*., Cadena *et al*., 2020; Buainain *et al*., 2021), el mejoramiento del entendimiento del papel de las barreras geográficas en los procesos de diferenciación de las poblaciones y la especiación (*e.g*., Lavinia *et al*., 2019), así como la detección de eventos de flujo genético e introgresión y evaluar su importancia en la dinámica actual de esas poblaciones (*e.g.,* Berv *et al*. 2021; Musher *et al*. 2019). Sin embargo, persisten varios sesgos y limitaciones conceptuales y económicas que pueden limitar el aprovechamiento de esta fuente de información para el estudio de los procesos evolutivos que han generado la elevada diversidad de aves y otros taxones neotropicales. Para superar estas deficiencias es necesario impulsar acciones tanto en el ámbito académico como en el entorno político y social. Las acciones académicas implican reorientar el estudio hacia grupos taxonómicos y ecosistemas poco analizados como aves acuáticas y desiertos y manglares respectivamente, así como la realización de trabajos con un enfoque comparativo multi-especies. Respecto al ámbito político-social, se requiere la toma de acciones para impulsar el desarrollo de políticas nacionales y regionales que permitan un mayor acceso a recursos, formación y divulgación por parte de las comunidades científicas de los países del sur global (*e.g*., Soares *et al*. 2023).

La información genómica analizada en este trabajo (Capítulos 3 y 4) permitió entender mejor la estructura poblacional, así como la inferencia de los procesos demográficos, en cuatro especies para las cuales se han descrito complejos patrones de variación morfológica y mitocondrial (*e.g*., García-Trejo *et al*. 2009; Benites *et al*., 2020). Específicamente para el Carpintero de Velázquez *Melanerpes santacruzi*, pudimos esclarecer las relaciones evolutivas de este taxón con otros cercanamente emparentados (*Melanerpes carolinus* y *M. aurifrons*) y que se incluyen dentro de un mismo grupo morfológico (*sensu* Short, 1982). Para estos, inferimos no sólo la estructura genética dentro de la especies, sino que identificamos zonas de contacto y de posible hibridación con *M. aurifrons*, lo que concuerda con los

patrones generales encontrados en otras especies de carpinteros (e.g., *M. carolinus* X *M. aurifrons*; Miller et al., 2020; *Dryobates*, Manthey et al. 2019; y *Sphyrapicus,* Billerman et al. 2019) y que representa una ventana de oportunidad para la adaptación de las especies y sus poblaciones a las variaciones ambientales presentes y futuras.

La integración de los resultados de los análisis genómicos con los modelos de distribución potencial de especies nos permitió inferir la intermitencia histórica de las áreas de idoneidad climática para los taxones analizados. Estos ciclos de continuidad/ discontinuidad en las áreas de distribución (generados por variaciones paleoclimáticas que produjeron a su vez cambios en los ensambles vegetales) probablemente han determinado la estructuración genética contemporánea, aun cuando actualmente existe continuidad entre las áreas que ocupan las especies de los BTM (*e.g*., Arbeláez-Cortés *et al*., 2014; Castillo-Chora *et al*., 2021). Esta explicación, que también ha sido sugerida para otras especies animales (*e.g*., Butler *et al*., 2023) y vegetales (*e.g*., DeNova *et al*., 2012; Ortiz-Rodríguez *et al*., 2020), apoyaría tanto los resultados de disimilitud de la composición de especies encontrados en el Capítulo 1, como la la hipótesis de trabajo enunciada en esta tesis de que los patrones de distribución similares de poblaciones diferenciadas de aves de los BTM, en ausencia de barreras aparentes a su dispersión, es producto de la divergencia genética derivada de eventos de vicarianza por la presencia de barreras ecológicas, que en el presente se han debilitado o han desaparecido.

Una vez identificados los patrones de divergencia dentro de cada taxón y los factores ambientales que pueden haber influido en estos, pusimos a prueba la hipótesis de sincronicidad de los eventos de divergencia y de las dinámicas demográficas de las aves co-distribuidas en los BTM. Esta es una problemática central de la biogeografía evolutiva, que busca, a través de diversas líneas de evidencia, encontrar patrones biogeográficos y evaluar los cambios históricos que las han moldeado (Morrone, 2009). Nuestros resultados sugieren que, aunque existe una congruencia espacial parcial en la estructura poblacional de las especies incluidas en el estudio, esta estructura parece ser consecuencia de respuestas diversas e idiosincráticas de cada una de las especies estudiadas a procesos comunes que han afectado la evolución de los BTM. Este tipo de patrones complejos ha sido encontrado previamente en varios grupos de aves distribuidos en el Neotrópico Mexicano (Arbeláez-Cortés *et al*., 2014; Castillo-Chora *et al*., 2021). La concordancia completa entre estos patrones es muchas veces difícil de encontrar, ya que las especies presentan características biológicas intrínsecas que provocan diferentes respuestas a eventos comunes, generando

historias evolutivas independientes que resultan en diferentes estructuras filogeográficas (Zink, 2002; Arbeláez-Cortés, 2012).

Nuestros análisis filogeográficos comparativos indican que el Istmo de Tehuantepec y los humedales de Tabasco constituyen zonas importantes en el aislamiento o la conectividad entre las poblaciones de aves. Los modelos de distribución potencial al pasado sugieren que estas áreas no presentaban condiciones de idoneidad climática para la distribución de las especies durante el último máximo glacial, sin embargo, al parecer las condiciones climáticas en los interglaciares permitieron la conexión de las poblaciones (*e.g*., Rocha-Moreira *et al*., 2020), con diversos resultados como la homogeneización genética encontrada en *M. santacruzi* a ambos lados del Istmo (Llanes-Quevedo *et al*., 2022, Cap. 3) y la diferenciación genética en *Saltator atriceps* y *Xiphorhynchus flavigaster* para las poblaciones distribuidas en Yucatán respecto a las de Veracruz. El diferente efecto como barrera o como conector entre poblaciones de estos accidentes geográficos ha sido estudiado principalmente en el Istmo de Tehuantepec, donde se ha encontrado que ha aislado poblaciones de especies de aves montanas como *Chlorospingus ophthalmicus* (Sánchez-González *et al*., 2007), *Lampornis amethystinus* (Cortés-Rodríguez *et al*., 2008) y *Picoides villosus* (Klicka *et al*., 2011), si bien para otras especies como *Lepidocolaptes affinis* (Arbeláez-Cortés *et al*., 2010) no ha tenido efectos apreciables en la diferenciación genética, por lo que se ha inferido que ha actuado como conector.

La integración de información ecológica nos permitió evaluar la asociación de la variación genética con la ambiental y tener una aproximación de los diferentes procesos que pueden influir en las dinámicas micro-evolutivas de las especies. Los resultados de estos análisis sugieren diferentes patrones, con algunas especies como *Icterus gularis* y *Xiphorhynchus flavigaster* para las cuales la distancia geográfica y el flujo limitado de genes entre las poblaciones parecen generar una diferenciación genética neutra, mientras que para otras como *Melanerpes santacruzi* y *Saltator atriceps* existe una correlación significativa entre la variación genómica y ambiental. Esto último podría ser indicio de procesos de adaptación local, los cuales tienen un papel relevante en el establecimiento y mantenimiento de las poblaciones bajo diversas presiones ambientales; sin embargo, la carencia de genomas de referencia de estas especies limita la realización de mejores inferencias.

Además de los aportes conceptuales al entendimiento de la evolución de las aves de los BTM, la información molecular analizada en este trabajo tiene implicaciones desde el punto de vista taxonómico y de conservación. Nuestros resultados indican una estructura genética que no apoya completamente las subespecies descritas y podría ser útil para la

designación de unidades evolutivas y de manejo. En todos los casos analizados, encontramos que las entidades diferenciadas morfológicamente (denominadas como subespecies) no son correspondientes con las unidades detectadas al analizar la estructura genética de las especies, con la excepción de las formas de Yucatán, que coincidieron con sus respectivas subespecies. Aunque la definición y empleo de esta categoría taxonómica ha sido polémica y no exenta de problemáticas (ver Zink, 2004; James, 2010), la conjunción de esas hipótesis basadas mayormente en caracteres morfológicos con los datos genómicos puede proporcionar pistas importantes sobre los procesos neutrales o adaptativos que están actuando sobre las poblaciones (Braby *et al*., 2012). El correcto conocimiento de estos procesos es vital para la planeación eficiente de la diversidad de las especies a largo plazo, de forma que se preserve en la mayor medida posible la variación genética importante para afrontar los cambios ambientales futuros.

En conclusión, los cuatro capítulos de esta tesis ofrecen un enfoque integrador al estudio evolutivo de la biodiversidad regional, con el empleo de datos geográficos, ecológicos y genómicos, provenientes de técnicas moleculares modernas, así como diversos métodos de análisis. Esto permite la generación y contraste de hipótesis biogeográficas y proveen de una guía para futuros estudios de la evolución de la diversidad biológica y su preservación. Así mismo, las limitaciones del presente trabajo pueden señalar algunas de las futuras prioridades de la investigación ornitológica en el país. Una de estas, es el mejoramiento del esfuerzo de muestreo para estudios genéticos dentro de las áreas de distribución de las especies (tanto para las estudiadas en esta tesis como para las no incluidas), especialmente en zonas de contacto entre formas con diferenciación morfológica (*e.g*., Rojas-Soto *et al*., 2002). También es importante proseguir los esfuerzos para obtener más y mejor información genómica de las especies, especialmente a través de la generación de genomas de referencia que permitan mapear adecuadamente la información obtenida por métodos de secuenciación de representaciones del genoma, así como realizar estudios más profundos sobre las bases genéticas de la adaptación, la estructura poblacional y los mecanismos epigenéticos (Thorburn *et al*., 2023). Finalmente, es recomendable el mejoramiento del estudio de ecosistemas poco abordados hasta la fecha en México (como manglares, matorrales, pastizales, etc.) que ayuden a complementar el panorama geográfico y ecológico de la diversificación de la avifauna (y la biota en general) de los BTM.

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