UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO

POSGRADO EN CIENCIAS BIOLÓGICAS

Facultad de Ciencias

EVOLUCIÓN DE LA COLORACIÓN DEL PLUMAJE Y LA IMPORTANCIA DE LA MORFOMETRÍA EN UN SUBCLADO DE LA FAMILIA CARDINALIDAE

TESIS

QUE PARA OPTAR POR EL GRADO DE:

DOCTORA EN CIENCIAS

PRESENTA:

SANDRA MARISOL RAMÍREZ BARRERA

TUTORA PRINCIPAL DE TESIS: DRA. BLANCA ESTELA HERNÁNDEZ BAÑOS
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Ciudad Universitaria, CD. MX. ABRIL, 2024

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COORDINACIÓN GENERAL DE ESTUDIOS DE POSGRADO
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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 **23 de enero de 2023** se aprobó el siguiente jurado para el examen de grado de **DOCTORA EN CIENCIAS** de la estudiante **RAMÍREZ BARRERA SANDRA MARISOL** con número de cuenta **301220576** con la tesis titulada: **"Evolución de la coloración del plumaje y la importancia de la morfometría en un subclado de la familia Cardinalidae",** realizada bajo la dirección de la **DRA. BLANCA ESTELA HERNÁNDEZ BAÑOS:**

Presidente:	**DR. JUAN JOSÉ MORRONE**
Vocal:	**DRA. LIVIA SOCORRO LEÓN PANIAGUA**
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Secretario:	**DR. ALEJANDRO EMMANUEL GONZÁLEZ VOYER**

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 17 de octubre de 2023

COORDINADOR DEL PROGRAMA

DR. ADOLFO GERARDO NAVARRO SIGÜENZA

POSGRADO EN CIENCIAS BIOLOGICAS
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RESUMEN

Los caracteres fenotípicos usualmente son utilizados para realizar diversos análisis que nos permitan comprender los patrones de variación que observamos en diversos grupos biológicos. La coloración (absorción y reflexión de luz) y la morfometría (tamaño y forma) son dos rasgos considerados de interés dentro del estudio de la ornitología, debido a que éstos tienden a generar patrones de variación que se agrupan a traves del espacio geográfico y el tiempo evolutivo de las especies. Ambas características han sido ampliamente exploradas y descritas dando como resultado la postulación de reglas ecogeográficas que pretenden explicar los principales mecanismos que dan forma a las tendencias generales de los patrones de variación que observamos en la actualidad, proporcionando algunas pistas sobre los procesos ecológicos y evolutivos que les permiten a las especies persistir en sus ambientes. En la primera parte de este proyecto se llevó a cabo un estudio utilizando datos de coloración y tamaño corporal de 23 especies pertenecientes a la familia Cardinalidae, para explorar que factores contribuyen o han contribuido con los procesos de diferenciación fenotípica en estas especies. Se obtuvieron resultados parcilmente consistentes con las reglas ecogeográficas, sin embargo, es necesario realizar análisis complementarios que nos permitan formular conclusiones generales respecto de este primer estudio. Posteriormente, se realizó un segundo estudio utilizando los datos de la especie *Habia rubica*, en el que se pusieron a prueba tres hipótesis de correlación entre datos genéticos y, datos geográficos (IBD: aislamiento por distancia), datos climáticos (IBE: aislamiento por ambiente) y datos fenotípicos (IBA: aislamiento por adaptación). Nuestros resultados muestran que la alta estructura genética de la especie *H. rubica* se encuentra influenciada principalmente por el efecto de la distancia geográfica, que responde a un modelo de aislamiento por distancia, en el que las poblaciones más cercanas genéticamente son aquellas que se encuentran separadas por menor distancia geográfica, mientras que las poblaciones genéticamente más diferentes se encuentran más alejadas. Los estudios realizados en este trabajo nos indican que la relación entre la variación genética, fenotípica y ambiental deberá ser explorada más exhaustivamente en análisis posteriores.

ABSTRACT

Phenotypic characters are usually used to carry out analyzes that allow us to understand the variation patterns that we observe in various biological groups. Coloration (absorption and reflection of light) and morphometry (size and shape) are two traits considered of interest within the study of ornithology, because these tend to generate patterns of variation that are grouped across geographic space and evolutionary time of the species. Both characteristics have been widely explored and described, resulting in the postulation of ecogeographic rules that aim to explain the main mechanisms that shape the general trends of the variation patterns that we observe today, providing some clues about the ecological and evolutionary processes that allow species to persist in their environments. In the first part of this project, a study was carried out using coloration and body size data from 23 species of the Cardinalidae family, to explore what factors contribute or have contributed to the phenotypic differentiation processes in these species. The results are partially consistent with the ecogeographic rules; however, it is necessary to carry out complementary analyzes that allow us to formulate general conclusions regarding this first study. Subsequently, a second study was carried out using data from the *Habia rubica* species, in which three correlation hypotheses were tested between genetic data and geographical data (IBD: isolation by distance), climatic data (IBE: isolation by environment) and phenotypic data (IBA: isolation by adaptation). Our results show that the high genetic structure of the species H. rubica is mainly influenced by the effect of geographical distance, which responds to an isolation by distance model, in which the most genetically close populations are those that are geographically closest, while the most genetically different populations are located further apart. The studies carried out in this work indicate that the relationship between genetic, phenotypic and environmental variation should be explored more exhaustively in subsequent analyses.

INTRODUCCIÓN

Muchas especies animales muestran considerables niveles de variación en sus caracteres fenotípicos. El estudio de dicha variación y su relación con diversos factores, como el medio ambiente, la geografía y la herencia genética, nos permite comprender y caracterizar el origen de la diversidad que existe en la naturaleza (Guayasamin et al. 2017). Un requisito indispensable de la evolución es la interacción entre la variación fenotípica de las especies y las fuerzas evolutivas, que potencialmente pueden influenciar la estructura poblacional, el aislamiento reproductivo y la especiación (McLean & Stuart-Fox 2014), dirigiendo los procesos de diversificación de las especies, o sus poblaciones. Algunas de estas fuerzas evolutivas son la deriva génica, la selección natural y la selección sexual; y desentrañar la contribución relativa que cada una de ellas ejerce sobre el proceso de divergencia entre y dentro de las especies, es uno de los temas centrales de la biología evolutiva actual (Wright 1931, Wright 1932, Fisher 1958, Thorpe et al. 2008, Mullen 2009, Wang & Summers 2010, Dreher & Pröhl 2014).

Durante mucho tiempo ha permanecido como una idea dominante considerar que la evolución de los caracteres fenotípicos está dirigida por la selección natural como principal fuerza evolutiva, ésto mediante un proceso gradual de reproducción diferencial no aleatoria entre unidades reproductivas, que favorece la continuidad de rasgos genéticamente heredables (genotipos), surgidos a partir variaciones generadas por mutación genética (Kull 2014, Zhang 2018); y se consideraba también a dicho mecanismo como la causa única del proceso mediante el cual los individuos se adaptan a sus entornos (Laland et al. 2014). Sin embargo, en la actualidad se reconoce que los caracteres fenotípicos pueden evolucionar además como consecuencia de cambios plásticos, cambios genéticos o una combinación de ambos (Laland et al. 2014, Ho & Zhang 2018, Zhang 2018); y que esta evolución también puede estar determinada por factores como contingencias históricas, limitaciones filéticas y ontogenéticas, asociadas a la producción de estructuras no adaptativas, por correlación con características seleccionadas (*e. g.* alometría, pleiotropía). Esta nueva información nos permite afirmar que, la evolución fenotípica de los organismos ocurre sobre unidades integrales no susceptibles de ser descompuestas en partes optimizadas de manera

independiente y separada (Gould & Lewontin 1979) , de manera que, diversos mecanismos pueden influenciar la evolución fenotípica.

La adaptación fenotípica a nuevos entornos se ha descrito como un proceso que puede comprender dos fases. Una primera fase en la que un cambio ambiental induce un cambio fenotípico a partir de la misma base genética, que es independiente de la adecuación en un inicio, dicha fase da como resultado un cambio fenotípico plástico que puede eventualmente ser adaptativo. La segunda fase altera un fenotipo preexistente a partir de la acumulación de variaciones genéticas que estabilizan dicho rasgo, después de su primera aparición, dicha fase da como resultado un cambio fenotípico con base genética (Gould & Lewontin 1979, Ho & Zhang 2018). Aunque los cambios fenotípicos con base genética han sido más ampliamente explorados, recientemente se ha comenzado a reconocer la importancia de la adaptación surgida a partir de cambios plásticos. Los avances tecnológicos recientes nos han permitido descubrir que existe un alto grado de plasticidad en la expresión génica como respuesta a diversas condiciones ambientales (Laland et al. 2014). Estudios realizados en aves, peces, anfibios e insectos sugieren que las adaptaciones, que en un principio fueron inducidas por cambios ambientales, tienen el potencial de promover la colonización de nuevos ambientes y facilitar la especiación (Pfenning 2010, Ho & Zhang 2018, Levis & Pfenning 2018). Dado que la evolución de los organismos a menudo sucede en ambientes ecológicamente variables, es posible que la oportunidad ecológica (*i. e.* condiciones ambientales que permiten la persistencia de un linaje por selección natural divergente) que surge de estas variaciones ambientales sea uno de los principales conductores de la evolución fenotípica que determina la tasa y la magnitud de las radiaciones en los linajes, influyendo directamente sobre el proceso de diversificación adaptativa de los linajes (Wellborn & Langerhans 2014). Sin embargo, el número de especies de un linaje no depende únicamente de la oportunidad ecológica que surge en los gradientes ambientales, sino también de las propiedades del desarrollo que contribuyen a su potencial evolutivo (Laland et al. 2014).

Divergencia morfológica y Diversificación de las especies

Comprender como surge y se mantiene la divergencia de los rasgos morfológicos, y cómo éstos contribuyen en el proceso de diversificación de las especies a lo largo de la trayectoria evolutiva de los linajes, ha sido uno una de las preguntas centrales de la biología evolutiva y la macroevolución. El *tempo* y *modo* de la diversificación de las especies y la evolución fenotípica puede variar ampliamente entre linajes y la relación entre ambos procesos ha sido explorada a través de múltiples estudios (Rabosky & Adams 2011). Algunas teorías han postulado una relación entre la tasa de evolución fenotípica y la tasa de diversificación de las especies, asumiendo que ambas podrían estar positivamente correlacionadas (Adams et al. 2009). La teoría ecológica de la radiación adaptativa (*i.e.* surgimiento de una diversidad de roles ecológicos y adaptaciones compatibles en diferentes especies dentro de un linaje, Givnish1997) predice que la divergencia de rasgos fenotípicos ecológicamente relevantes como resultado de un proceso gradual (*i. e.* relacionado al tiempo) promueve tasas aceleradas de especiación (*i. e.* rápida diversificación de un gran número de especies), al menos en el inicio del proceso de radiación (Schluter 2000, Adams et al. 2009). La hipótesis del equilibrio puntuado (Gould & Eldredge 1977) postula que la mayor parte de los cambios evolutivos que observamos en los linajes han surgido durante los eventos de especiación, y en consecuencia, predice una correlación entre la tasa divergencia morfológica y la tasa de diversificación de las especies y (Foote 1997, Harmon et al. 2003, Adams et al. 2009). Otros esfuerzos para abordar dicha relación han optado por analizar la cantidad de varianza morfológica entre clados, relacionándola posteriormente con la riqueza específica (Ricklefs 2004). El argumento principal de este método es que una relación positiva entre ambos factores implicaría una estrecha relación entre la divergencia morfológica y la especiación. Sin embargo, estudios posteriores mostraron que esta varianza morfológica más grande puede de hecho, deberse a que dichos clados pertenecen a linajes más antiguos que, por lo tanto, han tenido mayor tiempo de selección para la divergencia (Purvis 2004, Ricklefs 2006).

Explorar si la divergencia morfológica surge como resultado de un proceso evolutivo gradual o puntual permite postular ideas sobre aquellos mecanismos involucrados en el proceso de diversificación de las especies. Un aspecto que debe tenerse en cuenta al

establecer comparaciones entre hipótesis (gradual o puntual), es que la varianza morfológica de un clado podría ser muy diferente de la tasa de cambio morfológica en el mismo. Esto es porque, en la mayoría de los casos, las estimaciones de varianza morfológica no toman en consideración las relaciones de ancestría común entre las especies del clado (excepto en el modelo BM o Browniano). Por lo tanto, para poder abordar estimaciones de cambio morfológico, en términos de evolución entre especies, es necesario considerar su relación filogenética (O'Meara et al. 2006).

Algunas otras pistas sobre la trayectoria evolutiva de la morfología se refieren a la relación entre diferencias adaptativas y eventos de especiación. Es decir, la relación entre la divergencia ecológica y el aislamiento reproductivo en especies hermanas (Funk et al. 2006). Para poder abordar esta corriente de estudio es necesario primero definir la naturaleza de algunas correlaciones entre los factores involucrados. Por una parte, se tiene que las variables ambientales, son en su conjunto factores extrínsecos, que se correlacionan fuertemente con la riqueza específica (Hawkins et al. 2003). De manera que la variación en las condiciones ambientes que habitan los organismos puede considerarse un buen predictor de la riqueza, influyendo en ella a través de sus efectos sobre la productividad y complejidad del hábitat. Asimismo, la tasa de diversificación de un clado (*i. e.* acumulación de especies en el tiempo), se ha propuesto como parámetro clave para explicar factores tales como la riqueza de especies, a nivel de regiones geográficas y grupos de organismos (Cardillo et al. 2005, Ricklefs 2006, Mittelbach 2007, Wiens 2007, Svenning et al. 2008), la extensión geográfica de los clados, debido a la influencia de factores ecológicos que podrían limitar o regular la diversidad de los linajes (Rabosky 2009), la invasión de nuevas "zonas adaptativas", que pueden delimitarse de manera más rigurosa definiendolas a partir de criterios ecológicos, lo que explicarían la diversidad diferenciada entre linajes hermanos igualmente antiguos (Simpson 1944, Mitter et al. 1988, Kozak & Wiens 2010), la diversificación morfológica (Adams et al. 2009) y la diversificación de rasgos sexualmente seleccionados, como un mecanismo de aislamiento entre poblaciones y especies, que podría causar divergencia, y dar origen a nuevas variedades a partir de múltiples factores que

incluyen la mutación y la deriva, y que pueden explicar fenómenos como el desplazamiento de carácter (West-Eberh 1983, Seddon et al. 2008, Kozak & Wiens 2010).

Coloración, Morfometría y Patrones ecogeográficos

Dentro del reino animal, la morfología es un rasgo altamente diverso (Erwin 2007, Koch et al. 2017). Esta diversidad (*i. e.* disparidad) puede definirse como la distribución no homogénea de los patrones morfológicos, y está altamente agrupada a través de las jerarquías biológicas, desde los morfotipos diferenciados de las poblaciones hasta los *Baupläne* de categorías taxonómicas superiores (Erwin 2007, Koch et al. 2017). Uno de los objetivos principales de la ecología es comprender los mecanismos que generan y mantienen la diversidad fenotípica, a nivel de regiones geográficas y riqueza de especies (Hoekstra 2006, Kozak & Wiens 2010). La variación geográfica de las especies, y en el caso más extremo, la especiación, depende de una serie de interacciones complejas entre mecanismos de flujo génico, adaptación local y variación de gradientes ambientales (Coyne 2004, Nosil 2009, VanderWerf 2012).

Con frecuencia los animales exhiben variación geográfica de caracteres fenotípicos, específicamente en caracteres como la coloración y el tamaño corporal (VanderWerf 2012). Esta variación suele conformar patrones ecogeográficos que reflejan procesos de adaptación local frente a las condiciones ambientales variables, en escala temporal y/o espacial (Wallace 1876, Mayr 1956, James 1970, James 1991, Cooney et al. 2016, Wright et al. 2018).

La coloración es un rasgo de señalización ampliamente distribuido entre los clados animales (Protas & Patel 2008, Simpson & McGraw 2008, Friedman & Remeš 2015, Krause et al. 20017, Simpson & McGraw 2018). Como algunas otras señales sociales (*e. g.* acústicas, olfativas, eléctricas, quimio-sensoriales), la coloración presenta una amplia variedad de funciones evolutivamente importantes tales como, el reconocimiento intraespecíco que mantiene la diferenciación poblacional al tratarse de un carácter que puede estar sujeto a selección sexual (*e. g.* selección de pareja, competencia intrasexual), y la comunicación interespecífica (*e. g.* camuflaje críptico, aposematismo), (Hubbard et al. 2010). De esta manera, tanto los mecanismos de pigmentación, como los patrones de

coloración pueden ser ampliamente variables dentro y entre especies. Además de ello, también se ha sugerido que algunos colores y pigmentos pueden tener funciones adaptativas como fotoprotección (McGraw 2006a, McGraw 2006b, Protas & Patel 2008, Hubbard et al. 2010), resistencia microbiana (Goldstein et al. 2004) y termorregulación (Rosenblum 2004, McGraw 2006b, Protas & Patel 2008), que pueden ser notablemente afectados por la selección natural (Endler 1990, Caro 2005, Hoekstra 2006, Robertson & Rosenblum 2009). Este rasgo es expresado a través de la asimilación de pigmentos y/o arreglos nanoestructurales, y está determinado tanto por factores genéticos como ambientales (Hubbard et al. 2010).

La morfología, al igual que la coloración, se encuentra relacionada con una amplia variedad de funciones de carácter ecológico, etológico y fisiológico (Goldberg et al. 2018, Citadini et al. 2018). El estudio de la relación que guarda la morfología con cada uno de estos campos, ha proporcionado evidencia de múltiples y complejas adaptaciones en grupos como primates, aves y anfibios. Dada esta complejidad, es usual analizar la morfología a partir de propiedades particulares. Una de las propiedades morfológicas más ampliamente explorada es el tamaño corporal. Éste es considerado una aspecto clave de la evolución adaptativa y la diversificación de las especies (Blanckenhorn 2000, Huang et al. 2017). La existencia de clinas geográficas en el tamaño corporal puede ser reflejo de *trade-offs* que están determinados, al menos de manera parcial por la genética (*e.g.* en tamaños corporales grandes se espera: mayor éxito reproductivo/mayor depredación, parasitismo, intolerancia a la inanición, estrés por calor, costos de reproducción; mayor fecundidad de hembras/ mayor costo de viabilidad, desarrollo prolongado), y posteriormente estas tendencias pueden ser afectadas por la variación ambiental (Marangoni & Tejedo 2008). De manera que es importante distinguir entre las contribuciones genéticas y ambientales que determinan las características de un rasgo morfológico, para no comprometer las conclusiones de un estudio, o subestimar el papel de la plasticidad (*i.e.* variaciones sin base genética) en la determinación de las clinas geográficas (Stillwell 2010).

La variación latitudinal del tamaño corporal y la coloración puede ser explicada por dos importantes reglas biogeográficas (VanderWerf 2012), la regla de Bergmann (Bergmann

1847) y la regla de Gloger (Gloger 1833). La regla de Bergmann predice que dentro de los organismos homeotermos el tamaño corporal tenderá a ser más grande en aquellas especies distribuidas en ambientes más fríos (*e.g.* regiones polares), que en especies de climas tropicales (Bergmann 1847, Mayr 1956, Brown & Lee 1969, James 1970, McNab 1979). El mecanismo adaptativo más ampliamente aceptado para explicar dicha tendencia es una adaptación termorreguladora, gracias a la cual aquellos organismos de tamaño corporal más grande (*i.e.* menor área de superficie en proporción al volumen), perderían menos calor, en comparación con los organismos de tamaño corporal más pequeño. Esta tendencia ha sido reportada en diversas especies de animales homeotermos como las aves (Zink & Remsen 1986, Ashton 2002, Olson 2009), y los mamíferos (Ashton et al. 2000, Millien et al. 2006); así como en animales ectotermos como los reptiles (Olalla-Tárraga et al. 2006), anfibios y algunos invertebrados. Y aunque originalmente fue propuesta para explicar la relación del tamaño corporal entre especies, se ha comprobado que esta regla puede explicar patrones de variación entre poblaciones de una misma especie, así como también entre grupos taxonómicos superiores (Blackburn et al. 1999, Ashton et al. 2000, Freckleton et al. 2003, Ashton 2002, Millien et al. 2006, Olson 2009). Por otro lado, la regla de Gloger predice que aquellos organismos que habitan ambientes más cálidos y húmedos tienden a presentar pigmentación más oscura que aquellos habitantes de ambientes secos (VanderWerf 2012). Para explicar este patrón de correlación entre la pigmentación y el clima se han propuesto múltiples mecanismos que incluyen el camuflaje críptico, por reducción de la conspicuidad y, en consecuencia, de la depredación (Zink & Remsen 1986); la protección contra parásitos debido a cualidades antimicrobianas de la melanina, protección contra la radiación solar UV que no ha sido consistentemente soportado, efectos pleiotrópicos sobre la selección de los genes que codifican el receptor de melanocortina (MC1R) y sus ligandos que afectan la deposición de los pigmentos de melanina y pueden tener efectos fenotípicos alternos *(e.g.* mayor agresividad, mayor resistencia al estrés, mejor respuesta inmune); si estas características se encuentran bajo selección a lo largo de los gradientes de humedad, ésto significaría que los gradientes de coloración melánica podrían ser un subproducto de estas fuerzas de selección; también se ha propuesto que la coloración melánica podría conferir ventajas fisiológicas, ya que entre otras cosas, la coloración oscura es altamente costosa y

difícil de producir para organismos de ambientes extremos o secos (Burtt 1999). Finalmente, se ha mencionado una posible relación entre la regla de Gloger y funciones de termorregulación de la melanina, la cual se encuentra altamente conservada filogentéticamente a través del árbol de la vida (Pinkert & Zeuss 2018). Esta hipótesis de melanismo térmico predice que los organismos más oscuros se encontrarían en regiones más frías, donde la disponibilidad de luz ambiental es escasa, debido a que la coloración oscura tiende a absorber longitudes de onda corta que confiere mayor carga de calor solar para calentarse; mientras que los organismos más claros se distribuirían en ambientes más cálidos, absorbiendo menos radiación solar, para evitar el sobrecalentamiento y la pérdida de de agua por evapotranspiración (Delhey 2019). Basados en los principios de esta hipótesis, es posible considerar que en regiones más cálidas los organsmos oscuros puedan evitar los costros de termorregulación si éstas regiones corresponden con ambientes húmedos, con alta cobertura vegetal que confieren sombra, y presentan mayor disponibilidad de agua (Xing et al. 2016, Delhey 2019). En grupos como las aves, la alta concentración de pigmentos melánicos parecen ser una adaptación que favorece el mantenimiento de las plumas (*e. g.* dureza, resistencia a la abrasión), una característica que puede jugar un importante papel en la evolución de la coloración del plumaje (Gunderson et al. 2008). Estas reglas ecogeográficas (Bergmann, Gloger) han mostrado consistencia entre grupos cuya distribución abarca escalas continentales. Sin embargo, la interpretación de las correlaciones entre caracteres morfológicos y variables ambientales puede ser muy compleja debido a la alta covariación que existe entre éstas últimas (Millien et al. 2006, VanderWerf 2012).

Familia Cardinalidae

La familia Cardinalidae es un grupo taxonómico que junto a otras cuatro familias: Icteridae, Passerellidae, Thraupidae y Parulidae, forma parte de un clado más amplio que agrupa todas las aves oscines de nueve plumas primarias del Nuevo Mundo, identificado como superfamilia Emberizoidea (Selvatti et al. 2015); al igual que otros linajes, esta familia es producto de una radiación que evolucionó y se diversificó relativamente rápido en el continente americano, más probablemente desde la región de Norte América (Sibley &

Monroe 1990, Barker et al., 2004, Klicka et al. 2007, Barker et al. 2015), a partir de varias eventos de especiación identificados entre la segunda mitad del Mioceno e inicios del Plioceno (~10-5 ma, Barker et al. 2015). Actualmente esta familia está ampliamente distribuida e integra 13 géneros y 51 especies neárticas y neotropicales reconocidas que habitan una gran variedad de ambientes incluyendo: bosques boreales templados, selvas tropicales, pastizales y matorrales áridos (Winkler et al. 2020, Scott et al. 2023) y que presentan diferente estatus de desplazamiento geográfico (13 migratorias/ 38 residentes). Además de ser un grupo ecológicamente diverso, estas especies representan un ensamblaje heterogéneo de amplia diversidad fenotípica enfocada principalmente en el tamaño corporal que presenta amplia variación entre especies (Klicka et al. 2001), la coloración del plumaje, caracterizada por parches generalmente en tonos mate de: rojos, amarillos, azules vibrantes, hasta negros y marrones, dentro de la que se han reportado diferentes grados de dicromatismo (Winkler et al. 2020, Scott et al. 2023).

Los análisis de las relaciones filogenéticas en Cardinalidae han sido exhaustivos y nos han permitido aclarar algunas de las relaciones más controversiales entre los taxones superiores que la conforman (Burns 1997, Klicka et al. 2000, Lovette & Bermingham 2002, Yuri & Mindell 2002, Klicka et al. 2007, Barker et al. 2015); revelando la existencia de cinco subclados claramente identificados: 1) clado "enmascarado": integrado por las especies de los géneros *Piranga*, *Cardinalis*, *Caryothraustes*, *Periporphyrus* y *Rodothraupis*, este clado se caracteriza por presentar una coloración negra alrededor de ojos y pico que asemeja a un antifaz; 2) clado "tángara", que contiene todas las especies de los géneros *Habia* y *Chlorothraupis*: 3) clado "azul", integrado por las especies de los géneros *Amaurospiza*, *Cyanocompsa*, *Cyanoloxia*, *Passerina* y *Spiza*, llamado así debido a la coloración predominantemente azul de sus especies; 4) clado que contiene todas las especies del género *Granatellus*; y 5) clado que contiene todas las especies del género *Pheucticus*. Simultáneamente, algunas otras investigaciones han mostrado que varios complejos de especies dentro de Cardinalidae pueden estar constituidas por múltiples especies separadas (Pulgarín et al. 2013, Bryson et al. 2014, García et al. 2016, Tonetti et al. 2017, Ramírez-Barrera et al. 2018, Castillo-Chora et al. 2021), esto aunado a la amplia distribución

geográfica del grupo, sugiere una historia biogeográfica dinámica y compleja dentro y entre las especies de la familia (Klicka et al. 2007). Este contexto de Cardinalidae se presenta como un escenario ideal para evaluar los patrones espaciales de variación fenotípica, particularmente la coloración y el tamaño corporal, para identificar los principales mecanismos responsables de su diversificación a gran escala.

OBJETIVOS Y ESTRUCTURA DE LA TESIS

En el presente trabajo se pretende evaluar el efecto del clima sobre los patrones de variación espacial de dos rasgos fenotípicos: coloración del plumaje y tamaño corporal, utilizando para ello 23 especies de la familia Cardinalidae como sistema de estudio interespecífico, y la especie *Habia rubica* como sistema de estudio intraespecífco. En el primer capítulo, se utilizan dos aproximaciones metodológicas para evaluar si los patrones de variación del tamaño corporal y el brillo del plumaje son congruentes con tendencias de variación fenotípica descritas por las reglas ecogeográficas de Gloger (1833) y Bergmann (1847). En el segundo capítulo se evalúan las contribuciones de factores como la distancia geográfica y climática sobre los patrones geográficos de diferenciación genética (Ramírez-Barrera et al. 2018) y fenotípica (Winkler et al. 2020) de las poblaciones de *Habia rubica*. En este segundo estudio se realizaron análisis bajo dos enfoques metodológicos, uno a nivel de filogrupo y otro a nivel de individuos, para comparar la congruencia de ambos sistemas y determinar que modelo explica mejor los datos de diferenciación de la especie. Este trabajo permitió realizar inferencias sobre la influencia de algunos aspectos de la variación ambiental (*e.g.* geografía, temperatura, humedad) sobre la diferenciación fenotípica, las cuales son de importancia para comprender los patrones geográficos de la diferenciación fenotípica entre especies estrechamente relacionadas. De acuerdo con los resultados obtenidos de estos estudios se discute la relación entre los patrones de variación de variación fenotípica y diversos aspectos de la variación ambiental en las aves, Asimismo, se discute la importancia de la variación genética y su influencia sobre la variación fenotípica a nivel intraespecífico. Por último, se exploran diversas líneas de investigación que nos permitirían profundizar en el conocimiento sobre los patrones de variación fenotípica entre y dentro de las especies de aves de Cardinalidae.

CAPÍTULO UNO

INFERENCIAS MACROECOLÓGICAS Y MACROEVOLUTIVAS

Ramírez-Barrera SM, Velasco JA, Hernández-Baños BE (prep.)

Resumen. – La familia Cardinalidae es un grupo de especies surgida a partir de la radiación de lo que se conoce como las aves del Nuevo Mundo y forma parte de la superfamilia Passeroidea (oscines con nueve plumas primarias). Actualmente se reconoce que está integrada por 13 géneros y 51 especies, cuya distribución abarca desde la región noroeste de Canadá en el norte del continente, hasta la región sureste de Sudamérica, y en gran parte de esta distribución las especies son descritas como residentes todo el año. Estudios filogenéticos moleculares muestran que su familia hermana más probable es Thraupidae. En este trabajo seleccionamos un subclado de Cardinalidae integrado por 7 géneros y 23 especies. El objetivo principal de este estudio es analizar los patrones de variación geográfica en el tamaño corporal y la coloración del plumaje en ambos sexos de las especies muestreadas. Se contrastaron dichos patrones con las hipótesis postuladas en dos reglas ecogeográficas que describen tendencias generales de variación geográfica a gran escala. La regla de Bergmann, que describe una relación negativa entre el tamaño corporal de organismos endotermos y la temperatura ambiental; y la regla de Gloger, que describe una relación negativa entre el brillo de la superficie corporal de los animales y la humedad ambiental. Para ello se utilizaron dos aproximaciones estadísticas con bases teóricas complementarias. El análisis basado en ensamblaje, que es un enfoque macroecológico diseñado para considerar factores de variación de los rasgos a partir de un criterio geográfico-espacial; y el análisis entre especies, un enfoque macroevolutivo, que evalúa los patrones de variación de los rasgos considerando la no independencia de los datos analizados debido a la ancestría común de las especies analizadas. Nuestros resultados muestran que tanto el tamaño corporal como el brillo del plumaje presentan patrones de variación asociados a las regiones Neártica y Neotropical, e incluso son consistentes con patrones de variación geográfica previamente descritos en diversos grupos de aves. El análisis que mejor explica los datos obtenidos en este estudio es el análisis con enfoque

macroecológico (análisis autorregresivos simultáneos, SAR), donde la variable temperatura se observa más relacionada a los rasgos tamaño corporal y brillo del plumaje. Sin embargo, dicha relación es positiva, y contraria a lo esperado bajo la hipótesis de variación de la regla de Bergmann, mientras que el brillo y la temperatura al estar negativamente relacionados responden de manera parcial con lo esperado bajo la hipótesis de la regla de Gloger. Finalmente, los análisis macroevolutivos resultaron no significativos en todos los casos.

ARTÍCULO 1.

Phylogenetic and adaptive components of Gloger and Bergmann's rules in a subclade of the family Cardinalidae (Passeriformes: Aves)

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ABSTRACT

Ecogeographical rules have been postulated to describe general trends of geographic variation in both body size (Bergmann's rule) and various color attributes (Gloger's rule) in animals. In recent years, there has been renewed interest by researchers in exploring these trends from macroecological and macroevolutionary perspectives using various approaches that evaluate the contributions of geographic and climate factors. Body size and plumage coloration are two of the most widely studied phenotypic traits in birds. In this work, we used assemblage-based (SAR simultaneous autoregressive models) and cross-species (PGLS analysis) approaches to evaluate geographical variation patterns in body size and plumage brightness in 23 species of the Cardinalidae family (Passeriformes; Aves) with the aim of determining whether these patterns are consistent with Bergmann's and Gloger's rules. Our results showed that body size as well as plumage brightness present a geographic pattern that describes a gradient in a north-south direction (larger values to the north). However, these geographic patterns are not explained by the variables temperature

and average annual precipitation. This result is consistent across the different analysis criteria used in this study (SAR, PGLS). It is likely that additional geographic or climatic factors are influencing the processes of variation and evolution of these traits among the species of this family.

Keywords: Gloger's rule, Bergamnn's rule, spatial gradients, macroecology

INTRODUCTION

Knowing which ecological and evolutionary factors influence the differentiation process of phenotypic traits across spatial and temporal scales is one of the main research objectives in fields such as macroecology and macroevolution (Bromham & Cardillo 2019, McGill et al. 2019). The geographic patterns we see today in such phenotypic traits are the result of covariance between different morphological, physiological, or genetic traits and geographic (*e.g.* latitude, longitude, altitude, depth) or environmental conditions (*e.g*. temperature, precipitation, availability of ambient light) of the areas where the species or their populations are distributed (Olalla-Tárraga et al. 2010, Ferreira et al. 2019, Marcondes et al. 2020). Some of these spatial patterns are recognized as classic examples of ecogeographic rules because they describe systematic trends of variation in the form and function of biological attributes across the space and along climatic gradients (Delhey 2019). These ecogeographic rules provide us insights about the ecological and evolutionary processes that allow species to persist in their environments (Mayr 1956, Amado et al. 2019).

Bergmann's rule addresses the relationship between body size and environmental temperature. In its original postulation, this ecogeographical rule (Bergmann 1847) predicted that among closely related species of endothermic animals, those living in cooler regions at higher latitudes tend to be larger than those living in warmer environments as a result of their reduced surface-to-volume ratio (Mayr 1956, Ashton 2002, Salewski & Watt 2017). That is, because metabolic heat production is related to body volume and its dissipation occurs through the external body surface, it is expected that those animals with larger body size produce more heat than they lose (Peters 1983) and, in contrast, species

with smaller body size (*i.e.* larger surface-to-volume ratio) have more efficient body cooling in warmer and more humid environments (James 1970, Olson et al. 2009). The original adaptive mechanism of heat conservation proposed for Bergmann's rule is linked to the selective advantage of larger animals in cold climates (Olalla-Tárraga et al. 2009, Amado et al. 2019), and since this rule was formulated for endothermic vertebrate animals, there is ample reported evidence for spatial gradients body size at both intra and interspecific levels in birds and mammals (Ashton et al. 2000, Hawkins & Diniz-Filho 2006, Rodríguez et al. 2006, Olson et al. 2009).

Gloger's rule describes the spatial variation patterns of animal coloration, mainly in birds and mammals. Gloger (1833) suggested that the coloration differences observed in birds could be due to a plastic response of individuals to climate, and noted that pigmentation should become more intense at low latitudes, in warmer regions; therefore, tropical birds should not only have darker, but also more striking and contrasting ones. In addition, he noted that desert birds are generally paler even when they inhabit warmer regions, an observation that points to the potential effects of humidity on coloration (Delhey et al. 2017). This pattern often explains the variation in coloration between bird populations and subspecies (Zink & Remsen 1986, VanderWerf 2012). Later Joel Asaph Allen (1877) noted that birds and mammals that inhabit wetter regions tend to be darker than those from drier regions; and Karl Görnitz (1923) evaluated these variation patterns in detail, establishing a link between the different types of colors with their respective pigmentation mechanisms (*i.e.* melanin, carotenoids). Both authors agreed with what was pointed out by Gloger regarding the general pattern of color variation, however Allen established the possibility that these climate-related variation patterns are rather due to the differential responses of the two main types of melanin found in animals (*i.e.* eumelanin, pheomelanin). However, since animal coloration is a complex attribute that can have diverse functions in the life history of animals, the mechanisms proposed as drivers of the patterns described by Gloger are unclear and include camouflage, protection against parasites or abrasion, protection against solar radiation or pleiotropic effects. (Delhey 2019). Additionally, some definitions of Gloger's rule relate only humidity or temperature, while other approaches refer to other correlated variables such as latitude, solar radiation and vegetation cover, or

combinations thereof (Delhey 2019). This variety of interpretations complicates determining the generality and applicability of this geographic pattern, and while some studies suggest that this pattern of variation is more strongly supported than Bergmann's rule, other studies rule out the geographical applicability of this large-scale pattern.

Although these ecogeographic rules were coined at the beginning of the 20th century, the emergence of new tools that allow access to large-scale databases of geographic distribution, ecological variables and phenotypic variables, combined with the development of modern statistical methods, have driven a recent interest in verifying the validity of these trends (Meiri & Thomas 2007). Gaston et al. (2008) identified three approaches from which it is possible to analyze the special patterns of biological traits: intraspecific, interspecific (hereafter “cross-species”) and assemblage-based. Later studies (Ruggiero & Hawkins 2006, Olalla-Tárraga et al. 2010) have emphasized the importance of considering the methodological differences between both approaches (*i.e.* cross-species, assemblage-based) to make correct interpretations of the results. Macroecological studies such as assemblage-based approach (Stevens 1989), also known as the "community", "interspecific" and "grid-based" approach, are spatially explicit methods that consist of the subdivision of geographic space into grid-cells where the spatial distribution of traits is represented by the average value of all species that co-occur within each cell (*i.e.* distribution range), to then test whether geographic gradients of phenotypic traits are correlated with environmental variation across the geographic space where these assemblages are distributed (Terribile et al. 2009, Olalla-Tárraga 2010, Slavenko et al. 2019, Velasco et al. 2020). Many assemblage-based studies have reported evidence of an increase in body size with latitude (Lindsey 1966, Cousins 1989, Cushman et al. 1993, Blackburn & Gaston 1996). An alternative method of analyzing phenotypic variation patterns are cross-species or midpoint approaches (Rohde et al. 1993). These analyzes, unlike those based on assemblages, consider each species and its distribution range as an independent unit of analysis, from which midpoint values or unique spatial and phenotypic measurements are obtained, to perform covariation tests using scatter diagrams and they are able to consider the non-phylogenetic independence of the data.

Here, we use interspecific assemblage-based and cross-species approaches to assess whether patterns of variation in body size and coloration of 23 species of the family Cardinalidae are consistently correlated with the main climatic variables associated with the ecogeographic rules of Bergmann and Gloger. The Cardinalidae family (Passeriformes; Aves) is part of the New World radiation of nine-primaried oscines (Burns 1997) and is considered a diverse monophyletic group (Yuri & Mindell 2002, Ericson & Johansson 2003, Klicka et al. 2007, Barker et al. 2013, Winkler et al. 2020), whose distribution ranges encompass a wide variety of environments ranging from tropical forests to arid grasslands and scrublands, occupying a broad latitudinal range throughout the American continent (Winkler et a. 2020, Guallar et al. 2021). Interspecific comparative analyses are crucial to reveal how evolutionary processes operating within species can be generalized across macroevolutionary scales (Stoddard et al. 2019).

METHOD

Phylogenetic, coloration and body size data

We examined 2321 museum specimens of 23 species of the family Cardinalidae at the Museo de Zoología “Alfonso L. Herrera” (540, Facultad de Ciencias, UNAM), Colección Nacional de Aves (147, Instituto de Biología, UNAM), American Museum of Natural History (1486, New York, USA) and the Smithsonian Institution (148, Washington, D.C., USA). We included here data on all species for which both phylogenetic and phenotypic data were available. In most of the sampled species, we were able to measure at least five female specimens and five male specimens for each patch of the geographic distribution of each species. Only adult specimens that met the criteria of skull ossification, testis enlargement (for males) were selected.

We used reflectance spectrometry to measure plumage coloration, employing an Ocean Optics SpectraSuite (USB2000) spectrometer and an Ocean Optics DH2000 Tungsten Deuterium light source following established protocols. Measurements were standardized between each specimen using a WS-2 white reference, and included

reflectance values for the range of 300-700 nm (*i.e.* including the ultraviolet portion of the spectrum). For each specimen, we measured 9 plumage patches located on the crown, upper back, lower back, rump, tail, throat, breast, upper belly and lower belly. These measurements were visualized, corrected to remove negative values, and smoothed using the PAVO package (Maia et al. 2019) implemented in the RStudio platform. We obtained the descriptive variable of brightness (S8) from the nine body patches. This variable objectively characterizes the reflectance spectra without explicitly modeling the receiver's visual system (Andersson &Prager 2006, Friedman & Remeš 2015, Maia et al. 2019). Finally, we performed a Principal Components analysis, with the brightness metrics of the nine body patches, and obtained PC1 as a new variable used in subsequent analyzes and named from now on as "plumage brightness".

Morphometric data were taken from the same individuals sampled for plumage coloration. For each specimen, we recorded three external measurements (tarsus, wing and tail length) traditionally used as indicators of body size. We used a Mitutoyo digital caliper with 0.01 mm accuracy. These three morphometric measurements used have been evaluated in different studies as predictors of body size in birds, along with others (*e.g.* keel, culmen, beak length, beak width, first primary, third primary) and it has been recommended to use them together (*i.e.* multivariate measurements) in those cases where more precise measurements such as body mass cannot be accessed (Senar & Pascual 1997). For each measurement three repetitions were obtained per individual, and these repetitions were averaged to be used in subsequent analyzes. Prior to the main analyses, we tested whether there was sexual dimorphism in the morphometric measurements using *t* tests and corroborated the degree of within-individual correlation between variables using the *cor* function in R. Then we conducted a principal component analysis (PCA) of these three morphometric variables and extracted the scores of the first principal component (PC1) as a proxy of “body size” (Seeholzer & Brumfield 2017).

In summary, our analyzes followed the two interspecific approaches available to explore geographic gradients of biological traits in macroecology: assemblage-based analyses, where grid cells are the sampling units, and exploring which environmental

factors may influence the spatial distribution of a feature, and whether said feature can influence the structure of an assemblage; analysis between species, where the species are the sampling units, which allows us to investigate the factors that influence the determination of the traits of the species directly (Gaston et al. 2008, Olalla-Tárraga et al. 2010, Bishop et al. 2016).

Assemblage-based approach

We generated range maps for each of the 23 species sampled in this study using databases available and downloaded from the NatureServe online site in *shp* format. These distribution ranges were subsequently merged to obtain the distribution range on which the subsequent models were based. We rasterized the overall distribution range with a resolution of 1 x 1 degrees (latitude-longitude) and obtained an estimated presence-absence matrix from the overlap of the total number of species distributed in each cell (Appendix, Fig. S1). Subsequently, the attribute (body size, plumage brightness) was rasterized and mapped, following the method proposed by Olalla-Tárraga et al. (2010), to obtain the average value of all the species present within each cell for each of the analyzed attributes.

We tested the predictions derived from two ecogeographical relationships that describe trends in geographic variation: Bergmann's rule, which postulates an inverse relationship between environmental temperature and body size of endothermic animals; and Gloger's rule, which postulates an inverse relationship between ambient temperature and humidity and the surface brightness of animals. For the environmental variables, we used mean annual temperature (BIO1, WorldClim) and mean annual precipitation (BIO12, WorldClim, as a proxy for ambient humidity). Finally, we evaluated a series of spatial autoregressive (SAR) models to test the relationship between temperature and body size, and between precipitation and plumage brightness. For this we used the SPDEP and NCF packages implemented in the RStudio platform, in addition to the script by Kissling & Carl (2007). Spatial weights were estimated from a neighborhood matrix (presence-absence

matrix) throughout the general distribution range of the species, establishing a maximum distance of 100 km. Regression analyses were run and the Akaike Information Criterion (AIC) obtained for each model. We calculated ΔAIC to compare the models and select the one that best fit our data. Akaike weights were estimated to evaluate the degree of relative support for each model. We selected the model with the highest explanatory power based on AIC and calculated the Negelkerke pseudo-R^2 for each model. SAR models were chosen for this analysis because they have been reported to be able to control spatial autocorrelation effects on the residuals better than OLS models (Kissling & Carl 2007, Velasco et al. 2020). In order to compare this statement, OLS models were also run following the same analysis method described above.

Cross-species approach

After the spatial analyzes we performed a comparative analysis at the species level, to evaluate the congruence between the two types of interspecific analysis, and to check if the correlation between phenotypic and climatic variables remains the same. The coss-species approach is based on the species analysis, unlike the cells used by spatial analysis. To do this analysis, we obtained the distribution ranges of each species separately, then we calculated a centroid value (or midpoint) from which we obtained average values of the climatic variables mean annual temperature (BIO1, WorldClim) and mean annual precipitation (BIO12, WorldClim, as a proxy for ambient humidity). Subsequently, we performed regression analyzes between average value of body size and plumage brightness of each species against the environmental variables selected as possible predictors. Thus, the regressions were designed to analyze the effects of the average environmental values in the distribution range of each species on the average body size and brightness of the plumage. We performed a phylogenetic generalized least squares analyses (PGLS) which takes into account the non-independence of the data due to shared ancestry among species (Grafen 1989) and uses these phylogenetic relationships between species to generate an estimated of expected covariance in cross-species data. We selected the PGLS analysis because it has been reported that it does not lead to a problem

of overcorrection of the data for phylogeny, this is because if there is no phylogenetic signal in the data the parameter estimates will be equal to a regression of ordinary least squares (OLS) (Garamszegi 2014). To preform PGLS analysis we used a large-scale phylogeny which includes 791 of an estimated ~832 emberizoid species (Barker et al. 2015), this phylogeny is the most completephylogentic hypothesis to date and is suitable for our analysis because it does not present polytomies and includes all the species for which we have body size and coloration data. We adjusted all R^2 values for all variables. We ran PGLS analysis under a Brownian motion model using the CAPER package in R (Orme et al. 2012).

RESULTS

Geographical patterns of body size and plumage coloration

The assemblages of the 23 species of Cardinalidae showed that the highest species richness is concentrated in the region between Mexico and Central America. In this region, there was overlap in distribution among four to eight species with greatest richness in the region from southeastern Mexico to northern Guatemala and Belize (Figure 1A). Body size and plumage brightness values tended to be slightly higher in temperate regions and at higher latitudes (Figure 1B, Figure 2A, 2B). However, the relationship with the regions of higher humidity (mean annual rainfall) is clear only for plumage brightness, where it is also observed that females tend to be slightly brighter than males following a north-south gradient (Figure 1C, 2C, 2D,). This general pattern had a single exception in cells located in southeastern South America (a region with high humidity and temperature) where both body size and plumage brightness values tended to be higher than expected.

The study reported by Ramirez et al. (2008) present partially similar results to those obtained in our analyses, where tropical montane species tend to be smaller on average than those distributed in the far north of the continent (See Fig. 2A, 2B). The body size appear to be more similar (largest body size) between montane and lowland species in the Nearctic region than between montane and lowland species in the Neotropical region (large and small body size). These geographic patterns of body size found among the species of

the family Cardinalidae show that the variation of environmental gradients in the mountainous regions and the lowlands of the Neotropical area, among other factors such as topography, could be influencing the structure of the assemblages of species on a continental scale (Dorst & Vuilleumier 1986, Fjeldså & Krabbe 1990). The only exception to the pattern described above is found in the body size of species distributed in the southeastern region of South America, where the average body size is slightly larger than in species distributed in nearby regions. This pattern is consistent with the previously reported negative relationship between altitude and latitude, where a latitudinal tendency to be smaller in size towards the south, and an altitudinal tendency to be larger in lowland regions, has been described (Hawkins & Diniz-Filho 2006). Therefore, it is highly likely that the mechanisms and processes underlying body size differentiation in this group of birds are related to various factors operating along environmental, geographic, and evolutionary gradients.

Environmental correlates of body size and plumage coloration across phylogeny and geography

The ordinary least squares (OLS) analysis, showed significant negative relationships of body size and plumage brightness with temperature and mean annual precipitation. The results obtained from the SAR analyzes were consistent across all tested models. Although the *p* values of the regression models were significant in most of the models, the coefficient values for the temperature and average annual precipitation variables were very low in all cases. This result indicates that the environmental variables used in this study do not explain the geographic gradients in body size and plumage brightness described in the previous section. It is possible that these geographic patterns are associated with other different environmental factors or that the chosen variables are influencing the processes of variation of these phenotypic traits at a more local geographic scale. Finally, the results of the cross-species test (PGLS analysis) showed non-significant regressions in all cases, consistent with those obtained using the SAR models (Table 1).

DISCUSSION

Our analysis allowed us to test the predictions made by ecogeographical rules on body size (Bergmann's rule, Bergmann 1847) and plumage brightness (Gloger's rule, Gloger 1833) in 23 species of the family Cardinalidae. Mapping both attributes across geographic space shows a geographic pattern in gradient, with larger body size and brighter plumage to the north, where both temperature and humidity are lower in comparison with the southern region (Figure 1B, 1C). This pattern is consistent with that previously reported in some other groups of birds from the Americas, where body size tends to be smaller in the regions around the equator and increases towards the Polar regions (Blackburn & Gaston 1996, Ashton et al. 2002, Ramirez et al. 2008). It has also been reported that this geographic trend in bird body size is correlated with range size (Rapoport's rule) and species richness; and that these correlations are linked and could be one of the causes of the high diversity observed among the tropical bird species of the new world (Blackburn & Gaston 1996).

Nevertheless, the results of the SAR regression models and the PGLS analysis obtained in this study, are consistent in showing that the environmental variables temperature and average annual precipitation are not related to the values of body size and plumage brightness in this group of species (Table 1). Therefore, we do not find evidence on a spatial scale that the observed geographic patterns between body size and plumage brightness of Cardinalidae species follow a pattern consistent with Bergmann's rule and Gloger's rule, respectively. This result is also consistent with that obtained using the OLS models where, despite showing relatively high R^2 values and a significant *p*-value, the values of the coefficients show that the effects of the environmental variables on the variation of the phenotypic traits analyzed are very small. This low explanatory power of the tested models may be due, without a doubt, to the fact that the patterns of variation in body size and plumage brightness of the analyzed species are the result of mechanisms other than those suggested under the ecogeographical rules tested in this work. However, there are some other considerations that need to be specified and discussed, and that could be influencing our analyses.

On one hand, regarding the design of the analysis models, it is possible that both the geographic range of the species distribution (almost continental) and the coarse-grained scale used in SAR (macroecological) analyzes were not capable of capturing the interspecific differentiation of body size and plumage brightness, associated with environmental variation in the respective models. Similar complications have been reported, especially in analyzes carried out on continental scales (Ramirez et al. 2008, Velasco et al. 2020). Regarding the theoretical bases of the assemblage-based (SAR) model, the need to control the effects of species richness has been reported. This is because the assignment of species to cells is not random, and this may affect the estimates of central tendency estimated for each cell (Meiri & Thomas 2007). On the other hand, regarding the variables analyzed "body size" and "plumage brightness", these represent composite variables obtained from original linear metrics (PCA analysis). This characteristic of the variables used could be complex from an interpretive point of view, and it is important to consider that the different linear variables used as indicators of body size in birds (*e. g.* body mass, wing chord, tarsal length, tail length) could present different degrees of adjustment to Bergmann rule (Meiri & Dayan 2003); this is because although these are linear measurements usually used as relative estimators of body size, in some cases it has been pointed out that those external metrics based on feathers (such as wing length and tail length) may present measurement deficiencies or inaccuracies due to factors such as the natural wear of the feathers, and that the length of the wing tends to increase slightly in size with the age of the individuals, even when they are adults (skeleton completely ossified) (Rising & Somers 1989). Although no evidence of this differential adjustment between the species of the Cardinalidae family, the effect that the combination of linear variables may have for the estimation of body size is uncertain. Additional criteria such as the sedentary state of the species are related to the tendency to have patterns consistent with the Bergmann rule, unlike migratory species (Zink & Remsen 1986, Meiri & Dayan 2003). Although within the family Cardinalidae the proportion of migratory species is low (Guallar et al. 1997) it would be important to check the effect that the migratory species of this group have on the geographic patterns found.

Despite body size trends consistent with Bergmann's rule have been reported in intraspecific analyzes of Cardinalidae (DeVries et al. 2022, Robles-Bello et al. 2022), our results indicate that additional factors could be influencing patterns of variation in body size at the large-scale interspecific level. Additional variables such as latitude, altitude, size of geographic range and species richness has been found to be highly related to geographic patterns of body size in birds (Cousins 1989, Blackburn & Gaston 1996, Ramírez et al. 2008) and some hypothesis have been suggested to explain this relationship between variables. The starvation resistance hypothesis, postulates that those animals distributed in seasonal or drier environments tend to be larger because they metabolize fat at a lower rate than small species, and this allows them to withstand long periods of food shortage (Lindsey 1966, Boyce 1979, Calder 1984, Lindstedt & Boyce 1985, Cushman et al. 1993, Morales-Castilla et al. 2012). On the other hand, the inverse relationship between number of species and body size in New World birds has been reported by Blackburn & Gaston (1996) as a spatial pattern closely related to variation in range size across latitude (Rapoport's rule, Stevens 1989), variation expected under Bergmann´s rule and the higher concentration of energy (primary productivity) in tropical areas (Currie 1991). The complexity of these spatial patterns described for body size variation in birds suggests that several mechanisms are interrelated, and therefore, unraveling the geographic and environmental variables underlying a particular geographic pattern may require extensive analysis.

As for the variable "plumage brightness" used in this study, it was chosen with the aim of verifying Gloger's rule from its simplest hypothesis, that is, verifying a geographic trend of darker birds in more humid environments and warmer (Rensch 1929, Delhey 2017). However, although humidity appears to be the main climatic variable for geographic patterns of plumage coloration, multiple climatic factors associated with the tropics (*e. g.* vegetation, latitude, altitude, UV radiation, evapotranspiration, or combinations thereof) have been considered consistent with this ecogeographical rule (Delhey 2019). In addition to the wide selection of variables, various mechanisms have been proposed that could lead to the patterns described by Gloger's rule, including camouflage, protection against

parasites, protection against solar radiation or pleiotropic effects on the immune system in the fight against pathogens (Delhey 2017, Delhey et al. 2019, Goldenberg et al. 2022). Among the mechanisms most widely related to geographic patterns linked to Gloger's rule are camouflage, by which darker animals distributed in humid regions would be better camouflaged by dark backgrounds as a result of the scarce ambient light that manages to pass through the dense vegetation (McNaught & Owens 2002). However, although there is evidence of the importance of backgrounds in this mechanism, there is little evidence on the most appropriate variable between humidity or vegetation gradients (Delhey 2019). Resistance against parasites as an explanation for the geographical patterns of dark phenotypes distributed in more humid regions postulates that melanin deposits provide hardness to feathers and confer greater resistance to degradation by keratinolytic bacteria (Burtt & Ichida 2004). Resistance against parasites as an explanation for the geographical patterns of dark phenotypes distributed in more humid regions postulates that melanin deposits provide hardness to feathers and confer greater resistance to degradation by keratinolytic bacteria. Thus, the defense mechanisms against parasites and diseases could be linked to the variation of the coloration based on melanin. The pleiotropic hypothesis is built on the genetic foundations of melanin-based coloration (Mundy 2005), and knowledge about the genes that code for the melanocortin receptor (MC1R) and its ligands. In addition to influencing the deposition of melanin pigments, these genes give individuals greater aggressiveness and less sensitivity to stress, better anti-inflammatory, antipyretic, and antioxidant responses and a better energy balance. This complexity in determining the dark coloration of the plumage in birds means that if at least one of the characteristics associated with the MC1R genes is selected in more humid environments, the gradients of dark coloration are a byproduct of the selection on other characters (Ducrest et al. 2008). In general, it is undeniable that the knowledge acquired about the physiological and genetic processes that give rise to the dark coloration of bird’s gives a broader context to the study of the geographic patterns described by the Gloger rule. And it shows us that, like body size, plumage coloration in birds can be linked in complex ways to various aspects of geographic, climatic, and population variation in species.

Additional analyzes are needed to unravel the processes underlying geographic patterns of body size and plumage brightness in birds of the family Cardinalidae.

Perspectives: Additional analyzes such as Generalized Linear Mixed Models (GLMM, designed for data with non-normal distributions) could be carried out in order to estimate the contributions of the variables related to the ecogeographical rules of Bergmann and Gloger.

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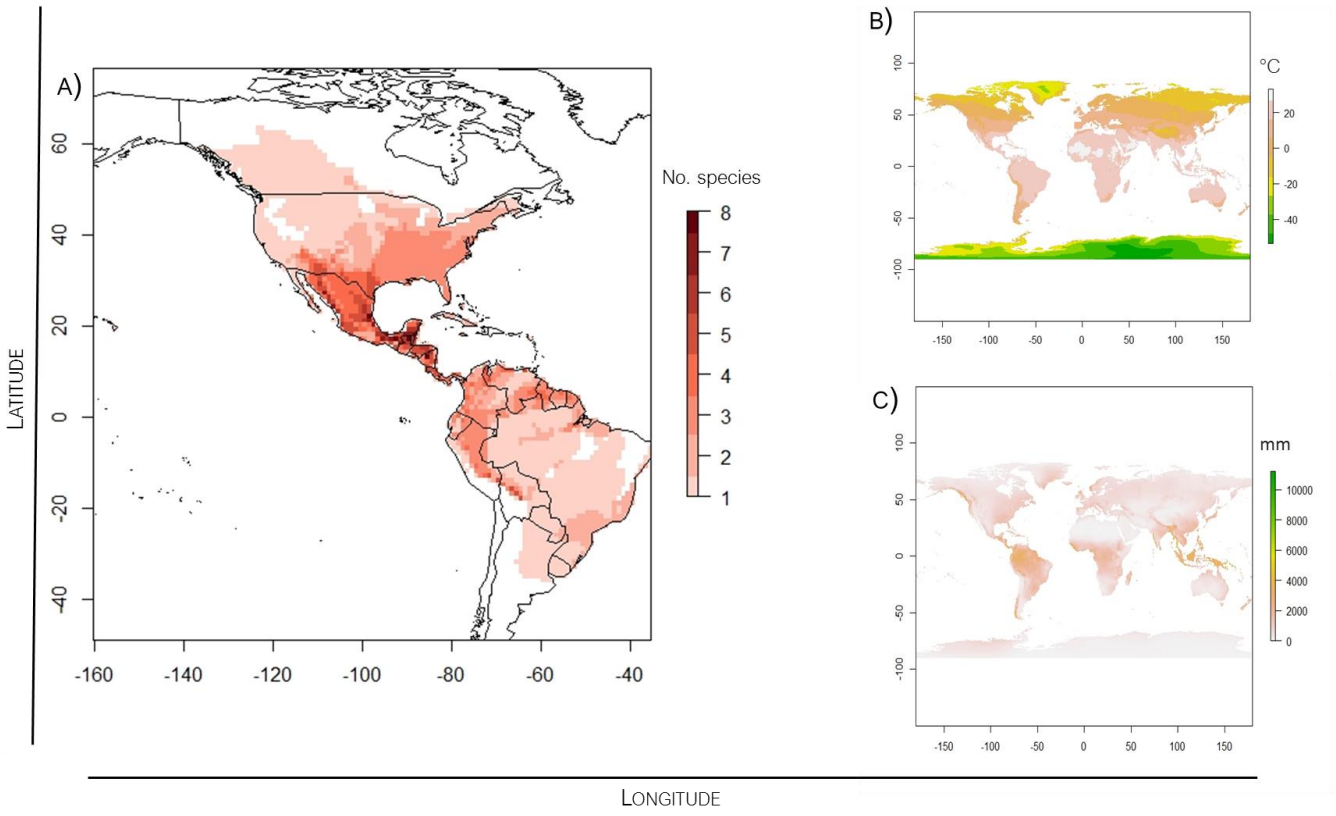

Figure 1. A) Presence-absence matrix of 23 species of the family Cardinalidae. B) World annual mean temperature records (BIO1 - WorldClim). c) World annual mean precipitation records (BIO12 - WorldClim).

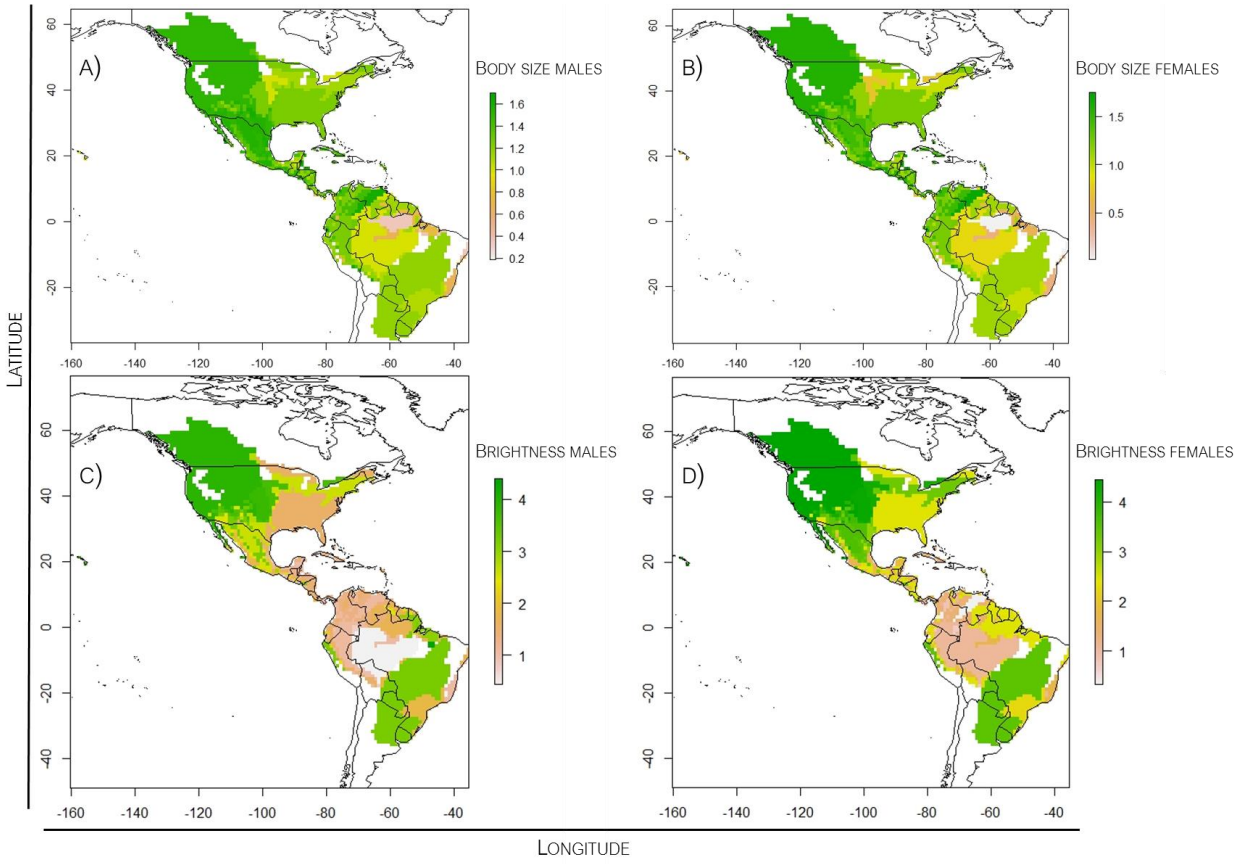

Figure 2. Geographic gradients of body size of A) males and B) females and plumage brightness of C) males and D) females from 23 species of the family Cardinalidae. Both phenotypic characters are represented in each cell by the PC1 of morphometric variables and reflectance spectrometry. El gradiente de color (de verde a marron claro) representa un gradiente decreciente de valores para cada atributo (tamaño corporal y brillo del plumaje).

Table 1. Results of ordinary least squares (OLS), spatial autoregressive models (SAR) and cross species analysis for the predictions derived from Bergmann's and Gloger's ecogeographical rules.

BERGMANN'S RULE

Body size

SAR_{ERR}	ASSEMBLAGE-BASED APPROACH					
	MALES			**FEMALES**		
Variables	**ANNUALTEMP**	**ANNUALPREC**	**SUMMARY**	**ANNUALTEMP**	**ANNUALPREC**	**SUMMARY**
Coefficients	0.00311	-0.00004	AIC -2468.6	0.00223	-0.00004	AIC -931.18
z-value	2.42	-5.12	AIC_W 0.87284	1.28	-3.73	AIC_W 0.45273
p value	0.02	< 0.001		0.20	< 0.001	
OLS						
Coefficients	-0.0072	-0.0001	R^2_{adj} = 0.33	-0.0123	-0.0001	R^2_{adj} = 0.32
t-value	-11.69	-17.09	AIC -778.02	-14.75	-13.11	AIC 751.17
p value	< 0.001	< 0.001		< 0.001	< 0.001	
PGLS	CROSS-SPECIES APROACH					
Coefficients	0.30882	-0.21797		0.021511	-0.243071	
t-value	0.3262	-0.7938	R^2_{adj} = -0.065	0.0207	-0.8063	R^2_{adj} = -0.050
p value	ns	ns		ns	ns	

GLOGER'S RULE

Plumage brightness

SAR_{ERR}	ASSEMBLAGE-BASED APPROACH					
Variables	**ANNUALTEMP**	**ANNUALPREC**	**SUMMARY**	**ANNUALTEMP**	**ANNUALPREC**	**SUMMARY**
Coefficients	-0.01522	-0.00024	AIC 4408.7	-0.01710	-0.00025	AIC 4198.7
z-value	-2.98	-7.46	AIC_W 0.96818	-3.49	-8.23	AIC_W 0.99377
p value	< 0.01	< 0.001		< 0.001	< 0.001	
OLS						
Coefficients	-0.0391	-0.0007	R^2_{adj} = 0.49	-0.0476	-0.0008	R^2_{adj} = 0.61
t-value	-15.66	-24.10	AIC 6211.8	-21.3	-29.06	AIC 5661.3
p value	< 0.001	< 0.001		< 0.001	< 0.001	
PGLS	CROSS-SPECIES APROACH					
Coefficients	1.55039	-0.47626		0.95353	-0.59931	
t-value	0.8145	-0.8627	R^2_{adj} = -0.053	0.5822	-1.218	R^2_{adj} = -0.024
p value	ns	ns		ns	ns	

CAPÍTULO DOS

DIVERGENCIA GENÉTICA Y FENOTÍPICA

Ramírez-Barrera SM, Velasco JA, Orozco-Téllez TM, Vázquez-López AM, Hernández-Baños BE (2019)

Resumen. – Habia rubica es una especie con amplia distribución geográfica, sus poblaciones habitan desde las selvas secas de la costa noroeste de México hasta las selvas tropicales del Amazonas y el sureste de Brasil. Debido a su amplia variación fenotípica y genética se han descrito hasta 17 subespecies, utilizando criterios como la variación en la coloración del plumaje y la distribución geográfica de sus poblaciones, y siete filogrupos bien definidos, limitados por barreras geográficas tales como el Istmo de Tehuantepec, el Arco Volcánico Centroamericano, el Istmo de Panamá y la Diagonal Árida Sudamericana. Estas características de la especie nos permitieron poner a prueba tres hipótesis sobre los factores que influyen en los procesos de diferenciación genética y fenotípica, utilizando el método de regresión múltiple de matrices con aleatorización (MMRR). La primera hipótesis a probar fue de aislamiento por distancia (IBD) donde la diferenciación estaría influenciada principalmente por la distancia geográfica. La segunda hipótesis fue el aislamiento por ambiente (IBE) donde el factor clima dirige los procesos de divergencia poblacional. Y por último se probó la hipótesis de aislamiento por adaptación (IBA) en donde el principal factor que dirige la diferenciación genética es la divergencia fenotípica entre las poblaciones, considerando como fenotipo los rasgos de tamaño corporal y coloración del plumaje (*hue*, *chroma*, brillo). Estas hipótesis fueron comprobadas a dos niveles dentro del análisis, el primero fue considerando el total de datos individuales disponibles de la especie; y el segundo se realizó promediando los datos individuales, utilizando para ello el criterio genético de filogrupos identificados y publicados previamente. El método MMRR está diseñado para realizar análisis de regresiones múltiples sobre matrices de distancias euclidianas, por lo que nuestros datos fueron convertidos con dicho criterio utilizando herramientas de análisis implementadas en la plataforma de análisis estadístico RStudio. Fueron observados distintos efectos de los factores geográficos, climáticos y fenotípicos, sin embargo, este trabajo concluye que el

factor geográfico es el que mejor explica la variación genética y fenotípica de la especie, y por lo tanto es posiblemente afirmar que la distancia geográfica entre poblaciones es el principal factor que dirige los procesos de diferenciación poblacional limitando el intercambio genético que promueve la variación fenotípica de la especie.

ARTÍCULO 2.

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ORIGINAL RESEARCH

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What drives genetic and phenotypic divergence in the Red-crowned Ant tanager (*Habia rubica*, Aves: Cardinalidae), a polytypic species?

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Abstract

Aim: The effects of geographic and environmental variables on patterns of genetic and phenotypic differentiation have been thoroughly studied. Ecological speciation involves reproductive isolation due to divergent natural selection that can result in a positive correlation between genetic divergence and adaptive phenotypic divergence (isolation by adaptation, IBA). If the phenotypic target of selection is unknown or not easily measured, environmental variation can be used as a proxy, expecting positive correlation between genetic and environmental distances, independent of geographic distances (isolation by environment, IBE). The null model is that the amount of gene flow between populations decreases as the geographic distance between them increases, and genetic divergence is due simply to the neutral effects of genetic drift (isolation by distance, IBD). However, since phenotypic differentiation in natural populations may be autocorrelated with geographic distance, it is often difficult to distinguish IBA from the neutral expectation of IBD. In this work, we test hypotheses of IBA, IBE, and IBD in the Red-crowned Ant tanager (*Habia rubica*).

Location: Mesoamerica (Mexico—Central America) and South America.

Taxon: *Habia rubica* (Aves: Cardinalidae).

Methods: We compiled genetic data, coloration, and morphometric data from specimens from collections in Mexico and the United States. We used the Multiple Matrix Regression with Randomization (MMRR) approach to evaluate the influence of geographic and environmental distances on genetic and phenotypic differentiation of *H. rubica* at both phylogroup and population levels.

Results: Our results provide strong evidence that geographic distance is the main driver of genetic variation in *H. rubica*. We did not find evidence that climate variation is driving population differentiation in this species across a widespread geographic region.

Main conclusions: Our data point to geographic isolation as the main factor structuring genetic variation within populations of *H. rubica* and suggest that climate is not playing a major role in genetic differentiation within this species.

KEYWORDS
ecological speciation, genetic structure, isolation by distance, landscape genetics, phenotypic variation, polytypic species

1 | INTRODUCTION

Many animal species show considerable levels of intraspecific variation that reflect the effects of selective and/or neutral evolution (Lande, 1976; Morales et al., 2017; Nosil, 2012; Seeholzer & Brumfield, 2017; Zamudio-Beltrán & Hernández-Baños, 2015). Within natural populations, genetic and phenotypic divergence may be influenced by factors such as sexual and natural selection, genetic drift, and geographic isolation (Bohonak, 1999; Slatkin, 1987; Wang & Summers, 2010; Wright, 1943). Although patterns of genetic differentiation often reflect spatial variation in gene flow, the landscape itself might influence this gene flow in at least two important ways: through geographic isolation and through ecological isolation (Coyne & Orr, 2004; Thorpe, Surget-Groba, & Johansson, 2008). Geographic isolation (Dobzhansky, 1937) proposes that geographic distances and barriers restrict gene flow among populations (Wang, 2013; Wang, Glor, & Losos, 2012), resulting in a positive correlation between genetic divergence and geographic factors (isolation by distance, IBD; Wright, 1943). Ecological isolation (Dobzhansky, 1937), on the other hand, occurs when gene flow is reduced among populations due to the effect of one or both of two different processes—isolation by adaptation and isolation by environment. Isolation by adaptation (IBA; Rundle & Nosil, 2005) is defined as the effect of environmental gradients that results in divergent natural selection that can lead to adaptive phenotypic divergence between populations, resulting in a positive correlation between genetic divergence and adaptive phenotypic differentiation (Funk, 1998; Guayasamin et al., 2017). Isolation by environment (IBE, Wang & Bradburd, 2014) is defined as the occupation of two populations in different points on the ecological gradient. This process is observed when the phenotypic target of selection is unknown or is not easily measured, and then, the environmental variation can be used as a proxy and a positive correlation between genetic divergence and environmental dissimilarity is expected. These hypotheses are not mutually exclusive; spatial genetic divergence among populations can result from reduced gene flow associated with both geographic and ecological factors (Figure 1).

Testing the associations between morphological, color, environmental, geographic, and genetic variation is the first step for understanding the relative contributions of these different

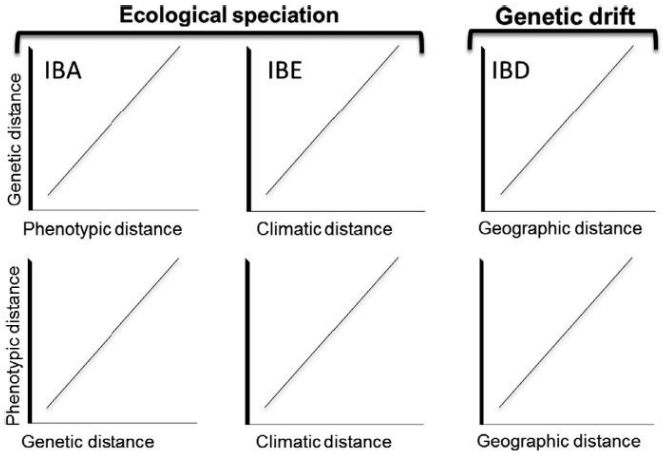

FIGURE 1 Simplified predictions of correlations between of genetic, phenotypic, climatic, and geographic distance matrices under the Isolation by adaptation, isolation by environment, and isolation by distance hypotheses. Isolation by adaptation (IBA) refers to a positive correlation between phenotypic differentiation (subject to sexual or natural selection) and genetic differentiation. This correlation occurs when the gene flow between populations is restricted by individual mate preferences or by increased mortality of immigrant phenotypes. Isolation by environment (IBE) refers to a positive effect of environmental differentiation on genetic or phenotypic differentiation, which occurs when the gene flow between populations is restricted by individual preferences to remain in a particular environment or by selection against dispersers moving between populations. Isolation by distance (IBD) refers to a positive effect of geographic separation on genetic or phenotypic differentiation as a consequence of restricted gene flow when the populations are isolated, either by geographic distances or by landscape barriers

potential drivers of genetic and phenotypic variation among populations within a species. Examining patterns of IBD and IBE is an important starting point for understanding how landscapes shape patterns of genetic variation in nature (Wang & Summers, 2010). Several factors make the Red-crowned Ant tanager (*Habia rubica*) a good model for performing tests of IBD and IBE. It is a highly polytypic species that is distributed from central Mexico to northeastern Argentina and southeastern Brazil (Figure 2a), and it has a continental distribution that encompasses a variety of suitable environments. It also has extensive geographically structured color variation that is well documented in species descriptions (Hilty, 2011), and plumage coloration differentiation among its genetic populations has been objectively measured using reflectance spectrometry (Lavinia et al., 2015). The most recent phylogeographic study indicated that the genetic variation of this species is geographically structured into seven phylogroups, which have been proposed for elevation to the category of species (Ramírez-Barrera, Hernández-Baños, Jaramillo-Correa, & Klicka, 2018). Five of these phylogroups are distributed in the Mesoamerica region (from Mexico to Panama), and two are from the western and eastern-northwestern parts of South America (Figure 2b), and these last two previously described by Lavinia et al. (2015). Finally, the relationship between the phylogroups in this species may be determined by the action of various historical processes that have promoted deep genetic structure (Lavinia et al., 2015; Ramírez-Barrera et al., 2018).

The *H. rubica* species complex contains up to 17 described subspecies, defined mainly by plumage color variation and geographic distribution. The geographic variation in plumage color of this species is mainly in the dorsal (from the crown to the tail) and ventral (from the throat to the lower belly) brightness. However, both hue and saturation also present some variation, ranging from pale pink in the populations from the Mexican Pacific to dark red in populations from eastern South America (Hilty, 2011). Populations from the eastern of Mexico to the Amazon are intermediate, with hues ranging from brown to brick red to salmon (Figure 2c–g; Hilty, 2011).

In this study, we use a multivariate approach to disentangle the relative influence of geographic and environmental distances on genetic and phenotypic differentiation of populations across the range of *H. rubica*. We test the IBA, IBE, and IBD hypotheses on the genetic structuring of the previously identified phylogroups (Lavinia et al., 2015; Ramírez-Barrera et al., 2018; see Figure 1c) and among populations across the entire distribution of this species. We estimated the "relative importance" of each predictor using standardized regression coefficients from MMRR (Multiple Matrix Regression with Randomization) analysis. We expected genetic divergence to be positively correlated with phenotypic divergence under IBA,

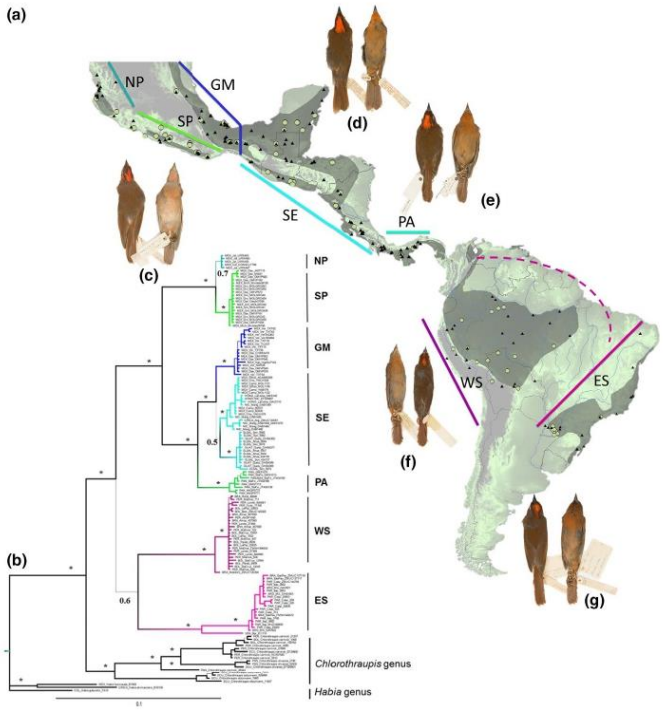

FIGURE 2 (a) Geographic distribution (gray shading) and sampling points of *Habia rubica* (green points in genetic sampling and black triangles in phenotypic sampling). (b) Phylogenetic consensus tree representing the relationship among *H. rubica* phylogroups based on Bayesian inference from a mitochondrial dataset obtained from Ramírez-Barrera et al. (2018). The values on the branches indicate posterior probability. Both the map and the phylogenetic tree show the geographic position of the sampled phylogroups: NP, northern pacific of Mexico; SP, southern pacific of Mexico; GM, Gulf of Mexico; SE, southeastern Mexico and northern Central America; PA, Panama; WS, western South America and ES, eastern-northwestern South America. (c–g) Color variation in plumage of phylogroups of *H. rubica* from coast of the Mexican Pacific (c); phylogroups from Gulf of Mexico to Costa Rica (d); phylogroup from Panama (e); phylogroups from western South America (f) and phylogroups from eastern-northwestern South America (g). Photographs by Sahid M. Robles

environmental divergence under IBE, and/or geographic distance under IBD (Figure 1).

2 | MATERIALS AND METHODS

2.1 | Genetic data

Our genetic sampling for this work comprised 124 mitochondrial DNA sequences (ND2 gene, ~1,041 bp) from *H. rubica* from a recent study (Ramírez-Barrera et al., 2018). This sample covers most of the geographic range of *H. rubica* and can therefore be considered a relatively good proxy for the total genetic diversity of the populations of this species (Figure 2a).

We generated two matrices of genetic distances using these molecular data. The first (124 sequences) was based on the affiliation to a given phylogroup as defined in Ramírez-Barrera et al. (2018; see Figure 2b), using the following groups: Mexican Northern Pacific (NP), Mexican Southern Pacific (SP), Gulf of Mexico (GM), southeastern Mexico and northern Central America (SE); Panama (PA); western South America (WS), and eastern/northwestern South America (ES, the northwest population is represented by a single sample from Venezuela). We used the program MEGA v7 (Kumar, Stecher, & Tamura, 2016) to generate this matrix, grouping individuals by phylogroup. The second matrix was generated using all possible pairs of individuals for which it was possible to match genetic and phenotypic data (110 males and 104 females, see "Data Matching" section below). Some sequences were used both in the database of males and females, and for this reason, a total database of 214 individuals were obtained from a genetic database of 124 sequences. This distinction between matrices allows us to identify the strength of the correlation between pairs of variables, so that if we obtained similar results, we could affirm that environmental variation is an important factor that influences the differentiation between populations on a continental scale. The data processing needed for this estimation was carried out using the *phyDat*, *modelTest*, and *dist.ml* functions of the "phangorn" package in R v3. 5. 0 (Schliep, 2011; R Foundation for Statistical Computing, Vienna, Austria). The Jukes–Cantor model was the nucleotide substitution model that best fit the data (Jukes & Cantor, 1969).

2.2 | Morphometric and color data

We obtained morphometric and color data for 339 adult specimens of *H. rubica* (see Appendix S1: Table S1.1). Our phenotypic sampling of *H. rubica* was conducted at the level of phylogroups including different numbers of females (NP = 8, SP = 17, GM = 27 SE = 32, PA = 15, WS = 28, ES = 20; total = 147) and males (NP = 10, SP = 15, GM = 32, SE = 59, PA = 12, WS = 33, ES = 31; total = 192), (Appendix S2: Figure S2.1). The morphometric and color data obtained were from specimens deposited in the following collections: Museo de Zoología "Alfonso L. Herrera," UNAM, Mexico (MZFC); Colección Nacional de Aves, UNAM, Mexico (CNAV-IB); the Ornithological Collection of the American Museum of Natural History, New York (AMNH), and the Ornithological Collection of the Smithsonian Institution, Washington D. C (SI). Sexual maturity was corroborated from collection data. The distribution of this sample covers the majority of the geographic and environmental range of the species (Figure 2a).

For each specimen, we recorded wing length, tarsus length, and tail length using a Mitutoyo digital caliper with 0.01 mm accuracy, taking the average of three independent repetitions of each measurement per individual for use in subsequent analyses. Prior to the main analyses, we tested whether there was sexual dimorphism in the morphometric measurements using *t* tests and corroborated the degree of within-individual correlation between variables using *cor* function in R. We conducted a principal component analysis—PCA—of these three morphometric variables and extracted the scores of the first principal component (PC1) as a proxy for body size (see Seeholzer & Brumfield, 2017 for a similar approach). PC1 explained 73% of the variation in body size among males and 75% among females in the phylogroup-level analysis and 66% among males and 64% among females in the individual-level analysis. We tested the relationship between PC1 and latitude to explore possible latitudinal trends in body size. Finally, we converted these body size values to a distance matrix using the *dist* function in R.

We obtained plumage reflectance spectra for the following nine plumage patches: crown, upper back, lower back, rump, tail, throat, breast, upper belly, and lower belly. We quantified plumage coloration for all specimens using a USB2000 spectrophotometer (Ocean Optics) with an Ocean Optics PX-2 pulsed xenon light source, connected to a bifurcated fiber-optic probe. The probe was fitted with a rubber stopper to exclude ambient light and maintain a constant distance and 90° angle between the probe tip and the plumage. Measurements were taken following standard procedures (Eaton, 2005) to record plumage reflectance for each wavelength within the avian visual spectrum, from 300 to 700 nm. We used Ocean Optics software to integrate the spectrophotometer data.

We analyzed reflectance spectra using Goldsmith's (1990) tetrahedral color space (Stoddard & Prum, 2008). This method quantifies color based on avian visual perception to be able to obtain a measure of total coloration, considering all the patches. We plotted all reflectance spectra in the avian tetrahedral color space (Stoddard & Prum, 2008), which represents the possible avian color space based on relative stimulations of the four retinal cone types. We processed the raw reflectance spectra using the "pavo" R package (Maia, Eliason, Bitton, Doucet, & Shawkey, 2013). We used the *visual model* function to determine the relative stimulation levels of the four avian cones using the *Sturnus vulgaris* (Common starling) visual model. The Common starling is the closest relative of *H. rubica* for which a spectral sensitivity function was available, and however, it is unlikely that changing the species on which the visual model is based would affect our analysis because the sensitivities of the avian cones are highly conserved (Hart, 2001). We converted the cone stimulation values (*u*, *s*, *m*, *l*) into a vector of three angles, which locates the color in the avian tetrahedral color space. We obtained three main measurements as a result of this processing: (a) the total volume occupied by the points across all body patches (color volume), (b) the mean of the hue span, and (c) mean saturation (chroma). The chroma

measurement was included to avoid the underestimation of color variation in uniformly colorful birds (see Friedman & Remeš, 2017 for a similar approach).

Three distance matrices were generated from the color measurements (volume, hue, and chroma) using the *dist* function implemented in R for each phylogroup, specimen, and sex. The first included all nine color patches, the second used only the dorsal patches (crown, upper back, lower back, rump, and tail patches), and the third used only the ventral patches (throat, breast, high belly, and low belly). We tested the relationships between hue span and latitude to explore the possibility of latitudinal color trends.

2.3 | Data matching

We used genetic and phenotypic data from the same individual (110 males, 104 females) whenever possible. When the two types of data were not available for the same individual, we matched phenotypic data to the genetic data from the closest individual available in terms of geographic proximity and membership in the same phylogroup. This association was conducted based on the georeferenced collection location of each sample (i.e., for each genetic and phenotypic sampled individual). Finally, since *H. rubica* is a species with evident sexual dimorphism in coloration and we found significant differences in body size between males and females ($p < .01$, Appendix S2: Table S2.1) genetic associations with morphometric, and color data were constructed separately for each sex. A list of full data associations is found in Appendix S1: Table S1.1.

2.4 | Geographic and climate data

We estimated geographic and climatic distances between pairs of phylogroups and individuals. For the geographic data, we assigned each individual to its respective phylogroup, and then, we estimated a minimum convex polygon from the georeferences of each genetic and phenotypic sample obtained of each phylogroup (110 males and 104 females), and estimated the geographic centroid for each group (see Appendix S2: Figure S2.2). We conducted this analysis using ArcGIS software (ArcMAP 10.2.2). Finally, we calculated a Euclidean distance matrix in meters among all phylogroups and all individuals using the *distm* function from "geosphere" in R (Hijmans, 2014). For the climate data, we follow these steps: 1. using the same minimum convex polygon from the geographic distances analysis; 2. then, a raster file of each polygon was obtained for each polygon (phylogroup) with a resolution of 2.5′ (compatible with the resolution of the database consulted in WorldClim); 3. we obtained the coordinates of each cell (raster) in ArcGIS software (ArcMAP 10.2.2); 4. we extracted data for 19 bioclimatic variables (Appendix S2: Table S2.2) from the WorldClim database (Hijmans, Cameron, Parra, Jones, & Jarvis, 2005) for all the coordinates that make up each polygon (the number of cells varied according to the size of each polygon); and 4. the mean and median values of each bioclimatic variable were estimated; 5. both values were compared using a graph, to verify they are very similar to each other, and therefore, it is possible to use the average value as a measure of central tendency of each variable (these graphs were incorporated into the Appendix S2: Figure S2.3 and S2.4). The average value of each variable was used to estimate the environmental dissimilarity matrices between each pair of polygons using the *dist* function in R.

2.5 | MMRR method

We used a MMRR approach to estimate the independent effects of environment and geography on genetic and phenotypic variation (Wang, 2013). This approximation is a similar to Mantel and partial Mantel test, but is extended to incorporate multiple regressions, can be extended to any numbers of variables that can be represented as distance matrices, and provides output in the form of a multiple regression equation (Wang, 2013). Thus, multiple regression analysis can estimate how a dependent variable changes with respect to multiple independent variables. A multiple regression equation for distance matrices can be estimated using standard multiple regression technique, with the exception that tests of significance must be performed using a randomized permutation because of the nonindependence of elements (Smouse, Long, & Sokal, 1986; Wang, 2013). Thus, MMRR analysis can quantify how genetic or phenotypic distances respond to changes in geographic and environmental distances (β = regression coefficients), the overall fit of the model (R^2 = coefficient of determination), and the significance of each variable (p-values). We used the *MRM* function implemented in the R package "ecodist" (Goslee & Urban, 2007) using 1,000 permutations of the genetic, geographic, environmental, color, and morphometric distance matrices. Before the analysis, we scaled and centered (mean = 0 and SD = 1) all distance matrices using the *scale* function in R to obtain comparable standardized linear regression coefficients.

To explore the relative importance of geographic and environmental distances as predictors of genetic and phenotypic (i.e., body size and plumage coloration) divergence, we constructed both a multivariate model and univariate models. In each model, the geographic and environmental distance were the linear predictors of the pairwise genetic or phenotypic difference between phylogroups or individuals.

Additionally, a second analysis was performed using a smaller database composed of individuals for whom it was possible to obtain both genetic and phenotypic data. The objective of including this second analysis was to be able to compare the effect that the assignment of individuals (without their own genetic data) could have on the phylogroup that, according to its distribution, belongs. Therefore, this analysis is limited to the distribution of *H. rubica* in Mexico (see Appendix S3: Table S3.6 and S3.7).

3 | RESULTS

3.1 | Genetic sampling

The corrected pairwise genetic distances (expressed in percentages) between phylogroups (124 sequences) and individuals (110

males, 104 females) ranged from 1% to 7%, showing a clear signal of geographic population structure. In the pairwise comparisons by phylogroup, the largest genetic distances were between the South American phylogroups and those distributed from Panama to Mexico. The Panama phylogroup had the smallest genetic distance from the Northern Central America and Gulf of Mexico phylogroups. In addition, we observed that phylogroups from the Northern Mexican Pacific and Southern Mexican Pacific exhibited the shortest genetic distances and were most closely related with the Gulf of Mexico and Southeastern Mexico phylogroups (Appendix S3: Table S3.1).

3.2 | Morphometric and color data

Within-individual correlation coefficients for each pair of morphometric measures ranged between 0.2 and 0.6 (Appendix S3: Table S3.2, Figure S3.1 and S3.2). The tarsus and tail measurements had the highest PC1 weights in both sexes (Appendix S3: Table S3.3 and S3.4, Figure S3.3). With respect to latitudinal trends, latitude correlated positively with both plumage hue and body size in both sexes, and though in all cases, the coefficient of determination was rather low (hue: R^2 = 0.10 and 0.06, body size: R^2 = 0.05 and 0.07 for males and females, respectively). See Appendix S3: Table S3.5 and Figure S3.4.

3.3 | MMR method for univariate analysis

The results of univariate MMRR analyses showed that geography was the best predictor of genetic distance in *H. rubica* (R^2 = 0.7, Figure 3 and Table 1a). The contribution of this variable was slightly stronger in analyses performed on individual data for males and females (β = 0.8), than when data were grouped by phylogroup (β = 0.6). Climate and body size were significant only for the individual data, although their contribution was notably lower than geographic distance in both sexes (climate: R^2 = 0.10, β = 0.1; body size: R^2 = 0.07, β = 0.33), (Table 1a). The univariate MMRR analysis of plumage color was not statistically significant in the phylogroup or individual-level analyses (Table 1b). Finally, the univariate analysis of body size was not significant for most of the variables (Table 1c), except for geography, though its contribution was very low in both sexes (males: R^2 = 0.05, β = 0.17; females: R^2 = 0.02, β = 0.12).

3.4 | MMR method for multivariate analysis

In general, the multivariate model explained a high percentage of the total variance in genetic distance at both the phylogroup (R^2 = 0.66) and individual levels (R^2 = 0.74, Figure 4). By far, the single most important predictor of genetic distance was geographic distance, and

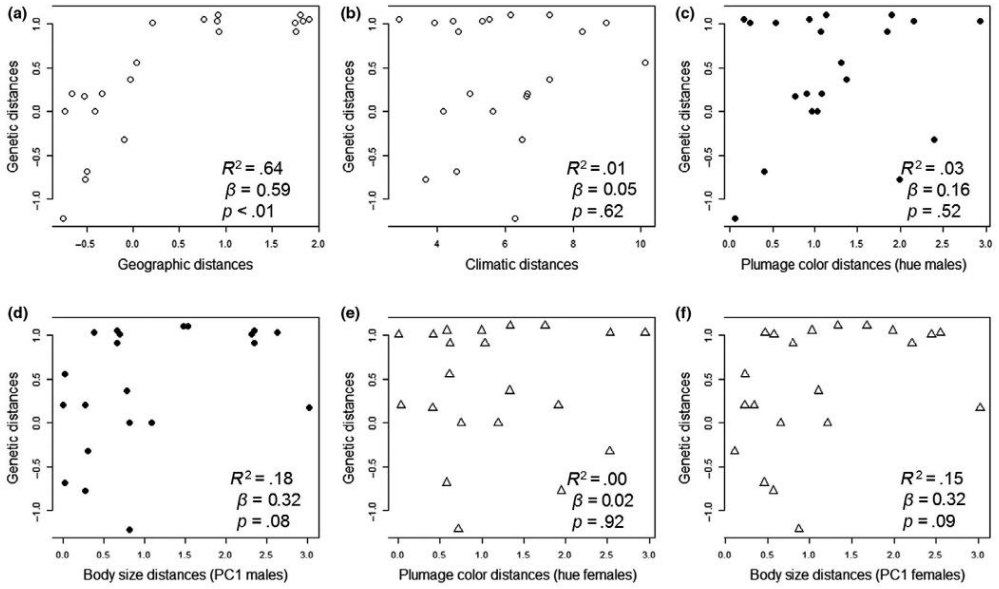

FIGURE 3 Pairwise distance matrices of mitochondrial DNA (mtDNA) against geographic, climatic, plumage color (hue), and body size distances for data grouping by phylogroups of *Habia rubica*. (a and b) geographic distances obtained with geographic centroids and environmental dissimilarity mean obtained from the coordinates per cell of the estimated raster polygon for each phylogroup (including males and females, hollow circles); (c and d) plumage color distances and body size from males (filled circles); (e and f) plumage color distances and body size from females (hollow triangles). Coefficient of determination (R^2), beta weights (β), and *p*-values (*p*) of each relationship tested are shown on the graph

TABLE 1 Results of univariate MMRR analysis grouping by sex for analysis between phylogroups and individuals of *Habia rubica*, testing three independent variables of distance: genetics, color, and body size. Here, we show the results of coefficient of determination (R^2), beta weights (β) and *p*-value (*p*) for each predictor. Because the genetic distances between phylogroups were the same, a single centroid was calculated per phylogroup and the same polygons were obtained for each phylogroup, and the first two results for analysis between phylogroups show the relationship between genetics, geography, and climate of both sexes

	Analysis by individuals						Analysis by phylogroups					
	Males			Females			Males			Females		
	R^2	β	*p*	R^2	β	*p*	R^2	β	*p*	R^2	β	*p*
(a) MRM (mtDNA ~ Predictor)												
Geography	**0.73**	**0.83**	**<.01**	**0.74**	**0.83**	**<.01**	**0.64**	**0.59**	**<.01**			
Climate	**0.09**	**0.13**	**<.01**	**0.10**	**0.16**	**<.01**	0.01	0.05	.62			
Hue total	0.00	0.02	.62	0.00	−0.03	.38	0.03	0.16	.52	0.00	0.02	.92
Hue dorsal	0.00	0.09	<.01	0.00	0.06	.06	0.08	0.27	.21	0.07	0.22	.37
Hue ventral	4E−07	−0.001	.97	0.00	−0.01	.76	0.05	−0.18	.50	0.08	0.26	.29
Chroma total	0.00	−0.04	.18	0.00	−0.06	.08	0.06	0.22	.30	0.01	0.097	.74
Chroma dorsal	0.00	−0.04	.20	0.00	−0.03	.31	0.05	0.20	.33	0.07	0.25	.32
Chroma ventral	0.00	−0.05	.10	0.00	−0.07	.04	0.05	0.20	.48	0.00	−0.03	.95
Volume total	0.00	−0.02	.54	0.00	−0.004	.94	0.01	−0.09	.72	0.02	−0.12	.40
Volume dorsal	0.00	−0.03	.39	0.01	0.068	.05	0.01	−0.10	.78	0.04	−0.15	.56
Volume ventral	3E−08	0.00	1.00	0.00	0.033	.27	0.05	−0.17	.45	0.01	−0.08	.73
Body size	**0.07**	**0.33**	**<.01**	**0.02**	**0.19**	**<.01**	0.18	0.32	.08	0.15	0.32	.09
(b) MRM (Hue ~ Predictor)												
Geography	0.00	0.02	.36	4E−07	−0.00	.99	0.11	0.26	.12	0.04	0.17	.44
Climate	1E−05	0.00	.95	0.01	−0.04	.10	3.88	0.00	.99	0.03	−0.08	.57
mtDNA	0.00	0.01	.63	0.00	−0.02	.42	0.03	0.18	.54	0.00	0.03	.91
Body size	0.01	0.10	.05	0.00	0.05	.39	0.02	0.10	.70	0.00	−0.01	.97
(c) MRM (Chroma ~ Predictor)												
Geography	0.00	−0.04	.26	0.00	−0.04	.30	0.13	0.30	.06	0.04	0.15	.45
Climate	0.00	−0.01	.78	4E−05	−0.00	.92	0.00	0.00	.94	0.00	0.01	.92
mtDNA	0.00	−0.04	.20	0.00	−0.05	.08	0.06	0.28	.31	0.01	0.11	.71
Body size	0.00	−0.03	.63	0.00	0.04	.51	0.00	0.02	.90	0.06	−0.22	.32
(d) MRM (Volume ~ Predictor)												
Geography	0.00	−0.02	.59	1E−05	−0.00	.91	0.00	−0.03	.88	0.00	0.06	.83
Climate	0.00	−0.00	.82	0.00	−0.03	.33	0.04	−0.10	.53	0.02	0.06	.52
mtDNA	0.00	−0.02	.55	1E−05	−0.00	.92	0.01	−0.14	.73	0.02	−0.18	.37
Body size	0.00	0.05	.51	0.01	0.16	.06	0.07	−0.23	.35	0.02	−0.13	.68
(e) MRM (Body size ~ Predictor)												
Geography	0.05	0.17	<.01	0.02	0.12	<.01	0.34	0.56	.05	0.29	0.48	.06
Climate	0.02	0.05	.02	0.00	0.02	.24	0.00	0.02	.93	0.00	0.02	.89
mtDNA	0.07	0.21	<.01	0.02	0.12	<.01	0.18	0.56	.09	0.15	0.48	.09
Hue total	0.01	0.09	.05	0.00	0.04	.41	0.02	0.16	.71	0.00	−0.01	.97
Hue dorsal	0.00	1.05	.16	0.00	0.01	.77	0.01	0.12	.77	0.00	0.04	.92
Hue ventral	0.00	0.02	.74	4E−05	−0.01	.89	0.01	−0.10	.75	0.13	0.39	.34
Chroma total	0.00	−0.02	.64	0.00	0.03	.52	0.00	0.03	.89	0.06	−0.27	.33
Chroma dorsal	0.00	−0.01	.77	0.00	0.05	.25	0.00	−0.05	.85	0.00	0.02	.95
Chroma ventral	0.00	−0.04	.37	0.00	0.05	.33	0.02	0.16	.70	0.12	−0.39	.21
Volume total	0.00	0.03	.53	0.01	0.08	.08	0.07	−0.27	.36	0.02	−0.13	.67
Volume dorsal	0.00	−0.02	.66	0.00	−0.01	.86	0.06	−0.27	.42	0.04	−0.17	.55
Volume ventral	0.00	−0.01	.82	0.00	−0.01	.78	0.07	−0.26	.35	0.02	−0.14	.61

In bold, high beta values were highlighted that were significant in the test.

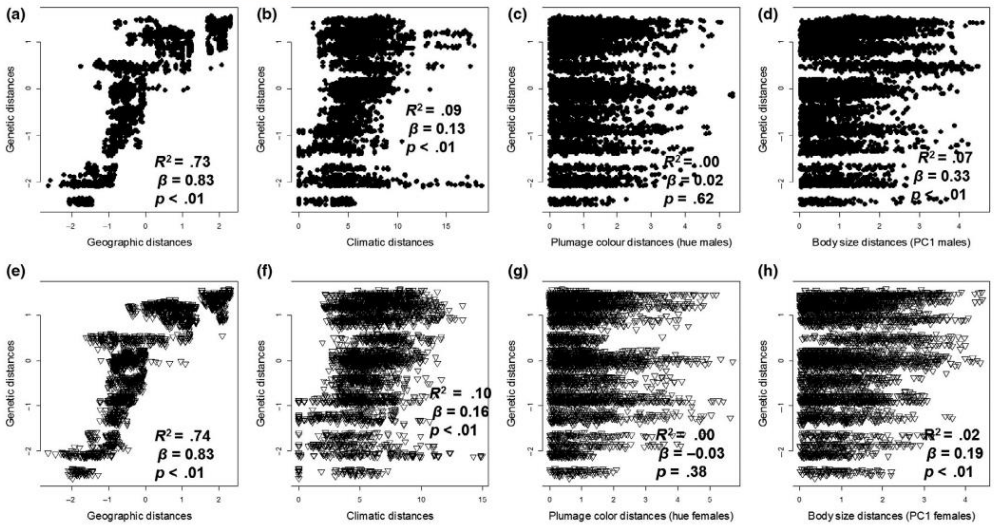

FIGURE 4 Pairwise distance matrices of mitochondrial DNA (mtDNA) against geographic distance, climate, color, and body size between individuals of *H. rubica* (black points (a–d): phenotypic data of males; hollow triangles (e–h): phenotypic data of females). Beta weights (β) and *p*-value (*p*) of each relationship tested are shown on the graph

in the phylogroup-level analysis, it was the only significant predictor variable. Geographic distance accounted for between 63% and 81% of the total variance explained by the multivariate models (Table 2a, Appendix S3: Table S3.6), while climate, plumage color, and body size variables each accounted for less than 10% of the variance explained by the overall model. In the individual-level analyses, climate and body size had significant effects on genetic variation in males and only climate variables affected genetic variation in female, but the effects were weak overall. Our results did not differ when different plumage color metrics were used (Table 2b).

In the comparison between the univariate and multivariate results obtained through the data matching (Tables 1 and 2) and through the matrices obtained for Mexico (Appendix: Table S3.3 and S3.4), no significant differences were observed, given that in this last analysis also was the geographic distance the factor that explains the greatest proportion of the genetic variation found in *H. rubica* (R^2 = 0.31, β = 0.50 for males and R^2 = 0.48, β = 0.86 for females). However, the environmental difference also proved to be an important factor (R^2 = 0.34, β = 0.18 for males and R^2 = 0.23, β = 0.22 for females). In the case of the regressions made with data grouped by phylogroups, the contribution of the factors was not significant.

4 | DISCUSSION

Our results provide strong evidence that geographic distance is a major driver of genetic variation in *H. rubica*. We did not find evidence that climate variation or phenotypic variation (i.e., body size and plumage coloration) is driving population differentiation in this species complex over its large geographic distribution.

4.1 | Isolation by distance

Multiple Matrix Regression with Randomization analyses revealed that geographic distance was the predominant factor explaining patterns of deep genetic differentiation across populations of *H. rubica* (Ramírez-Barrera et al., 2018). This result is consistent between the phylogroup-level and individual-level analyses (Tables 1 and 2). These results suggest that population differentiation in *H. rubica* might be explained mostly by a process of isolation by distance (IBD, Wright, 1943). Under this process, the observed genetic structure suggests equilibrium between gene flow and drift (Hutchison & Templeton, 1999) that could be explained by two processes: long-distance movement and local dispersal (Malpica & Ornelas, 2013).

It is generally accepted that IBD is one of the main factors driving genetic divergence in natural populations (Wu, Yu, & Xu, 2016). Since IBD considers the role of geographic barriers in the process of genetic differentiation among populations in addition to distance per se, patterns of differentiation can provide information on the historical patterns of dispersal by the taxon (Garrido-Garduño & Vázquez-Domínguez, 2013; Slatkin, 1994). Species diversification can therefore be strongly influenced by processes such as plate tectonics and climate change that promote speciation by vicariance, as well as speciation by dispersal events. The effects of the paleogeographic changes in the Miocene and Pliocene on

TABLE 2 Results of multivariate MMRR analysis grouped by sex for analysis between phylogroups and individuals of *Habia rubica*. We show the results of coefficient of determination (R^2) Beta weights (β) and *p*-value (*p*) and of each predictor from the overall model

	Analysis by individuals						Analysis by phylogroups					
	Males			Females			Males			Females		
	R^2	β	*p*	R^2	*B*	*p*	R^2	β	*p*	R^2	β	*p*
(a) MRM (mtDNA ~ Geography + Clime + Hue + Body size)												
Geography	0.74	0.80	<.01	0.74	0.81	<.01	0.66	0.64	<0.01	0.66	0.64	<.01
Climate		0.03	<.01		0.03	<.01		0.01	0.86		0.00	.99
Hue		−0.02	.17		−0.02	.19		−0.11	0.56		−0.13	.46
Body size		0.09	<.01		0.02	.14		−0.06	0.64		−0.06	.67
(b) MRM (Hue ~ Geography + Clime + mtDNA + Body size)												
Geography	0.01	0.06	.35	0.012	0.07	.24	0.15	0.50	0.17	0.15	0.54	.17
Climate		−0.003	.88		−0.04	.12		−0.01	0.93		−0.09	.56
mtDNA		−0.06	.27		−0.06	.19		−0.30	0.52		−0.44	.38
Body size		0.10	.06		0.05	.37		−0.10	0.70		−0.18	.52
(c) MRM (Chroma ~ Geography + Clime + mtDNA + Body size)												
Geography	0.00	−0.03	.70	0.00	0.02	.82	0.20	0.54	0.13	0.23	0.50	.14
Climate		−0.00	.97		0.00	.86		−0.01	0.91		0.00	.97
mtDNA		−0.00	.93		−0.07	.22		−0.17	0.70		−0.22	.57
Body size		−0.02	.75		0.05	.42		−0.24	0.33		−0.45	.07
(d) MRM (Volume ~ Geography + Clime + mtDNA + Body size)												
Geography	0.00	−0.01	.85	0.01	−0.01	.90	0.15	0.36	0.34	0.20	0.62	.11
Climate		−0.00	.86		−0.04	.33		−0.09	0.58		0.07	.51
mtDNA		−0.02	.73		0.00	.93		−0.31	0.52		−0.75	.09
Body size		0.07	.45		0.17	.10		−0.35	0.26		−0.27	.27
(e) MRM (Body size ~ Geography + Clime + mtDNA + Hue)												
Geography	0.08	0.01	.88	0.027	0.07	.16	0.35	0.71	0.16	0.31	0.62	.19
Climate		0.02	.24		0.00	.86		−0.01	0.95		−0.02	.90
mtDNA		0.18	<.01		0.06	.17		−0.19	0.70		−0.19	.72
Hue		0.09	.05		0.04	.35		−0.11	0.74		−0.16	.63

speciation trends in neotropical birds are related to the formation and disappearance of barriers and bridges, which influence and even change migration and isolation patterns that favor vicariance (Coyne & Orr, 2004; Rull, 2008). The complicated phylogeographic structure of *H. rubica* is consistent with some geological and biogeographic characteristics of their distribution that could limit gene flow between remote populations (Lavinia et al., 2015; Ramírez-Barrera et al., 2018).

The phylogeographic structure of the *H. rubica* species complex can be grouped in seven phylogroups (Ramírez-Barrera et al., 2018), five of which are distributed in Mesoamerica (i.e., the region between Central Mexico and Western Panama; García-Moreno, Cortés, García-Deras, & Hernández-Baños, 2006) and two of which are in South America (Figure 2a,g). Given the large number of phylogroups in a comparatively small area, Mesoamerica can be considered a hot spot for this species, where differentiation among populations has occurred in a relatively short time period. Molecular evidence has shown a similar pattern in the plants of Central America, which originated more recently than South America taxa (Pennington et al., 2004; Pennington, Prado, & Pendry, 2000). The five Mesoamerican phylogroups of *H. rubica* are distributed in the northern (NP) and southern (SP) regions of the Mexican Pacific coast, on the slope of the Gulf of Mexico (GM), from Southeastern Mexico to Costa Rica (SE), and Panama (PA). Mesoamerica has been described as a highly fragmented topographic complex where the composition of flora and fauna has been strongly influenced by both climatic and geological events (Burnham & Graham, 1999; Coates & Obando, 1996). These events have given rise to geographic characteristics such as the Balsas River and the Isthmus of Tehuantepec in Mexico and the Central American Volcanic Arc and the Isthmus of Panama in Central America, which could drive the high ecological diversity of this region.

The other two phylogroups of *H. rubica* are distributed in Western (WS) and Eastern/Northwestern (ES) South America (Lavinia et al., 2015; Ramírez-Barrera et al., 2018). We suggest that the association between the populations from Atlantic forests and

northwestern South America (phylogroup ES) could indicate that the evolutionary history of these populations is deeply associated with those reported for the seasonal forests of South America (Banda et al., 2016; Lavinia et al., 2015; Pennington, Lavin, & Oliveira-Filho, 2009; Pennington et al., 2004, 2000; Prates et al., 2017). The rainforests of the Amazon basin and the tropical forests of the Atlantic are two of the most important morphoclimatic domains of South America (Ab'Saber, 1977). These two forests are separated by a diagonal strip of dry vegetation, a corridor considered an important barrier for the migration of species between the two forest regions (Por, 1992). However, vegetation maps show that gallery forests and forests distributed in patches across the dry diagonal constitute an interconnected network (Oliveira-Filho & Ratter, 1995). In addition, several lines of evidence support the hypothesis of old contact between the two regions through this strip of dry vegetation (Auler et al., 2004; Costa, 2003; Oliveira, Barreto, & Suguio, 1999; Por, 1992; Wang et al., 2004). To explain this contact between the eastern and western regions of South America, at least two main routes have been suggested. The first, which arose during the middle to late Miocene, extended through the current Cerrado and Mato Grosso regions of Brazil (Hulka, Grafe, Sames, Uba, & Heubeck, 2006; Roddaz et al., 2006); the second, during the Pliocene and Pleistocene, extended through the current Cerrado and Caatinga regions of northeastern Brazil (Auler et al., 2004; Costa, 2003; Por, 1992; Wang et al., 2004), as a result of the expansion of the gallery forests during the Quaternary climate changes. Some studies have suggested the existence of these old connections in lizard species (Pellegrino, Rodriguez, Harris, Yonenaga-Yassuda, & Sites, 2011; Prates et al., 2017), mammals (Galewski, Mauffrey, Leite, Patton, & Douzery, 2005), and birds (Lavinia et al., 2015). In support of the latter, there is evidence of genetic divergence during the Pleistocene, following the route of expansion of dry habitats between the two biomes (Martins, Templeton, Pavan, Kohlbach, & Morgante, 2009). This hypothesis of the evolution of the vegetation in South American could explain the pattern in *H. rubica* where, as mentioned before, two phylogroups are defined in this area and coincide with the separation of the Amazonian forest from the Atlantic forest as well as the connection between the Atlantic forest and the northwest populations (Banda et al., 2016; Pennington et al., 2009, 2004, 2000; Prates et al., 2017; Ramírez-Barrera et al., 2018). All of the geographic features mentioned above are considered important barriers to dispersal in several animal taxa (Amman & Bradley, 2004; Bryson, García-Vázquez, & Riddle, 2011; BrysonJr, Nieto-Montes de Oca, & Reyes, 2008; Daza, Castoe, & Parkinson, 2010; Devitt, 2006; Gutiérrez-García & Vázquez-Domínguez, 2012, 2013; Navarro-Sigüenza, Peterson, Nyari, García-Deras, & García-Moreno, 2008; Suárez-Atilano, Burbrink, & Vázquez-Domínguez, 2014).

4.2 | Isolation by environment

Environmental dissimilarity did not have a significant effect on genetic differentiation of *H. rubica* after controlling for geographic distance (Table 2). This suggests that geographic isolation (i.e., isolation by distance, IBD; Wright, 1943) but not adaptation to local climatic environments (i.e., isolation by environmental, IBE; Wang & Bradburd, 2014) was the underlying process of the observed patterns of genetic structure (Figure 2g).

This suggests that climate likely is not playing a major role in genetic differentiation within *H. rubica*. However, climatic fluctuations seem to have played a major role in the diversification history of the species. This is also evidenced by the fact that there is a single phylogroup formed in the Gulf of Mexico region, despite a vast range of environmental conditions, from dry forests in Tamaulipas to Rainforest in Veracruz. This might be explained if we consider that even through a very broad distribution area. Therefore, there was little association between genetic differentiation and climatic differentiation. This phylogeographic pattern of *H. rubica* has been previously reported in several species from regions with more contrasting climatic fluctuations in South America (Carnaval, Hickerson, Haddad, Rodrigues, & Moritz, 2009; Yannik et al., 2014).

On the other hand, the lack of signal in isolation by environment analyses in *H. rubica* could occur for other reasons, including adaptation to local environments through phenotypic plasticity (Ramírez-Valiente, Sanchez-Gomez, Aranda, & Valladares, 2010), positive selection on immigrant genotypes from distant populations mediated by heterosis (Bensch et al., 2006), or as a consequence of long-distance gene flow counteracting the effects of natural selection and impeding or attenuating local adaptation processes (Buschbom, Yanbaev, & Degen, 2011). It should be noted that we cannot rule out all hypothesis of isolation by adaptation and isolation by environment, given that other parameters (e.g., vocal variation, other attributes of coloration, vegetation) were not considered in our study and could potentially affect genetic differentiation within the *H. rubica* complex (Lavinia et al., 2015; Ramírez-Barrera et al., 2018).

4.3 | Local adaptation

While our results show a positive correlation between biogeographic patterns of diversification and phenotypic divergence (plumage coloration and body size), the MMRR analysis does not provide enough evidence to support ecological speciation.

Even though plumage differentiation is often considered a relevant character for species delimitation in avian taxonomy, in some cases it does not provide enough evidence for the correct discrimination of species. *Habia rubica* has considerable plumage color differentiation, but this appears to be a result of neutral processes (e.g., genetic drift). We found little support for the role of plumage divergence in explaining genetic divergence (i.e., isolation by adaptation).

Body size clines that are correlated with temperature gradients are common in nature, particularly in birds (Friedman & Remeš, 2016; Meiri & Dayan, 2003). These correlations are often taken as evidence of local thermoregulatory adaptation (Friedman & Remeš, 2017). However, the precise selective agent is debatable, as many variables that plausibly correlate with variation in body mass also covary with elevation, temperature gradients, or latitude (Seeholzer &

Brumfield, 2017). Although body size divergence is likely influenced by environmental divergence, it has not impacted genetic structure in *H. rubica* and, in practice, body size is generally not ultimately an important character in avian species delimitation (Price, 2007). However, phenotypic variation at the intraspecific level may present high correlation with genetic variation (García, Barreira, Lavinia, & Tubaro, 2016).

Plumage and vocal differences are expected to play a more important role in conspecific recognition and mate choice in birds than body size (Hilty, 2011; Lavinia et al., 2015; Price, 2007) and thus may be important in structuring genetic variation (Seeholzer & Brumfield, 2017). It is expected that rapid local adaptation and phenotypic divergence will occur at the edges of range expansions (García-Ramos & Kirkpatrik, 1997), which Mayr proposed as an important driver of incipient speciation (Mayr, 1982).

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CONFLICT OF INTEREST

None declared.

AUTHOR CONTRIBUTIONS

SM Ramírez-Barrera conceived the ideas, designed and performed the research, analyzed data, prepared figures and/or tables and reviewed drafts of the paper. JA Velasco analyzed, interpreted data and reviewed drafts of the paper. T Orozco-Tellez collected part of the data. AM Vázquez-López collected the data. BE Hernández-Baños conceived the ideas, designed the research, contributed reagents/materials/analysis tools and reviewed drafts of the paper.

DATA AVAILABILITY STATEMENT

Sampling locations, morphological, and coloration data: We have curation plans before archiving the data. These curation plans include editing and organizing the morphology and coloration data. The data were grouped by filogroup (geographic region) and is now available for consultation at: *DNA sequences*: https://figshare.com/s/fe9f9f6fed1686782f62; *Morphological data*: https://doi.org/10.6084/m9.figshare.8023565; *Coloration data by phylogroups*: *NP*, northern pacific of Mexico (https://doi.org/10.6084/m9.figshare.9883382); *SP*, southern pacific of Mexico (https://doi.org/10.6084/m9.figshare.9883388); *GM*, Gulf of Mexico (https://doi.org/10.6084/m9.figshare.9883391); *SE*, southeastern Mexico and northern Central America (https://doi.org/10.6084/m9.figshare.9883430; https://doi.org/10.6084/m9.figshare.9883511); *PA*, Panama (https://doi.org/10.6084/m9.figshare.9883514); *WS*, western South America (https://doi.org/10.6084/m9.figshare.9883556; https://doi.org/10.6084/m9.figshare.9883562); *ES*, eastern-northwestern South America (https://doi.org/10.6084/m9.figshare.9883523).

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SUPPORTING INFORMATION

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DISCUSIÓN

En este trabajo se analizaron los patrones de variación geográfica del tamaño corporal y brillo del plumaje de 23 especies de la familia Cardinalidae (Ramírez-Barrera et al. En prep). Se utilizaron las aproximaciones metodológicas basada en ensamblaje (*assemblage-based*) y entre especies (*cross-species*), para comprobar si dichos patrones de variación fenotípica se ajustan a las tendencias geográficas portuladas por las reglas ecogeográficas de Bergmann (Bergmann 1847) y Gloger (Gloger 1833). Se observa que ambos rasgos fenotípicos (tamaño corporal, brillo del plumaje) presentan un patrón geográfico de variación através del rango de distribución de las especies, en dirección norte–sur, con las especies más grandes y más brillantes en la región norte, comparado con aquellas especies de la región sur. Sin embargo, dicho patrón geográfico no fue explicado satisfactoriamente por las variables climáticas temperatura promedio anual y precipitación promedio anual (utilizada como *proxy* de la variable humedad). Es posible que, debido a la compleja red de correlaciones entre los patrones de variación fenotípica y aquellos de variación climática, geográfica y genética (Blackburn & Gaston 1996, Delhey 2019), sea necesario realizar análisis complementarios que nos permitan describir y comprender los mecanismos que subyacen a los patrones geográficos de variación de estos rasgos fenotípicos en las especies de Cardinalidae.

Simultáneamente se analizó el grado de influencia de factores geográficos y climáticos sobre los patrones de diferenciación genética y fenotípica en siete filogrupos identificados dentro de la espacie *Habia rubica* (Ramírez-Barrera et al. 2019). Utilizando el método de regresión múltiple de matrices con aleatorización (MMRR), se pusieron a prueba las hipótesis de aislamiento por distancia (IBD), aislamiento por ambiente (IBE) y aislamiento por adaptación (IBA), para comprobar sus respectivas predicciones sobre el efecto que la distancia geográfica, variables ambientales y variación fenotípica tienen sobre la diferenciación genética poblacional de la especie. Debido al amplio rango de distribución de *H. rubica*, se esperaba que su estructura genética estuviera estrechamente vinculada con una amplia variación climática esperada para los distintos rangos de distribución de sus poblaciones. Lo reportado en este trabajo es que, contrario a lo esperado, la distancia geográfica es el principal factor que explica los patrones de diferenciación genética entre filogrupos e individuos de esta especie. Y por lo tanto, no hay evidencia de que el bajo intercambio génico entre filogrupos se deba a procesos adaptativos relacionados con la variación ambiental de los rangos de distribucón de las poblaciones, o procesos de diferenciación fenotípica poblacional.

INFERENCIAS MACROECOLÓGICAS Y MACROEVOLUTIVAS

Conocer y comprender cuáles son los patrones y procesos que subyacen y dan forma a la variación geográfica de los rasgo fenotípicos que observamos actualmente en los organismos, es uno de los principales objetivos en campos de conocimiento como la macroecología y la macroevolución (McGill et al. 2019). Los estudios macroecológicos han sido utilizados para analizar cómo se relacionan los gradientes espaciales de los rasgos fenotípicos (*e. g.* tamaño corporal, coloración, canto) con la variación ambiental pasada y actual (*e .g.* geografía, clima), en un contexto espacial, y utilizando para ello aproximaciones *assemblage-based* (Olalla-Tárraga et al. 2010). Sin embargo, estos análisis no resultan adecuados para realizar inferencias sobre los procesos evolutivos que pudieron haber dirigido la evolución fenotípica, a través de escalas temporales profundas o más antiguas (Slavenko et al. 2019). En contraste, las aproximaciones macroevolutivas describen los procesos históricos (*i. e.* filogenéticos) que produjeron los patrones macroecológicos que vemos en la actualidad (McGill et al. 2019). Ésto, nos permite identificar cambios en el *tempo* (*i.e.* tasas de evolución; o que tan rápido evolucionan los rasgos y cómo cambia esta tasa a lo largo de la historia de un clado) y *modo* (i. *e.* procesos; es decir, "vía, manera o patron" que subyace a la evolución de los rasgos) de la evolución fenotípica dentro de un contexto temporal, utilizando aproximaciones *cross-species* (Clavel & Morlon 2017, Matysioková et al. 2017). Esta aproximación puede evaluar las diferencias en la evolución de los rasgos asociadas a regiones o ambientes determinados, pero es ineficiente para considerar factores espacialmente explícitos (*e. g.* geográficos, climáticos) que podrían tener un efecto sobre los procesos de diferenciación de los rasgos (Velasco et al. 2020). Debido a que ambas perspectivas exploran diferentes dimensiones de los patrones fenotípicos, su integración simultánea nos permitiría comprender cómo surgen y evolucionan los patrones de biodiversidad fenotípica a través del tiempo y el espacio (McGill et al. 2019).

Los rasgos fenotípicos son las características observables de los organismos, y éstos son regulados por factores como la expresión génica, modificaciones epigenéticas, variación ambiental e historia de vida (Goldenberg et al. 2022). En las aves, esta enorme diversidad fenotípica abarca aspectos tales como tamaño corporal, forma corporal, coloración del

plumaje, canto y comportamiento (Töpfer 2018), mediante los cuales transmiten diversos tipos de información a través de un conjunto de señales (*e. g.* visuales, acústicas). Estas señales son emitidas y recibidas por los individuos a través del ambiente, y diversos factores pueden influir de manera importante sobre la evolución de las señalizaciones, entre ellas las condiciones ambientales particulares, la morfología de los individuos y las interacciones intra (*e. g.* selección sexual) e interesecíficas (*e. g.* competencia, reforzamiento), (González-Voyer et al. 2013). Sin embargo, nuestra comprensión sobre los procesos evolutivos que dan origen a la diversidad fenotípica a traves de grandes radiaciones, y el grado de influencia que tienen los efectos combinados de las interacciones bióticos (*e. g.* depredadores, competidores, elección de pareja) y los factores abióticos (*e. g.* geográficos, climáticos), aún es reducido (Dale et al. 2015, Cooney et al. 2017).

Algunos patrones de variación espacial a gran escala en la forma o función de los rasgos fenotípicos son descritos por las reglas ecogeográficas (Delhey 2019). Las reglas ecogeográficas describen correlaciones entre las variables fenotípicas de los organismos y las variables ambientales asociadas a su rango de distribución geográfica, que se mantienen a través de los taxa y el espacio geográfico; éstas son consideradas evidencia sólida sobre el papel de la adaptación a través de mecanismos ecológicos y evolutivos, que permiten a las especies mantenerse en su ambiente mediante la variación (Mayr 1956, Gaston et al. 2008, Marcondes et al. 2020). Estos patrones de variación geográfica en las aves han sido asociados con factores como el clima, latitud, altitud y disponibilidad de recursos, por mencionar algunos (Graves 1991, Roulin 2014).

La coloración animal, al igual que otras adaptaciones fisiológicas, estructurales y de comportamiento, presenta múltiples funciones, entre las que se encuentran el camuflaje, señalización intra y enterespecífica, termorregulación, protección UV, y protección antimicrobiana (Goldenberg et al. 2022). Se puede esperar que la fuerza y el óptimo de selección en cada uno de estos mecanismos varíe espacialmente entre hábitats debido a las diferencias en el comportamiento, el clima, y las condiciones de iluminación (Hill & McGraw, 2006). Entre estas funciones de la coloración, la termorregulación y el camuflaje críptico se reconocen como las más dependientes de la variación ambiental, debido a que éstas

implican mecanismos de absorción y reflexión selectiva la rediación solar, una dinámica que afecta directamente la temperatura corporal y la conspicuidad de las especies (Crusella-Trullas et al. 2011). Entre los pigmentos biológicos que determinan el grado de oscuridad de la coloración animal se encuentra la melanina, una macromolécula multifuncional que determina la coloración más oscura mediante la mayor deposición de dicho pigmento (Protas 1992). De esta manera, se espera que, si asumimos condiciones ambientales, radiación solar y material (*i. e.* individuo) constantes e iguales, un plumaje más brillante (o pobre en melanina) reflejará más luz y absorverá menos energía solar, que un color oscuro (o rico en melanina) que, se espera, absorberá más energía y la transformará en calor (Mader et al. 2022). El tamaño corporal por su parte, también realiza funciones biológicas clave relacionadas con la regulación de la temperatura corporal a través de un mecanismo conocido como inercia térmica (*i. e.* velocidad de intercambio de calor de un material, en relación a su masa, con respecto al ambiente externo) de los organismos, que representa la relación superficie/volumen (S:V; Sears & Angilletta 2015). Así, se espera una ventaja evolutiva de las especies pequeñas distribuidas en ambientes cálidos, entre la cuales suponemos debería haber un intercambio de calor más rápido con el ambiente; contrario a lo que se espera en especies grandes distribuidas en ambientes fríos, donde la velocidad de ganancia y pérdida de calor deberá ser más lenta (Goldenberg et al. 2022).

En este contexto, existen dos reglas ecogeográficas que puntualmente postulan tendencias de variación geográfica sobre la coloración y el tamaño corporal de los animales endotérmicos. La regla de Gloger (Gloger 1833) inicialmente describió la variación en los patrones de coloración de las aves en función de la temperatura, donde las especies más oscuras tenderán a distribuirse en regiones tropicales cálidas (Figura 1A). Posteriormente, esta regla fue acuñada y revisada casi 100 años después por Bernhard Rensch (1929) postulando, en su forma más simple, que los ambientes cálidos y húmedos tenderán a seleccionar fenotipos más oscuros (*i. e*. mayor deposición de pigmentos de melanina) en animales endotérmicos. Una versión más compleja de esta regla examina los efectos diferenciales de la temperatura y la humedad a partir de dos tipos de melaninas (*i. e.* eumelaninas, feomelaninas). Las eumelaninas son pigmentos que proporcionan los tonos

que van del negro al gris, mientras que las feomelaninas confieren tonos que van del marrón al rojizo (Delhey 2017, 2019). Se sabe que ambos tipos de melaninas aumentan en respuesta a climas húmedos y las feomelaninas aumentan en respuesta a climas secos (Figuras 1B, 2). Como resultado de estas tendencias se espera que los colores feomelánicos (*i. e.* marrones a rojizos) predominen en condiciones secas y cálidas, mientras que los colores eumelánicos (*i. e.*negros a grises) lo hagan en condiciones húmedas y cálidas.

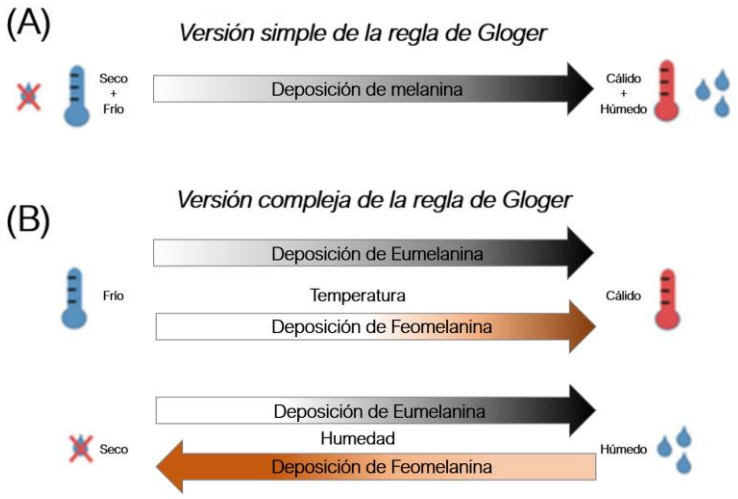

Figura 1. Presentación resumida de las versiones simple (A; Gloger 1833) y compleja (B; Rensch 1929) de la regla de Gloger. Imagen tomada y modificada de Delhey (2019).

En contraste con algunas otras reglas ecogeográficas, la regla de Gloger no se encuentra asociada a una explicación mecanisista clara (Delhey 2019). Aunque se han propuesto cuatro posibles explicaciones, la primera, indica que la melanina posee propiedades antimicrobianas que estarían siendo seleccionadas en especies distribuidas en ambientes cálidos/humedos donde las condiciones son propicias para el crecimiento bacteriano (Burtt Jr. & Ichida 2004); la segunda, postula la eficacia de protección UV de la melanina, al dispersar y reducir la penetración de radiaciones UV (Chaplin 2004); la tercera, sugiere un efecto pleiotrópico de los genes que regulan la producción de melanina, influyendo sobre la variación de la oscuridad del color, y la selección de caracteres fisiológicos y de comportamiento como efectos secundarios (Ducrest et al. 2008); y la cuarta

explicación postula un mejor camuflaje en hábitats húmedos, relacionados con estructura de dosel que limita la exposición de luz del sotobosque (Cheng et al. 2018). Finalmente, a pesar de que la regla de Gloger no propone explícitamente mecanismos de regulación térmica detrás de la variación de la coloración, se ha propuesto que el plumaje más oscuro puede tener efectos refrescantes en ambientes más cálidos y húmedos, sobre la temperatura corporal. Esto debido a que la carga de calor por radiación solar de dichos plumajes llega a ser más baja comparada con aquella de plumajes más claros, cuando se consideran factores tales como la velocidad del viento y el tipo de plumaje de las especies (*i. e.* rígido, suave); de manera que aquellos plumajes negros en el desierto y los blancos en regiones polares podrían resultar térmicamente ventajosos (Walsberg et al. 1978).

En general, ningún mecanismo de adaptación propuesto para la versión simple de la regla de Gloger ha obtenido evidencia a traves de las especies de aves analizadas. Inlcuso, análisis recientes han mostrado resultados contradictorios (Delhey 2017, Delhey et al. 2019), en los que se observan tendencias de variación de poblaciones más oscuras en regiones más húmedas (consistentes con la regla de Gloger) y más frías (consistente con la hipótesis de melanismo térmico; Bogert 1949). Y en cuanto a la versión compleja, no se ha propuesto algún mecanismo específico. Esto, aunado al hecho de que existen muy pocos estudios que hayan explorado los patrones de variación geográfica en términos de la regla de Gloger, utilizando para ello datos de cuantificación objetiva del color, como los obtenidos por espectrometría de reflectancia, o datos de variables bioclimáticas actuales basadas en tendencias anuales como las disponibles en WorldClim; dado que la mayoría de los estudios han sido realizados a partir de las comparaciones de color entre individuos distribuidos en regiones categorizadas como “áridas” y “húmedas”, mientras que el color ha sido evaluado de manera subjetiva a partir de categorías hechas a simple vista utilizando tablas de color (Burtt & Ichida 2004, Marcondes et al. 2020), lo que hace evidente que la exploración de reglas ecogeográficas postuladas sobre los últimos 150 años deben seguir siendo exploradas en el contexto de temas de actualidad, como el fenómeno del calentamiento global, y nuevos métodos de análisis que consideren el complejo escenario de los procesos de evolución de lo caracteres fenotípicos.

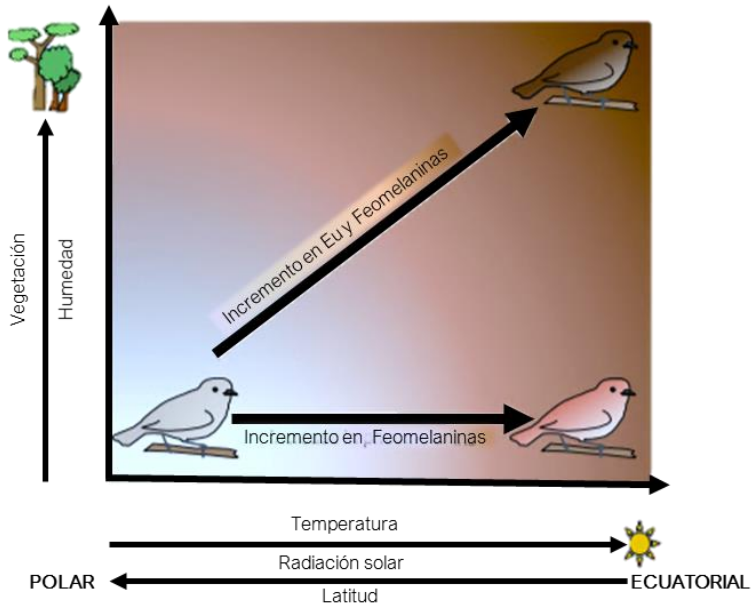

Figura 2. Representación gráfica de la regla de Gloger postulada por Rensch (1929). Imagen tomada y modificada de Delhey (2019).

La regla de Bergmann (Bergmann 1947) es una de las reglas ecogeográficas más conocidas y mejor estudiadas. Esta regla establece que entre especies estrechamente relacionadas el tamaño corporal tiende a aumentar hacia ambientes más fríos en latitudes altas. Originalmente, este gradiente de variación del tamaño corporal fue propuesto para animales vertebrados endotérmicos, postulando que aquellas especies de cuerpo más grande deberían conservar el calor corporal de manera mas eficiente debido a la reducida proporción superficie/volumen (Gastón et al. 2007). Este mecanismo de termorregulación en los endotermos está basado en el conocimiento de que en este grupo de animales la producción de calor está relacionada con el volumen corporal, mientras que la discipación se produce a través de la superficie externa del cuerpo (Goldenberg et al. 2022). Esta relación permite que los animales más grandes (*i. e.* baja relación superficie/volumen) produzcan más calor, a través de un metabolismo más eficiente, del que pierden o discipan (Peters 1983) y, por lo tanto, este mecanismo adaptativo de la conservación del calor está estrechamente asociado a la ventaja selectiva de los animales más grandes en climas más fríos, como estrategia para conservar mejor el calor metabólico (Lindsey 1966, James 1970).

Incluso, de manera contraria al supuesto común, la idea central de la regla de Bergamnn se refiere a la producción de calor de las especies más grandes en ambientes contrastantes; y no al mantenimiento de la temperatura corporal a través de gradientes ambientales (Salewski & Watt 2017). Blackburn y Gaston (1996) analizaron los patrones de variación de la masa corporal de las aves del Nuevo Mundo y reportaron que las tendencias de variación parecen espacialmente ordenadas. Con tamaños corporales más pequeños (*i. e.* masa corporal) en las regiones cercanas al ecuador, que van aumentando conforme se alejan hacia lo polos (Figura 3). Este patrón geográfico descrito, implica que son las regiones polares, y su contraste con las regiones tropicales, lo que determina los patrones geográficos de variación del tamaño corporal, y más aún sugiere que el mecanismo de termorregulación podría estar efectvamente vinculado (al menos en las aves) con estrategias de tolerancia fisiológica hacia las regiones geográficas que habitan.

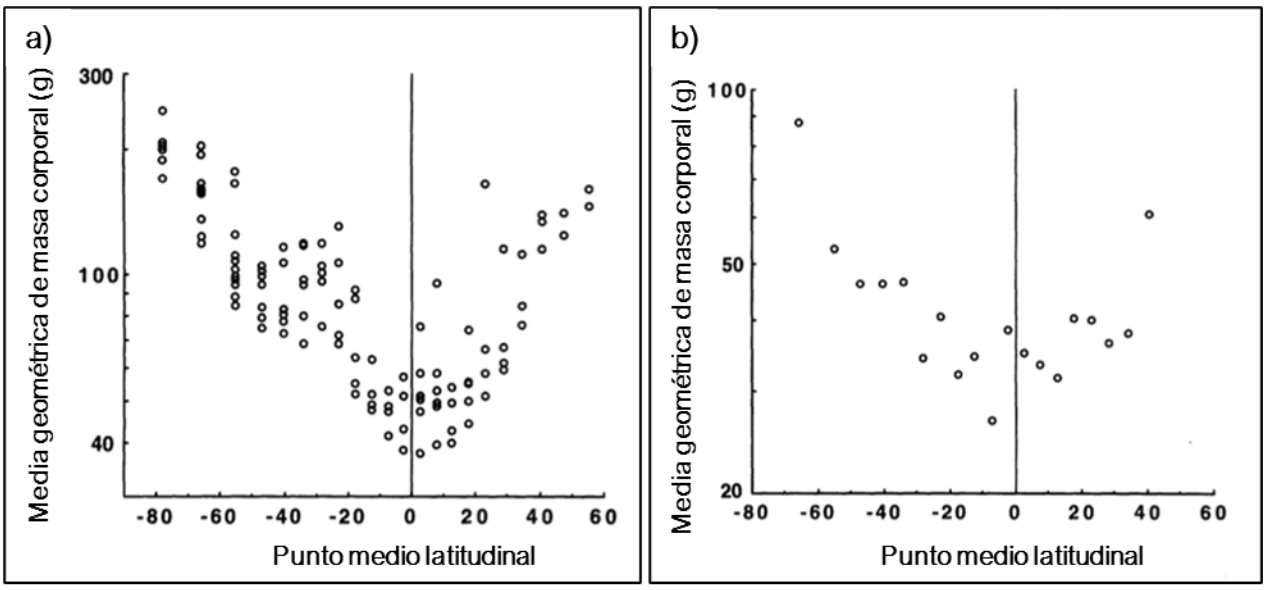

Figura 3. Relación entre masa corporal promedio (g) y latitud de distribución de las aves del Nuevo Mundo, calculada (a) a través de cuadrícula WORLDMAP, usando el método el método de celdas, y (b) utilizando el método de punto medio (promedios). Imagen tomada y modificada de Blackburn & Gaston 1996.

Los resultados obtenidos en este estudio muestran que los patrones de variación del tamaño corporal y el brillo del plumaje entre las especies de Cardinalidae parece estar espacialmente agrupados. Este patrón muestra una tendencia a presentar tamaños

corporales más pequeños y plumajes menos brillantes en las regiones cercanas al ecuador (más cálidas y húmedas) mientras que los tamaños corporales más grandes y plumajes más brillantes se encuentran en las regiones más cercanas a ambos polos donde predomina una temperatura y humedad más baja (Ramírez-Barrera et al. En prep.). Las variables ambientales temperatura promedio anual y precipitación promedio anual, no explican la variación de los rasgos fenotípicos probados para regla de Bergmann (*i.e.* tamaño corporal) y regla de Gloger (*i.e.* brillo del plumaje). La complejidad de relaciones entre variables ambientales, geográficas y evolutivas que determinan los procesos de diversificación de los rasgos fenotípicos indica que es necesario implementar nuevos fundamentos teóricos que podrían explicar de manera más eficaz los mecanismos que subyacen los patrones de la variación fenotípica entre las especies de esta familia de aves. Además, identificar las razones por las que las reglas ecogeográficas aplican en algunos casos pero en otros no es importante para establecer la validez general y la utilidad de dichas reglas, así como de los mecanismos adaptativos que las respaldan.

Divergencia genética y fenotípica

Comprender los patrones y procesos que determinan la estructura de la variación genética en las poblaciones naturales es uno de los objetivos de la ecología y la evolución (Yannik et al. 2017). A pesar de que los campos de conocimiento de ambas disciplinas comienzan con análisis a micro y macroescala, es durante el transcurso del siglo XX que las principales tendencias comienzan a promover y señalar la necesidad de fundar un campo de conocimiento a nivel micro en ambas disciplinas (Brown & Maurer 1989, Stanley 1975). De esta manera, dentro del campo de conocimiento de la evolución el estudio de los procesos que ocurren dentro de una especie se denomina microevolución, y se diferencia de la macroevolución en que ésta se encarga de analizar cuestiones por encima del nivel de especie; por su parte, la microecología estudia los procesos a pequeña escala que involucran mecanismos fisiológicos, y de comportamiento a nivel de poblaciones y de comunidades, diferenciándose de la macroecología en que ésta última aborda los estudios a gran escala espacial, temporal y taxonómica (McGill et al. 2019). Es en este contexto de estudio a nivel microevolutivo y microecológico, que surge el campo de conocimiento de la genética del

paisaje (Manel et al. 2003). Esta disciplina busca comprender los patrones y procesos que originan la estructura genética de las poblaciones, analizando la inlfuencia de diferentes características del paisaje sobre los patrones de flujo génico y procesos de adaptación local, dentro de un marco teórico espacialmente explícito (Segelbacher et al. 2010); y utiliza para ello métodos combinados de ecología del paisaje y genética de poblaciones.

Los patrones espaciales de diferenciación genética con frecuencia reflejan procesos de variación espacial en la interrupción del flujo génico (Wang 2013). Es bien conocido que tanto la distancia geográfica como las características del paisaje son los dos factores principales que dirigen los procesos de diferenciación genética poblacional (Wright 1943, Manel et al. 2003). Por un lado, las características del paisajes pueden restringir el flujo génico de las poblaciones de dos maneras importantes, que dan origen a dos patrones de diferenciación genética que se explican a continuación. La primera forma ocurre mediante procesos de "aislamiento ecológico" entre poblaciones (Dobzhansky 1937), en los que se interrumpe o se limita el flujo génico entre poblaciones que habitan diferentes ambientes ecológicos. Este proceso dará como resultado una correlación positiva entre las distancias genéticas y las distancias climáticas o ambientales de las poblaciones; dicho fenómeno es conocido con el nombre de hipótesis de aislamiento por ambiente (IBE, Wang & Summers 2010, Wang & Bradburd 2014). El IBE puede surgir bajo distintos escenarios, entre los que se pueden señalar cuatro principales: 1. Por preferencias de dispersión sesgadas hacia ambientes particulares o hábitats determinados por el fenotipo, genotipo o el comportamiento de los individuos (Nosoil 2004, Nosil et al. 2005); 2. Por efecto de la selección que actúa negativamente contra los genotipos inmigrantes que están adaptados a sus entornos locales originales; 3. Por selección sexual que reduce el éxito reproductivo de los individuos "dispersores" con rasgos sexuales divergentes, o debido a las preferencias de elección de pareja; y finalmente, 4. Por el surgimiento de híbridos entre individuos nativos e inmigrantes adaptados a diferentes condiciones ambientales que, por lo tanto, tienen una adecuación reducida (e.g. selección en contra de rasgos intermedios o menos adaptados).

Una segunda consecuencia del "aislamiento ecológico" de las poblaciones se da cuando estos mismos gradientes ambientales que generan procesos de IBE, producen

selección natural divergente que deriva en la diferenciación de rasgos adaptativos entre poblaciones (Endler 1977). Esto debido al efecto de presiones selectivas locales que varian a través del espacio y que están vinculadas a condiciones ambientales particulares. Además de esto, la evolución de rasgos adaptados localmente que proporcionan ventajas en la adecuación de los individiduos también es capaz de inluenciar el flujo génico entre y dentro de las poblaciones, y potencialmente la diferenciación genética. Este proceso dará como resultado una correlación positiva entre la diferenciación genética y la diferenciación adaptativa de las poblaciones (*i. e.* fenotípica), y se conoce con el nombre de hipótesis de aislamiento por adaptación (IBA, Rundle & Nosil 2005, Shafer & Wolf 2013).

Por otro lado, la distancia geográfica genera procesos de "aislamiento geográfico" entre poblaciones por efecto de distancias y barreras geográficas, que producen movimientos de dispersión espacialmente limitados. Este aislamiento de las poblaciones y el bajo o nulo intercambio génico que presentan genera que los efectos de la deriva génica sean más fuertes por acumulación de mutaciones neutrales que resultan en su diferenciación genética (Wright 1943, Manel et al. 2003). Este proceso dará como resultado una correlación positiva entre las distancias genéticas y las distancias geográficas de las poblaciones, este fenómeo es conocido con el nombre de hipótesis de aislamiento por distancia (IBD, Wright 1943, Slatkin 1994). Este patrón de diferenciación por procesos de IBD representa un modelo nulo, donde la cantidad de flujo génico entre poblaciones disminuye conforme aumenta la distancia geográfica que las separa (IBD). Y esto significa que la diferenciación genética entre poblaciones es resultado únicamente de los efectos neutrales de la deriva génica, y no de procesos que implican mecanismos adaptativos dirigidos por presiones de selección específicas (Wright 1943). Sin embargo, al examinar los efectos que los factores geográficos y ecológicos tienen sobre los procesos de reducción del flujo génico entre poblaciones, podemos contribuir a una comprensión más completa e integral sobre cómo los paisajes dan forma a los patrones de variación genética en la naturaleza (Wang & Summers 2010). Los distintos procesos de IBD, IBE e IBA representan trayectorias evolutivas clave en las que la distancia geográfica y la heterogeneidad del paisaje pueden influir la diferenciación genética entre poblaciones (Wang et al. 2013). Explorar la importancia

relativa de estos procesos, puede ayudarnos a comprender las características del paisaje que limitan el flujo génico, y cómo las poblaciones de adaptan a sus entornos locales. Esto tiene importantes implicaciones evolutivas que nos permitirán dilucidar los mecanismos mediante los cuales las especies se adaptan a sus ambientes.

En este estudio se analizaron los patrones de variación genética espacial de las poblaciones de la especie *Habia rubica*, dentro de las cuales se ha reportado una profunda estructura genética (Ramírez-Barrera et al. 2018). El objetivo fue determinar si dichos patrones de diferenciación genética poblacional son resultado de procesos adaptativos asociados al aislamiento ecológico de sus poblaciones (*i. e.* IBE, IBA); o si, por el contrario, obedecen más bien al efecto de la deriva génica, como resultado del aislamiento por distancia geográfica (*i. e.* IBD). Se reportó que es el aislamiento geográfico, y no el climático, el factor que mejor explica el patrón de diferenciación genética entre las poblaciones de esta especie. Por lo tanto, no hay evidencia de que la variación ambiental entre los rangos de distribucón de las poblaciones de *H. rubica* se encuentre asociada con los mecanismos de diferenciación poblacional en esta especie.

CONCLUSIONES

La diferenciación fenotípica observada en las especies de la familia Cardinalidae es altamente variable respecto a la coloración del plumaje, el canto, el tamaño corporal, la distribución geográfica, los movimientos de migración y el grado de dimorfismo sexual (Winkler et al. 2020, Scott et al. 2024). Esto representa un escenario ideal para la evaluación de patrones de variación fenotípica en aves, así como los principales mecanismos involucrados en sus procesos de diversificación a gran escala. Mediante el estudio de las variables que regulan la evolución de la coloración dentro de la familia se han observado patrones interesantes (Scott et al. 2023). Por ejemplo, Stoddard & Prum (2008) observaron que dentro del subclado "azul" de Cardinalidae la diversidad de coloración es producida por una compleja combinación de mecanismos estructurales y pigmentarios, en el que todas las especies presentan al menos un parche de coloración azul estructural que refleja longitudes de onda dentro del espectro ultravioleta, visible para las aves. Esta coloración domina los patrones de las especies del género *Cyanocompsa*, junto con la especie *Passerina cyanea*. Adicionalmente,

identificó dos subclados internos conformados por las especies del género *Passerina*, con rutas independientes de coloración que incluyen tonos de rojo, amarillo y verde, dentro del primer subclado la coloración está determinada por la expresión de feomelaninas, mientras que dentro del segundo subclado fue identificada la expresión de pigmentos carotenoides. Adicionalmente, Scott et al. (2024) observó que la complejidad y el brillo del plumaje de las hembras y los machos en las especies de esta familia parecen estar determinadas diferencialmente por factores como la disponibilidad de luz ambiental, asociada a distintas estructuras del hábitat.

A nivel intraespecífico, Tonetti et al. (2017) observaron que entre las poblaciones de C*aryothraustes canadensis* existe diferenciación genética y de coloración entre poblaciones geográficamente disyuntas; esta misma diferenciación de coloración también discrimina entre subespecies distribuidas en simpatría entre las poblaciones de los bosques del Atlántico en Sudamérica. Estudios adicionales indican que los patrones de variación geográfica de la coloración y el tamaño corporal entre poblaciones de las especies *Piranga bidentata* (Robles-Bello et al. 2022) y *Había rubica* (Ramírez-Barrera et al. 2019) se encuentran asociados a diferentes mecanismos de divergencia a nivel poblacional; pues mientras el primero reporta baja estructura genética, con diferenciación de la coloración relacionada a gradientes de humedad, y diferencias en el tamaño corporal asociadas a un gradiente de temperatura entre poblaciones geográficamente disyuntas; en el segundo estudio se reporta que la alta diferenciación genética, que describe siete filogrupos bien diferenciados (Ramírez-Barrera et al. 2018) y fenotípica que describe hasta 17 subespecies con base en la variación de sus patrones de coloración y distribución geográfica principalmente (Hilty 2020), se encuentran asociados más probablemente a procesos de divergencia dirigidos por factores neutrales producto del aislamiento geográfico que separa sus poblaciones. Los resultados de estos estudios muestran la importancia de cuantificar la proporción de la variación fenotípica explicada por la geografía o el ambiente para evaluar su influencia e interacciones relativas, considerando la diversificación de los linajes como un proceso que puede involucrar factores tanto selectivos como neutrales, y comprender así los mecanismos que subyacen a las primeras etapas de la especiación ¿Pero qué pasa con otras especies de esta familia?

Las características geográficas y ecológicas de las especies de Cardinalidae permiten probar la congruencia del efecto de distintos factores genéticos, geográficos y ambientales a los patrones de variación fenotípica de este grupo. Por un lado, se trata de un grupo de especies estrechamente emparentado con amplia variación fenotípica, geográfica y ecológica entre las que es posible evaluar respuestas y patrones compartidos entre especies. Se espera que las distintas especies muestren

patrones de variación fenotípica (e.g. coloración del plumaje, tamaño corporal) acoplados con la variación geográfica y ecológica de las regiones donde éstas se distribuyen. Por otro lado, los análisis de Scott et al. (2024) muestran evidencia de que la variación en la coloración de machos y hembras en Cardinalidae se encuentra asociada diferencialmente con métricas categóricas del hábitat y criterios ecológicos. El estudio de los patrones de variación fenotípica espacial en especies con amplia distribución también puede ayudar a comprender algunos patrones observados dentro en la familia. Por ejemplo, entre las especies *Habia rubica* y *Habia fuscicauda* existen poblaciones simpátricas (desde la región noreste de México hasta la vertiente del Atlántico en Honduras) y alopátricas (desde Nicaragua hasta Panamá y la vertiente del Pacífico en México), entre las que existe una aparente semejanza de coloración, uniformemente rojiza (Winkler et al. 2020), que a menudo conduce a errores de identificación taxonómica en campo. Esto podría representar un buen modelo que permita probar hipótesis de aislamiento de las especies para evitar hibridación vs. hipótesis de variación fenotípica asociada a factores ambientales (McNaught & Owens 2002). Se esperaría encontrar mayor diferenciación de coloración entre aquellas poblaciones en simpatría, o bien una correlación de dicha variación fenotípica a factores ambientales diferentes entre especies; puesto que también hay evidencia del efecto de factores como la disponibilidad de luz ambiental como director de la variación del color entre especies de esta familia (Scott et al. 2023). También es posible considerar que las tendencias de variación fenotípica descritas por reglas ecogeográficas, al señalar tendencias de variación latitudinal desde los polos hacia el ecuador (Mota et al. 2017), deberían comprobarse acotando las especies según su distribución, asegurando así comprobar tendencias del norte hacia el sur vs. tendencias del sur hacia el norte; tomando como referencia el ecuador como el punto más sureño. Adicionalmente, a nivel intraespecífico se cuenta con datos de diferenciación genética en especies como: *Cardinalis cardinalis*, *Piranga erythrocephala* y *Habia fuscicauda*, entre las que es posible comprobar el efecto de factores genéticos, geográficos y ambientales sobre los distintos patrones de diferenciación fenotípica poblacional; considerando también que existe evidencia de que las tendencias de variación fenotípica descritas por reglas ecogeográficas a menudo se ajustan a los patrones de variación a nivel de población.

Muchos linajes de aves Neárticas y Neotropicales presentan mecanismos de divergencia fenotípica alternativos a los producidos por la variación ambiental (McNaught & Owens 2002, Friedman et al. 2009, Dale et al. 2015, Dunn et al. 2015, Simpson et al. 2015, Friedman & Remes 2016, Cuthill et al. 2017, Doutrelant et al. 2020). La variación ambiental a lo largo de un gradiente de distribución geográfica no es necesaria para la generación de divergencia fenotípica (). En este

sentido, el estudio de la variación fenotípica en las especies de Cardinalidae sugiere que existe un efecto significativo de la disponibilidad de luz ambiental y los hábitos de migración en estas especies. Las diferencias en coloración asociadas con efectos de la disponibilidad de luz ambiental y hábitos de migración nos indica que existe influencia de la variación ambiental sobre los procesos de diversificación entre las especies de Cardinalidae.

Los resultados de estudios que consideran la influencia de variables geográficas y ambientales sobre los patrones de diversificación fenotípica de las especies nos ayudan a comprender el origen de la diversidad biológica considerando la importancia relativa de esos componentes. El estudio de la distribución espacial de rasgos fenotípicos dentro y entre especies puede llevar a una mejor comprensión de los mecanismos de diferenciación involucrados en los procesos de diferenciación de las especies en Cardinalidae.

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APÉNDICE 1. MATERIAL SUPLEMENTARIO: ARTÍCULO 1

PENDIENTE

SUPPLEMENTARY MATERIAL

Phylogenetic and adaptive components of Gloger's and Bergmann's rules in a subclade of the family Cardinalidae (PASSERIFORMES; AVES)

Sandra M. Ramírez-Barrera, Julián A. Velasco, Blanca E. Hernández-Baños

DESCRIPTIVE STATISTICS OF MORPHOMETRY VARIABLES

Table 1. Morphometric measurements and normality tests by species in males.

																	Normality test					
				Wing_length				Tarsus_length				Tail_length				Wing_length		Tarsus_length		Tail_length	
Total	AOU_names	Phylogenetic_names	Males	Range	Mean	Median	Variance	Range	Mean	Median	Variance	Range	Mean	Median	Variance	W	*p*-value	W	*p*-value	W	*p*-value
325	Cardinalis_cardinalis	Cardinalis_cardinalis	189	74.35 - 98.19	88.21	88.19	22.38	21.45 - 28.90	24.7	24.56	2.08	82.00 - 120.33	100.31	99	54.07	0.99	0.31	0.97	**0.00**	0.99	0.11
40	Cardinalis_phoeniceus	Cardinalis_phoeniceus	23	72.42 - 86.78	81.43	81.96	13.22	20.41 - 24.79	23.1	23.16	0.92	79.00 - 96.67	85.81	84	23.73	0.95	0.28	0.96	0.49	0.95	0.34
88	Cardinalis_sinuatus	Cardinalis_sinuatus	49	85.35 - 99.16	91.96	91.44	13.80	19.26 - 25.44	23.22	23.38	1.82	91.33 - 109.33	99.52	100.33	21.90	0.95	0.03	0.94	**0.02**	0.97	0.33
75	Caryothraustes_poliogaster	Caryothraustes_poliogaster	44	76.85 - 99.33	88.5	87.81	24.89	16.70 22.96	20.61	20.81	1.67	63.33 - 74.67	68.3	68	10.54	0.99	0.95	0.95	0.05	0.96	0.10
58	Caryothraustes_canadensis	Caryothraustes_canadensis	33	81.94 - 99.64	91	90.85	18.30	17.88 - 22.94	20.57	20.57	0.95	60 - 81	70.84	68.33	35.99	0.98	0.68	0.98	0.72	0.93	0.03
40	**Caryothraustes_celaeno**	Rhodothraupis_celaeno	22	99.84 - 109.90	104	104.67	8.97	21.78 - 25.03	23.53	23.41	0.64	87 - 100	93.97	93.67	13.29	0.95	0.27	0.94	0.24	0.96	0.59
18	**Caryothraustes_erythromelas**	Periporphyrus_erythromelas	13	93.08 - 109.93	98.6	97.22	22.25	17.123 - 21.73	19.86	19.79	1.15	81.33 - 95.00	87.08	86	21.63	0.91	0.17	0.88	0.06	0.92	0.27
71	Chlorothraupis_carmioli	Chlorothraupis_carmioli	40	82.41 - 92.91	87.17	86.96	7.36	19.81 - 23.93	22.2	22.43	0.82	60.00 - 69.33	64.52	64.67	6.96	0.98	0.51	0.98	0.59	0.96	0.15
27	Chlorothraupis_olivacea	Chlorothraupis_olivacea	14	84.23 - 92.93	87.75	87.79	6.39	19.88 - 23.95	21.99	22.16	1.10	57 - 65	60.17	59.67	5.29	0.96	0.79	0.97	0.89	0.95	0.58
15	Chlorothraupis_stolzmanni	Chlorothraupis_stolzmanni	8	85.46 - 89.69	87.35	87.22	2.37	22.34 - 24.91	23.35	23.07	0.87	60.67 - 70.67	66.33	66	9.43	0.93	0.50	0.90	0.31	0.95	0.76
10	Habia_atrimaxillaris	Habia_atrimaxillaris	7	85.64 - 99.62	92.83	93.04	27.76	22.35 - 24.98	24.2	24.38	0.80	74.67 - 90.00	84.1	85.67	30.17	0.91	0.41	0.82	0.06	0.93	0.56
202	Habia_fuscicauda	Habia_fuscicauda	102	88.25 - 104.76	97.13	97.41	12.42	21.87 - 29.75	26.1	26.03	1.53	80.67 - 103.67	91.72	92	17.23	0.99	0.50	0.98	0.25	0.98	**0.27**
26	Habia_gutturalis	Habia_gutturalis	12	90.03 - 95.16	92.58	92.48	4.21	22.38 - 25.29	23.88	23.75	0.92	87.67 - 92.00	89.58	89.83	3.01	0.86	0.05	0.95	0.66	0.85	**0.04**
293	Habia_rubica	Habia_rubica	172	77.02 - 104.00	90.03	90.16	20.72	20.33 - 27.57	23.75	23.79	2.16	67.67 - 95.00	81.38	80.5	31.52	0.99	0.71	0.99	0.36	0.98	0.05
112	Piranga_bidentata	Piranga_bidentata	70	90.90 - 102.84	96.38	96.17	8.99	17.73 - 23.84	21.48	21.5	1.52	70.33 - 84.67	79.23	79.33	7.73	0.98	0.19	0.98	0.45	0.98	0.44
44	Piranga_erythrocephala	Piranga_erythrocephala	27	65.47 - 75.67	71.49	72	6.74	15.97 - 21.07	18.78	18.73	1.69	59.67 - 73.00	65.91	65.33	10.45	0.97	0.55	0.97	0.59	0.96	0.46
276	Piranga_flava	Piranga_flava	137	83.74 - 106.74	94.25	94.25	19.14	18.23 - 25.57	21.38	21.32	1.16	69.00 - 87.33	76.95	76.67	13.48	0.99	0.70	0.99	0.17	0.99	0.50
103	Piranga_leucoptera	Piranga_leucoptera	62	63.81 - 73.96	68.76	68.71	6.19	15.44 - 19.61	17.88	17.98	0.57	52.33 63.00	57.76	57.83	6.71	0.98	0.54	0.98	0.27	0.98	0.43
195	Piranga_ludoviciana	Piranga_ludoviciana	113	80.92 - 98.94	93.38	93.35	8.86	17.46 - 22.44	19.91	19.93	0.71	63.00 - 76.33	68.14	67.67	6.66	0.96	0.00	0.99	0.34	0.98	0.08
81	Piranga_olivacea	Piranga_olivacea	47	71.58 - 97.38	92.22	92.24	17.65	17.65 - 20.58	19.13	19.13	0.51	56.67 - 71.00	65.92	66	5.96	0.79	**<0.5**	0.98	0.64	0.94	**0.02**
30	Piranga_roseogularis	Piranga_roseogularis	16	74.67 - 80.75	78.21	78.08	2.86	17.42 - 22.61	20.09	20.08	2.69	61.67 - 69.33	64.96	65	4.38	0.96	0.71	0.95	0.53	0.95	0.44
175	Piranga_rubra	Piranga_rubra	105	83.71 - 101.90	92.31	92.33	10.38	17.06 - 21.48	19.03	18.93	0.55	64.33 - 80.00	71.46	70.67	11.50	0.99	0.43	0.98	0.20	0.94	**0.00**
17	Piranga_rubriceps	Piranga_rubriceps	9	89.36 - 96.03	93.26	93.97	5.63	20.70 - 23.74	22.29	22.5	1.45	71.33 - 80.00	78.19	78.67	7.86	0.84	0.05	0.89	0.21	0.70	**0.00**
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Table 2. Morphometric measurements and normality tests by species in females.

																Normality test					
				Wing_length				Tarsus_length				Tail_length				Wing_length		Tarsus_length		Tail_length	
Total	AOU_names	Phylogenetic_names	Females	Range	Mean	Median	Variance	Range	Mean	Median	Variance	Range	Mean	Median	Variance	W	*p*-value	W	*p*-value	W	*p*-value
325	Cardinalis_cardinalis	Cardinalis_cardinalis	136	72.67 - 96.66	85.43	85.21	20.28	21.04 - 29.73	24.25	24.21	1.76	77.67 - 119.33	96.48	96	59.43	0.99	0.82	0.97	0.01	0.98	0.04
40	Cardinalis_phoeniceus	Cardinalis_phoeniceus	17	74.52 - 83.43	79.05	79.26	6.03	20.73 - 24.58	22.77	22.75	1.18	72.33 - 89.67	82.1	80	31.14	0.98	0.91	0.98	0.91	0.91	0.09
88	Cardinalis_sinuatus	Cardinalis_sinuatus	39	80.56 - 95.00	87.76	87.67	11.48	18.95 - 25.92	87.76	87.67	2.02	87.33 - 107.00	95.2	94	19.45	0.99	0.94	0.93	0.02	0.95	0.09
75	Caryothraustes_poliogaster	Caryothraustes_poliogaster	31	75.82 - 96.81	86.43	85.48	30.13	18.67 - 25.01	20.83	20.78	2.11	63.33 - 74.67	67.97	67.33	6.55	0.98	0.79	0.93	0.06	0.97	0.42
58	Caryothraustes_canadensis	Caryothraustes_canadensis	25	80.41 - 93.31	86.76	86.15	13.56	17.40 - 21.41	19.84	19.96	0.92	60.00 - 77.33	66.64	66	19.11	0.97	0.58	0.96	0.46	0.93	0.08
40	**Caryothraustes_celaeno**	Rhodothraupis_celaeno	18	95.40 - 108.99	100.14	99.94	11.77	21.38 - 25.20	23.26	23.4	0.99	83.33 - 94.00	89.17	89.83	9.21	0.94	0.25	0.98	0.95	0.95	0.37
18	**Caryothraustes_erythromelas**	Periporphyrus_erythromelas	5	92.61 - 100.38	97.12	97.46	7.95	19.37 - 21.01	20.21	20.16	0.50	84.67 - 89.67	87.13	86.33	4.48	0.90	0.43	0.94	0.64	0.91	0.50
71	Chlorothraupis_carmioli	Chlorothraupis_carmioli	31	76.48 - 90.98	84.17	84.18	11.03	20.35 - 24.31	22.5	22.64	0.72	56 - 67	63.12	63.67	7.29	0.98	0.83	0.98	0.77	0.95	0.12
27	Chlorothraupis_olivacea	Chlorothraupis_olivacea	13	77.05 - 87.95	82.84	83.72	13.49	21.14 - 23.37	22.09	22.07	0.54	52.00 - 60.67	57.05	57.67	5.92	0.90	0.12	0.94	0.40	0.95	0.63
15	Chlorothraupis_stolzmanni	Chlorothraupis_stolzmanni	7	83.15 - 88.29	85.56	85.28	3.33	22.79 - 25.37	23.85	23.41	0.96	64.33 - 70.00	67.1	67.67	5.66	0.97	0.91	0.87	0.18	0.86	0.16
10	Habia_atrimaxillaris	Habia_atrimaxillaris	3	86.16 - 89.85	88.46	89.37	4.03	22.36 - 24.97	23.32	22.62	2.07	76 - 80	77.78	77.33	4.15	0.85	0.23	0.82	0.17	0.96	0.64
202	Habia_fuscicauda	Habia_fuscicauda	100	80.19 - 99.10	88.29	88.53	15.96	22.24 - 28.62	25.45	25.44	1.86	73 - 91	82.13	81.83	15.68	0.99	0.55	0.99	0.96	0.99	0.47
26	Habia_gutturalis	Habia_gutturalis	14	80.44 - 100.42	85.38	84.18	22.67	22.29 - 25.00	23.67	23.46	0.65	75 - 87	80.67	80.83	8.48	0.68	0.00	0.96	0.77	0.97	0.86
293	Habia_rubica	Habia_rubica	121	76.43 - 95.70	84.38	84.28	17.11	19.96 - 26.49	23.21	23.51	2.00	64.33 - 89.00	76.08	76	26.87	0.98	0.05	0.98	0.02	0.99	0.33
112	Piranga_bidentata	Piranga_bidentata	42	86.57 - 102.05	92.14	92.26	11.24	18.20 - 23.78	21.37	21.14	1.52	64.67 - 83.00	76.08	76.33	13.03	0.97	0.31	0.98	0.56	0.96	0.14
44	Piranga_erythrocephala	Piranga_erythrocephala	17	63.54 - 71.00	67.66	68.33	4.96	17.26 - 21.52	18.83	18.67	1.76	57.67 - 68.33	63.29	64	9.43	0.93	0.22	0.92	0.13	0.97	0.77
276	Piranga_flava	Piranga_flava	139	67.70 - 106.95	90.77	90.86	26.65	18.30 - 23.37	21.25	21.39	1.07	65.67 - 85.00	75.22	75.33	13.08	0.97	0.01	0.98	0.07	0.99	0.24
103	Piranga_leucoptera	Piranga_leucoptera	41	59.79 - 73.18	65.95	65.9	9.40	16.00 - 19.87	17.83	17.91	0.78	50.00 - 61.33	56.01	56	-0.01	0.97	0.45	0.99	0.97	0.95	0.08
195	Piranga_ludoviciana	Piranga_ludoviciana	82	84.56 - 98.57	91.22	91.38	7.50	15.90 - 23.17	19.9	19.94	1.16	61.67 - 74.67	67.93	68	5.44	0.99	0.91	0.96	0.01	0.98	0.26
81	Piranga_olivacea	Piranga_olivacea	34	83.87 - 92.37	88.44	88.62	5.34	16.16 - 20.72	19.13	19.41	1.53	58.00 - 68.67	63.64	63.5	5.85	0.96	0.28	0.89	0.00	0.98	0.91
30	Piranga_roseogularis	Piranga_roseogularis	14	68.35 - 76.00	73.25	73.58	4.07	18.46 - 22.21	20.61	20.63	0.85	61.00 - 65.33	63.19	63.17	2.10	0.91	0.15	0.96	0.67	0.91	0.18
175	Piranga_rubra	Piranga_rubra	70	81.37 - 97.19	89.11	88.77	9.42	14.68 - 20.77	19.05	19.03	0.86	58.67 - 80.00	69.9	69	14.71	0.98	0.51	0.91	< 0-05	0.96	0.05
17	Piranga_rubriceps	Piranga_rubriceps	8	83.91 - 95.87	89.46	89.69	17.88	21.40 - 22.67	22.13	22.29	0.26	73.33 - 80.00	75.87	75.5	4.16	0.95	0.70	0.88	0.18	0.92	0.44
2321			1007																		

Table 3. Statistical tests of sexual dimorphism (*t*-test) by species. Values highlighted in bold represent taxonomic changes in species names (AOU), and tests without sexual dimorphism.

AOU_names	Phylogenetic_names	Wing		Tarsus		Tail	
		Homoscedasticity *p*-value	*t* Test	Homoscedastici *p*-value	*t* Test	Homoscedastici *p*-value	*t* Test
Cardinalis_cardinalis	Cardinalis_cardinalis	0.54	0.00	0.30	0.00	0.55	0.00
Cardinalis_phoeniceus	Cardinalis_phoeniceus	0.11	0.02	0.58	**0.33**	0.55	0.04
Cardinalis_sinuatus	Cardinalis_sinuatus	0.56	0.00	0.73	**0.82**	0.71	0.00
Caryothraustes_poliogaster	Caryothraustes_poliogaster	0.56	**0.10**	0.47	**0.51**	0.17	**0.63**
Caryothraustes_canadensis	Caryothraustes_canadensis	0.45	0.00	0.94	0.01	0.11	0.00
Caryothraustes_celaeno	Rhodothraupis_celaeno	0.55	0.00	0.35	**0.37**	0.45	0.00
Caryothraustes_erythromelas	Periporphyrus_erythromelas	0.33	**0.43**	0.45	**0.45**	0.14	**0.97**
Chlorothraupis_carmioli	Chlorothraupis_carmioli	0.23	0.00	0.71	**0.16**	0.88	0.03
Chlorothraupis_olivacea	Chlorothraupis_olivacea	0.20	0.00	0.23	**0.79**	0.84	0.00
Chlorothraupis_stolzmanni	Chlorothraupis_stolzmanni	0.66	**0.06**	0.89	**0.33**	0.55	**0.60**
Habia_atrimaxillaris	Habia_atrimaxillaris	0.26	**0.09**	0.31	**0.40**	0.25	0.03
Habia_fuscicauda	Habia_fuscicauda*	0.21	0.00	0.33	0.00	0.64	0.00
Habia_gutturalis	Habia_gutturalis	**0.01**	0.00	0.55	**0.55**	0.09	0.00
Habia_rubica	Habia_rubica*	0.26	0.00	0.65	0.00	0.35	0.00
Piranga_bidentata	Piranga_bidentata	0.41	0.00	0.98	**0.65**	0.05	0.00
Piranga_erythrocephala	Piranga_erythrocephala	0.53	0.00	0.90	**0.92**	0.85	0.01
Piranga_flava	Piranga_flava	0.05	0.00	0.64	**0.30**	0.86	0.00
Piranga_leucoptera	Piranga_leucoptera	0.14	0.00	0.25	**0.77**	1.00	0.00
Piranga_ludoviciana	Piranga_ludoviciana	0.43	0.00	0.02	**0.94**	0.34	**0.56**
Piranga_olivacea	Piranga_olivacea	**0.00**	0.00	**0.00**	0.99	0.97	0.00
Piranga_roseogularis	Piranga_roseogularis	0.51	0.00	**0.04**	**0.29**	0.19	0.01
Piranga_rubra	Piranga_rubra	0.67	0.00	**0.04**	**0.85**	0.25	0.01
Piranga_rubriceps	Piranga_rubriceps	0.13	0.05	0.04	**0.73**	0.42	**0.07**

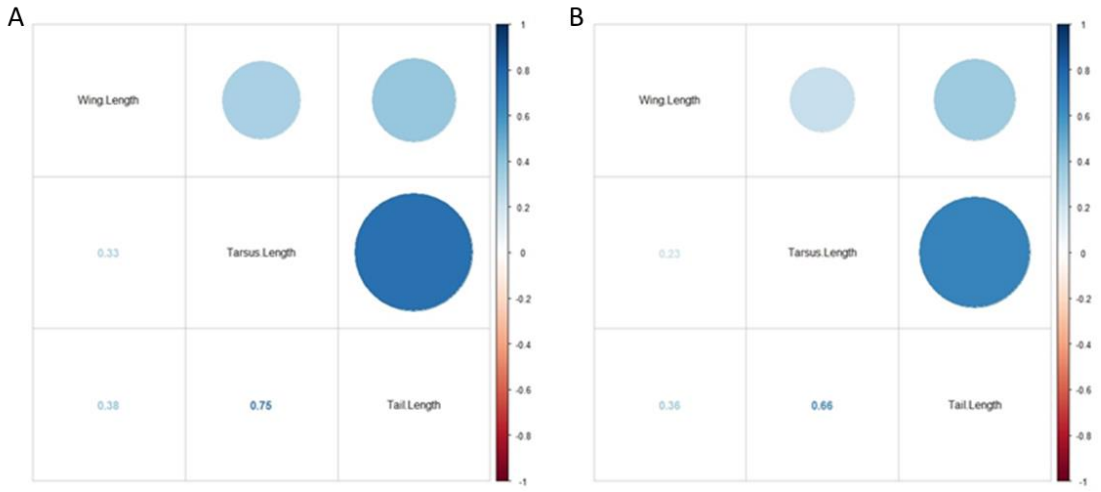

Figure 1. Correlation graph between morphometric variables in A) males and B) females.

Table 4. Principal component analysis performed on highly correlated morphometric variables (Figure 2. Tarsus Length, Tail Length), considering 1314 male data.

Principal Component Analysis - MALES		
BODY SIZE	PC1	PC2
Standard deviation	1.32	0.50
Proportion of Variance	0.87	0.13

Cumulative Proportion	0.87	1.00

1314 Individuals

Table 5. Principal component analysis performed on highly correlated morphometric variables (Figure 5. Tarsus Length, Tail Length), considering 1007 female data.

Principal Component Analysis - FEMALES		
BODY SIZE	PC1	PC2
Standard deviation	1.29	0.58
Proportion of Variance	0.83	0.17
Cumulative Proportion	0.83	1.00

1007 Individuals

DESCRIPTIVE STATISTICS OF COLOR VARIABLES

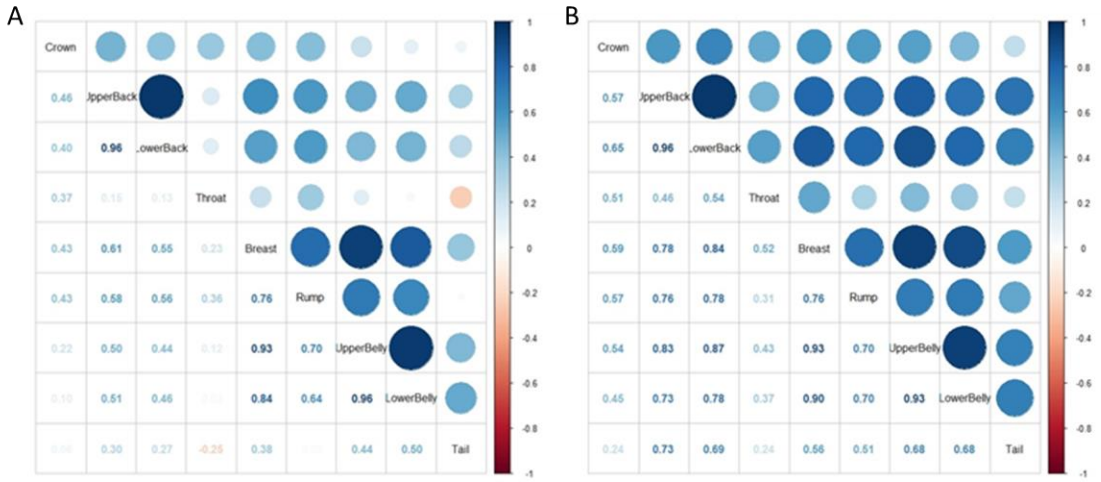

Figure 2. Correlation between color variables in A) males and B) females.

Table 6. Principal component analysis performed on the average brightness of nine body patches in males.

Principal Component Analysis - MALES									
COLORATION	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9
Standard deviation	2.18	1.28	1.05	0.85	0.71	0.39	0.30	0.18	0.11
Proportion of Variance	0.53	0.18	0.12	0.08	0.06	0.02	0.01	0.00	0.00
Cumulative Proportion	**0.53**	0.71	0.83	0.91	0.97	0.99	1.00	1.00	1.00

Averaged variables of 23 species

Table 7. Principal component analysis performed on the brightness averages of nine body patches in females.

Principal Component Analysis - FEMALES									
COLORATION	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9
Standard deviation	2.51	1.00	0.78	0.69	0.56	0.45	0.26	0.19	0.15
Proportion of Variance	0.70	0.11	0.07	0.05	0.03	0.02	0.01	0.00	0.00
Cumulative Proportion	0.70	0.81	0.88	0.93	0.96	0.99	0.99	1.00	1.00

Averaged variables of 23 species

SAR MODELS

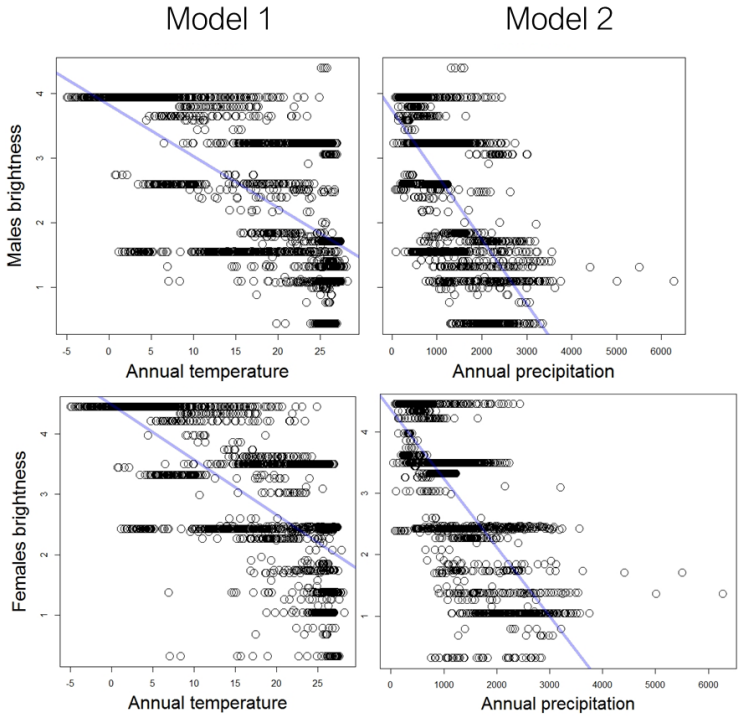

Figure 3. Brightness of A) males and B) females.

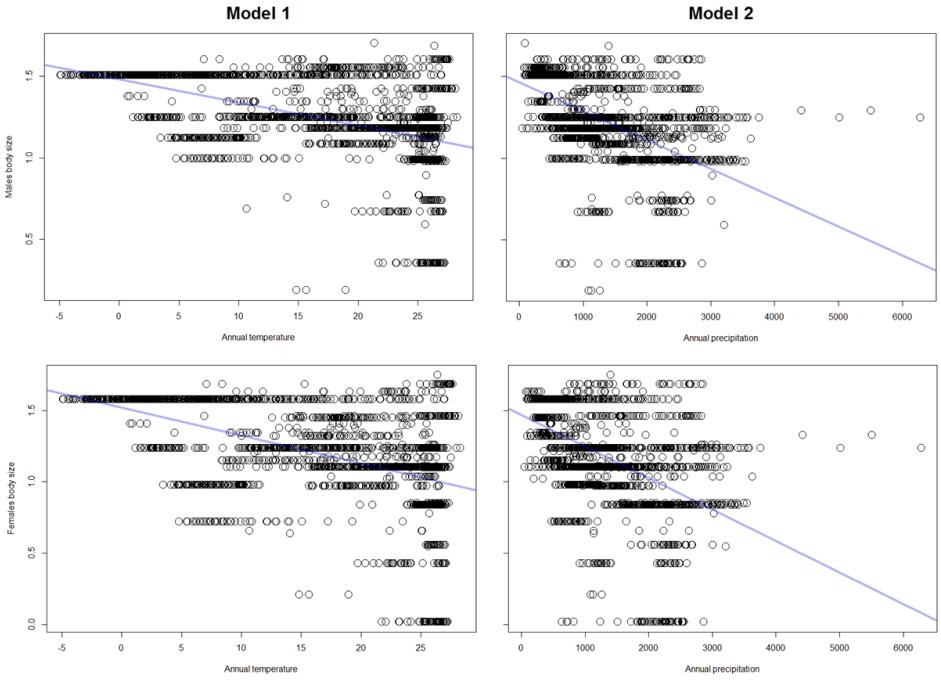

Figure 4. Body size of A) males and B) females.

APÉNDICE 2. MATERIAL SUPLEMENTARIO: ARTÍCULO 2

Ecology & Evolution

SUPPLEMENTARY MATERIAL

What drives genetic and phenotypic divergence in the Red-crowned Ant-tanager (*Habia rubica*, Aves: Cardinalidae), a polytypic species?

Sandra M. Ramírez-Barrera, Julián A. Velasco, Tania Orozco-Téllez, Alma M. Vázquez-López & Blanca E. Hernández-Baños

Supporting information on the material used in the study

Table S1.1. List of total samples used in this study and pairing of genetic and phenotypic data (column genetic association). The collections of origin of each sample are: Museo de Zoología "Alfonso L. Herrera", UNAM, Mexico (MZFC); Colección Nacional de Aves, UNAM, Mexico (CNAV); El Colegio de la Frontera Sur, Chetumal, México (ECOSUR-Ch);

the Ornithological Collection of the American Museum of Natural History, New York (AMNH), the Ornithological Collection of the Smithsonian Institution, Washington D. C (SI); The Burke Museum, University of Washington (UWBM); Natural History Museum of The University of Kansas (KU) and Museum of Natural Science of Louisiana State University (LSU). The abbreviation SEC responds with sequences of the mitochondrial gene ND2 provided by John Klicka. The phylogroup column refers to the names of each genetic groups identified in *Habia rubica*: NP, northern pacific of Mexico; SP, southern pacific of Mexico; GM, Gulf of Mexico; SE, southeastern Mexico and northern Central America; PA, Panama; WS, western South America and ES, eastern-northwestern South America.

Specimen data							Phenotypic data	Genetic asociation	
Collection number	Sex	Collection	Latitude	Longitude	Country	State		Phylogroup	Collection number
156119	F	SI	20.76	-104.85	Mexico	Jalisco	x	NP	URRA67
156120	F	SI	20.76	-104.85	Mexico	Jalisco	x	NP	
510473	M	AMNH	20.81	-105.25	Mexico	Nayarit	x	NP	CONA799
510475	F	AMNH	21.83	-103.78	Mexico	Jalisco	x	NP	
510476	M	AMNH	21.83	-103.78	Mexico	Jalisco	x	NP	
P9771	F	CNAV	21.15	-105.12	Mexico	Nayarit	x	NP	URRA63
P9772	M	CNAV	21.15	-105.12	Mexico	Nayarit	x	NP	URRA63
P9773	F	CNAV	21.30	-105.10	Mexico	Nayarit	x	NP	
P9774	F	CNAV	20.22	-103.70	Mexico	Jalisco	x	NP	
P22391	M	CNAV	22.39	-105.27	Mexico	Nayarit	x	NP	
P22392	M	CNAV	20.61	-105.23	Mexico	Jalisco	x	NP	URRA55
P22393	M	CNAV	19.17	-103.94	Mexico	Colima	x	NP	
PEP459	M	MZFC	20.75	-105.38	Mexico	Nayarit	x	NP	URRA60
URRA55	F	MZFC	20.47	-105.29	Mexico	Jalisco	x	NP	URRA55
URRA60	F	MZFC	20.47	-105.29	Mexico	Jalisco	x	NP	URRA60
URRA67	M	MZFC	20.47	-105.29	Mexico	Jalisco	x	NP	URRA67
URRA63	ND	MZFC	20.47	-105.29	Mexico	Jalisco	x	NP	
CONACYT799	F	MZFC	19.46	-103.71	Mexico	Colima	x	NP	CONA799
FRG116	M	MZFC	21.58	-105.23	Mexico	Nayarit	x	NP	
FRG76	M	MZFC	21.44	-105.00	Mexico	Nayarit	x	NP	
185673	F	SI	17.04	-100.25	Mexico	Guerrero	x	SP	MOLGRO454
185674	M	SI	17.04	-100.25	Mexico	Guerrero	x	SP	MOLGRO455
P9775	M	CNAV	18.75	-102.37	Mexico	Michoacán	x	SP	MOLGRO454
P9776	F	CNAV	18.75	-102.37	Mexico	Michoacán	x	SP	MICH196
P9777	M	CNAV	16.17	-97.10	Mexico	Oaxaca	x	SP	OMVP161
P9778	F	CNAV	16.17	-97.10	Mexico	Oaxaca	x	SP	
P9779	M	CNAV	16.16	-97.13	Mexico	Oaxaca	x	SP	OMVP672
P9780	M	CNAV	16.16	-97.13	Mexico	Oaxaca	x	SP	OAX58

P9781	F	CNAV	16.16	-97.13	Mexico	Oaxaca	x	SP	
P13206	M	CNAV	16.22	-97.23	Mexico	Oaxaca	x	SP	OMVP160
P13207	F	CNAV	16.22	-97.23	Mexico	Oaxaca	x	SP	OMVP682
P15757	M	CNAV	16.13	-97.10	Mexico	Oaxaca	x	SP	
P28151	F	CNAV	18.28	-102.58	Mexico	Michoacán	x	SP	MICH126
PLU17	M	MZFC	15.89	-96.40	Mexico	Oaxaca	x	SP	MIA81
PLU18	F	MZFC	15.89	-96.40	Mexico	Oaxaca	x	SP	OMVP160
PLU23	M	MZFC	15.89	-96.40	Mexico	Oaxaca	x	SP	OAX115
SRSC024	M	MZFC	17.23	-99.86	Mexico	Guerrero	x	SP	MOLGRO41
AGNS404	M	MZFC	17.25	-100.30	Mexico	Guerrero	x	SP	MOLGRO43
AGNS405	F	MZFC	17.25	-100.30	Mexico	Guerrero	x	SP	MOLGRO40
AMT382	F	MZFC	15.93	-96.42	Mexico	Oaxaca	x	SP	MOLGRO1008
AZAR09	M	MZFC	18.19	-100.16	Mexico	Guerrero	x	SP	MOLGRO262
MOLGRO40	M	MZFC	17.36	-99.46	Mexico	Guerrero	x	SP	MOLGRO40
MOLGRO41	F	MZFC	17.36	-99.46	Mexico	Guerrero	x	SP	MOLGRO41
MOLGRO42	F	MZFC	17.36	-99.46	Mexico	Guerrero	x	SP	MOLGRO42
MOLGRO43	F	MZFC	17.36	-99.46	Mexico	Guerrero	x	SP	MOLGRO43
MOLGRO261	M	MZFC	17.59	-99.84	Mexico	Guerrero	x	SP	MOLGRO261
MOLGRO262	F	MZFC	17.59	-99.84	Mexico	Guerrero	x	SP	MOLGRO262
MOLGRO438	F	MZFC	17.53	-100.56	Mexico	Guerrero	x	SP	
MOLGRO440	F	MZFC	17.53	-100.56	Mexico	Guerrero	x	SP	MOLGRO261
molgro454	ND	MZFC	17.53	-101.44	Mexico	Guerrero	x	SP	
MOLGRO455	F	MZFC	17.18	-99.49	Mexico	Guerrero	x	SP	MOLGRO455
MOLGRO591	M	MZFC	17.36	-99.54	Mexico	Guerrero		SP	
omvp160	ND	MZFC	16.96	-97.91	Mexico	Oaxaca		SP	
mia81/amt390	ND	MZFC	15.93	-96.42	Mexico	Oaxaca		SP	
OMVP161	F	MZFC	16.96	-97.91	Mexico	Oaxaca	x	SP	OMVP161
OMVP672	F	MZFC	16.83	-97.88	Mexico	Oaxaca	x	SP	OMVP672
OMVP682	M	MZFC	16.82	-97.89	Mexico	Oaxaca	x	SP	OMVP682
OMVP1008	M	MZFC	16.24	-97.29	Mexico	Oaxaca	x	SP	MOLGRO1008
oaxjk07115	ND	UWBM	16.20	-97.15	Mexico	Oaxaca		SP	
oaxjk07058	ND	UWBM	16.20	-97.15	Mexico	Oaxaca		SP	
michbts08126	ND	UWBM	18.10	-102.40	Mexico	Michoacán		SP	
michbts08196	ND	UWBM	18.17	-102.31	Mexico	Michoacán		SP	
143576	M	SI	18.64	-96.73	Mexico	Veracruz	x	GM	
158612	M	SI	20.73	-97.85	Mexico	Puebla	x	GM	
158614	F	SI	20.73	-97.85	Mexico	Puebla	x	GM	
158615	F	SI	20.73	-97.85	Mexico	Puebla	x	GM	
370781	F	SI	19.20	-96.16	Mexico	Veracruz	x	GM	
95901	M	AMNH	18.64	-96.73	Mexico	Veracruz	x	GM	NAR28
153421	F	AMNH	19.54	-96.91	Mexico	Veracruz	x	GM	

707207	M	AMNH	19.15	-96.17	Mexico	Veracruz	x	GM	
P7664	F	CNAV	18.45	-95.22	Mexico	Veracruz	x	GM	
P7677	M	CNAV	18.58	-95.07	Mexico	Veracruz	x	GM	
P9782	F	CNAV	18.60	-95.07	Mexico	Veracruz	x	GM	TUX37
P9783	M	CNAV	18.58	-95.07	Mexico	Veracruz	x	GM	TXT15
P15699	M	CNAV	17.11	-95.03	Mexico	Oaxaca	x	GM	
P15711	F	CNAV	18.42	-95.07	Mexico	Veracruz	x	GM	28txt15
P15712	F	CNAV	18.47	-95.35	Mexico	Veracruz		GM	
P15730	F	CNAV	18.97	-97.07	Mexico	Veracruz	x	GM	NAR28
P15735	F	CNAV	17.50	-94.92	Mexico	Veracruz		GM	
P15736	F	CNAV	17.17	-95.03	Mexico	Oaxaca	x	GM	
P15737	F	CNAV	17.17	-95.03	Mexico	Oaxaca	x	GM	OMVP574
P15738	M	CNAV	17.17	-95.03	Mexico	Oaxaca	x	GM	
P15739	M	CNAV	17.37	-95.05	Mexico	Veracruz	x	GM	
P15741	M	CNAV	17.17	-95.03	Mexico	Oaxaca	x	GM	
P15742	F	CNAV	17.83	-95.82	Mexico	Veracruz	x	GM	29txt19
P15745	M	CNAV	17.37	-95.05	Mexico	Veracruz	x	GM	
P15760	M	CNAV	17.17	-95.03	Mexico	Oaxaca	x	GM	
P15761	M	CNAV	17.17	-95.03	Mexico	Oaxaca	x	GM	
P15762	M	CNAV	17.17	-95.03	Mexico	Oaxaca	x	GM	
P24436	F	CNAV	18.43	-94.91	Mexico	Veracruz		GM	
P24437	F	CNAV	18.42	-95.12	Mexico	Veracruz	x	GM	
P24438	M	CNAV	18.34	-94.89	Mexico	Veracruz	x	GM	
P24441	M	CNAV	18.34	-94.89	Mexico	Veracruz	x	GM	
P24442	F	CNAV	18.25	-94.86	Mexico	Veracruz	x	GM	
P24443	F	CNAV	18.27	-95.70	Mexico	Veracruz	x	GM	
P24445	M	CNAV	18.58	-95.05	Mexico	Veracruz	x	GM	
P24497	M	CNAV	18.58	-95.05	Mexico	Veracruz	x	GM	
P24593	F	CNAV	18.29	-93.86	Mexico	Veracruz	x	GM	
P24594	M	CNAV	18.29	-93.86	Mexico	Veracruz	x	GM	
P27809	M	CNAV	17.17	-95.03	Mexico	Oaxaca		GM	
P29301	M	CNAV	18.43	-94.91	Mexico	Veracruz	x	GM	TXT19
P29491	M	CNAV	18.43	-94.91	Mexico	Veracruz	x	GM	VER343363
P29494	M	CNAV	18.43	-94.91	Mexico	Veracruz	x	GM	VER393884
P29507	M	CNAV	18.43	94.91	Mexico	Veracruz	x	GM	
P29530	F	CNAV	18.43	94.91	Mexico	Veracruz	x	GM	VER393884
P29535	M	CNAV	18.43	94.91	Mexico	Veracruz	x	GM	
P29542	M	CNAV	18.43	94.91	Mexico	Veracruz	x	GM	
P29543	M	CNAV	18.43	94.91	Mexico	Veracruz	x	GM	
P29547	M	CNAV	18.43	-94.97	Mexico	Veracruz	x	GM	
P29548	M	CNAV	18.43	-94.97	Mexico	Veracruz	x	GM	

11095	M	MZFC	18.58	-95.07	Mexico	Veracruz	x	GM	
AGNS1004	M	MZFC	17.71	-96.41	Mexico	Oaxaca	x	GM	
AGNS1007	F	MZFC	17.71	-96.41	Mexico	Oaxaca	x	GM	
AGNS1010	M	MZFC	17.71	-96.41	Mexico	Oaxaca	x	GM	
AMT169	F	MZFC	21.07	-98.99	Mexico	Hidalgo	x	GM	
AMT241	M	MZFC	20.73	-98.16	Mexico	Veracruz	x	GM	HGOSLP143
AV328	M	MZFC	19.18	-96.1429000	Mexico	Veracruz	x	GM	
CHIMA86	F	MZFC	17.07	-94.12	Mexico	Oaxaca	x	GM	
CHIMA162	F	MZFC	17.07	-94.58	Mexico	Oaxaca	x	GM	OMVP563
CHIMA164	M	MZFC	17.07	-94.58	Mexico	Oaxaca	x	GM	OMVP545
CHIMA416	F	MZFC	17.07	-94.05	Mexico	Oaxaca	x	GM	CHIMA416
CHIMA516	M	MZFC	17.01	-94.69	Mexico	Oaxaca	x	GM	OMVP546
CHIMA526	M	MZFC	17.01	-94.69	Mexico	Oaxaca	x	GM	
CHIMAS112	M	MZFC	17.07	-94.12	Mexico	Oaxaca	x	GM	CHIMA416
HGO-SLP143	F	MZFC	21.08	-98.96	Mexico	Hidalgo	x	GM	HGOSLP143
MEX035	F	MZFC	18.32	-94.83	Mexico	Veracruz	x	GM	TXT58
MEX056	F	MZFC	18.32	-94.83	Mexico	Veracruz	x	GM	VER343363
MEX123	M	MZFC	18.32	-94.83	Mexico	Veracruz	x	GM	TXT62
MEX133	F	MZFC	18.32	-94.83	Mexico	Veracruz	x	GM	TXT63
MEX134	F	MZFC	18.32	-94.83	Mexico	Veracruz	x	GM	TXT64
OMVP545	F	MZFC	17.07	-94.58	Mexico	Oaxaca	x	GM	OMVP545
OMVP546	F	MZFC	17.07	-94.58	Mexico	Oaxaca	x	GM	OMVP546
OMVP563	M	MZFC	17.02	-94.66	Mexico	Oaxaca	x	GM	OMVP563
OMVP574	M	MZFC	17.02	-94.66	Mexico	Oaxaca	x	GM	OMVP574
NAR 28	ND	MZFC	18.80	-96.96	Mexico	Veracruz		GM	
TUXFPO37	M	MZFC	18.59	-95.10	Mexico	Veracruz	x	GM	TUXFPO37
TXT58	M	MZFC	18.31	-94.88	Mexico	Veracruz	x	GM	TXT58
TXT62	F	MZFC	18.31	-94.88	Mexico	Veracruz	x	GM	TXT62
TXT63	M	MZFC	18.31	-94.88	Mexico	Veracruz	x	GM	TXT63
TXT64	M	MZFC	18.31	-94.88	Mexico	Veracruz	x	GM	TXT64
TXT15	ND	MZFC	18.55	-95.12	Mexico	Veracruz		GM	
TXT19	ND	MZFC	18.55	-95.12	Mexico	Veracruz		GM	
Ver343363	ND	UWBM	18.32	-94.83	Mexico	Veracruz		GM	
Ver393884	ND	UWBM	18.32	-94.83	Mexico	Veracruz		GM	
35249	M	SI	10.07	-84.31	Costa Rica		x	SE	
35256	F	SI	10.07	-84.31	Costa Rica		x	SE	
55935	M	SI	9.93	-84.09	Costa Rica		x	SE	
161650	M	SI	15.51	-87.99	Honduras		x	SE	434137
166580	M	SI	17.51	-91.99	Mexico	Chiapas	x	SE	
199431	M	SI	9.79	-84.13	Costa Rica		x	SE	
199963	M	SI	9.73	-85.02	Costa Rica		x	SE	

199964	M	SI	9.80	-84.23	Costa Rica		x	SE	
302815	M	SI	16.99	-89.69	Guatemala		x	SE	
302816	F	SI	16.91	-90.30	Guatemala		x	SE	B2037
302818	M	SI	17.27	-90.19	Guatemala		x	SE	B2037
302819	M	SI	16.91	-90.30	Guatemala		x	SE	
40893	M	AMNH	15.78	-90.23	Guatemala		x	SE	DHB4362
95318	M	AMNH	10.67	-85.01	Costa Rica		x	SE	ZMUC130351
95319	F	AMNH	10.67	-85.01	Costa Rica		x	SE	ZMUC130351
101458	M	AMNH	13.21	-86.11	Nicaragua	Jinotega	x	SE	DAB1349
101459	M	AMNH	13.21	-86.11	Nicaragua	Jinotega	x	SE	
101460	F	AMNH	13.21	-86.11	Nicaragua	Jinotega	x	SE	DAB1484
101461	F	AMNH	13.21	-86.11	Nicaragua	Jinotega	x	SE	DAB1508
101462	M	AMNH	12.93	-85.92	Nicaragua	Matagalpa	x	SE	DAB1486
101463	F	AMNH	12.93	-85.92	Nicaragua	Matagalpa	x	SE	DAB1486
102359	M	AMNH	9.00	-83.33	Costa Rica		x	SE	
102361	F	AMNH	9.00	-83.33	Costa Rica			SE	
144707	M	AMNH	12.93	-85.92	Nicaragua	Matagalpa	x	SE	DAB1508
144708	M	AMNH	11.83	-85.98	Nicaragua		x	SE	DAB1485
144710	F	AMNH	11.83	-85.98	Nicaragua			SE	
254746	M	AMNH	20.36	-87.34	Mexico	Quintara Roo	x	SE	
254747	F	AMNH	20.36	-87.34	Mexico	Quintara Roo	x	SE	MOL1121
326507	M	AMNH	15.51	-87.99	Honduras	Cortes	x	SE	5985
326508	F	AMNH	15.51	-87.99	Honduras	Cortes	x	SE	DAB1349
328527	M	AMNH	15.51	-87.99	Honduras	Cortes	x	SE	5982
328529	M	AMNH	15.51	-87.99	Honduras	Cortes	x	SE	5978
328530	F	AMNH	15.51	-87.99	Honduras	Cortes	x	SE	5974
328533	M	AMNH	15.42	-88.16	Honduras	Cortes	x	SE	5974
328535	F	AMNH	15.49	-87.93	Honduras	Cortes	x	SE	GAV2110
328538	M	AMNH	15.49	-87.93	Honduras	Cortes	x	SE	GAV2110
328539	M	AMNH	15.49	-87.93	Honduras	Cortes	x	SE	GMS145
328540	M	AMNH	15.49	-87.93	Honduras	Cortes	x	SE	434136
328541	F	AMNH	15.49	-87.93	Honduras	Cortes	x	SE	GMS145
328542	F	AMNH	15.49	-87.93	Honduras		x	SE	434136
328544	M	AMNH	14.07	-87.19	Honduras		x	SE	5934
328545	M	AMNH	14.07	-87.19	Honduras		x	SE	5937
328546	F	AMNH	14.07	-87.19	Honduras		x	SE	5934
328549	F	AMNH	14.07	-87.19	Honduras		x	SE	5937
328550	M	AMNH	14.07	-87.19	Honduras		x	SE	5949
328551	F	AMNH	14.07	-87.19	Honduras		x	SE	5949
392420	F	AMNH	10.75	-85.15	Costa Rica		x	SE	5985
392422	M	AMNH	10.75	-85.15	Costa Rica		x	SE	

392424	M	AMNH	10.75	-85.15	Costa Rica		x	SE	
392425	F	AMNH	10.75	-85.15	Costa Rica		x	SE	434137
392426	F	AMNH	10.75	-85.15	Costa Rica		x	SE	DAB1485
398444	M	AMNH	15.53	-89.77	Guatemala		x	SE	DHB4377
398447	M	AMNH	15.53	-89.77	Guatemala		x	SE	DHB4399
398448	F	AMNH	15.53	-89.77	Guatemala		x	SE	MOL1122
398452	F	AMNH	15.53	-89.77	Guatemala		x	SE	DHB4377
398454	F	AMNH	15.53	-89.77	Guatemala		x	SE	DHB4369
423582	M	AMNH	12.93	-85.92	Nicaragua	Matagalpa	x	SE	DAB1484
510449	M	AMNH	9.93	-84.18	Costa Rica	San José	x	SE	
510450	F	AMNH	9.00	-83.33	Costa Rica			SE	
510451	M	AMNH	10.66	-84.26	Costa Rica		x	SE	
510463	M	AMNH	15.59	-90.15	Guatemala	Alta Verapaz	x	SE	
510471	M	AMNH	10.75	-85.15	Costa Rica		x	SE	
813870	M	AMNH	14.30	-90.79	Guatemala	Escuintla	x	SE	DHB4369
813871	F	AMNH	14.30	-90.79	Guatemala	Escuintla	x	SE	DHB4362
813872	F	AMNH	14.20	-90.43	Guatemala			SE	
P15740	M	CNAV	16.95	-93.45	Mexico	Chiapas	x	SE	
P15743	M	CNAV	16.95	-93.45	Mexico	Chiapas	x	SE	
P15756	M	CNAV	16.95	-93.45	Mexico	Chiapas	x	SE	
P15763	M	CNAV	16.90	-93.30	Mexico	Chiapas	x	SE	
P15766	M	CNAV	16.95	-93.45	Mexico	Chiapas	x	SE	
P22395	F	CNAV	16.48	-92.23	Mexico	Chiapas	x	SE	5978
P22397	M	CNAV	15.51	-92.89	Mexico	Chiapas	x	SE	
P25054	M	CNAV	17.99	-92.93	Mexico	Tabasco	x	SE	
P29570	F	CNAV	15.93	-90.66	Guatemala	Alta Verapaz	x	SE	
P29586	M	CNAV	15.93	-90.66	Guatemala	Alta Verapaz	x	SE	
P29599	M	CNAV	15.64	-88.83	Guatemala		x	SE	
P29600	F	CNAV	15.64	-88.83	Guatemala		x	SE	
P29614	M	CNAV	15.64	-88.83	Guatemala		x	SE	
P29615	M	CNAV	15.64	-88.83	Guatemala		x	SE	
P29616	F	CNAV	15.64	-88.83	Guatemala		x	SE	
P29617	M	CNAV	15.64	-88.83	Guatemala		x	SE	
A-0075	M	ECOSUR-CH	18.53	-88.30	Mexico	Quintara Roo	x	SE	ADAB95289
ADAB 55	M	ECOSUR-CH	18.53	-88.30	Mexico	Quintara Roo	x	SE	
ADAB 163	F	ECOSUR-CH	18.53	-88.30	Mexico	Quintara Roo	x	SE	Y408176
ADAB 164	F	ECOSUR-CH	18.53	-88.30	Mexico	Quintara Roo	x	SE	B2000
ADAB 95099	F	ECOSUR-CH	19.39	-88.08	Mexico	Quintara Roo	x	SE	ADAB95289
ADAB 95289	ND	ECOSUR-CH	19.16	-87.89	Mexico	Quintana Roo		SE	
ADAB 96465	M	ECOSUR-CH	18.53	-88.30	Mexico	Quintara Roo	x	SE	

EMFE 80	M	ECOSUR-CH	18.53	-88.30	Mexico	Quintara Roo	x	SE	
EMFE 389	M	ECOSUR-CH	18.53	-88.30	Mexico	Quintara Roo	x	SE	
EMFE 403	M	ECOSUR-CH	18.53	-88.30	Mexico	Quintara Roo	x	SE	
b2000	M	MZF	18.59	-90.26	Mexico	Campeche	x	SE	
b2037	ND	MZF	18.59	-90.26	Mexico	Campeche		SE	
Y408 176	M	MZF	18.02	-90.32	Mexico	Campeche	x	SE	Y408176
HBD053	M	MZF	17.97	-88.89	Mexico	Quintana Roo	x	SE	
MOL1121	M	MZF	18.60	-89.28	Mexico	Campeche	x	SE	MOL1121
MOL1122	M	MZF	18.59	-89.26	Mexico	Campeche	x	SE	MOL1122
MOL1149	M	MZF	18.60	-89.28	Mexico	Quintana Roo	x	SE	MOL1149
PGD038	F	MZF	15.48	-93.04	Mexico	Chiapas	x	SE	5982
QROO017	M	MZF	21.21	-87.19	Mexico	Quintana Roo		SE	
YACH330	M	MZF	16.91	-90.98	Mexico	Chiapas	x	SE	DAB1485
YACH378	M	MZF	16.08	-90.98	Mexico	Chiapas	x	SE	YACH378
YACH528	F	MZF	16.91	-90.98	Mexico	Chiapas	x	SE	YACH528
13725	M	MZF						SE	
dab1485	ND	UWBM	11.99	-86.26	Nicaragua			SE	
dab1486	ND	UWBM	11.99	-86.26	Nicaragua			SE	
dhb4369	ND	UWBM	14.65	-91.60	Guatemala			SE	
5934	ND	KU	13.81	-89.81	El Salvador			SE	
5937	ND	KU	13.81	-89.81	El Salvador			SE	
5949	ND	KU	13.81	-89.81	El Salvador			SE	
5974	ND	KU	13.83	-89.57	El Salvador			SE	
5978	ND	KU	13.83	-89.57	El Salvador			SE	
5982	ND	KU	13.83	-89.57	El Salvador			SE	
5985	ND	KU	13.83	-89.57	El Salvador			SE	
dab1484	ND	SEC	11.99	-86.26	Nicaragua			SE	
dab1508	ND	SEC	11.99	-86.26	Nicaragua			SE	
dab1349	ND	SEC	13.02	-85.92	Nicaragua			SE	
zmuc130351	ND	SEC	10.31	-84.81	Costa Rica			SE	
gav2110	ND	SEC	15.43	-86.52	Honduras			SE	
gms145	ND	SEC	15.43	-86.52	Honduras			SE	
dhb4362	ND	SEC	14.65	-91.60	Guatemala			SE	
dhb4377	ND	SEC	14.65	-91.60	Guatemala			SE	
dhb4399	ND	SEC	14.65	-91.60	Guatemala			SE	
434136	ND	SEC	13.93	-89.84	El Salvador			SE	
434137	ND	SEC	13.68	-89.66	El Salvador			SE	
229144	F	SI	9.14	-79.72	Panama		x	PA	
433980	M	SI	8.71	-79.91	Panama		x	PA	GMS1070
459208	M	SI	8.73	-82.66	Panama		x	PA	
471747	F	SI	8.25	-81.87	Panama		x	PA	JK04166

477183	M	SI	8.62	-80.58	Panama		x	PA	GMS1113
477184	F	SI	8.62	-80.58	Panama		x	PA	GMS1113
534190	M	SI	8.99	-79.54	Panama		x	PA	
77897	F	AMNH	8.78	-82.45	Panama	Chiriqui	x	PA	
77943	M	AMNH	8.54	-82.58	Panama	Chiriqui	x	PA	
136341	F	AMNH	8.79	-80.01	Panama		x	PA	GMS1070
183029	F	AMNH	8.12	-81.08	Panama			PA	
183032	M	AMNH	8.12	-81.08	Panama	Veraguas	x	PA	JK160
183042	F	AMNH	8.30	-81.82	Panama			PA	
186977	M	AMNH	7.96	-80.99	Panama		x	PA	JK04138
186983	M	AMNH	7.96	-80.99	Panama		x	PA	JK04166
187939	F	AMNH	8.51	-81.08	Panama		x	PA	JK04138
187942	F	AMNH	8.51	-81.08	Panama		x	PA	GMS1013
187946	F	AMNH	8.51	-81.08	Panama		x	PA	ANSP5772
233529	F	AMNH	8.79	-80.01	Panama		x	PA	
233530	F	AMNH	8.79	-80.01	Panama		x	PA	
246532	M	AMNH	7.95	-80.44	Panama		x	PA	ANSP5772
246533	M	AMNH	7.95	-80.44	Panama		x	PA	ANSP5771
246534	F	AMNH	7.95	-80.44	Panama		x	PA	ANSP5771
510442	M	AMNH	8.58	-82.39	Panama			PA	
510444	F	AMNH	8.48	-82.62	Panama			PA	
510445	M	AMNH	8.58	-82.39	Panama	Chiriqui	x	PA	GMS1013
510446	F	AMNH	8.58	-82.39	Panama		x	PA	JK160
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gms1113	ND	SEC	8.62	-80.10	Panama			PA	
gms1013	ND	SEC	8.05	-81.10	Panama			PA	
gms1070	ND	SEC	8.62	-80.10	Panama			PA	
jtk04138	ND	SEC	8.05	-81.10	Panama			PA	
jtk04166	ND	SEC	8.05	-81.10	Panama			PA	
ansp5772	ND	SEC	8.10	-80.98	Panama			PA	
ansp5771	ND	SEC	8.10	-80.98	Panama			PA	
308390	M	SI	-17.41	-63.85	Bolivia		x	WS	12591
308391	F	SI	-17.41	-63.85	Bolivia		x	WS	12591
308392	M	SI	-6.82	-66.15	W Brazil		x	WS	ANSP1495
308393	F	SI	-4.78	-56.60	W Brazil		x	WS	ZMUC120394
327553	F	SI	0.56	-68.14	W Brazil		x	WS	457560
138395	M	AMNH	-17.41	-66.17	Bolivia		x	WS	22625
138407	F	AMNH	-17.41	-66.17	Bolivia		x	WS	12594
138408	F	AMNH	-17.41	-66.17	Bolivia		x	WS	B18345
138410	F	AMNH	-17.41	-66.17	Bolivia		x	WS	22625
138413	M	AMNH	-17.42	-66.16	Bolivia		x	WS	B1052

138414	M	AMNH	-17.42	-66.16	Bolivia		x	WS	B22623
138415	F	AMNH	-17.42	-66.16	Bolivia		x	WS	ZMUC145305
147199	M	AMNH	-3.13	-60.00	W Brazil		x	WS	
147200	F	AMNH	-3.13	-60.00	W Brazil			WS	
148916	M	AMNH	-16.95	-65.38	Bolivia		x	WS	ZMUC145305
169529	F	AMNH	-11.06	-75.33	Peru		x	WS	B11166
179777	M	AMNH	-0.75	-80.37	Ecuador		x	WS	
179778	M	AMNH	-0.75	-80.37	Ecuador		x	WS	
179779	F	AMNH	-0.75	-80.37	Ecuador		x	WS	
183281	M	AMNH	-0.70	-77.1333	Ecuador		x	WS	
183283	F	AMNH	-0.70	-77.1333	Ecuador		x	WS	
183796	F	AMNH	-0.70	-77.1333	Ecuador		x	WS	
232876	F							WS	
235325	M	AMNH	-6.11	-77.21	Peru		x	WS	B40061
235326	F	AMNH	-6.11	-77.21	Peru		x	WS	B40060
239566	M	AMNH	-9.83	-73.09	Peru			WS	
239568	M	AMNH	-9.83	-73.09	Peru			WS	628
239569	F	AMNH	-9.83	-73.09	Peru		x	WS	636
240789	M	AMNH	-11.81	-77.17	Peru			WS	
256968	F	AMNH	-12.05	-77.03	Peru		x	WS	ANSP1495
278369	M							WS	
278364	M	AMNH	-10.42	-65.40	Bolivia		x	WS	8909
278367	F	AMNH	-10.42	-65.40	Bolivia		x	WS	8909
278371	F	AMNH	-10.42	-65.40	Bolivia		x	WS	8959
278854	M	AMNH	-10.42	-65.40	Bolivia		x	WS	8959
280309	M	AMNH	-6.90	-62.11	W Brazil		x	WS	457
282930	M	AMNH	-6.90	-62.11	W Brazil		x	WS	ZMUC120394
288002	M	AMNH	-4.78	-56.60	W Brazil		x	WS	
288012	F	AMNH	-2.40	-54.68	W Brazil		x	WS	
309714	M	AMNH	-3.35	-64.71	W Brazil		x	WS	457558
309716	M	AMNH	-3.11	-43.22	W Brazil		x	WS	
309719	F	AMNH	-5.00	-63.00	W Brazil		x	WS	457559
428939	M	AMNH	-4.78	-56.60	W Brazil			WS	
428945	F	AMNH	-2.45	-54.70	W Brazil			WS	
430092	F	AMNH	-7.27	-52.61	W Brazil			WS	
430093	M	AMNH	-7.27	-52.61	W Brazil		x	WS	
435435	M	AMNH	-0.10	-67.47	W Brazil		x	WS	457560
435436	M	AMNH	0.03	-67.27	W Brazil		x	WS	457559
510397	F	AMNH	-16.33	-59.62	Bolivia		x	WS	B1052
510398	M	AMNH	-16.33	-59.62	Bolivia			WS	
510399	M	AMNH	-16.29	-63.59	Bolivia		x	WS	12594

510401	M	AMNH	-16.93	-63.63	Bolivia		x	WS	B18345
510403	F	AMNH	-3.39	-64.94	W Brazil		x	WS	457558
510404	F	AMNH	-8.75	-63.88	W Brazil		x	WS	36646
510406	F	AMNH	-6.90	-62.11	W Brazil		x	WS	
510410	M	AMNH	-6.90	-62.11	W Brazil		x	WS	36646
510411	M	AMNH	-5.24	-75.66	Peru		x	WS	27368
510415	F	AMNH	-5.90	-76.11	Peru		x	WS	27369
819679	M	AMNH	-13.73	-72.71	Peru		x	WS	722
819878	M	AMNH	-12.15	-73.23	Peru		x	WS	FMNH398440
819879	F	AMNH	-12.15	-73.23	Peru		x	WS	711
22625	ND	LSU	-16.50	-68.15	Bolivia			WS	
8909	ND	LSU	-10.80	-67.00	Bolivia			WS	
8959	ND	LSU	-10.80	-67.00	Bolivia			WS	
12591	ND	LSU	-17.87	-63.00	Bolivia			WS	
12594	ND	LSU	-17.87	-63.00	Bolivia			WS	
36646	ND	LSU	-11.51	-63.58	W Brazil			WS	
27368	ND	LSU	-4.23	-74.22	Peru			WS	
27369	ND	LSU	-4.23	-74.22	Peru			WS	
457	ND	KU	-12.33	-69.03	Peru			WS	
628	ND	KU	-12.33	-69.03	Peru			WS	
636	ND	KU	-12.33	-69.03	Peru			WS	
711	ND	KU	-12.33	-69.03	Peru			WS	
722	ND	KU	-12.33	-69.03	Peru			WS	
1052	ND	SEC	-16.50	-68.15	Bolivia			WS	
22623	ND	SEC	-16.50	-68.15	Bolivia			WS	
18345	ND	SEC	-17.87	-63.00	Bolivia			WS	
zmuc145305	ND	SEC	-14.38	-65.10	Bolivia			WS	
zmuc120394	ND	SEC	-9.45	-55.86	W Brazil			WS	
457560	ND	SEC	-1.68	-65.83	W Brazil			WS	
457559	ND	SEC	-1.88	-66.93	W Brazil			WS	
457558	ND	SEC	-2.49	-68.26	W Brazil			WS	
40060	ND	SEC	-4.23	-74.22	Peru			WS	
40061	ND	SEC	-4.23	-74.22	Peru			WS	
11166	ND	SEC	-9.83	-73.09	Peru			WS	
fmnh398440	ND	SEC	-12.67	-71.27	Peru			WS	
ANSP 1495	ND	SEC	-10.00	-76.00	Peru			WS	
173431	M	SI	-25.66	-56.96	Paraguay		x	ES	205
368410	M	SI	-19.56	-40.44	E Brasil		x	ES	
515988	M	SI	-22.93	-43.24	E Brasil		x	ES	
128944	M	AMNH	-23.57	-46.96	E Brasil		x	ES	ZMUC137118
146798	M	AMNH	-25.60	-54.58	Argentina		x	ES	313

154264	M	AMNH	-25.99	-54.63	Argentina			ES	
156397	M	AMNH	-18.92	-48.31	E Brazil		x	ES	
245524	M	AMNH	-6.89	-38.56	E Brazil		x	ES	
245525	F	AMNH	-6.89	-38.56	E Brazil		x	ES	
245527	F	AMNH	-6.89	-38.56	E Brazil		x	ES	
245528	M	AMNH	-13.69	-40.09	E Brazil		x	ES	
316394	M	AMNH	-27.24	-50.22	E Brazil		x	ES	
316395	M	AMNH	-27.24	-50.22	E Brazil		x	ES	
316396	F	AMNH	-27.24	-50.22	E Brazil		x	ES	B25830
316401	M	AMNH	-27.24	-50.22	E Brazil		x	ES	DHB1801
316402	F	AMNH	-27.24	-50.22	E Brazil			ES	
316403	F	AMNH	-20.36	-50.70	E Brazil		x	ES	ZMUC137117
316406	M	AMNH	-30.03	-51.22	E Brazil		x	ES	3785
316407	M	AMNH	-30.03	-51.22	E Brazil		x	ES	3852
316408	F	AMNH	-30.03	-51.22	E Brazil			ES	
316409	F	AMNH	-30.03	-51.22	E Brazil		x	ES	3852
316412	F	AMNH	-30.03	-51.22	E Brazil		x	ES	3853
316413	M	AMNH	-30.03	-51.22	E Brazil		x	ES	3853
316414	M	AMNH	-30.03	-51.22	E Brazil		x	ES	MVZ168909
316415	F	AMNH	-30.03	-51.22	E Brazil		x	ES	MVZ168909
318029	F	AMNH	-19.71	-40.48	E Brazil		x	ES	
318030	M	AMNH	-19.38	-40.07	E Brazil		x	ES	
318031	M	AMNH	-19.38	-40.07	E Brazil		x	ES	
318032	M	AMNH	-19.38	-40.07	E Brazil		x	ES	
318033	M	AMNH	-19.38	-40.07	E Brazil		x	ES	
318038	F	AMNH	-19.71	-40.48	E Brazil		x	ES	
318230	M	AMNH	-19.82	-43.95	E Brazil		x	ES	
318231	F	AMNH	-19.82	-43.95	E Brazil		x	ES	FMNH345472
319282	M	AMNH	-23.89	-55.43	E Brazil		x	ES	ZMUC144784
320262	M	AMNH	-25.69	-56.26	Paraguay		x	ES	B25909
320711	M	AMNH	-23.44	-58.44	Paraguay		x	ES	
320713	F	AMNH	-23.44	-58.44	Paraguay		x	ES	3785
320714	M	AMNH	-25.26	-57.56	Paraguay		x	ES	25853
510390	M	AMNH	-23.55	-46.63	E Brazil		x	ES	ZMUC137117
510391	M	AMNH	-23.55	-46.63	E Brazil		x	ES	FMNH345472
510393	M	AMNH	-23.55	-46.63	E Brazil		x	ES	
510395	F	AMNH	-23.55	-46.63	E Brazil		x	ES	ZMUC137118
774270	M	AMNH	-25.90	-54.61	Argentina		x	ES	GAV821
774272	M	AMNH	-25.90	-54.61	Argentina		x	ES	GAV822
774276	F	AMNH	-25.90	-54.61	Argentina		x	ES	GAV821
774277	F	AMNH	-25.90	-54.61	Argentina		x	ES	GAV822

774278	F	AMNH	-25.90	-54.61	Argentina		x	ES	226
774280	F	AMNH	-25.90	-54.61	Argentina		x	ES	259
774281	F	AMNH	-25.90	-54.61	Argentina		x	ES	DHB1801
813091	F	AMNH	-29.44	-49.80	E Brazil		x	ES	3662
dhb1801	ND	UWBM	-26.96	-55.09	Argentina			ES	
gav821	ND	UWBM	-26.96	-55.09	Argentina			ES	
gav822	ND	UWBM	-26.96	-55.09	Argentina			ES	
25853	ND	LSU	-26.23	-56.02	Paraguay			ES	
205	ND	KU	-26.35	-55.52	Paraguay			ES	
226	ND	KU	-26.35	-55.52	Paraguay			ES	
259	ND	KU	-26.35	-55.52	Paraguay			ES	
313	ND	KU	-26.35	-55.52	Paraguay			ES	
3662	ND	KU	-26.52	-55.80	Paraguay			ES	
3785	ND	KU	-26.52	-55.80	Paraguay			ES	
3852	ND	KU	-26.52	-55.80	Paraguay			ES	
3853	ND	KU	-26.52	-55.80	Paraguay			ES	
ic1110	ND	SEC	8.31	-70.05	Venezuela			ES	
zmuc137118	ND	SEC	-23.61	-46.46	E Brazil			ES	
zmuc137117	ND	SEC	-23.61	-46.46	E Brazil			ES	
fmnh345472	ND	SEC	-23.61	-46.46	E Brazil			ES	
25909	ND	SEC	-25.46	-56.02	Paraguay			ES	
25830	ND	SEC	-26.23	-56.02	Paraguay			ES	
zmuc144784	ND	SEC	-26.07	-55.75	Paraguay			ES	
mvz168909	ND	SEC	-27.18	-55.78	Paraguay			ES	
176576	M							ES	

APÉNDICE 3. MATERIAL SUPLEMENTARIO: ARTÍCULO 2

Ecology & Evolution

SUPPLEMENTARY MATERIAL

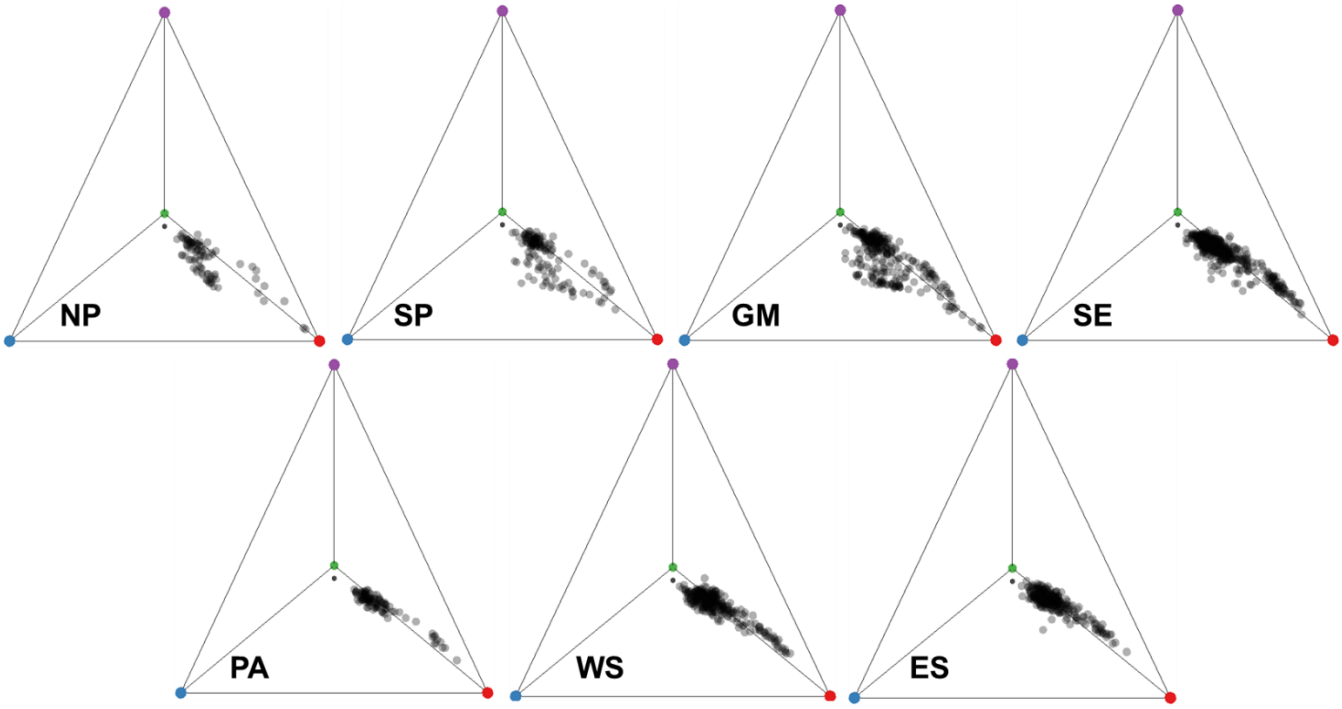
What drives genetic and phenotypic divergence in the Red-crowned Ant-tanager (*Habia rubica*, Aves: Cardinalidae), a polytypic species?

Sandra M. Ramírez-Barrera, Julián A. Velasco, Tania Orozco-Téllez, Alma M. Vázquez-López & Blanca E. Hernández-Baños

Supporting information for method of this study

Figure S2.1 Tetrahedral color space plots occupied by nine corporal patches measured in 339 males and females of the species *Habia rubica*. This sampling is divided by recently described phylogroups (complete sampling). These phylogroups were named as follows: NP, northern pacific of Mexico; SP, southern pacific of Mexico; GM, Gulf of Mexico; SE, southeastern

Males



Females

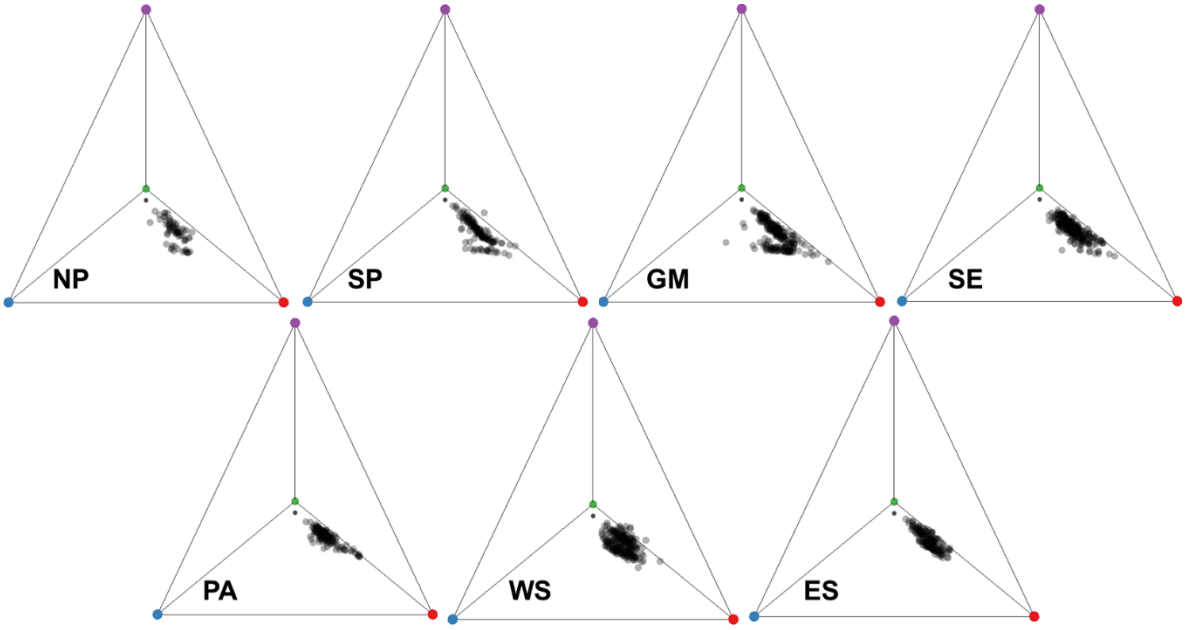


Figure S2.2 (a) Map showing the position of the polygons in raster format for the seven phylogroups that make up the *Habia rubica* sampling: NP, northern pacific of Mexico; SP, southern pacific of Mexico; GM, Gulf of Mexico; SE, southeastern Mexico. (b) Map showing the position of some of the delimited phylogroups from individuals with genetic and phenotypic sampling: M_NP, males from northern pacific of Mexico; M_SP, males from southern pacific of Mexico; F_GM, females from Gulf of Mexico; M_SE, males from southeastern Mexico.

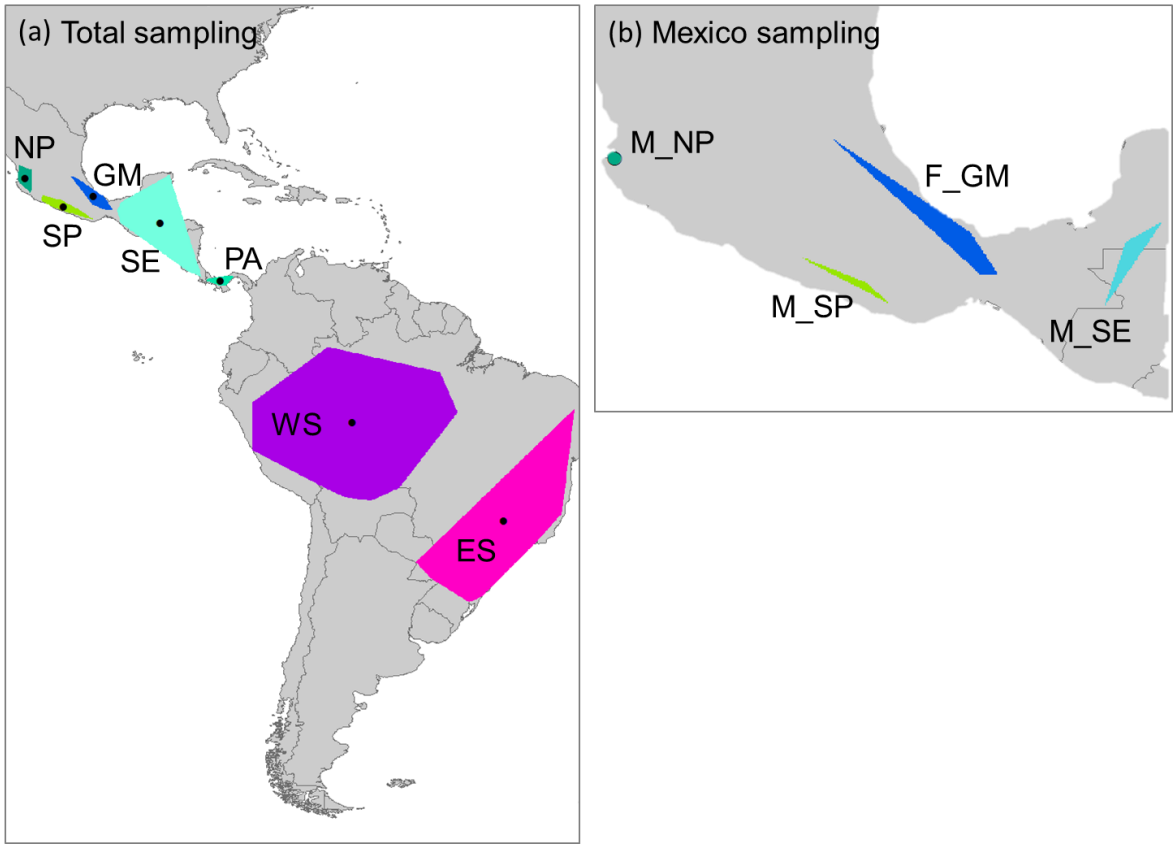


Figure S2.3 Comparison graph between mean and median values of 19 bioclimatic variables obtained for raster cell coordinates for each polygon (phylogroup) of the complete distribution of *Habia rubica*.

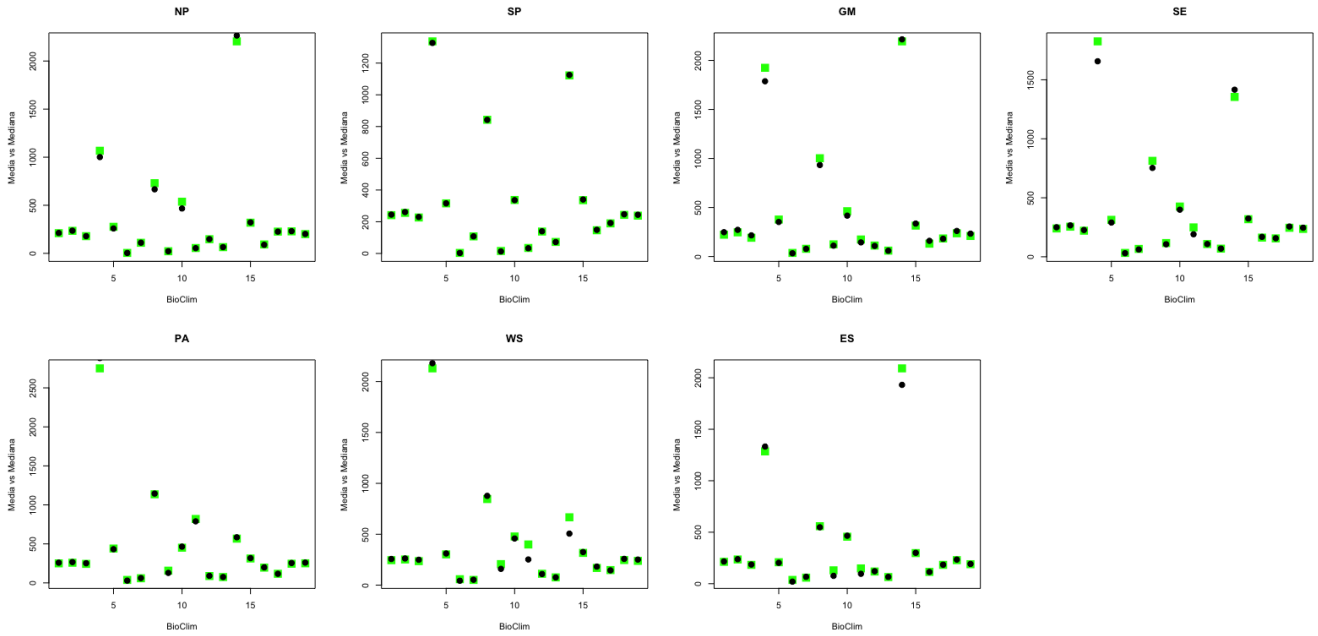


Figure S2.4 Comparison graph between mean and median values of 19 bioclimatic variables obtained for raster cell coordinates for each polygon (phylogroup) of the distribution in Mexico of *Habia rubica*.

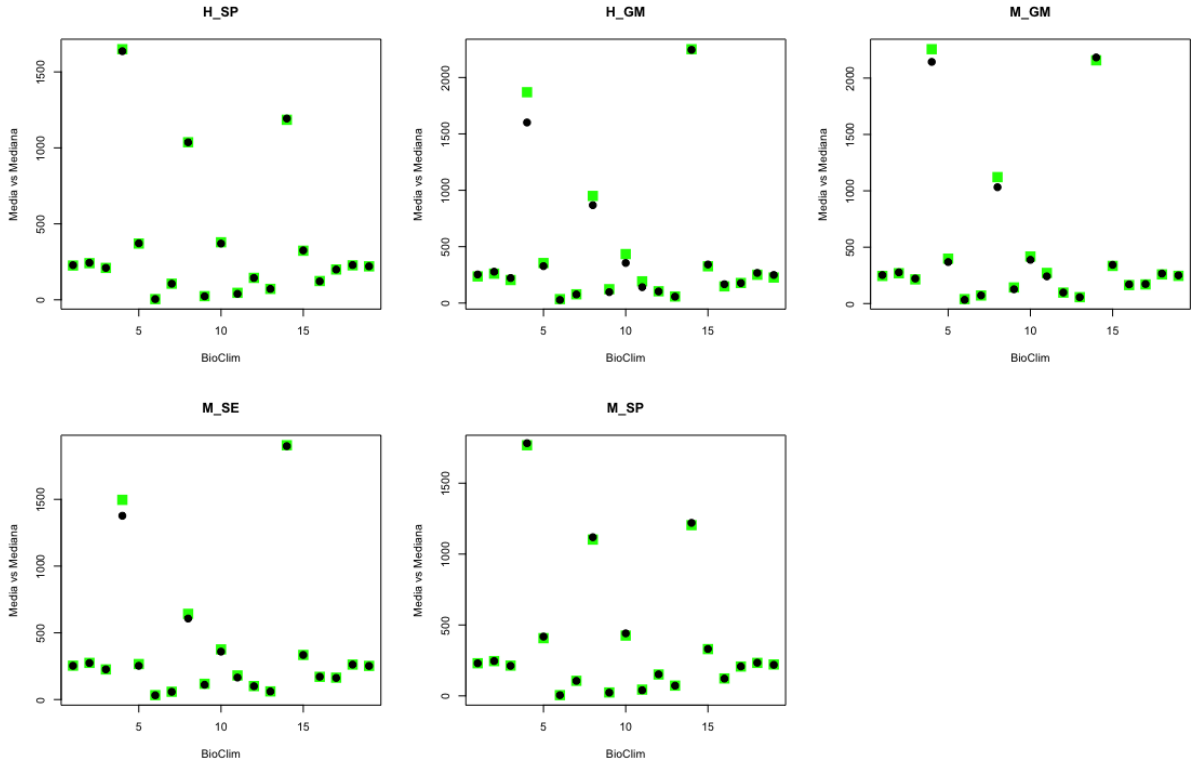


Table S2.1 Test for to sexual dimorphism for three morphometric measurements of individuals from *Habia rubica* populations.

	n (males)	n (females)	Males mean ± sd	Females mean ± sd	Mean difference	95% CI Lower	95% CI Upper	*t*	df	*p*-value
Wing	110	104	90.35 ± 4.07	84.04 ± 4.32	6.31	-7.44	-5.17	-10.97	209.21	<0.05
Tarsus	110	104	23.73 ± 1.32	23.20 ± 1.47	0.53	-0.91	-0.16	-2.80	206.63	<0.05
Tail	110	104	81.39 ± 5.16	75.91 ± 5.17	5.48	-6.87	-4.08	-7.75	211.28	<0.05
H0: Difference in means = 0										

Table S2.2 Bioclimatic variables obtained from WorldClim. The bioclimatic variables represent annual trends, seasonality and extreme or limiting environmental factors. A quarter is a period of three months (1/4 of the year).

Name of layer	Varibles
BIOO1	Annual Mean Temperature
BIOO2	Mean Diurnal Range (Mean of monthly (max temp - min temp))
BIOO3	Isothermality (BIO2/BIO7) (* 100)
BIOO4	Temperature Seasonality (standard deviation *100)
BIOO5	Max Temperature of Warmest Month
BIOO6	Min Temperature of Coldest Month
BIOO7	Temperature Annual Range (BIO5-BIO6)
BIOO8	Mean Temperature of Wettest Quarter
BIOO9	Mean Temperature of Driest Quarter
BIO10	Mean Temperature of Warmest Quarter
BIO11	Mean Temperature of Coldest Quarter
BIO12	Annual Precipitation
BIO13	Precipitation of Wettest Month
BIO14	Precipitation of Driest Month
BIO15	Precipitation Seasonality (Coefficient of Variation)
BIO16	Precipitation of Wettest Quarter
BIO17	Precipitation of Driest Quarter
BIO18	Precipitation of Warmest Quarter
BIO19	Precipitation of Coldest Quarter

APÉNDICE 4. MATERIAL SUPLEMENTARIO: ARTÍCULO 2

Ecology & Evolution

SUPPLEMENTARY MATERIAL

What drives genetic and phenotypic divergence in the Red-crowned Ant-tanager (*Habia rubica*, Aves: Cardinalidae), a polytypic species?

Sandra M. Ramírez-Barrera, Julián A. Velasco, Tania Orozco-Téllez, Alma M. Vázquez-López & Blanca E. Hernández-Baños

Supporting information for results of this study

Table S3.1 Paired corrected genetic distances (percentage of differentiation), estimated using the nucleotide model Jukes-Cantor (Jukes & Cantor, 1969). The names of rows and columns correspond to the names of each identified group within the species had rubica (Ramírez-Barrera et al., 2018).

	NP	SP	GM	SE	PA	WS	ES
NP							
SP	1.0						
GM	5.1	4.4					
SE	5.1	4.5	1.7				
PA	6.1	5.5	2.7	2.4			
WS	7.4	7.4	5.7	5.8	6.6		
ES	6.8	7.6	5.7	5.8	6.7	6.4	

Table S3.2 Correlation values estimated between three morphometric variables (wing length, tarsus length, tail length) obtained from individuals of *Habia rubica*.

	Wing length	Tarsus length	Tail length
Females			
Wing length	1.00	0.21	0.49
Tarsus length	0.21	1.00	0.65
Tail length	0.49	0.65	1.00
Males			
Wing length	1.00	0.32	0.56
Tarsus length	0.32	1.00	0.57
Tail length	0.56	0.57	1.00

Figure S3.2 Graphs of correlation between three morphometric variables (wing length, tarsus length, tail length) obtained from males of *Habia rubica*.

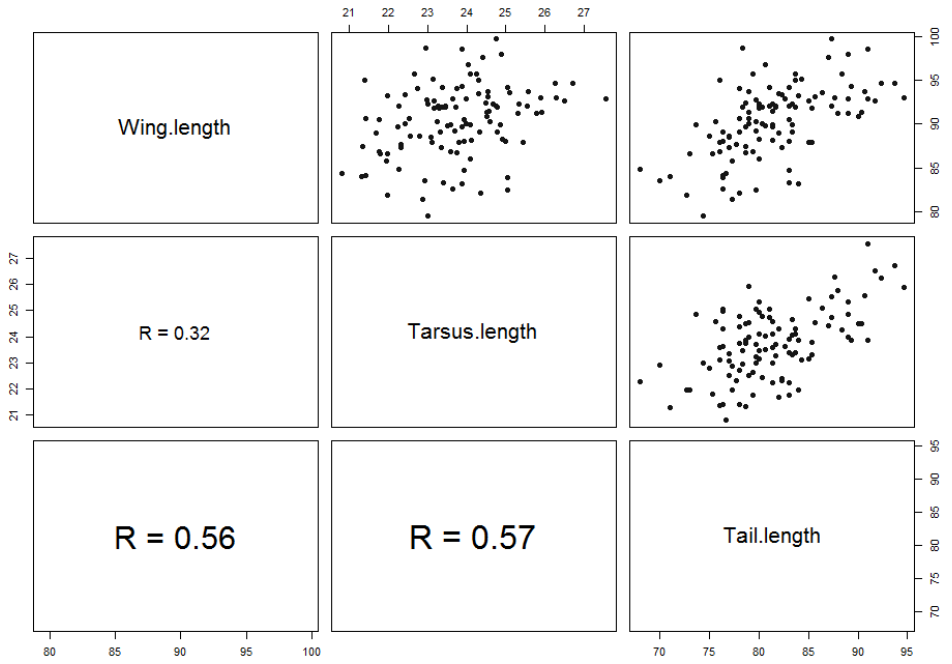


Figure S3.3 Correlation graphs between three morphometric variables (wing length, tarsal length, tail length) obtained from the female individuals of *Habia rubica*.

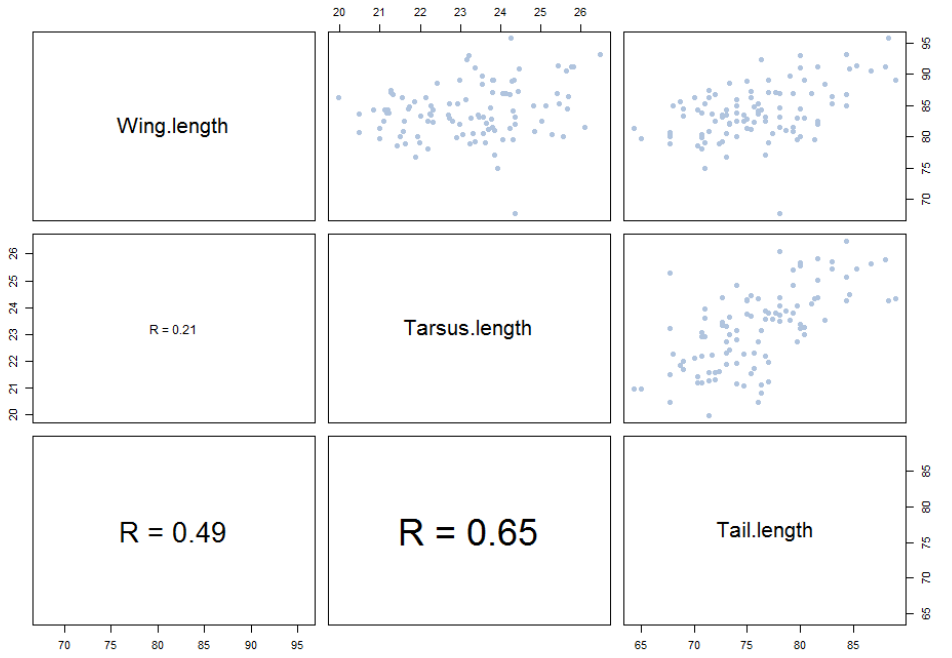


Table S3.3 Results of the PCA analysis carried out to summarize morphometric variables used as estimation of body size of males and females of the species *Habia rubica* in subsequent analyzes. The data were grouped by phylogroups according to Ramírez-Barrera et al. (2018).

PHYLOGROUPS MALES	PC1	PC2	PC3
Wing length	0.4194101	0.9074694	0.02438163
Tarsus length	0.644541	-0.2787628	-0.71193977
Tail length	0.6392669	-0.3143097	0.70181714
PHYLOGROUPS FEMALES			
Wing length	0.4540808	-0.8909452	-0.00522271
Tarsus length	0.6294597	0.3249492	-0.70582473
Tail length	0.6305483	0.317214	0.70836726

PHYLOGROUPS MALES	PC1	PC2	PC3
Standard deviation	1.4826	0.863	0.23894
Proportion of Variance	0.7327	0.2482	0.01903
Cumulative Proportion	0.7327	0.981	1
PHYLOGROUPS FEMALES			
Standard deviation	1.5037	0.82	0.25776
Proportion of Variance	0.7537	0.2241	0.02215
Cumulative Proportion	0.7537	0.9778	1

Table S3.4 Results of the PCA analysis carried out to summarize morphometric variables used as estimation of body size of males and females of the species *Habia rubica* in subsequent analyzes. This analysis was done by taking the individual data.

MALES	PC1	PC2	PC3
Wing length	0.544	-0.715	0.437
Tarsus length	0.548	0.698	0.459
Tail length	0.634	0.009	-0.772
FEMALES			
Wing length	0.481	-0.809	0.335
Tarsus length	0.576	0.580	0.575
Tail length	0.660	0.083	-0.746

MALES			
Standard deviation	1.406	0.825	0.583
Proportion of Variance	0.659	0.227	0.113
Cumulative Proportion	0.659	0.886	1

FEMALES			
Standard deviation	1.387	0.893	0.531
Proportion of Variance	0.640	0.265	0.094
Cumulative Proportion	0.640	0.906	1

Figure S3.4 Plots of the analysis of PCA made with the total individual data of males and females of the species *Habia rubica*.

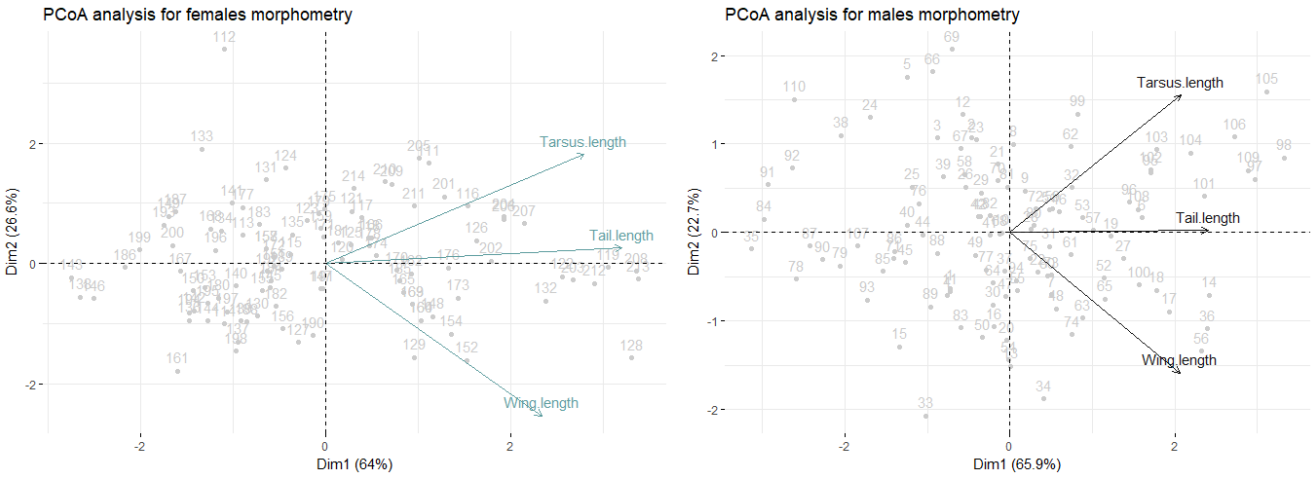


Table S3.5 Results of the linear regression analysis carried out taking the values of coloration of the plumage (variable hue) and body size (PC1) of males and females of the species *Habia rubica*.

MALES	Estimate	Std. Error	t value	p value	R^2
Plumaje coloration	0.020	0.005	3.582	<0.05	0.098
Body size	-0.015	0.006	-2.628	<0.05	0.05
FEMALES					
Plumaje coloration	0.016	0.006	2.836	<0.05	0.06
Body size	-0.017	0.006	0.006	<0.05	0.07

Figure S3.5 Plots of plumage coloration values (variable hue) and body size (PC1) against latitude. Dashed line for females and solid line for males of *Habia rubica* species.

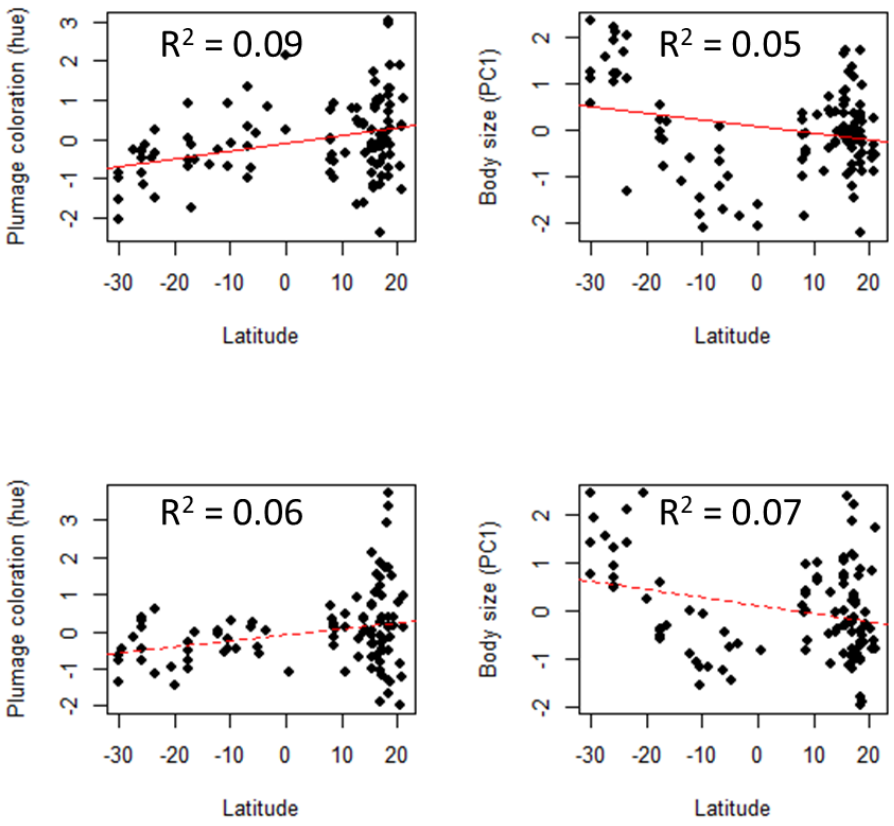


Table S3.6 Results of univariate MMRR analysis grouping by sex for analysis between phylogroups and individuals of distribution in Mexico of *H. rubica,* testing three independent variables of distance: genetics, color and body size. Here, we show the results of coefficient of determination (R^2), beta-weights (β) and *p*-value (*p*) for each predictor.

	Analysis by individuals						Analysis by phylogroups					
	Males			Females			Males			Females		
a) MRM (**mtDNA** ~ Predictor)												
	R^2	β	*p*	R^2	β	*p*	R^2	β	*p*	R^2	β	*p*
Geography	**0.31**	**0.50**	**<0.01**	**0.48**	**0.86**	**<0.01**	0.36	0.79	0.04	0.17	0.54	0.17
Climate	**0.34**	**0.18**	**<0.01**	**0.23**	**0.22**	**<0.01**	0.90	0.51	0.09	0.53	0.41	0.16
Hue total	0.10	0.28	**<0.01**	0.00	0.05	0.63	0.63	0.79	0.24	0.06	-0.26	1.00
Chroma total	0.00	-0.03	0.57	0.00	-0.01	0.88	0.01	-0.06	0.53	0.01	-0.10	0.15
Volume total	0.03	0.16	0.03	5.5E-05	0.01	0.94	0.73	0.81	0.20	0.14	-0.43	0.84
Body size	**0.10**	**0.29**	**0.01**	0.03	0.21	0.05	0.15	-0.44	0.75	0.44	0.66	0.17
b) MRM (**Hue** ~ Predictor)												
	R^2	β	*p*	R^2	β	*p*	R^2	β	*p*	R^2	β	*p*

Geography	0.013	0.11	0.32	0.05	0.20	0.06	0.09	0.40	0.44	0.28	0.70	0.29
Climate	0.01	0.03	0.52	0.01	-0.03	0.53	0.85	0.50	0.03	0.09	0.17	0.58
mtDNA	0.10	0.36	0.01	0.00	0.03	0.67	0.63	0.80	0.24	0.06	-0.26	1.00
Body size	0.21	0.47	**<0.01**	0.01	-0.08	0.56	0.04	-0.23	0.75	0.17	0.42	0.48
c) MRM (**Chroma** ~ Predictor)												
Geography	3.0E-06	0.00	0.99	0.01	-0.10	0.38	0.13	0.60	0.65	0.08	-0.40	0.63
Climate	0.02	-0.05	0.37	0.01	-0.04	0.45	0.05	-0.15	0.27	0.00	-0.04	0.95
mtDNA	0.00	-0.04	0.60	0.00	-0.01	0.91	0.01	-0.10	0.57	0.01	-0.11	0.15
Body size	0.00	-0.03	0.86	0.00	-0.05	0.77	0.11	0.48	0.56	0.12	-0.37	0.70
d) MRM (**Volume** ~ Predictor)												
Geography	0.01	0.09	0.42	0.00	-0.04	0.72	0.35	0.82	0.24	0.07	0.29	0.43
Climate	0.00	0.02	0.70	0.01	-0.03	0.53	0.56	0.42	0.27	0.05	0.11	0.67
mtDNA	0.03	0.20	0.04	5.5E-05	0.00	0.92	0.73	0.89	0.20	0.14	-0.31	0.82
Body size	0.02	0.13	0.38	0.01	-0.08	0.50	0.03	-0.22	0.96	0.03	0.15	0.61
e) MRM (**Body size** ~ Predictor)												
	R^2	*β*	*p*	R^2	*β*	*p*	R^2	*β*	*p*	R^2	*β*	*p*
Geography	0.03	0.16	0.07	0.04	0.20	0.07	0.01	-0.09	0.85	0.69	1.09	0.08
Climate	0.05	0.08	0.08	0.00	0.01	0.85	0.12	-0.17	0.84	0.91	0.53	0.03
mtDNA	0.10	0.36	0.01	0.03	0.13	0.05	0.15	-0.34	0.75	0.44	0.65	0.17
Hue total	0.21	0.45	**<0.01**	0.01	-0.09	0.57	0.04	-0.18	0.74	0.17	0.41	0.44
Chroma total	0.00	-0.03	0.84	0.00	-0.04	0.77	0.11	0.22	0.57	0.12	-0.32	0.70
Volume total	0.02	0.12	0.37	0.01	-0.09	0.52	0.03	-0.16	0.96	0.03	0.20	0.61

Table S3.7 Results of multivariate MMRR analysis grouped by sex for analysis between phylogroups and individuals of distribution in Mexico of *H. rubica*. We show the results of coefficient of determination (R^2) Beta-weights (*β*) and *p*-value (*p*) and of each predictor from the overall model.

	Analysis by individuals						Analysis by phylogroups					
	Males			Females			Males			Females		
a) MRM (**mtDNA** ~ Geography + Clime + Hue + Body size)												
	R^2	*β*	*p*	R^2	*B*	*p*	R^2	*β*	*p*	R^2	*β*	*p*
Geography		0.32	<0.01		0.76	<0.01		-0.38	0.69		-0.14	0.50
Climate	0.52	0.12	<0.01	0.52	0.09	<0.01	0.96	1.19	0.39	0.79	0.27	1.00
Hue		0.18	0.01		-0.10	0.17		-1.12	0.58		-0.56	0.67
Body size		0.08	0.16		0.03	0.63		0.16	0.77		0.52	0.83
b) MRM (**Hue** ~ Geography + Clime + mtDNA + Body size)												
	R^2	*β*	*p*	R^2	*B*	*p*	R^2	*β*	*p*	R^2	*β*	*p*
Geography		-0.06	0.75		0.41	0.01		-0.40	0.46		0.31	0.87
Climate	0.26	-0.06	0.31	0.12	-0.07	0.23	0.98	0.89	0.21	0.68	-0.07	1.00
mtDNA		0.36	<0.01		-0.10	0.30		-0.51	0.38		-0.86	0.54
Body size		0.41	<0.01		-0.14	0.27		0.17	0.64		0.92	0.65
c) MRM (**Chroma** ~ Geography + Clime + mtDNA + Body size)												
Geography	0.02	0.06	0.71	0.028	-0.14	0.43	0.51	0.94	0.79	0.90	-0.32	0.87

Climate		-0.06	0.40		-0.05	0.56		-0.99	0.67		1.92	0.41
mtDNA		0.03	0.81		0.12	0.28		1.34	0.89		-0.41	0.70
Body size		-0.01	0.94		-0.04	0.85		0.39	1.00		-3.19	0.31
d) MRM (**Volume** ~ Geography + Clime + mtDNA + Body size)												
Geography		0.00	0.99		-0.05	0.79		0.12	0.96		-0.23	0.83
Climate	0.04	-0.03	0.66	0.02	-0.04	0.54	0.79	-0.35	0.79	0.72	0.93	0.45
mtDNA		0.22	0.13		0.09	0.40		1.52	0.49		-1.06	0.32
Body size		0.08	0.63		-0.09	0.51		0.19	1.00		-0.59	0.74
e) MRM (**Body size** ~ Geography + Clime + mtDNA + Hue)												
	R^2	β	p	R^2	B	p	R^2	β	p	R^2	β	P
Geography		-0.00	0.98		0.25	0.15		1.32	0.43		0.16	0.95
Climate	0.25	0.04	0.45	0.07	-0.05	0.43	0.61	-2.64	0.39	0.93	0.38	0.57
mtDNA		0.15	0.28		0.04	0.71		1.27	0.73		0.16	0.82
Hue		0.40	<0.01		-0.16	0.23		2.98	0.38		0.18	0.61