UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO

POSGRADO EN CIENCIAS BIOLÓGICAS

FACULTAD DE CIENCIAS

SISTEMÁTICA

Variación genética, morfológica y de coloración en *Diglossa baritula* (Aves: Thraupidae)

TESIS

(POR ARTÍCULO CIENTÍFICO)

Recent genetic, phenetic and ecological divergence across the Mesoamerican highlands: a study case with *Diglossa baritula* (Aves: Thraupidae)

QUE PARA OPTAR POR EL GRADO DE:

MAESTRA EN CIENCIAS BIOLÓGICAS

PRESENTA:

Alondra Karina Terrones Ramírez

TUTOR(A) PRINCIPAL DE TESIS: Dra. Blanca Estela Hernández Baños, Facultad de Ciencias, UNAM
COMITÉ TUTOR: Dr. Adrián Nieto Montes de Oca, Facultad de Ciencias, UNAM
Dra. María del Coro Arimendi Arriaga, Facultad de Estudios Superiores Iztacala, UNAM

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

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COORDINACIÓN DEL POSGRADO EN CIENCIAS BIOLÓGICAS
FACULTAD DE CIENCIAS
DIVISIÓN ACADÉMICA DE INVESTIGACIÓN Y POSGRADO
OFICIO FCIE/DAIP/083/2023

ASUNTO: Oficio de Jurado

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

Me permito informar a usted que en la reunión del Comité Académico del Posgrado en Ciencias Biológicas, celebrada el día **28 de noviembre de 2022** se aprobó el siguiente jurado para el examen de grado de **MAESTRA EN CIENCIAS BIOLÓGICAS** en el campo de conocimiento de **Sistemática** de la alumna **TERRONES RAMÍREZ ALONDRA KARINA** con número de cuenta **308138603** por la modalidad de graduación de **tesis por artículo científico** titulado: **"Recent genetic, phenetic and ecological divergence across the Mesoamerican highlands: a study case with *Diglossa baritula* (Aves: Thraupidae)",** que es producto del proyecto realizado en la maestría que lleva por título "**Variación genética, morfológica y de coloración en *Diglossa baritula* (Aves: Thraupidae)**" ambos realizados bajo la dirección de la **DRA. BLANCA ESTELA HERNÁNDEZ BAÑOS**, quedando integrado de la siguiente manera:

Presidente:	DR. LUIS ENRIQUE EGUIARTE FRUNS
Vocal:	DR. JOSÉ JAIME ZÚÑIGA VEGA
Vocal:	DRA. BERTHA PATRICIA ESCALANTE PLIEGO
Vocal:	DR. LUIS ANTONIO SÁNCHEZ GONZÁLEZ
Secretario:	DR. ADRIÁN NIETO MONTES DE OCA

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 13 de marzo de 2023

COORDINADOR DEL PROGRAMA

DR. ADOLFO GERARDO NAVARRO SIGÜENZA

POSGRADO EN CIENCIAS BIOLOGICAS
COORDINACIÓN

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"Lo importante es no dejar de hacerse preguntas."

- Albert Einstein

"La ciencia es una magia que funciona."

- Kurt Vonnegut

"The universe is made of stories, not of atoms".

- Muriel Rukeyser

"La evolución es multidimensional, dinámica y abundante como un fractal eterno, también así es el universo"

- Alondra Terrones (Morning pages, febrero de 2023)

ÍNDICE

LISTA DE FIGURAS Y TABLAS

LISTA DE FIGURAS

LISTA DE TABLAS

RESUMEN

La complejidad topográfica, geológica, climática y de biodiversidad de Mesoamérica la ha convertido en un foco de atención para la investigación. Las tierras altas mesoamericanas son una región con una alta riqueza de especies y variación intraespecífica. El pinchaflor ventricanelo, *Diglossa baritula* (Wagler, 1832), es una especie de ave endémica de las tierras altas de Mesoamérica, con tres subespecies alopátricas actualmente reconocidas. Para caracterizar la divergencia en esta especie, integramos diferentes enfoques, como datos genómicos, morfológicos, de coloración y de nicho ecológico, muestreando individuos a lo largo de toda la distribución geográfica de la especie. Nuestros resultados revelaron una clara divergencia genómica entre las poblaciones del este del Istmo de Tehuantepec y las poblaciones del oeste del Istmo. En contraste con los resultados genómicos, los análisis de morfología y coloración mostraron niveles intermedios de diferenciación, lo que indica que probablemente las agrupaciones poblacionales dentro de *D. baritula* no han estado sujetas a diferentes presiones evolutivas. Finalmente, los análisis de sobrelapamiento de nicho ecológico sugieren que hay moderado sobrelapamiento de nicho entre las tres subespecies, indicando que hay divergencia de nicho en respuesta a diferentes condiciones ambientales. El presente trabajo representa el primer estudio multidisciplinario de las poblaciones del complejo *D. baritula*, y nuestros datos sugieren que *D. baritula* podría contener dos o más linajes evolutivos independientes. Estos resultados resaltan la importancia del Istmo de Tehuantepec como la principal barrera geográfica de las poblaciones de *D. baritula*. La presente investigación ilustra el potencial de especiación del complejo *D. baritula* y la capacidad de las tierras altas de Mesoamérica para crear linajes incipientes y endemismo.

ABSTRACT

The topographical, geological, climatic and biodiversity complexity of Mesoamerica has made it a primary research focus. The Mesoamerican highlands is a region with particularly high species richness and within-species variation. The Cinnamon-bellied Flowerpiercer, *Diglossa baritula* (Wagler, 1832), is a bird species endemic to the Mesoamerican highlands, with three allopatric subspecies currently recognized. To characterize divergence in this species, we integrated different approaches including genomics, morphological, coloration, and ecological niche analysis, obtained from sampling individuals across the entire geographic distribution of the species. Our results revealed a clear genomic divergence between the populations east of the Isthmus of Tehuantepec and the populations of the west of the Isthmus. In contrast to the genomic results, morphology and coloration analyses showed intermediate levels of differentiation, indicating that population groups within *D. baritula* probably have not been under different evolutionary pressures. Finally, the ecological niche overlap data indicated that there is moderate niche overlap between the three subspecies, indicating that there is niche divergence in response to different environmental conditions. This work represents the first multidisciplinary study of the populations of the *D. baritula* complex. Our data suggest that *D. baritula* could contain two or more independent evolutionary lineages. These results highlight the importance of the Isthmus of Tehuantepec as the main geographic barrier for *D. baritula* populations. The present investigation illustrates the speciation potential of *D. baritula* complex and the capacity of Mesoamerican highlands to create incipient lineages and endemisms.

INTRODUCCIÓN

La gran complejidad y diversidad de Mesoamérica es el resultado de distintos factores históricos, físicos y ecológicos (Myers et al., 2000; Winker, 2011; Gutiérrez-García & Vázquez-Domínguez, 2013; Hufnagel & Mics, 2021). Grandes eventos geológicos, como el cierre del Istmo de Panamá (Gutiérrez-García & Vázquez-Domínguez, 2013), la formación de una orografía accidentada (Marshall, 2007; Mastretta-Yanes et al., 2015) y las glaciaciones del Cuaternario (Ramírez-Barahona & Eguiarte, 2013) han generado la presencia de una variedad de tipos de vegetación y climas (Rzedowski, 1978) en Mesoamérica. Esta heterogeneidad ambiental ha favorecido una amplia diversidad de taxones y un gran número de especies endémicas tanto de plantas como de animales (Hufnagel & Mics, 2021).

El pinchaflor ventricanelo, *Diglossa baritula* (Wagler, 1832), *Cinnamon-bellied Flowerpiercer* en inglés es una especie endémica de las tierras altas de Mesoamérica. La riqueza de especies y el endemismo son característicos de las regiones montanas mesoamericanas (Myers et al., 2000). La vasta biodiversidad que ha evolucionado en las tierras altas mesoamericanas se debe a su distribución discontinua que está moldeada por el requerimiento de determinadas características ambientales relacionadas con la altitud, temperatura, y humedad, entre otras (Hernández-Baños et al., 1995; Weir, 2009). Las tierras altas mesoamericanas han permitido la persistencia de taxones montanos *in situ* por largos períodos de tiempo. Al carecer de las condiciones ambientales presentes en los sistemas montanos, las tierras bajas actúan como efectivas barreras geográficas para especies de tierras altas. Estas barreras climáticas pueden generar las condiciones para la especiación alopátrica (Weir, 2009). La variación genética y geográfica en especies politípicas de tierras altas

mesoamericanas ha sido utilizada para explorar hipótesis de adaptación y evolución, y detectar la presencia de linajes previamente no reconocidos (Weir, 2009).

Diglossa baritula es una especie de ave politípica que se distribuye desde el centro-sur de México hasta Nicaragua. Es un ave residente que se encuentra entre los 1500 a 3500 metros sobre el nivel del mar, donde habita en diversos tipos de hábitats incluyendo bosques de pino, bosques de pino-encino, bosques mesófilos de montaña, ecotonos de bosques, pastizales y jardines en áreas urbanas (Howell y Webb, 1995; Lauck, 2020). Actualmente, se reconocen tres subespecies alopátricas dentro de *D. baritula* con base en sus distribuciones discontinuas y de descripciones cualitativas del plumaje de machos (Howell & Webb, 1995; Lauck, 2020).

Esta tesis es el primer estudio multidisciplinario de las poblaciones de *D. baritula* y los resultados obtenidos permiten tener una primera perspectiva sobre la historia natural de esta especie. El objetivo del estudio fue obtener una filogenia de las poblaciones para *D. baritula*, describir sus patrones de morfología, coloración y variación ambiental y relacionar estos patrones con la filogenia. Esperábamos encontrar altos niveles de variación genética, fenética y ambiental, ya que el complejo *D. baritula* es una especie residente con distribución alopátrica y pertenece a una radiación adaptativa.

ARTÍCULO

Recent genetic, phenetic and ecological divergence across the Mesoamerican highlands: a study case with Diglossa baritula (Aves: Thraupidae)

Recent genetic, phenetic and ecological divergence across the Mesoamerican highlands: a study case with *Diglossa baritula* (Aves: Thraupidae)

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ABSTRACT

Mesoamerica's topographical, geological, climatic and biodiversity complexity has made it a primary research focus. The Mesoamerican highlands is a region with particularly high species richness and within-species variation. The Cinnamon-bellied Flowerpiercer, *Diglossa baritula* (Wagler, 1832), is a species endemic to the Mesoamerican highlands, with three allopatric subspecies currently recognized. To characterize divergence in this species, we integrated different approaches such as genomic, morphology, coloration and ecological niche approaches, sampling individuals from across the species' geographical distribution. Our results revealed a clear genomic divergence between the populations east of the Isthmus of Tehuantepec and the populations of the west of the Isthmus. In contrast to the genomic results, morphology and coloration analyses showed intermediate levels of differentiation, indicating that phenotypic traits were poor indicators of intraspecific divergence in this species. Finally, ecological data indicated that there are differences in ecological niche within *D. baritula*. The present study represents the first multidisciplinary study of the populations of *D. baritula* complex, and our data suggest that *D. baritula* could contain two or more incipient species lineages in the intermediate phase of the speciation continuum. These results highlight the importance of the Isthmus of Tehuantepec as a geographical barrier and Pleistocene climatic events in driving isolation and population divergence in *D. baritula*. The present investigation illustrates the speciation potential of *D. baritula* complex and the capacity of Mesoamerican highlands to create cryptic biodiversity and endemism.

Key words: coloration, ecological niche, Flowerpiercer, Isthmus of Tehuantepec, Mexico, morphology, NextRAD, Pleistocene climatic events, speciation.

INTRODUCTION

The Cinnamon-bellied Flowerpiercer, *Diglossa baritula* (Wagler, 1832), belongs to a genus that is considered one of the Neotropical evolutionary radiations (Vuilleumier, 1969). The flowerpiecers (*Diglossa*, Aves: Thraupidae) present a high variation in (i) plumage coloration patterns (Mauck & Burns, 2009; Lauck, 2020), (ii) presence and absence of sexual dimorphism, (iii) body size (Lauck, 2020), (iv) bill size and shape (Mauck & Burns, 2009), (v) diet composition (Schondube & Martínez del Río, 2003; Lauck, 2020) and (vi) altitudinal range (from coast to highlands) (Gutiérrez-Zuluaga et al., 2021). Furthermore, there are species complexes whose phylogenetic relationships remain unresolved (Gutiérrez-Zuluaga et al., 2021). This biological heterogeneity has evolved on a reduced time scale and geographic range (Barker et al, 2015; Fig. S1).

The Mesoamerican highlands, where *D. baritula* (Wagler, 1832) is distributed (Lauck, 2020, Fig. 1A), has high species richness (Myers et al., 2000). The great biodiversity of the Mesoamerican highlands is due to its discontinuous distribution, which is shaped by environmental characteristics related to altitude, temperature, humidity, etc. (Hernández-Baños et al., 1995). The fact that high-altitude taxa are distributed in isolated patches favors high degrees of phenetic and genetic divergence among populations (García-Moreno et al, 2004).

Both the *Diglossa* genus and the *D. baritula* complex have had an array of taxonomic arrangements (Hellmayr, 1935; Friedmann et al., 1950; Skutch, 1954; Monroe, 1968; Vuilleumier, 1969; Bock, 1985; Isler & Isler, 1987; Sibley & Monroe, 1990; Dickinson, 2003; Remsen *et al*., 2008; Table S1 and Table S2). Three allopatric subspecies are currently recognized based on disjunct distributions and qualitative descriptions of male

plumage (Howell & Webb, 1995; Lauck, 2020). *Diglossa baritula baritula* (Wagler, 1832) occurs in Mexico, from southern Jalisco to west of the Isthmus of Tehuantepec; it has cinnamon-rufous ventral coloration that extends from the throat to the tail coverts. *Diglossa baritula montana* (Dearborn, 1907) is distributed from east of the Isthmus of Tehuantepec to southern El Salvador; the throat is gray and ventral underparts are deeper cinnamon-rufous. *Diglossa baritula parva* (Griscom, 1932) is restricted to Honduras and northwestern Nicaragua; it is similar to *D. b. montana*, but smaller, with a shorter and slenderer bill (Fig. 1). In addition, most studies on *D. baritula* have been ecological and behavioral investigations, mainly due to the feeding behavior of the species, which extracts nectar from flowers that are generally pollinated by hummingbirds (Arizmendi et al., 1996; Schondube et al., 2003). This is the first multidisciplinary study of the populations of *D. baritula* and the results obtained allow us to have a first perspective about the natural history of this species. The aim is to obtain a phylogeny of the populations; describe the pattern of morphology, coloration and environmental variation; and relate these patterns with the phylogeny. We expected to find high levels of genetic, phenetic and environmental variation, since the *D. baritula* complex is a resident species with allopatric distribution and belongs to an adaptive radiation.

MATERIALS AND METHODS

Genomic data

We extracted total genomic DNA from 76 tissue samples comprising the three subspecies currently recognized using the Epicentre MasterPure kit (Epibio) or Qiagen DNAeasy Blood & Tissue kit (Qiagen, Valencia, CA, USA). Tissue samples were taken from MZFC

collection (Instituto Nacional de Ecologia, SEMARNAT, Mexico FAUT-0169), as well as samples donated by other institutions. We verified the DNA quality with gel electrophoresis and quantified the DNA concentration using a Qubit 3 fluorometer. We amplified two mitochondrial genes (mtDNA), NADH dehydrogenase subunit 2 (ND2) and cytochrome b (cyt b). We used six samples (3 from *D. b. baritula*, 2 from *D. b. montana* and 1 from *D. b. parva*; Table 1) for the genomic analysis. We included one sample from *Diglossa plumbea* as the sister group and one sample from *Euphonia hirundinacea* as the outgroup. Genomic DNA was submitted to SNPsaurus (http://snpsaurus.com/) to construct NextRAD genotyping-by-sequencing libraries as in Russello et al., (2015). First, genomic DNA was fragmented with Nextera reagent (Illumina, Inc.), and short adapter sequences were ligated to the ends of the fragments. Fragmented DNA was then amplified for 27 cycles at 74 °C, with one of the primers matching the adapter and extending 10 nucleotides into the genomic DNA with the selective sequence GTGTAGAGCC. Sequencing was performed on a HiSeq 4000 with a lane of 150 pb reads (University of Oregon). Raw sequence reads are available at https://figshare.com/articles/dataset/fastq_baritula_tar/21540072 and Genbank accession number PRJNA901463.

Raw reads quality was verified with FastQC v. 0.11.9 (Andrews, 2019). We assembled demultiplexed reads into sequence alignment through ipyrad v 0.9.53 (Eaton & Overcast 2020**)** with default setting with the following exceptions: reads were mapped to a *D. b. baritula* draft genome assembly (Licona-Vera, et al., in progress; Table 1), CACATCTCGG for restriction overhang, the cluster threshold was set to 0.93 (McCartney-Melstad et al., 2019) and the parameters used to filter out poor quality reads were changed according to the results from FastQC. To assess the phylogenetic relationships among

individuals we used Maximum Likelihood (ML) approach, under the GTR+Gamma evolutionary model, and nodal supports were accessed through 1,000 ultrafast bootstrap replicates using IQTree v. 1.6.12 (Nguyen et al., 2015) on the CIPRES Science Gateway v.3.3 (Miller et al., 2010). FigTree v. 1.4.4 (Rambaut, 2018) was used to display the tree and the bootstrap values of each node.

Coloration and morphological data

To quantify color variation, we measured the reflectance spectra of six plumage patches (upper back, lower back, throat, breast, upper belly and lower belly) of 85 males (Table 1) over the avian visual range (300–700 nm). Three measurements were taken per patch. We used an Ocean Optics USB2000 spectrophotometer with a PX-2 pulsed xenon light source and a bifurcated fiber optic probe (Ocean Optics, Dunedin, Florida, USA). The probe was fitted with a rubber tip to maintain the probe fixed perpendicularly to the feather surface and to block out ambient light. The spectrophotometer was calibrated with a white standard (WS-2, Ocean Optics). Prior to analysis the three measurements were averaged and smoothed (smoothing parameter of 0.25) to remove electrical noise. To visually assess variation, we constructed reflectance curves comparing each plumage patch of the subspecies. Lastly, to determine significant differences we calculate chromatic just noticeable distances (JND; Vorobyev & Osorio, 1998) for each patch assuming a Weber fraction of 0.1 for the long-wavelength sensitive cone. The visual system we used was the spectral sensitivity data from the Blue Tit *Cyanistes caeruleus* (based on Hart et al., 2000), since it is the phylogenetically closest species available in the package. A value of 1 JND is considered the threshold that represents the distance in the perceptual color space at which

two colors would be visually discernible, thus if the distances (including their 95% confidence intervals) have a value > 1 JND, color differences are discernible. All spectral processing was done using package pavo 2.1.0 (Maia et al., 2019) in R v. 4.0.2 (R Core Team 2020). Since the ventral coloration of females is patchy (without homogeneous coloration) and therefore measurements repeatability is difficult, we focused our color study on male coloration.

To examine the morphological variation in *D. baritula*, we obtained five standard body measurements from 163 adult specimens (93 males and 70 females) from 75 localities housed in nine skin collections (Table 1). Measurements were taken with a digital caliper to the nearest 0.01 mm by the first author. The measurements were: bill length (BL, from anterior edge of skull to the tip of the culmen), bill width (BW, culmen width at the nostril), tarsus length (TL) and wing cord (WC, from the carpal joint to the tip of the longest primary) following Baldwin et al., (1931), and bill hook length (BHL; HL in Mauck & Burns (2009)). Wing and tarsus measurements were always taken from the specimens' right side. We included only adult individuals of known sex. All individuals were measured three times and those values were averaged. We first evaluated the normality and the homogeneity of variances with Shapiro-Wilk test and F tests, respectively. Then, we determined if variables varied between males and females with t-tests. Since we only found significant differences between sexes in WC, we analyzed males and females separately for that variable but considered the sexes together for the rest of the morphological traits. We evaluated whether the variables were correlated to each other with Pearson tests. Differences among subspecies in the morphological variables were examined through one-way ANOVAs followed by Tukey´s post hoc comparisons. Descriptive statistics included

means and standard deviations. We performed a Principal Components Analysis (PCA) and obtained PC scores for each individual. We also plotted individuals' scores on PC1 versus PC2 to visualize placement of the subspecies in morphospace. All tests were performed in R v. 4.0.2 (R Core Team 2020) and the significance level was set at 0.05.

Ecological Niche Modeling

We used ecological niche models (ENM) to estimate the current potential geographic distribution of *D. baritula* subspecies and to simulate past refugia. We obtained records of occurrence from the collection locations of the specimens used for our genomic, coloration and morphology sampling, as well as from the Global Biodiversity Information Facility (www.gbif.org/). To avoid spatial autocorrelation, we filtered the occurrence data with the R package Nichetoolbox (Osorio-Olvera et al., 2016). This resulted in a total of 173 records for *D. b. baritula*, 72 for *D. b. montana* and 43 for *D. b. parva*.

We calculated niche overlap between subspecies to compare their niche similarity and equivalence. We used a Principal Component Analysis of the environmental space (PCA-env), an approach proposed by Broennimann et al. (2012), to transform the environmental space of the selected environmental variables into a two-dimensional climatic space defined by the first two principal components, using the ecospat R package (Di Cola et al., 2017). Then, we estimated niche similarity using two standard indices: Schoener's D (1968) and Hellinger's-based I (Warren et al., 2008), which range from 0 (no overlap) to 1 (complete overlap). Finally, we determined niche equivalence by comparing the observed D and I values to a null distribution of 100 randomly simulated values. ENMs

generated with occurrence data were compared with pseudo-replicate models generated with randomly redistributed occurrence data. In this procedure, the smaller the empirically observed D and I values compared to those generated by the pseudo-replicates, the more significant the niche difference, thus the null hypothesis of niche equivalence is rejected.

RESULTS

Genomics

After quality filtering, our data set contained 1,278,209 to 5,344,377 reads for eight samples with an average of 3,897,579 reads per individual. Using the reference assembly approach on ipyrad produced 43,704 consensus loci, 178,160 SNPs and 5,827,151 pb. The missing sites were 18.15% for the SNPs matrix and 22.73% for the sequence matrix. The results for mtDNA are analyzed in Terrones-Ramírez et al., (in progress).

The ML tree recovered the *D. baritula* complex as a well-supported monophyletic lineage (100). The deepest split was between two strongly supported clades: The West-IT Group (100) included all individuals of *D. b. baritula* which are distributed west of the Isthmus of Tehuantepec, and the East-IT Group (100), which included the *D. b. montana* and *D. b. parva* individuals, which are both distributed east of the Isthmus of Tehuantepec, (See Fig. 2). Within the West-IT Group there was a strongly supported subclade (91), while the subclade within the East-IT Group had middling support (62). Individuals from the subspecies with “cinnamon throat males” clustered together, and samples from the subspecies with “gray-throated males” group together as well. In general, the topology revealed phylogeographic structure within the *D. baritula* complex.

Coloration and morphological analyses

In our JNDs analyses we found statistically significant differences in plumage color among the three named subspecies in both sexes for most feather patches we measured. These differences are likely to be biologically significant, since the magnitude of the difference is greater than the 1 JDN threshold for the colors to be perceived as different under the noise-constrained receptor visual model (Vorobyev & Osorio 1998).

Differences in color on the slate-colored back feathers are borderline nonsignificant, with the bulk of the variation being observed on the reddish ventral patches (Fig. 3). This is particularly noticeable in the *baritula* subspecies, where the reddish ventral coloration extends up to the throat and shows a distinct slope in the corresponding spectral shape. The throat patch was significantly different between the eastern group and the western group.

We found no significant morphological sexual dimorphism in this complex in four of the five variables measured. Only wing cord (WC) showed statistically significant differences between sexes (Table 2). For the measurements of bill length (BL), bill width (BW), bill hook length (BHL) and tarsus length (TL) we found significant variation among subspecies. However, the only measurement that showed a significant difference between the East-IT and West-IT Groups was TL (Fig. 4A, Table 2).

The PCA analyses indicated that the first two Principal Components explained 54.1% of the observed variation. Bill hook length and bill length had the highest correlation with principal component 1 (PC1), and tarsus length was correlated with principal component 2 (PC2, Table 2). The PCA scores showed that the three subspecies had a high

degree of overlap. The analyses did not show differences between the East-IT Group and the West-IT Group (Fig. 4B).

Ecological niche modeling

The PCA of environmental data indicated that the first two niche axes explained 80.75% of the environmental variation. The first axis explained 43.62% of the variation and was associated with Min Temperature of coldest month (BIO 6) and Mean Temperature of coldest quarter (BIO 11). The second axis explained 37.13% of the variation and was associated with Isothermality (BIO 3). The occurrence density surfaces showed broad ecological niches in all three subspecies, and the current occupied niche space varied among the three subspecies (Fig. 5A).

Observed values of Schoener's D and Hellinger's-based I were lower than expected from a random distribution of values (D = 0.4760 and I = 0.6873 for *D. baritula*/*D. montana*, D = 0.2224 and I = 0.3698 for *D. baritula*/*D. parva*, and D = 0.5219 and I = 0.7219 for *D. montana*/*D. parva*), and D values fell outside the density of 95% of the random distribution (Fig. 5B). Therefore, the null hypothesis of niche equivalency could be rejected, showing that the three subspecies occupy distinct environmental niches. In addition, niche similarity tests among subspecies showed that they were less similar than random (Fig. 5C). This indicates that they had similar, but not identical ecological niches, thus allowing us to reject the null hypothesis of niche conservatism. This result is evidence of climatic niche conservatism in this complex.

DISCUSSION

Our phylogenetic tree shows a well-supported deep divergence between populations from either side of the Isthmus of Tehuantepec. The West-IT Group is a monophyletic clade that comprises all individuals of *Diglossa baritula baritula*, while the East-IT Group is another monophyletic lineage that contains *Diglossa baritula montana* and *Diglossa baritula parva*. Individuals from the subspecies with "cinnamon-throated males" forms one group, and samples from the subspecies with "gray-throated males" clusters into another. Overall, this result suggests that the two clades are independent lineages with substantial speciation potential, since evolutionary forces can act rapidly on each isolated gene pool. Meanwhile, the morphometric data show weak phenotypic differentiation between the two lineages (West-IT and East-IT), likely because they are lineages that have recently undergone diversification that have had insufficient time to have a clear morphometric pattern. We found significant color differences among the three groups for most feather patches that we measured; the largest difference is in the throat patch. Finally, ecological data indicate that there are differences in ecological niche within *D. baritula*.

Well-supported divergence in *D. baritula* complex

Our phylogenomic tree, based on 176,609 SNPs, shows a split with 100% bootstrap support between the Isthmus of Tehuantepec western populations and the eastern ones. The short branches and limited phenotypic divergence (see following sections) suggest that this split was very recent (Fig. 2; Barker et al., 2015). The *D. baritula* complex originated during the Pleistocene, less than one million years ago (Barker et al., 2015, Fig. S1). Since *D. baritula*

is a resident species restricted to the Mesoamerican highlands, the Pleistocene climatic oscillations were likely a relevant factor in its current allopatric geographical distribution, and therefore, in its evolutionary history. Its movements to suitable habitats that fulfill its biogeographic affinities and ecological requirements determined the response of *D. baritula* to the glacial and interglacial periods.

The Pleistocene was a geological epoch with severe global alternations between glacial and interglacial climates (Hewitt, 2004). During warmer interglacial periods, the highlands forests were reduced as they moved up to higher elevation. Consequently, the potential distribution of *D. baritula* was reduced and populations were isolated, facilitating genetic differentiation. Conversely, in cooler glacial periods the highlands descended to lower altitudes, which promoted their connectivity. This enabled geographic contact and gene flow between *D. baritula* populations. The occurrence of two allopatric lineages in *D. baritula* provides evidence for its long-term persistence within separate refugia during Pleistocene glaciations. Such temporal and spatial population dynamics have impacted the patterns of genetic and phenotypic variation in our study species.

The genus *Diglossa*, to which *D. baritula* belongs, represents one of many evolutionary radiations that have accumulated species during the last hundreds and millions of years (Fig. S1, Mauck & Burns, 2009; Barker et al., 2015). This genus has a neotropical distribution, with most species inhabiting South America. The early-diverging branches are distributed in the Northern Andes, suggesting that the *Diglossa* lineage originated in the Andean biogeographic origin. There were two major dispersal events, one of them from the Northern Andes to the tepuis of Venezuela, and the other from the Northern Andes to Central America (Hackett, 1995; Mauck & Burns, 2009). This second dispersal event can

explain the presence of the only two Mesoamerican species—*D. baritula* and its sister species, *D. plumbea*. Thus, *Diglossa* species and their populations have a wide dispersal capacity as well as potential for speciation.

The Isthmus of Tehuantepec as a biogeographical barrier

The Isthmus of Tehuantepec (IT) is the current topographic and ecological barrier between the West-IT Group and East-IT Group. The marked genetic divergence between populations (and subspecies) separated by the IT suggests that gene flow across this barrier has been reduced or interrupted owing to the low-elevation area of the IT. This geographic isolation is the major driver of intraspecific genetic divergence in *D. baritula*.

The IT is a lowland valley that appeared during the late Miocene (Barrier et al., 1998). This timing confirms again that the genetic divergence found here is a consequence of Pleistocene climatic oscillations, and not due to the emergence of the IT. The IT has played the role of an important geographic barrier in southern Mexico shaping the Mesoamerican highlands biodiversity, both, in birds (Barber & Klicka, 2010; Zamudio-Beltrán et al., 2020) and other taxa such as reptiles (Bryson et al., 2011), mammals (León-Paniagua et al., 2007) and plants (Hernández-Langford et al., 2020).

Partial congruence between phylogeny and phenotype (coloration and morphology)

Despite the phylogenetic inference of two well-supported clades in the *D. baritula* complex, we found weak phenotypic variation between them (Fig. 3 and Fig. 4). The two

lineages observed here have diverged recently with insufficient time for clear a phenotypic divergence pattern to emerge. Some possible explanations are that the West-IT Group and East-IT Group have not yet fully diverged and continue to share ancestral alleles (i.e., incomplete lineage sorting). Alternatively, in the past, *D. baritula* could have encompassed two incipient species lineages in the intermediate phase of the speciation continuum that subsequently reentered into contact and admixture due to geographic population expansions during glacial periods. In either case, it can result in shared polymorphism across lineages, which may explain the partial phenotypic split within *D. baritula*.

The coloration of the Cinnamon-bellied Flowerpiercer is consistent with the findings of Shultz & Burns (2017), who describe differences in the evolution of female and male plumages corresponding to differences in their selective pressures. It is likely that the males' ventral cinnamon plumage is a sexually selected trait, and thus the observed differences between subspecies might be due to the effect of diversifying selection. In birds, the ventral part is often involved in mate choice (Shultz & Burns, 2017, Merwin et al., 2020). This suggests that different sexes and body parts are subjected to different intensities of sexual selection.

We found significant color differences among the three groups for most of the feather patches we measured, many of those clearing the 1 JND threshold for being likely biologically significant. The throat patch was the most strongly differentiated (i.e., had the highest JND values). The most obviously diagnosable color trait was the difference in throat color between the West-IT and East-IT Group, i.e. the two genomic lineages. In *D. b. baritula* the cinnamon ventral coloration extends up to the throat while in *D. b. montana* and *D. b. parva* the throat is gray. The sister group of the *D. baritul*a complex, *Diglossa*

plumbea is gray-throated, which could be the ancestral state for the group, with the cinnamon throat of *D. baritula* being a derived state. An ancestral state reconstruction including other related taxa would be of interest to clarify the evolution of color in these tanagers.

The throat patch coloration is more divergent between the subspecies isolated by IT (cinnamon in *baritula* vs. gray in *montana* and *parva*) than between the *montana* and *parva* subspecies (both gray). Melanin is the most abundant pigment in bird feathers (Roulin et al. 2011) and consists of two types: eumelanin, which gives rise to black and grey colorations, and pheomelanin, which gives rise to yellowish to reddish colorations (McGraw, 2006; Galván & Solano, 2016). Both pigments are present in most melanic feathers (McGraw, 2004), but the dominant pigment type can be reliably assessed based on the color's appearance and spectral curve (Galván & Wakamatsu, 2016). The West-IT Group throat color is predominantly due to pheomelanin pigmentation, while the East-IT Group the color is mainly eumelanin derived. Different melanin ratios are implicated in pleiotropic associations providing fitness benefits under different selective conditions (Roulin et al. 2011; Roulin, 2015). For example, there is a positive correlation between eumelanin coloration and plasma testosterone levels (Arai et al., 2018), while pheomelanin pigmentation is related to resistance to oxidative stress (Roulin et al., 2011) and antioxidant level (Arai et al., 2017). Melanogenesis produces a mix of pheomelanin and eumelanin (Roulin & Ducrest, 2013), since pheomelanogenesis and eumelanogenesis share the early stage of their production pathway and both are derived from a common precursor (Ducrest et al., 2008). This biomolecular pathway is considerably conserved in the vertebrates (Galván & Solano 2016). Inter and intraspecific variation in melanin-based coloration is

due to polymorphisms at the Mc1R gene (Roulin & Ducrest, 2013). Thus, a study with this gene would help us to solve the evolutionary history of *D. barirula*.

In species-delimiting assessments, priority should be given to traits that are relevant as sexual signals, such as plumage (Sibley & Monroe, 1990; Price 2008). Melanic plumage patterns are common sexually selected characters (Ducrest et al. 2008, Roulin 2015). However, although color differentiation is usually considered an important character in alpha taxonomy studies, in some cases it does not contribute sufficient evidence as a species-delimiting criterion (Ramírez-Barrera et. al., 2019).

Some patterns related to melanin-based pigments and the environment have been found. A complex version of Gloger's rule predicts that pheomelanin is deposited more in warm/dry climates and eumelanin tends to be found in warm/humid areas (Delhey 2017, 2019; Marcondes et al. 2020). In many species with 'drab' melanin-based coloration, the light environment (e.g., the light that filters through vegetation cover) is an important factor in determining plumage brightness, especially in dorsal patches (Marcondes & Brumfield, 2019). The small amount of variation found in the dorsal plumage of both males and females suggests that color in that body part might be constrained by their light environments. Moreover, their low-reflectance dorsal parts may decrease detection and aggression from conspecifics, competitors, and/or predators (Lyon & Montgomerie, 1986). Together, these results show support for the idea that plumage evolution occurs in a patchwork fashion, with different parts of the plumage being subject to different selective pressures, as well as to the hypothesis that ventral-dorsal variation is an important driver of sexual dichromatism (Marcondes & Brumfield, 2019).

In the morphological analyses, the first principal component (PC1) was most strongly related to bill length and beak hook length (BL and BHL), and moderately related to bill width (BW). Thus, we can consider PC1 to be a proxy for bill size. The bill is a highly variable character in our study species as well as in the genus *Diglossa* (Mauck & Burns, 2009; Fig. S1) The second principal component (PC2) was positively related to wing cord and tarsus length (WC and TL), which we considered to be a proxy of overall body size. In the scatterplot, we find a large degree of overlap between the *baritula* and *montana* groups, and weak differentiation between those two and *parva*. As in the individual measurement comparisons, this supports the trend of *parva* being smaller than the other two subspecies. These differences might indicate differences in thermal or feeding ecology. However, as the observed variation appears to be phylogenetically structured, it could be the result of isolation by distance, as has been observed in other highland bird taxa (Seeholzer & Brumfield, 2018).

Our morphological results revealed that the only sexually dimorphic morphological trait is wing cord (WC), with males having longer WC than females. The wing is related to locomotion and flight performance in the environment (Tobias, 2022). The longer wings in males decrease the flight energy required in courtship display (Møller, 1991; Sun et al., 2017). However, it may not only by the force of sexual selection solely but might also have an ecological cause. For example, if females are more vulnerable to predation when they are incubating eggs, shorter wings may improve their maneuverability and help them evade predators (Swaddle & Lockwood 2003; Bomberger & Brown 2011). If wing length is used as a good indicator of body size (Ashton, 2002), we provide the first evidence for sexual

dimorphism in body size in *D. baritula*. This pattern of differences between sexes in wing cord have been reported in other birds (Robles-Bello, 2022; Thom, 2018; Sun et al., 2017).

Within the East-IT Group, we found geographical differentiation for bill length (BL) in the form of clinal variation from large BL in *D. b. montana* to small BL in *D. b. parva*. Since geographic variation refers to intraspecific phenotypic differentiation (Mayr, 1969) and the bill is related to foraging (Tobias, 2022), populations of *D. b. montana* and *D. b. parva* may be adapted to their specific local diets. Another example of geographic variation within the East-IT Group could be total length. Although we did not measure total length, photographs of skins show that *D. b. parva* has a smaller total length than *D. b. montana* (Fig. 1), this variation could be evidence of local thermal adaptation (Friedman & Remeš, 2017).

The tarsus length (TL) has a congruence with the phylogenetic inference; the East-IT Group has a shorter TL than the West-IT Group. TL is generally used as a proxy of body size (Zink & Remsen, 1986; Yom-Tov, 2001, Töpfer, 2018). Since the intraspecific variation in body size represent a thermoregulatory adaption to local environmental niches (Mayr, 1956; Pigot et al., 2020) and the *D. baritula* complex is sedentary, its populations must adapt to their habitats and are likely to follow patterns such as Bergmann´s (1847) rule, which postulates the tendency of animals to have larger individuals in higher latitudes (Graves, 1991), or in cooler areas, given that climate is correlated with latitude (Ashton, 2002). Therefore, we propose that vicariance plays an important role in the *D. baritula* complex. The trend of smaller individuals to the east than to the west of the IT has been found in other birds (e. g. Rodríguez-Gómez, 2021).

The interpretation of morphological variability as the main or only species-delimiting criterion provides a challenge to taxonomists because cryptic diversity at the species level is not diagnosable by morphological characters (Töpfer, 2018). However, studies of morphology are a crucial element in the understanding of bird evolution and ecology (James, 2017). For example, macroecological patterns have been reported, such as tarsus length increasing in less vegetated biomes (i.e. steppes, grasslands and deserts), reflecting adaptation to a more terrestrial lifestyle, and bill length increases in well-vegetated regions such as tropical rainforests (Tobias et. al., 2022). Our study illustrates the importance of incorporating a genetic approach in alpha taxonomy.

Differences in ecological niche within the *D. baritula* complex

Ecological niche differentiation, one of the drivers of lineage divergence, was confirmed by the PCA-env and niche overlap tests, which suggest that the three subspecies occupy different environmental spaces (Fig. 5). Equivalency tests were performed to compare the observed and expected niche spaces, revealing that the three subspecies share a very small niche overlap. By conducting similarity tests, we found that pairs of subspecies' realized niches were more different than expected by chance, since they occupy areas with different environmental conditions. Differentiation among the niches may indicate that each subspecies has different ecological tolerances and their own distribution range. Thus, climatic variables have played an important role in promote adaptive divergence of *D. baritula* complex.

Our results revealed that *D. b. baritula* and *D. b. parva* have the widest niche divergence. This may be because these two subspecies inhabit different climatic conditions. The habitats of *D. b. baritula* have less rainfall, for example pine-oak forests, compared to *D. b. parva*'s habit in montane regions with greater seasonality in both temperature and precipitation, for example in cloud forests (Ruiz-Sánchez & Ornelas, 2014; Ortiz-Rodriguez et al. 2018). We also found distinct niches between *D. b. montana* and *D. b. parva* because *D. b. montana* inhabits both montane cloud forests and subalpine cloud forests, while the main ecosystem of *D. b. parva* is montane cloud forest (Helmer et al., 2019). The geographical (and ecological) barriers between these two last subspecies are the Motagua Fault in Guatemala and the central plateau in El Salvador. In general, the *D. baritula* complex has the capacity for rapid dispersal and to occupy a variety of habitats.

CONCLUSION

Within the *D. baritula* complex, current taxonomic limits (i.e., subspecies) do not correspond to the genomic and phenotypic splits presented herein. We detected two evolutionarily independent genomic units: one includes only *D. b. baritula,* while the other contains both the *D. b. montana* and *D. b. parva* subspecies. In contrast to the genomic results, phenotypic data showed intermediate levels of differentiation, indicating that in this study phenotypic traits are poor indicators of intraspecific divergence. On the other hand, ecological data revealed that each subspecies has a different ecological niche, suggesting environmental factors have influenced the evolution within this complex.

This is the first study at the genomic, phenetic and ecological levels within *D. baritula* to include samples of all three subspecies. Our data demonstrated that there is intraspecific genetic divergence, which might suggest that *D. baritula* is made up of two different species. Alternatively, the cinnamon-bellied flowerpiercer is in the process of incipient speciation, a "gray zone" of speciation (De Queiroz, 2008) where genetic variation occurs before notable phenotypic variation has developed and both lineages fall on the speciation continuum. Making inferences about species limits is difficult because our genomic sampling had low representation, and phenotypes have not completely diverged. More extensive genomic sampling is necessary to fully resolve the taxonomic status of two lineages.

In particular, the present investigation illustrates the evolutionary potential of the *D. baritula* complex, and in general, highlights the capacity of the Mesoamerican highlands create cryptic biodiversity and endemism. Climatic fluctuations during the Pleistocene facilitated the diversification and distribution in our study group. Hence, Mesoamerican highlands might act as future refugia for species faced with the present climate change, and we emphasize the necessity of their conservation. Moreover, recognition of the different genetic stocks is a useful basis to recognize and treat differentiated lineages as separate units for conservation management.

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Figures and tables

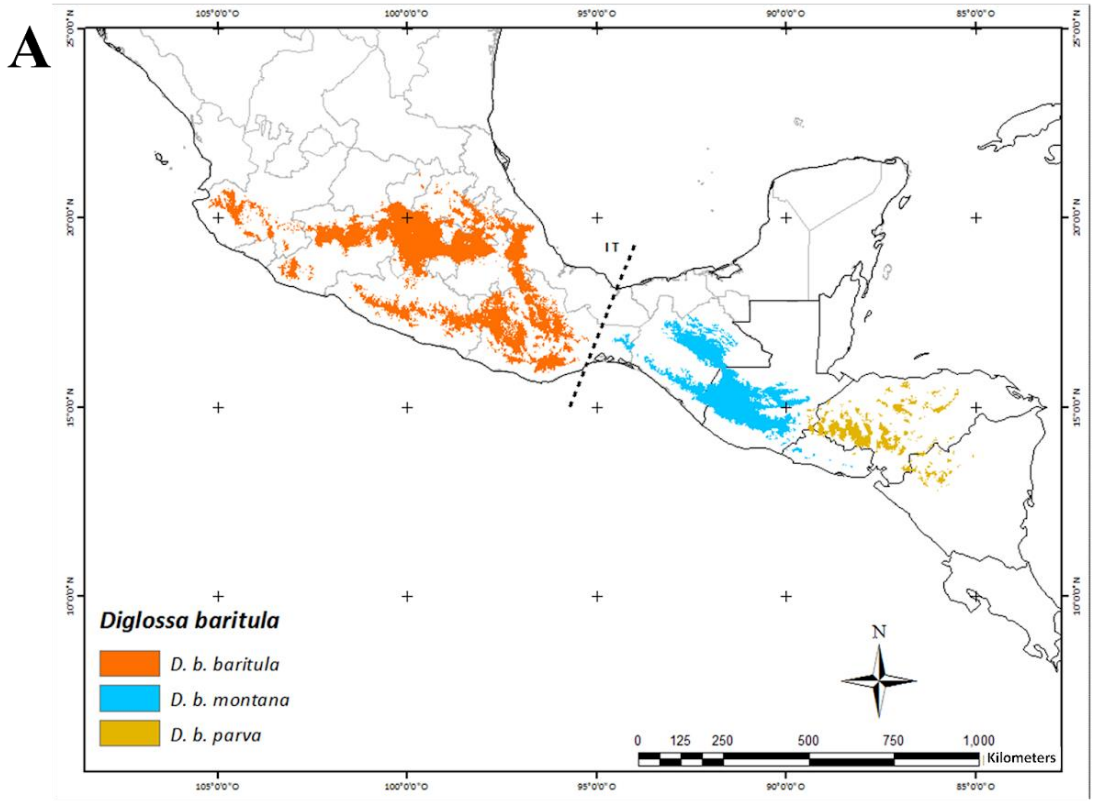


Figure 1. Distribution of *Diglossa baritula*. (A) Geographic distribution of *D. baritula* showing the allopatric subspecies represented by different colors, in orange *D. b. baritula,* in blue *D. b. montana* and in yellow *D. b. parva*. (B) and (C) Male specimens of each subspecies in lateral and ventral position, respectively. Photographs by Sahid M. Robles-Bello.

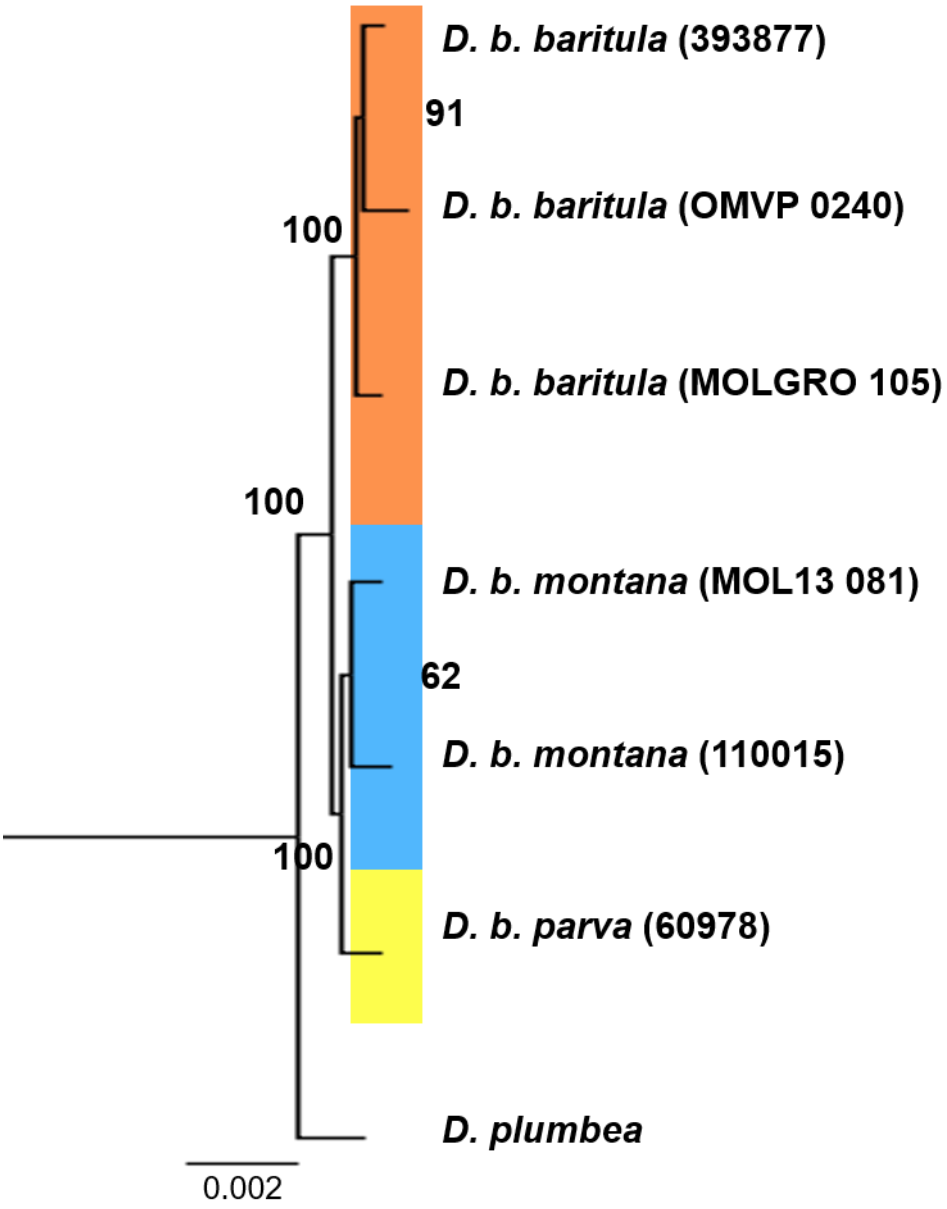


Figure 2. Maximum likelihood phylogenomic tree obtained from 44,739 RADseq loci representing phylogenetic relationships among clades in the *Diglossa baritula* complex, in orange *D. b. baritula*, in blue *D. b. montana* and in yellow *D. b. parva*. Numbers indicate bootstrap node supports. *Diglossa plumbea* and *Euphonia hirundinacea* are used as sister group and outgroup, respectively.

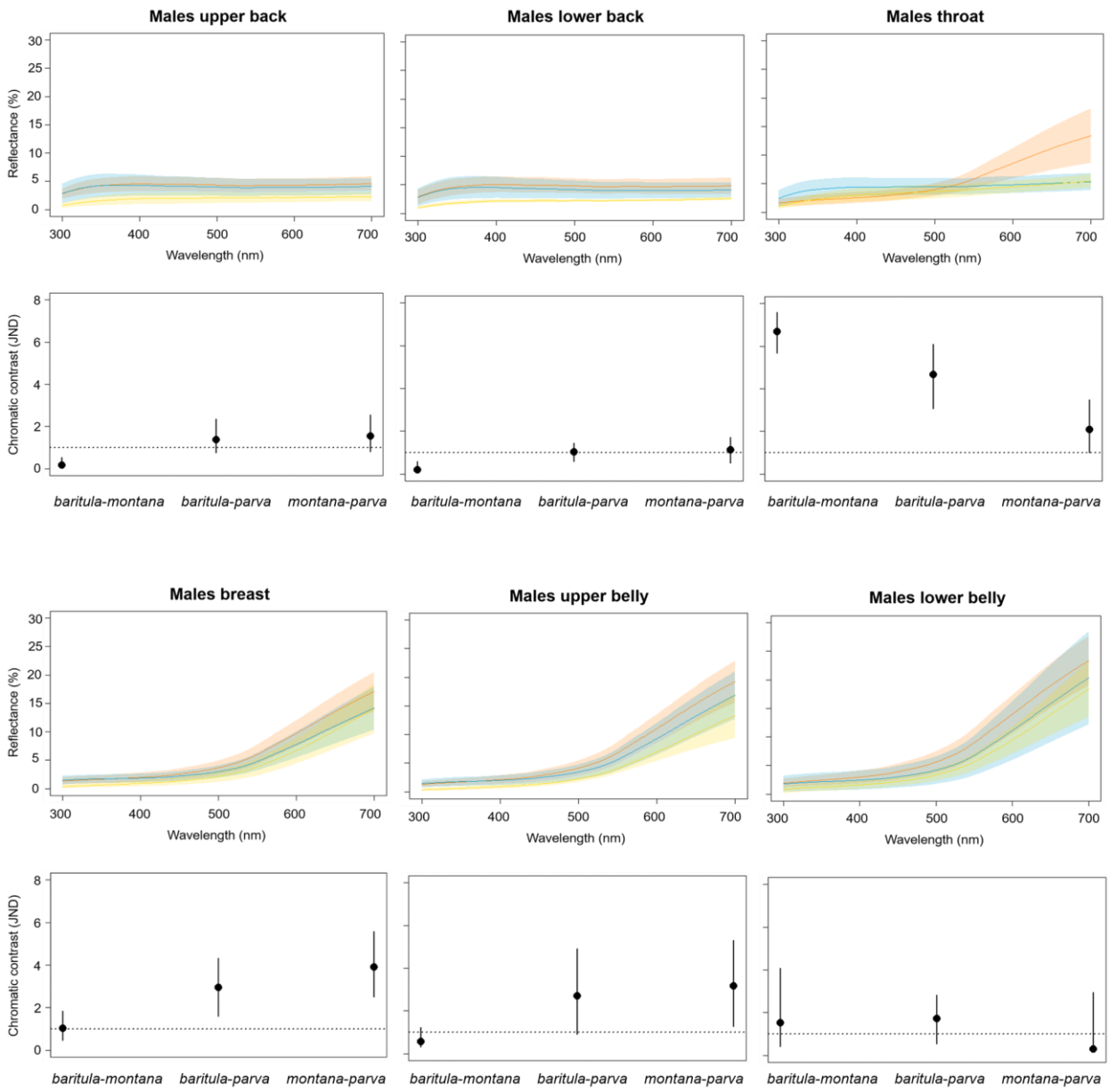
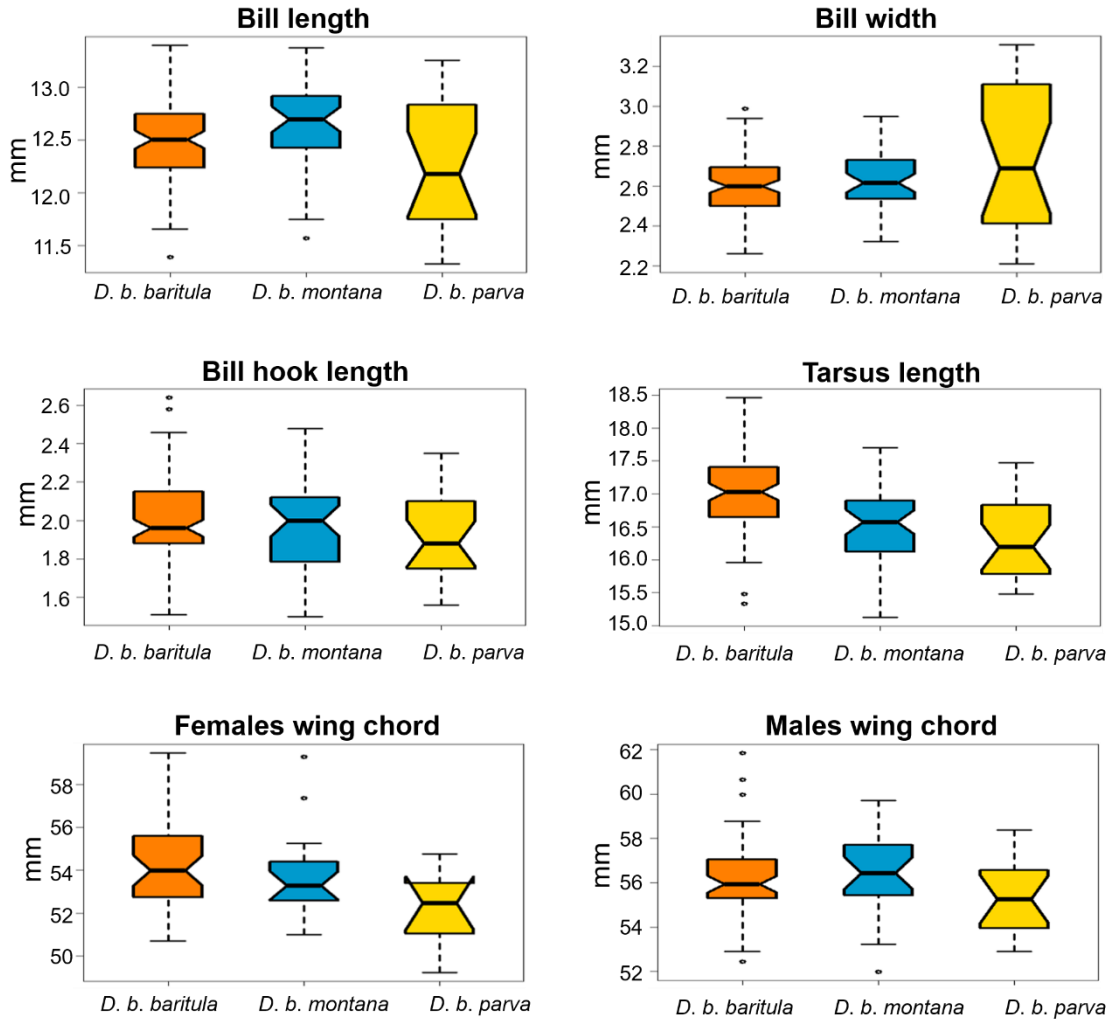


Figure 3. Color differences of *Diglossa baritula* males. Above are shown the mean spectral reflectance curves for six plumage patches of adult males. The shaded area around each line indicates standard error of the mean, in orange *D. b. baritula*, in blue *D. b. montana* and in yellow *D. b. parva*. Below are shown the color distances in units of chromatic contrast (Just Noticeable Differences, JND). Points and bars represent the mean values and 95% confidence intervals, respectively. The horizontal dashed line indicates the discriminability threshold

(JND of 1), colors with JND greater than 1 are considered perceptually distinguishable among subspecies.

A


Bill length
mm
13.0
12.5
12.0
11.5
D. b. baritula
D. b. montana
D. b. parva
Bill width
mm
3.2
3.0
2.8
2.6
2.4
2.2
D. b. baritula
D. b. montana
D. b. parva
Bill hook length
mm
2.6
2.4
2.2
2.0
1.8
1.6
D. b. baritula
D. b. montana
D. b. parva
Tarsus length
mm
18.5
18.0
17.5
17.0
16.5
16.0
15.5
15.0
D. b. baritula
D. b. montana
D. b. parva
Females wing chord
mm
58
56
54
52
50
D. b. baritula
D. b. montana
D. b. parva
Males wing chord
mm
62
60
58
56
54
52
D. b. baritula
D. b. montana
D. b. parva

B

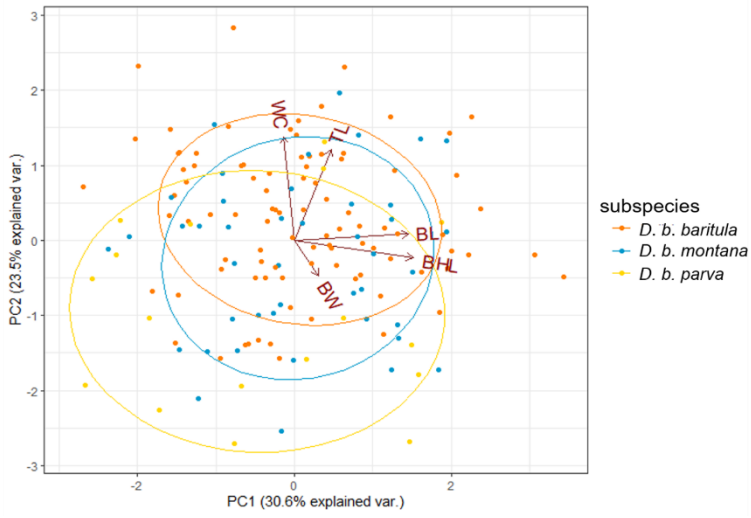

PC2 (23.5% explained var.)
PC1 (30.6% explained var.)
WC
TL
BL
BHL
BW
subspecies
D. b. baritula
D. b. montana
D. b. parva

Figure 4. Morphological variation for *D. baritula* based on five measured morphological variables. (A) Boxplots of morphological variables. Wing cord was analyzed separately for each sex due to our results indicate differences between sexes (see results and Table 2). Boxes span the first and third quartile of data with the median as a horizontal line, and whiskers (vertical dashed lines) represent range of data excluding outliers. (B) Principal Component biplot of morphological variables for the first two axes. Ellipses are graphical tools that represent the 95% confidence intervals of the principal component scores. Arrowed lines show direction and magnitude of each variable. For summary statistics, see Table 2. In orange *D. b. baritula*, in blue *D. b. montana* and in yellow *D. b. parva*. BL=bill length, BW=bill width, BHL=beak hook length, TL=tarsus length and WC=wing cord.

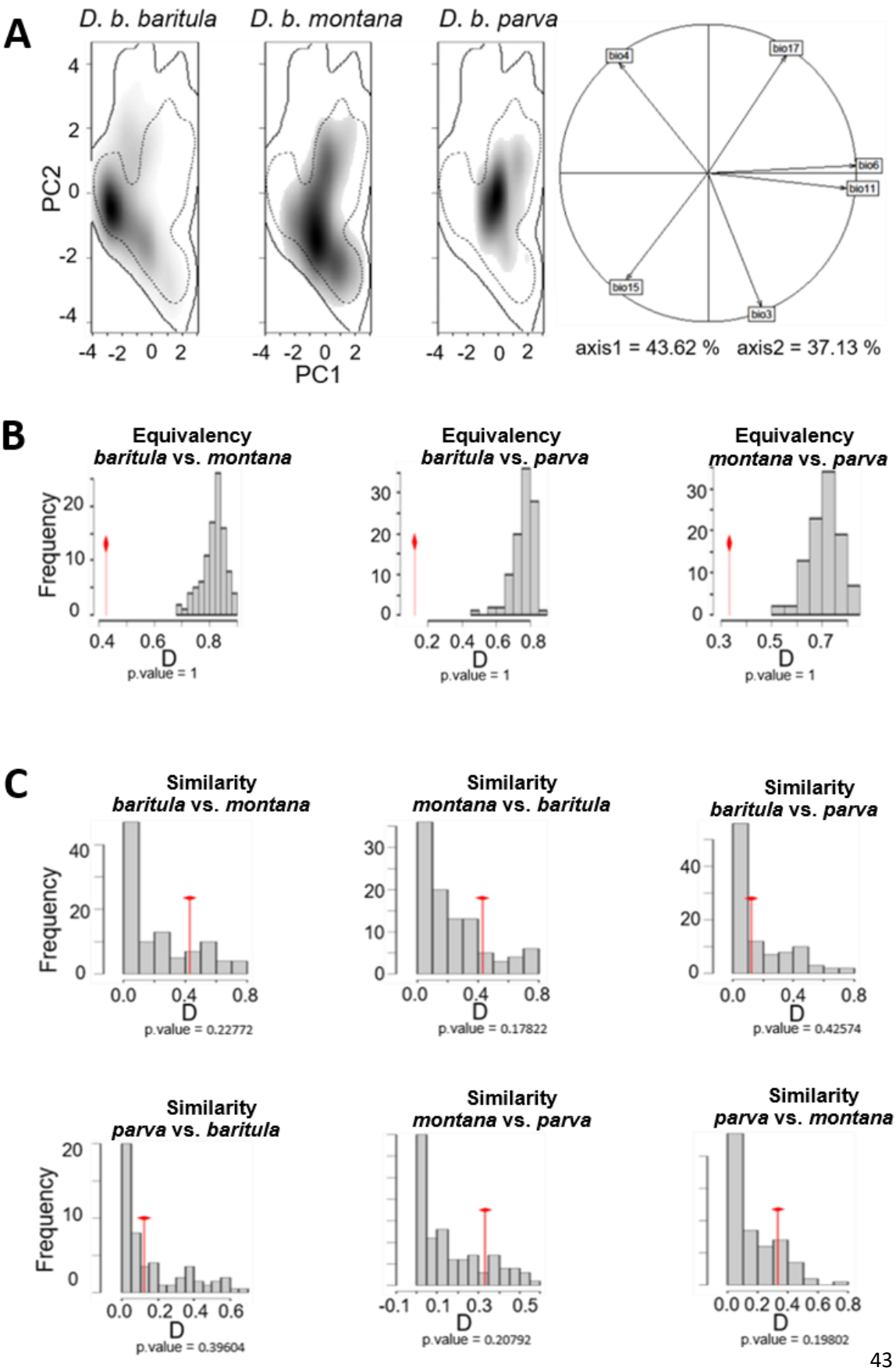

A
D. b. baritula
D. b. montana
D. b. parva
PC2
PC1
bio4
bio17
bio6
bio11
bio15
bio3
axis1 = 43.62 %
axis2 = 37.13 %
B
Equivalency
baritula vs. montana
Equivalency
baritula vs. parva
Equivalency
montana vs. parva
Frequency
D
p.value = 1
C
Similarity
baritula vs. montana
Similarity
montana vs. baritula
Similarity
baritula vs. parva
Similarity
parva vs. baritula
Similarity
montana vs. parva
Similarity
parva vs. montana
p.value = 0.22772
p.value = 0.17822
p.value = 0.42574
p.value = 0.39604
p.value = 0.20792
p.value = 0.19802

Figure 5. Results of ecological niche overlap analyses for the *Diglossa baritula* complex. (A) Principal Component Analysis biplots of the environmental space (PCA-env). Grey shadings show the density of individual occurrences by cell, with darker cells showing the highest density. The solid and dashed contour lines illustrate the 100% and 50% of the available environment, respectively. The correlation circle on the right indicates the magnitude (arrow length) and direction of the correlation of each bioclimatic variable on the niche space defined by the first two principal components. (B) and (C) Histograms showing results of the equivalency tests and the similarity tests, respectively. For both tests, we only presented values for the Schoener's D metrics, gray columns represent null distributions of D values. Red diamonds and lines represent observed values of D.

Table 1 Information for the *Diglossa baritula* specimens included in this study.

Subspecies	Collection	ID sample	Gender	Latitude	Longitude	Locality	Genomic	Morphology	Coloration
D. b. baritula	CNAV	11045	F	19.8	-104.32333	Mexico, Jalisco, Reserva de la Biosfera de Miahuatlan		X	
D. b. baritula	CNAV	11047	F	18.15	-99.9	Mexico, Guerrero, Tepoxtepec		X	
D. b. baritula	CNAV	11048	F	17.41666	-100.11666	Mexico, Guerrero, Atoyac de Álvarez		X	
D. b. baritula	CNAV	11050	F	17.01666	-97.75	Mexico, Oaxaca, Santiago Nuyoo		X	
D. b. baritula	CNAV	11054	M	19.26166	-99.99166	Mexico, Mexico state, Amanalco		X	X
D. b. baritula	CNAV	11055	F	19.706934	-99.786552	Mexico, Mexico state, Jocotitlán		X	
D. b. baritula	CNAV	11057	F	19.11666	-100.01666	Mexico, Mexico state, Temascaltepec		X	
D. b. baritula	CNAV	11058	M	19.11666	-100.01666	Mexico, Mexico state, Temascaltepec		X	X
D. b. baritula	CNAV	11059	F	19.29527	-99.24	Mexico, Mexico city, La Magdalena Contreras		X	
D. b. baritula	CNAV	11060	M	19.29527	-99.24	Mexico, Mexico city, La Magdalena Contreras		X	X
D. b. baritula	CNAV	11061	M	19.29527	-99.24	Mexico, Mexico city, La Magdalena Contreras		X	X
D. b. baritula	CNAV	11062	M	19.6	-98.08333	Mexico, Tlaxcala, Tlaxco		X	X
D. b. baritula	CNAV	11063	M	19.6	-98.08333	Mexico, Tlaxcala, Tlaxco		X	X
D. b. baritula	CNAV	11064	M	19.6	-98.08333	Mexico, Tlaxcala, Tlaxco		X	X
D. b. baritula	CNAV	11065	M	18.89166	-98.72833	Mexico, Morelos, Tetela		X	X
D. b. baritula	CNAV	11066	M	19.01666	-99.09333	Mexico, Morelos, Tetela		X	X
D. b. baritula	CNAV	14180	F	19.16833	-99.90333	Mexico, Morelos, Tetela		X	
D. b. baritula	CNAV	14687	M	16.21666	-97.11666	Mexico, Oaxaca, La Cima		X	X
D. b. baritula	CNAV	14688	M	18.15	-99.9	Mexico, Guerrero, Tepoxtepec		X	X
D. b. baritula	CNAV	14689	M	17.16666	-96.61666	Mexico, Oaxaca, Cerro San Felipe		X	X
D. b. baritula	CNAV	14690	M	17.16333	-96.675	Mexico, Oaxaca, Cerro San Felipe		X	X
D. b. baritula	CNAV	14719	F	17.16666	-96.61666	Mexico, Oaxaca, Cerro San Felipe		X	
D. b. parva	MLZ	16844	F	14.4	-89.23333	Honduras, Ocotepeque, El Chorro		X	
D. b. baritula	CNAV	17555	M	19.03333	-99.205	Mexico, Morelos, Coajomulco		X	X
D. b. parva	MLZ	18353	F	14.516667	-88.75	Honduras, Ocotepeque, Cerro Verde		X	

D. b. parva	MLZ	18355	F	14.516667	-88.75	Honduras, Ocotepeque, Cerro Verde		X	
D. b. parva	MLZ	18356	F	14.516667	-88.75	Honduras, Ocotepeque, Cerro Verde		X	
D. b. parva	MLZ	18361	M	14.516667	-88.75	Honduras, Ocotepeque, Cerro Verde		X	X
D. b. parva	MLZ	18362	M	14.516667	-88.75	Honduras, Ocotepeque, Cerro Verde		X	X
D. b. parva	MLZ	18363	M	14.516667	-88.75	Honduras, Ocotepeque, Cerro Verde		X	X
D. b. baritula	CNAV	22951	M	19.2647	-99.299	Mexico, Mexico city, Dinamo 4		X	X
D. b. baritula	CNAV	22952	M	19.2647	-99.299	Mexico, Mexico city, Dinamo 4		X	X
D. b. baritula	CNAV	24849	M	17.3970306	-96.428366	Mexico, Oaxaca, Cerro de los Pozuelos		X	X
D. b. baritula	CNAV	24850	F	17.3970306	-96.428366	Mexico, Oaxaca, Cerro de los Pozuelos		X	
D. b. parva	MLZ	26635	M	14.33333	-87.4	Honduras, Francisco Morazán, Cantoral		X	X
D. b. parva	MLZ	26636	F	14.33333	-87.4	Honduras, Francisco Morazán, Cantoral		X	
D. b. parva	MLZ	26637	M	14.58333	-88	Honduras, Francisco Morazán, San Marcos de Guaimaca		X	
D. b. baritula	CNAV	27132	M	19.42241	-102.088	Mexico, Michoacán, Parque Nacional del Cupatitzio		X	X
D. b. montana	USNM	30724	M	14.8	-91.0167	Guatemala, Chimaltenango, Tecpán		X	X
D. b. baritula	AMNH	40345	M	19.4326009	-99.1333416	Mexico, Mexico city		X	
D. b. parva	LSUMZ	60978	ND	13.9388	-87.1691	Honduras, Francisco Morazán, Santa Ana	X		
D. b. parva	MVZ	86316	M	14.38306	-89.12899	Honduras, Ocotepeque, El Pital		X	X
D. b. parva	MVZ	86322	F	14.38306	-89.12899	Honduras, Ocotepeque, El Pital		X	
D. b. baritula	AMNH	105891	M	19.579	-103.623	Mexico, Jalisco, Volcán de Nieve		X	
D. b. baritula	AMNH	105892	M	19.579	-103.623	Mexico, Jalisco, Volcán de Nieve		X	
D. b. baritula	AMNH	105893	F	19.579	-103.623	Mexico, Jalisco, Volcán de Nieve		X	
D. b. montana	UWBM	110015	ND	14.7166667	-91.535	Guatemala, Quetzaltenango, Santa María de Jesús	X		
D. b. parva	AMNH	326509	M	14.220453	-87.913411	Honduras, La Paz, Muye		X	X
D. b. parva	AMNH	326510	F	14.236378	-87.957242	Honduras, Tegucigalpa, Archaga		X	
D. b. parva	AMNH	328423	M	14.33333	-87.4	Honduras, Francisco Morazán, Cantoral		X	X
D. b. parva	AMNH	328424	M	14.33333	-87.4	Honduras, Francisco Morazán, Cantoral		X	X
D. b. parva	AMNH	328425	M	14.33333	-87.4	Honduras, Francisco Morazán, Cantoral		X	X
D. b. parva	AMNH	328426	M	14.33333	-87.4	Honduras, Francisco Morazán, Cantoral		X	X
D. b. parva	AMNH	328427	M	14.33333	-87.4	Honduras, Francisco Morazán, Cantoral		X	X
D. b. parva	AMNH	328428	M	14.3423	-87.2946	Honduras, Tegucigalpa, Archaga		X	X

D. b. parva	AMNH	328429	F	14.3423	-87.2946	Honduras, Tegucigalpa, Archaga		X	
D. b. parva	AMNH	328430	M	14.33333	-87.4	Honduras, Francisco Morazán, Cantoral		X	X
D. b. parva	USNM	348152	M	14.43333	-87.78333	Honduras, Francisco Morazán, Cantoral		X	X
D. b. parva	USNM	348172	F	14.43333	-87.78333	Honduras, Francisco Morazán, Cantoral		X	
D. b. montana	USNM	349723	M	14.86666	-91.21666	Guatemala, Totonicapán, María Tecun		X	X
D. b. montana	USNM	349724	M	14.8	-91.0167	Guatemala, Chimaltenango, Tecpán		X	X
D. b. montana	USNM	349725	F	14.8	-91.0167	Guatemala, Chimaltenango, Tecpán		X	
D. b. baritula	FMNH	393877	ND	19.591827	-104.265508	Mexico, Jalisco, Las Joyas	X		
D. b. montana	AMNH	398054	M	14.76667	-91	Guatemala, Chimaltenango, Tecpán		X	X
D. b. montana	AMNH	398055	M	14.76667	-91	Guatemala, Chimaltenango, Tecpán		X	
D. b. montana	AMNH	398056	F	14.76667	-91	Guatemala, Chimaltenango, Tecpán		X	
D. b. montana	AMNH	398057	F	14.8	-91.0167	Guatemala, Chimaltenango, Tecpán		X	
D. b. montana	AMNH	398062	M	14.61667	-90.66667	Guatemala, Sacatepéquez, San Lucas		X	X
D. b. montana	AMNH	398063	M	14.61667	-90.66667	Guatemala, Sacatepéquez, San Lucas		X	X
D. b. montana	AMNH	398064	M	14.61667	-90.66667	Guatemala, Sacatepéquez, San Lucas		X	X
D. b. montana	AMNH	398065	F	14.61667	-90.66667	Guatemala, Sacatepéquez, San Lucas		X	
D. b. baritula	AMNH	508149	M	20.537108	-104.81302	Mexico, Jalisco, Mascota		X	
D. b. baritula	AMNH	508150	M	20.628395	-104.739202	Mexico, Jalisco, Juanacatlán		X	
D. b. montana	AMNH	748477	M	17.1599	-92.8999	Mexico, Chiapas, Pueblo Nuevo Solistahuacán		X	X
D. b. baritula	AMNH	778545	M	19.018976	-99.26216	Mexico, Morelos, Cuernavaca		X	X
D. b. baritula	MZFC	AGH 032	M	17.065	-100.066667	Mexico, Guerrero, Omiltemi		X	X
D. b. baritula	MZFC	AGH SN 5025	M	17.065	-100.066667	Mexico, Guerrero, Omiltemi		X	X
D. b. baritula	MZFC	AGNS 0175	M	19.295	-99.24	Mexico, Mexico city, Primer Dinamo		X	X
D. b. baritula	MZFC	AGNS 0206	M	17.5	-100.266667	Mexico, Guerrero, Toro Muerto		X	X
D. b. baritula	MZFC	AGNS 0216	F	17.5	-100.266667	Mexico, Guerrero, Toro Muerto		X	
D. b. baritula	MZFC	AGNS 0366	M	17.4833333	-100.2	Mexico, Guerrero, El Iris		X	X
D. b. baritula	MZFC	AGNS 1023	F	17.515	-96.505	Mexico, Oaxaca, La Esperanza		X	
D. b. baritula	MZFC	AGNS 1024	M	17.51	-96.5033333	Mexico, Oaxaca, La Esperanza		X	X
D. b. baritula	MZFC	AGNS SN 02391	F	19.295	-99.24	Mexico, Mexico city, Primer Dinamo		X	
D. b. baritula	MZFC	AGNS SN 03996	F	17.4833333	-100.2	Mexico, Guerrero, El Iris		X	
D. b. baritula	MZFC	AGNS SN 04983	F	17.5	-100.266667	Mexico, Guerrero, El Iris		X	

D. b. baritula	MZFC	AGNS SN 05026	M	17.065	-100.066667	Mexico, Guerrero, Omiltemi		X	X
D. b. baritula	MZFC	AGNS SN 05027	M	17.7416666	-99.7266667	Mexico, Guerrero, Omiltemi		X	X
D. b. baritula	MZFC	AGNS SN 05030	F	17.7416666	-99.7266667	Mexico, Guerrero, Omiltemi		X	
D. b. baritula	MZFC	AGNS SN 05032	F	17.065	-100.066667	Mexico, Guerrero, Omiltemi		X	
D. b. baritula	MZFC	AGNS SN 05034	F	17.065	-100.066667	Mexico, Guerrero, Omiltemi		X	
D. b. baritula	MZFC	AHC 099	M	19.79948	-97.80345	Mexico, Puebla, Chopilco Alto		X	X
D. b. baritula	MZFC	ATP2002 18	F	19.25715	-99.0347333	Mexico, Mexico city, Ajusco medio	*		
D. b. baritula	MZFC	BEHB 009	M	17.705	-96.4116666	Mexico, Oaxaca, Puerto Eligio		X	X
D. b. montana	MZFC	BEHB08 14	F	16.7278528	-92.6963889	Mexico, Chiapas, Cerro Huitepec		X	
D. b. montana	MZFC	BEHB08 26	F	16.7278528	-92.6963889	Mexico, Chiapas, Cerro Huitepec		X	
D. b. montana	MZFC	BEHB08 38	M	16.7278528	-92.6963889	Mexico, Chiapas, Cerro Huitepec		X	X
D. b. montana	MZFC	BEHB08 46	M	16.7278528	-92.6963889	Mexico, Chiapas, Cerro Huitepec		X	X
D. b. baritula	MZFC	BIODF 060	F	19.2182493	-99.0630278	Mexico, Mexico city, Axomulco		X	
D. b. montana	MZFC	BMM 866	M	15.1316667	-92.1083333	Mexico, Chiapas, Volcán Tacaná		X	X
D. b. montana	MZFC	BMM 879	F	15.1316667	-92.1083333	Mexico, Chiapas, Volcán Tacaná		X	
D. b. montana	MZFC	BONA 37	M	15.0666667	-92.0833333	Mexico, Chiapas, Volcán Tacaná		X	X
D. b. montana	MZFC	BONA 50	M	15.0666667	-92.0833333	Mexico, Chiapas, Volcán Tacaná		X	X
D. b. montana	MZFC	BONA 70	F	15.0666667	-92.0833333	Mexico, Chiapas, Volcán Tacaná		X	
D. b. montana	MZFC	BONA 71	M	15.0666667	-92.0833333	Mexico, Chiapas, Volcán Tacaná		X	X
D. b. montana	MZFC	BONA 73	F	15.0666667	-92.0833333	Mexico, Chiapas, Volcán Tacaná		X	
D. b. montana	MZFC	BONA 86	F	15.0666667	-92.0833333	Mexico, Chiapas, Volcán Tacaná		X	
D. b. montana	CNAV	BRB 782	F	16.5026	-93.0193	Mexico, Chiapas, Chiapa de Corzo		X	
D. b. baritula	MZFC	CONACYT 770	F	18.165	-96.9966667	Mexico, Oaxaca, Puerto de la Soledad		X	
D. b. montana	MZFC	CRGA 24	F	15.23541	-92.30463	Mexico, Chiapas, Cerro Boquerón		X	
D. b. montana	MZFC	CRGA 45	F	15.23541	-92.30463	Mexico, Chiapas, Cerro Boquerón		X	
D. b. montana	MZFC	CRH 024	F	17.16613	-93.14163	Mexico, Chiapas, Coapilla		X	
D. b. montana	MZFC	CRH 031	M	17.16613	-93.14163	Mexico, Chiapas, Coapilla		X	X
D. b. baritula	MZFC	DONAD 059	F	19.3901944	-99.2042222	Mexico, Mexico city, UNAM		X	
D. b. montana	MZFC	EAGT 830	M	15.23541	-92.30463	Mexico, Chiapas, Cerro Boquerón		X	X
D. b. montana	MZFC	EAGT 841	M	15.23541	-92.30463	Mexico, Chiapas, Cerro Boquerón		X	X
D. b. baritula	MZFC	GUER 17	M	17.61583	-99.83861	Mexico, Guerrero, Carrizal de Bravo		X	X
D. b. baritula	MZFC	GUER 18	F	17.61583	-99.83861	Mexico, Guerrero, Carrizal de Bravo		X	

D. b. baritula	MZFC	IFFD 0018	M	17.8074167	-100.915467	Mexico, Guerrero, Los Vergeles		X	X
D. b. baritula	MZFC	INECOL 073	F	17.2054056	-97.8365611	Mexico, Oaxaca, Chicahuaxtla		X	
D. b. baritula	MZFC	INECOL 074	F	17.2054056	-97.8365611	Mexico, Oaxaca, Chicahuaxtla		X	
D. b. baritula	MZFC	INECOL 075	M	17.2054056	-97.8365611	Mexico, Oaxaca, Chicahuaxtla		X	X
D. b. baritula	MZFC	JEMP 360	F	18.566	-99.6	Mexico, Guerrero, El Huizteco		X	
D. b. baritula	MZFC	JEMP 503	F	18.65	-99.7833333	Mexico, Guerrero, Los Jarillos		X	
D. b. baritula	MZFC	JEMP 505	M	18.65	-99.7833333	Mexico, Guerrero, Los Jarillos		X	X
D. b. baritula	MZFC	JEMP 506	M	18.65	-99.7833333	Mexico, Guerrero, Los Jarillos		X	X
D. b. baritula	MZFC	JEMP 507	M	18.65	-99.7833333	Mexico, Guerrero, Los Jarillos		X	X
D. b. baritula	MZFC	JK04 111	F	17.816713	-99.967595	Mexico, Guerrero, Carrizal de Bravo		X	
D. b. baritula	MZFC	JK04 112	F	17.816713	-99.967595	Mexico, Guerrero, Carrizal de Bravo		X	
D. b. baritula	MZFC	JK04 164	M	17.816713	-99.967595	Mexico, Guerrero, Carrizal de Bravo		X	X
D. b. baritula	MZFC	JK04 174	F	17.816713	-99.967595	Mexico, Guerrero, Carrizal de Bravo		X	
D. b. baritula	MZFC	JK04 176	F	17.816713	-99.967595	Mexico, Guerrero, Carrizal de Bravo		X	
D. b. baritula	CNAV	JK11 201	M	17.5741	-99.69	Mexico, Guerrero, Omiltemi		X	X
D. b. baritula	CNAV	JK11 206	M	17.5741	-99.69	Mexico, Guerrero, Omiltemi		X	X
D. b. baritula	CNAV	JMD 437	F	17.614166	-99.851	Mexico, Guerrero, Leonardo Bravo		X	
D. b. baritula	CNAV	LGM 08	M	21.46262	-100.99472	Mexico, Guanajuato, San Diego de la Unión		X	X
D. b. baritula	CNAV	LGM 12	F	21.46262	-100.99472	Mexico, Guanajuato, San Diego de la Unión		X	
D. b. montana	MZFC	MFOR 736	M	17.18758	-93.12125	Mexico, Chiapas, Tapalapa		X	X
D. b. montana	MZFC	MFOR 744	M	17.18758	-93.12125	Mexico, Chiapas, Tapalapa		X	X
D. b. baritula	CNAV	MM 458	M	19.4231644	-102.233	Mexico, Michoacán, Nuevo Parangaricutiro		X	X
D. b. montana	MZFC	MOL13 008	F	16.7380556	-92.6880556	Mexico, Chiapas, Cerro Huitepec		X	
D. b. montana	MZFC	MOL13 014	F	16.7380556	-92.6880556	Mexico, Chiapas, Cerro Huitepec		X	
D. b. montana	MZFC	MOL13 037	M	16.7380556	-92.6880556	Mexico, Chiapas, Cerro Huitepec		X	X
D. b. montana	MZFC	MOL13 038	M	16.7380556	-92.6880556	Mexico, Chiapas, Cerro Huitepec		X	X
D. b. montana	MZFC	MOL13 071	M	16.7380556	-92.6880556	Mexico, Chiapas, Cerro Huitepec		X	
D. b. montana	MZFC	MOL13 080	F	16.7380556	-92.6880556	Mexico, Chiapas, Cerro Huitepec		X	
D. b. montana	MZFC	MOL13 081	M	16.7380556	-92.6880556	Mexico, Chiapas, Cerro Huitepec	X	X	X
D. b. montana	MZFC	MOL13 083	F	16.7380556	-92.6880556	Mexico, Chiapas, Cerro Huitepec		X	

D. b. montana	MZFC	MOL13 093	F	16.7380556	-92.6880556	Mexico, Chiapas, Cerro Huitepec		X	
D. b. montana	MZFC	MOL13 131	M	16.7380556	-92.6880556	Mexico, Chiapas, Cerro Huitepec		X	X
D. b. baritula	MZFC	MOL15 31	F	16.08972	-96.48548	Mexico, Oaxaca, Puente Río Molino		X	
D. b. baritula	MZFC	MOLGRO 105	M	17.55	-99.6666667	Mexico, Guerrero, Omiltemi	X		
D. b. baritula	MZFC	MOLGRO 143	F	17.55	-99.6666667	Mexico, Guerrero, Omiltemi		X	
D. b. baritula	MZFC	MOLGRO 150	F	17.55	-99.6666667	Mexico, Guerrero, Omiltemi		X	
D. b. baritula	MZFC	MOLGRO 193	M	17.58668	-99.83707	Mexico, Guerrero, Carrizal de Bravo		X	X
D. b. baritula	MZFC	MOLGRO 242	M	17.58668	-99.83707	Mexico, Guerrero, Carrizal de Bravo		X	X
D. b. baritula	MZFC	MOLGRO 243	F	17.58668	-99.83707	Mexico, Guerrero, Carrizal de Bravo		X	
D. b. baritula	MZFC	MOLGRO 244	F	17.58668	-99.83707	Mexico, Guerrero, Carrizal de Bravo		X	
D. b. baritula	MZFC	MOLGRO 245	M	17.58668	-99.83707	Mexico, Guerrero, Carrizal de Bravo		X	X
D. b. baritula	MZFC	MT 157	M	17.515	-96.505	Mexico, Oaxaca, La Esperanza		X	X
D. b. baritula	MZFC	OMVP 0090	F	18.17	-96.8466667	Mexico, Oaxaca, Sierra de Huautla		X	
D. b. baritula	MZFC	OMVP 0240	ND	17.025	-97.795	Mexico, Oaxaca, Santa María Yucuhiti	X		
D. b. baritula	MZFC	OMVP 0972	F	17.845	-96.74	Mexico, Oaxaca, Peña Verde		X	
D. b. baritula	MZFC	PEP 352	M	17.4666667	-100.166667	Mexico, Guerrero, Puerto Gallo		X	X
D. b. baritula	MZFC	PEP 364	M	17.4666667	-100.166667	Mexico, Guerrero, Puerto Gallo		X	X
D. b. baritula	MZFC	PEP 368	M	17.4666667	-100.166667	Mexico, Guerrero, Puerto Gallo		X	X
D. b. baritula	MZFC	PEP 371	F	17.4666667	-100.166667	Mexico, Guerrero, Puerto Gallo		X	
D. b. baritula	MZFC	PEP 396	F	17.4666667	-100.166667	Mexico, Guerrero, Puerto Gallo		X	
D. b. baritula	MZFC	PEP 602	M	17.4666667	-100.166667	Mexico, Guerrero, Puerto Gallo		X	X
D. b. baritula	MZFC	PEP 762	F	17.55	-99.6666667	Mexico, Guerrero, Omiltemi		X	

Notes:

Abbreviations for collections of voucher specimens: Colección Nacional de Aves, Instituto de Biología, UNAM (CNAV); Moore Laboratory of Zoology, Occidental College (MLZ); National Museum of Natural History, Smithsonian Institution (USNM), American

Museum of Natural History (AMNH); Louisiana State University Museum of Natural Science (LSUMZ); Museum of Vertebrate Zoology, University of California, Berkeley (MVZ); University of Washington Burke Museum of Natural History and Culture (UWBM); Field Museum of Natural History (FMNH); Museo de Zoología "Alfonso L. Herrera", Facultad de Ciencias, Universidad Nacional Autónoma de México (MZFC).

Abbreviations for sex: female (F) and male (M).

The asterisk represents the reference genome used.

Table 2 Mean and ANOVA to test differences between subspecies and PCA values.

Measurement	Sex	Mean ± SD			*F*	*df*	*p*-value	PC1	PC2
		D. b. baritula	*D. b. montana*	*D. b. parva*				30.60%	23.50%
Bill length	Both	12.48 ± 0.40	12.65 ± 0.41	12.33 ± 0.60	4.163	2	<**0.05**	0.67	0.04
Bill width	Both	2.61 ± 0.15	2.63 ± 0.15	2.74 ± 0.37	4.467	2	<**0.05**	0.14	-0.24
Bill hook length	Both	2 ± 0.20	1.97 ± 0.22	1.92 ± 0.22	1.523	2	0.221	0.69	-0.12
Tarsus length	Both	17.05 ± 0.57	16.55 ± 0.58	16.35 ± 0.61	20.42	2	<**0.001**	0.21	0.63
Wing chord	Females	54.09 ± 2.1	53.72 ± 2.04	52.22 ± 1.81	3.063	2	0.053	-0.06	0.72
Wing chord	Males	56.28 ± 1.77	56.46 ± 2	55.51 ± 1.72	1.381	2	0.257		

Significant differences are indicated in bold.
df = degrees of freedom.

Supplemental material

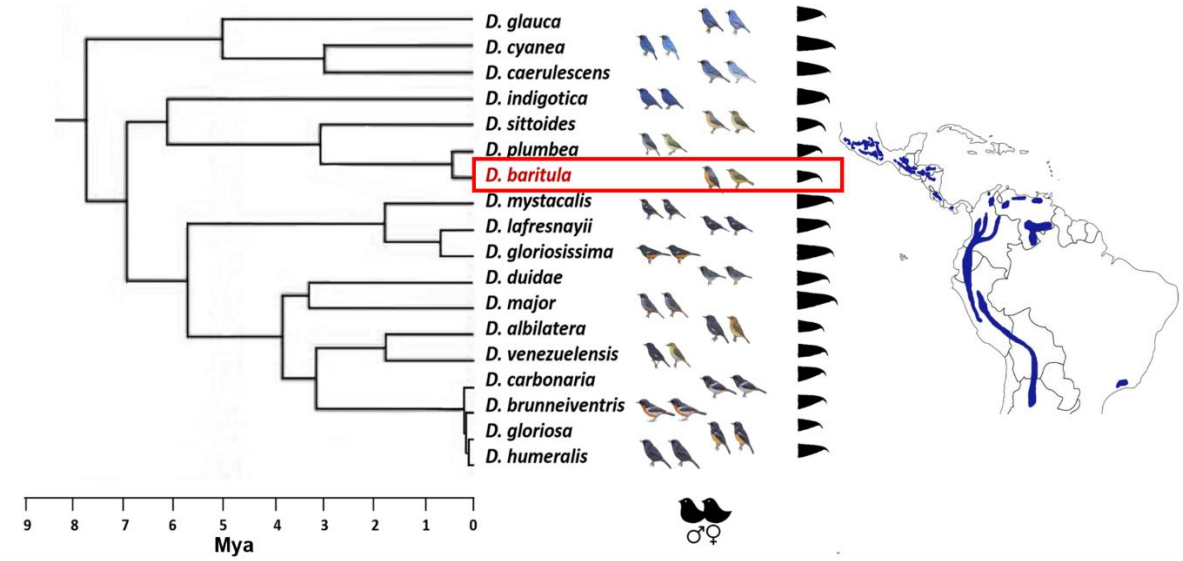


Figure S1. Evolutionary radiation of the *Diglossa* genus, showing that in less than 10 million years there has been a great diversification in patterns of coloration, bill shape and size, etc. In red the species of this study. Image modified from Mauck & Burns, 2009; Baker et al., 2015; Lauk, 2020.

Table S1. Taxonomic proposals for *Diglossa* species.

Reference	Taxonomic hypothesis	Data used
Cassin (1864) & Sclater (1875)	The species were included in two genera, *Diglossa* and *Diglossopis*, the latter being a monotypic genus containing only *Diglossopis* caerulescens, which has the smallest bill.	Bill size and coloration
Hellmayr (1935)	Some closely related species were combined, reducing the number of species.	Morphology
Vuilleumier (1969)	Two ranges of taxonomic classification were proposed: four "species groups" (*major*, *lafresnayii*, *albilatera* and *caerulescens*) and four "superspecies" (*lafresnayii*, *carbonaria*, *baritula*, and *albilatera*).	Bill and tongue morphology, coloration
Bock (1985)	The genus *Diglossopis* was returned, where *D. caerulescens*, *D. cyanea* and *D. glauca* were included.	Skull, tongue and bill morphology
Sibley & Monroe (1990)	*D. indigotica* was moved to *Diglossopis.*	DNA-DNA hybridization
Dickinson (2003) and Remsen et al. (2008)	*Diglossopis* was absorbed by *Diglossa*.	Not specified

Table S2. Taxonomic positions for the *D. baritula* complex.

Reference	Taxonomic classification
Wagler (1832)	*D. baritula* species was named, which is the type species of the genus.
Dearbon (1907)	*D. b. montana* subspecies was described.
Griscom (1932)	The third subspecies, *D. b. parva*, was named
Hellmayr (1935)	*D. baritula, D. plumbea* and *D. sittoides*grouped as a single species.
Friedmann et al. (1950)	Two subspecies were reported: *D. b. baritula* (pico chueco mexicano) and *D. b. montana* (pico chueco chiapaneco).
Skutch (1954)	*D. baritula* and *D. plumbea* combined as a single species with three subspecies: *D. b. baritula, D. b. montana* and *D. b. plumbea.*
Vuilleumier (1969)	*D. baritula* was grouped with *D. montana* and *D. sittoides* in the *baritula*superspecies.
Monroe (1968) and Isler & Isler (1987)	Three subspecies were mentioned: *D. b. baritula*, *D. b. montana* and *D. b. parva.*

DISCUSIÓN GENERAL

Nuestro árbol filogenómico muestra una divergencia estadísticamente robusta (i.e., un valor de *bootstrap* = 100) entre las poblaciones de ambos lados del Istmo de Tehuantepec (IT). Los individuos de *Diglossa baritula baritula* que se encuentran al oeste del IT conforman un clado monofilético que llamamos Grupo Oeste-IT, mientras que el otro linaje monofilético contiene a los individuos de las otras dos subespecies, *Diglossa baritula montana* y *Diglossa baritula parva*, los cuales están presentes al este del IT, que vamos a llamar el Grupo Este-IT. Los individuos de la subespecie con "machos de garganta canela" forman el grupo Grupo Oeste-IT, mientras que los individuos de las subespecies con "machos de garganta gris" se agrupan en el Grupo Oeste-IT. En general, este resultado genómico sugiere que los dos clados tienden a ser linajes independientes, con un claro potencial para la especiación, ya que las fuerzas evolutivas podrían actuar rápidamente en cada acervo genético aislado de manera independiente, generando divergencia genética. En contraste con los resultados genómicos, nuestros análisis de morfología y coloración mostraron niveles intermedios de diferenciación entre los dos clados encontrados. Este resultado, probablemente se deba a que son clados que divergieron recientemente y no han tenido suficiente tiempo para mostrar un patrón claro de divergencia fenética, y además a que los clados dentro de *D. baritula* no han estado sujetos a presiones evolutivas diferentes. Finalmente, los datos ecológicos indicaron que hay moderado sobrelapamiento de nicho ecológico en las agrupaciones poblacionales de *D. baritula*.

Las ramas cortas de la filogenia y la débil divergencia fenotípica sugieren que la divergencia ha sido reciente. Efectivamente, hasta donde sabemos el complejo *D. baritula* se originó durante el Pleistoceno, hace menos de un millón de años (Barker et al., 2015). Dado

que *D. baritula* es una especie residente restringida a las tierras altas de Mesoamérica, las oscilaciones climáticas del Pleistoceno probablemente fueron un factor relevante en su distribución geográfica alopátrica actual y, por lo tanto, en su historia evolutiva. Sus movimientos hacia hábitats adecuados que cumplían con sus afinidades biogeográficas y requerimientos ecológicos determinaron su respuesta a los periodos glaciales e interglaciares.

Distintos factores, tales como fluctuaciones climáticas y la presencia de barreras geográficas promueven la diversificación. El Istmo de Tehuantepec es la barrera topográfica y ecológica que divide al Grupo Oeste-IT del Grupo Este-IT. La marcada divergencia genética entre poblaciones separadas por el IT sugiere que el flujo de genes a través de esta barrera se ha reducido o interrumpido debido a la baja elevación del IT. Consideramos que este aislamiento geográfico ha impulsado la divergencia genética intraespecífica en nuestra especie de estudio.

A pesar de la inferencia filogenética de que en la especie *D. baritula* se presentan dos clados bien soportados, sólo encontramos parcial divergencia fenotípica entre ambos clados. Las variables fenotípicas que presentan una correspondencia con la filogenia son el color de la garganta y la longitud del tarso. Por un lado, el Grupo Este-IT tiene la garganta color gris y la longitud del tarso es menor. Por otro lado, el Grupo Oeste-IT presenta la garganta con coloración canela y una mayor longitud del tarso. Una posible explicación de los niveles intermedios de divergencia fenotípica es que probablemente los grupos poblacionales (Grupo Este-IT y Grupo Oeste-IT) han estado sujetos a presiones selectivas similares.

La divergencia de nichos ecológicos, uno de los impulsores de la divergencia de linajes, fue confirmada por el PCA de datos ambientales y las pruebas de sobrelapamiento de nicho, que sugieren que las tres subespecies ocupan diferentes espacios ambientales. Las

superficies de densidad de ocurrencia ambiental indican que el espacio de nicho ocupado actual varía entre las subespecies. La divergencia de nichos es plausible debido a que las subespecies habitan en distintos ecosistemas, lo cuál posiblemente ha promovido las diferencias fenotípicas en el complejo *D. baritula*. *Diglossa b. baritula* habita en ecosistemas con menos precipitación --por ejemplo, bosques de pino-encino--, mientras que *D. b. parva* se encuentra en regiones montanas con mayor estacionalidad --tanto en temperatura como en precipitación--, por ejemplo, en bosques nubosos (Ruiz-Sánchez & Ornelas, 2014; Ortiz-Rodríguez et al. 2018) y *D. b. montana* habita tanto en bosques nubosos montanos como en bosques nubosos subalpinos (Helmer et al., 2019). El moderado sobrelapamiento de nicho puede indicar que las subespecies que conforman al complejo *D. baritula* tienen diferentes tolerancias ecológicas y su propia área de distribución, y además que los nichos han ido adquiriendo identidad en respuesta a diferentes condiciones ambientales.

CONCLUSIONES

- Se encontraron dos unidades genómicas (clados): una incluye sólo a la subespecie *D. b. baritula*, mientras que la otra contiene tanto a *D. b. montana* como a *D. b. parva*.

- La divergencia genética intraespecífica podría sugerir que los dos clados que componen a *D. baritula* tienden a ser linajes evolutivamente independientes o linajes incipientes.

- Los datos fenotípicos (morfología y coloración) mostraron niveles intermedios de diferenciación, lo que indica que las agrupaciones poblacionales dentro de *D. baritula* no han estado sujetas a diferentes presiones evolutivas.

- El color de la garganta de los machos tiene correspondencia con la filogenia: el Grupo Este-IT tiene la garganta color gris y el Grupo Oeste-IT color canela.

- Hay congruencia entre la longitud del tarso y el árbol filogenómico: la longitud del tarso del Grupo Este-IT es menor, mientras que el Grupo Oeste-IT tiene una mayor longitud del tarso.

- Nuestros resultados de morfología indican que la única variable morfológica sexualmente dimórfica es la cuerda alar: los machos tienen una cuerda alar más larga que las hembras.

- Los análisis de sobrelapamiento de nicho revelaron que hay moderado sobrelapamiento de nicho entre las subespecies, lo que sugiere que los factores ambientales han influido en la divergencia dentro del complejo *D. baritula*.

- Se necesita un muestreo genómico más extenso para resolver el estado taxonómico dentro de *D. baritula*.

- El Istmo de Tehuantepec es la principal barrera geográfica que ha determinado la evolución de los dos clados independientes dentro de *D. baritula*.

PERSPECTIVAS

Se necesita un muestreo genómico más amplio para poder llevar a cabo diferentes análisis y estadísticos tales como tasas de migración, tiempos de expansión, tamaños efectivos de las poblaciones, estructuración genética con el programa *Structure*, tiempos de divergencia, etc.

Relacionar los resultados de los análisis de genómica, coloración, morfología y sobrelapamiento de nicho con los datos de genes mitocondriales: heteroplasmia y/o DNA mitocondrial nuclear (NUMTs, del inglés *nuclear mitochondrial DNA segment*), lo cual actualmente se está procesando. Así como plantear una hipótesis del papel de la heteroplasmia y/o el DNA mitocondrial nuclear en el complejo *D. baritula*.

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