UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO
POSGRADO EN CIENCIAS BIOLÓGICAS

INSTITUTO DE BIOLOGÍA

BIOLOGÍA EVOLUTIVA

VARIACIÓN GEOGRÁFICA EN LA MORFOLOGÍA Y VOCALIZACIONES DEL COMPLEJO *CAMPYLORHYNCHUS BRUNNEICAPILLUS* (AVES: TROGLODYTIDAE)

TESIS

(**POR ARTÍCULO CIENTÍFICO**)

ECOLOGICAL FACTORS DRIVE THE DIVERGENCE OF MORPHOLOGICAL, COLOR AND BEHAVIORAL TRAITS IN THE CACTUS WREN (*CAMPYLORHYNCHUS BRUNNEICAPILLUS*).

QUE PARA OPTAR POR EL GRADO DE:

MAESTRA EN CIENCIAS BIOLÓGICAS

PRESENTA: Violeta Monserrath Andrade González

TUTOR PRINCIPAL DE TESIS: DR. JOSÉ ROBERTO SOSA LÓPEZ
CIIDIR UNIDAD OAXACA, INSTITUTO POLITÉCNICO NACIONAL
COTUTOR DE TESIS: DR. HERNÁN VÁZQUEZ MIRANDA
INSTITUTO DE BIOLOGÍA, UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO
COMITÉ TUTOR: DRA. CLAUDIA PATRICIA ORNELAS GARCÍA
INSTITUTO DE BIOLOGÍA, UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO

Ciudad Universitaria, CD.MX. 2023

UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO
POSGRADO EN CIENCIAS BIOLÓGICAS
INSTITUTO DE BIOLOGÍA
BIOLOGÍA EVOLUTIVA

VARIACIÓN GEOGRÁFICA EN LA MORFOLOGÍA Y VOCALIZACIONES DEL COMPLEJO *CAMPYLORHYNCHUS BRUNNEICAPILLUS* (AVES: TROGLODYTIDAE)

TESIS

(**POR ARTÍCULO CIENTÍFICO**)

ECOLOGICAL FACTORS DRIVE THE DIVERGENCE OF MORPHOLOGICAL, COLOR AND BEHAVIORAL TRAITS IN THE CACTUS WREN (*CAMPYLORHYNCHUS BRUNNEICAPILLUS*).

QUE PARA OPTAR POR EL GRADO DE:

MAESTRA EN CIENCIAS BIOLÓGICAS

PRESENTA: Violeta Monserrath Andrade González

TUTOR PRINCIPAL DE TESIS: DR. JOSÉ ROBERTO SOSA LÓPEZ
CIIDIR UNIDAD OAXACA, INSTITUTO POLITÉCNICO NACIONAL
COTUTOR DE TESIS: DR. HERNÁN VÁZQUEZ MIRANDA
INSTITUTO DE BIOLOGÍA, UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO
COMITÉ TUTOR: DRA. CLAUDIA PATRICIA ORNELAS GARCÍA
INSTITUTO DE BIOLOGÍA, UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO

Ciudad Universitaria, CD.MX. 2023

UNAM POSGRADO

POSGRADO CIENCIAS BIOLÓGICAS

COORDINACIÓN DEL POSGRADO EN CIENCIAS BIOLÓGICAS
INSTITUTO DE BIOLOGÍA

OFICIO CPCB/139/2023

ASUNTO: Oficio de Jurado

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

Presente

Me permito informar a usted que en la reunión ordinaria del Comité Académico del Posgrado en Ciencias Biológicas, celebrada el 28 de noviembre del 2022, se aprobó el siguiente jurado para el examen de grado de **MAESTRA EN CIENCIAS BIOLÓGICAS** en el campo de conocimiento de **BIOLOGÍA EVOLUTIVA** de la alumna **ANDRADE GONZÁLEZ VIOLETA MONSERRATH** con número de cuenta **310087346** por la modalidad de graduación de **tesis por artículo científico** titulado: **"Ecological factors drive the divergence of morphological,color and behavioral traits in the cactus wren (*Campylorhynchus brunneicapillus*)"**, que es producto del proyecto realizado en la maestría que lleva por título: **"VARIACIÓN GEOGRÁFICA EN LA MORFOLOGÍA Y VOCALIZACIONES DEL COMPLEJO *CAMPYLORHYNCHUS BRUNNEICAPILLUS* (AVES: TROGLODYTIDAE)"**, ambos realizados bajo la dirección del **DR. JOSÉ ROBERTO SOSA LÓPEZ**, quedando integrado de la siguiente manera:

Presidente:	**DRA. MARÍA DEL CORO ARIZMENDI ARRIAGA**
Vocal:	**DRA. CLEMENTINA GONZÁLEZ ZARAGOZA**
Vocal:	**DR. ADOLFO GERARDO NAVARRO SIGÜENZA**
Vocal:	**DR. LUIS ANDRÉS SANDOVAL VARGAS**
Secretario:	**DRA. CLAUDIA PATRICIA ORNELAS GARCÍA**

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

ATENTAMENTE
"POR MI RAZA HABLARÁ EL ESPÍRITU"
Ciudad Universitaria, Cd. Mx., a 31 de enero de 2023

COORDINADOR DEL PROGRAMA

DR. ADOLFO GERARDO NAVARRO SIGÜENZA

Agradecimientos institucionales

Este trabajo se realizó gracias al Posgrado en Ciencias Biológicas de la Universidad Nacional Autónoma de México. Gracias a la beca extraoridinaria otorgada por la Universidad Nacional Autónoma de México. Gracias a DGAPA-PAPIIT 2020 (IA204220), DGAPA-PAPIIT 2022 (IA205422) y The Frank M. Chapman Memorial Fund of the American Museum of Natural History por el fininciamiento otorgado para la elaboración de este proyecto. Gracias al apoyo económico PAEP que me fue concedido.

Agradezco a los miembros de mi comité tutor por guiarme en la elaboración de este proyecto, por enseñarme de forma tan detallada y mostrar su interés en mi aprendizaje:

Dr. José Roberto Sosa López
Dr. Hernán Vázquez Miranda
Dra. Claudia Patricia Ornelas García

Agradecimientos a título personal

Gracias a Dios por estar conmigo, guiarme y darme la fortaleza necesaria.

Agradezco a los miembros del jurado de examen: Dra. María del Coro Arizmendi Arriaga, Dra. Clementina González Zaragoza, Dr. Adolfo Navarro Sigüenza, Dr. Luis Sandoval por la revisión y comentarios para mejorar este trabajo.

Gracias a los miembros de mi comité tutoral: Dra. Claudia Patricia Ornelas García, Dr. Hernán Vázquez Miranda y Dr. José Roberto Sosa López porque sin ellos esto no hubiera sido posible.

A Alejandro Gordillo, por siempre estar conmigo y brindarme su apoyo en cada momento. Gracias por cada consejo y dejarme seguir en mi lugar favorito.

A Ronald Fernández, Wiliam Ku y Esaú Toaki por su apoyo y enseñarme a realizar análisis acústicos.

A Cami por hacer que mi estancia en CNAVlab fuera de lo más agradable, por tantas pláticas, risas y por siempre apoyarme.

A Vero, Caro, Donají y Arturo por siempre ser un gran apoyo para mí, por todo su cariño y amistad.

A mis padres Raúl Andrade, Hortencia González y a mi Oliver por todo el apoyo, su amor y por siempre creer en mí. Son mi mayor motivación.

A mi tía Lala y mi tío Dionisio por estar siempre.

A mis tíos, primas y sobrinos por todo su apoyo.

Índice

Resumen en español

El estudio de los mecanismos ecológicos que influyen en la variación fenotípica de los organismos es un tema central dentro de la biología evolutiva. En este estudio caracterizamos la variación morfológica, de color y acústica en la matraca del desierto (*Campylorhynchus brunneicapillus*) a lo largo de su distribución y evaluamos si las reglas ecogeográficas de Gloger, Allen, Bergmann y la Hipótesis de Adaptación Acústica explican la variación geográfica de los caracteres. Analizamos fotografías de 88 especímenes para obtener valores de coloración RGB en el plumaje del vientre y la corona. Realizamos morfometría tradicional con 73 individuos, morfometría geométrica del pico y analizamos las características estructurales del canto. Probamos si la clasificación taxonómica o los grupos peninsulares y continentales reflejaban la variación geográfica en los fenotipos y probamos si los patrones de variación de los rasgos se explicaban por factores ecológicos como temperatura, precipitación y densidad de la vegetación. Nuestros resultados sugieren que los rasgos morfológicos, de color y acústicos varían a lo largo de la distribución y reflejan dos grupos que corresponden a los linajes sugeridos previamente con datos genéticos. También encontramos que la variación en algunos rasgos de color y morfología entre los grupos peninsulares y continentales se explica por las versiones compleja y simple de las reglas de Gloger y Allen. Por el contrario, los patrones de variación fenotípica no fueron respaldados por la regla de Bergmann. La variación en los cantos fue respaldada por la hipótesis de adaptación acústica para rasgos relacionados con la frecuencia. Concluimos que la variación en los rasgos morfológicos, de color y vocales refleja el análisis genético previo, lo que sugiere la existencia de dos especies (*C. affinis* en la península de Baja California y *C. brunneicapillus* en el continente). La divergencia en algunos rasgos fenotípicos fue explicada por reglas ecogeográficas, sugiriendo que la divergencia entre linajes podría ser el resultado de la selección ecológica.

Resumen en inglés

The study of ecological mechanisms influencing organisms’ phenotypic variation is a central subject of evolutionary biology. In this study, we characterized morphological, color, and acoustic variation in cactus wrens *Campylorhynchus brunneicapillus* throughout its distribution and assessed whether ecogeographic rules of Gloger, Allen, Bergmann, and the Acoustic Adaptation Hypothesis explain trait geographic variation.

We analyzed specimen photographs of 88 individuals to obtain RGB coloration values in belly and crown plumage, and geometric morphometric of the beak in 73 individuals and analyzed structural song characteristics. We tested whether the taxonomical classification or the peninsular/mainland groups mirrored the geographic variation in phenotypes and tested whether patterns of traitsvariation were explained by ecological factors.

Our results suggest that morphological, color, and acoustic traits varied across the distribution range of the species complex, correlated with two lineages suggested by genetics. Our results suggest that variation in some color traits and morphology between the peninsular/mainland groups are explained by the complex and simple versions of Gloger´s and the Allen´s rule. Conversely, patterns of phenotypic variation were not supported by the Bergmann´s rule. We also found that variation in songs was supported by the acoustic adaptation hypothesis for frequency related traits.

We conclude that the variation in morphological, color and vocal traits mirrored previous genetic analysis, suggesting the existence of two species (*C. affinis* in Baja California peninsula and *C. brunneicapillus* in mainland). Divergence in some phenotypic traits were explained by ecogeographic rules, suggesting that divergence between lineages could be the result of Ecological Selection.

Introducción general

Comprender el papel de los rasgos ecológicos en la especiación como resultado de la adaptación local a ambientes divergentes es una cuestión fundamental en biología (Rundle & Nosil, 2005; Neto et al., 2013). De acuerdo la especiación ecológica, la divergencia ocurre cuando la selección disruptiva actúa sobre fenotipos que transmiten una ventaja de aptitud en un entorno pero no en otro (Shafer & Wolf, 2013).Por lo tanto, la divergencia ecológica entre poblaciones podría surgir debido a las diferencias en la estructura del hábitat, el clima, los recursos alimentarios y/o la presencia tanto de depredadores como de competidores (Rundle & Nosil, 2005; Schluter & Conte, 2009). En aves, varios estudios muestran que la diferencia ambiental actúa sobre la divergencia de color, morfología y cantos (Simpson & McGraw, 2018; FernándezGómez et al., 2020), haciendo referencia a reglas ecológicas como las reglas de Gloger, Bergmann y Allen para rasgos morfológicos o la hipótesis de la adaptación acústica – para vocalizaciones–(e.g. Fanet al., 2019; Marcondes, Nations, et al., 2020; Marcondes, Stryjewski, et al., 2020; Acero-Murcia et al. , 2021; Malpica et al., 2022).

La regla de Gloger explica los patrones de variación espacial en la coloración de los animales endotérmicos (Delhey & Delhey, 2019). En particular, se refiere a patronesde variación acromática (es decir, variación de color pálido a oscuro, comúnmente relacionada con la deposición de pigmentos de melanina; Delhey & Delhey, 2019). Predice que el color oscuro en animales endotérmicos debería surgir en regiones cálidasy húmedas, mientras que la coloración más pálida debería surgir en animales que habitan en áreas secas y cálidas (Delhey & Delhey, 2019). Esta regla se ha comprobado que ocurre en mamíferos y aves (Marcondes & Brumfield, 2019; Ribot et al., 2019; Cerezer et al., 2020; Newell et al., 2021). En ambientes húmedos, la coloración oscura debería beneficiarse de una mayor protección contra las bacterias que degradan las plumas, debido al aumento de melanina (Burtt & Ichida, 2004). Asimismo, la coloración más oscura podría verse favorecida por el camuflaje en una vegetación densa, que también se asocia con condiciones climáticas cálidas y húmedas (McNaught & Owens, 2002). Según la regla de Gloger, la temperatura y la humedad deben actuar juntas; sin embargo, se ha sugerido que solo es necesaria la humedad o la temperatura (Delhey & Delhey, 2019). Rensch (1929) reformuló esta regla sugiriendo una versión compleja para evaluar los efectos climáticos sobre los dos tipos de melanina: eumelaninas y feomelaninas. La eumelanina aumenta con la humedad, disminuye solo a temperaturasbajas y es responsable de los colores blanco y negro. Mientras que la feomelanina prevalece en regiones secas y cálidas, disminuye rápidamente con las bajas temperaturas y es responsable de los colores rojizos oxidados (Delhey & Delhey, 2019). La versión compleja de la regla de Gloger se ha probado en aves y mamíferos (Marcondes, Nations, et al., 2020; Newell et al., 2021). Particularmente en las aves del complejo de choca corona negruzca (*Thamnophilus caerulescens*), se sugiere que los machos de las subespecies con color rufo en la mayor parte de la parte inferior se encuentran en regiones cálidas, mientras que los machos de subespecies con color blanco, negro y gris se encuentran en regiones húmedas (Marcondes et al., 2020). Se han encontrado patrones similares en otras especies de aves y mamíferos que respaldanla idea de que la humedad y la temperatura son factores importantes que impulsan la evolución de los rasgos fenotípicos (Delhey, 2017; Marcondes et al., 2020; Malpica et al., 2022).

La regla de Bergmann se basa en la hipótesis de conservación del calor, que supone que el tamaño corporal de animales endotérmos es un factor limitante para la producción de calor, mientras que el área de superficie es un factor limitante para la disipación de calor (Fan et al., 2019). Bajo estos supuestos, Bergmann (1847) propuso una relación entre el tamaño corporal y las condiciones ambientales, donde el tamaño corporal aumenta con la latitud, y los individuos más pequeños se encuentran típicamente en latitudes más bajas donde los climas son generalmente más cálidos. La regla de Bergmann ha sido demostrada en copépodos marinos, peces, anfibios, mamíferos y aves (Fernández-Torres et al., 2018; Yu et al., 2019; Suzuki et al., 2020; Campbell et al., 2021). Específicamente en aves, la regla de Bergmann se ha demostrado en búhos del género Tyto, la urraca oriental (*Pica serica*) y el carbonero chino (*Parus minor*) (Fan et al., 2019; Romano et al., 2020a). Sin embargo, hay algunas excepciones que sugieren cambios en el tamaño corporal como resultado de bases genéticas o restricciones fisiológicas y no en respuesta al entorno o la geografía (Salewski et al., 2010; Kirchman & Schneider, 2014; Lee et al., 2021; Malpica et al., 2022).

La regla de Allen –una extensión de la regla de Bergmann– predice que los apéndices más pequeños ayudan a mantener una temperatura constante en ambientesmás fríos, mientras que los apéndices más grandes favorecen la pérdida de calor en climas más cálidos (Symonds & Tattersall, 2010). La regla de Allen se ha demostrado en ranas, mamíferos y aves (Alho et al., 2011; Fan et al., 2019; Alhajeri et al., 2020). En las aves, a menudo se utiliza la regla de Allen para explicar las variaciones en el tamaño delpico (Danner & Greenberg, 2015; Gardner et al., 2016).El pico es una estructura aislada y bien vascularizada, lo que sirve como una ventana térmica esencial para el intercambio de calor (Friedman et al., 2019; Ryeland et al., 2019). De manera similar, también se ha informado que la longitud del tarso influye en la disipación de calor (por ejemplo, Laiolo & Rolando, 2001; VanderWerf, 2012; Fan et al., 2019). Sin embargo, la regla de Allen no explica el tamaño de los apéndices en todas las especies estudiadas (p.ej., Malpica et al 2022), porque en los rasgos multifuncionales (p. ej., la longitud del pico) otras fuerzas selectivas como la ecología alimentaria pueden estar involucradas en la evolución morfológica (Olsen, 2017; Friedman et al. al., 2019).

La Hipótesis de Adaptación Acústica sugiere que las características espectrotemporales de las señales acústicas se adaptan a las condiciones locales para optimizar la transmisión de la señal en el entorno (Morton, 1975). En general, se espera que ocurra un fenomeno de atenuación. La atenuación se refiere a la pérdida de intensidad de una onda sonora, que puede ocurrir por el incremento de la distancia de la fuente sonora o por refracción (Bradbury & Vehrencamp, 2011). De acuerdo con la Hipótesis de Adaptación Acústica, la densidad de la vegetación atenúa las señales acústicas, con frecuencias más altasatenuándose más rápido que las frecuencias más bajas, la vegetación densa favorece las señales con frecuencias más bajas en general y las áreas abiertas favorecen las señales con frecuencias más altas (Acero-Murcia et al., 2021). Bajo esta hipótesis, también se espera que la atenuación por absorción aumente con la temperatura y se reduzca con el aumento de la humedad (Bradbury & Vehrencamp, 2011). La atenuación por absorción se refiere a la pérdida de energía que ocurre cuando el sonido entra en contacto con el medio (Bradbury & Vehrencamp, 2011). La hipótesis de adaptación acústica se ha utilizado para explicar variaciones en los rasgos acústicos de anuros, mamíferos y aves (p. ej., Ey & Fisher, 2009; Sebastián-González et al., 2018; Acero-Murcia et al., 2021). Muchos de los estudios publicados hasta la fecha explican patrones de divergencia en los rasgos fenotípicos utilizando algunas de las reglas ecológicas para la morfología y el color, o la hipótesis de adaptación acústica para vocalizaciones (p. ej., VanderWerf,2012; Malpica et al., 2022). Sin embargo, pocos estudios consideran todas estas reglas e hipótesis para explicar la variación en múltiples rasgos en un solo organismo.

La matraca del desierto (*Campylorhynchus brunneicapillus*) es un ave insectívora que habita los desiertos de California, Arizona, Nuevo México y Texas hasta la Península de Baja California, Chihuahua, Durango, Tamaulipas, Sonora, Sinaloa e Hidalgo en México, mostrando una marcada variación geográfica en la coloración y comportamiento (Howell y Webb 1995, Brewer y MacKay 2001). Según el tamaño corporal y la coloración del plumaje, algunas autoridades taxonómicas reconocieron de siete a ocho subespecies: *brunneicapillus*, *anthonyi*, *bryanti*, *affinis*, *guttatus*, *sandiegensis*, *seri* y *couesi* (Rea & Weaver, 1990; Gill et al. 2021; Hamilton et al. 2020) mientras que otros consideran el complejo como monotípico (Chesser et al. 2020). Selander (1964), Atwood et al. (2007) y Navarro-Sigüenza & Peterson (2004), con base en rasgos vocales y de coloración, sugieren que existen diferencias entre las poblaciones de la península de Baja Californiay las poblaciones continentales. En cuanto a la coloración, las aves continentales posen un parche pectoral de color negro y flancos más obscuros, mientras las aves peninsulares solo presentan un patrón bandeado en el área pectoral y los flancos de color beige (Hamilton et al. 2020). Para las vocalizaciones se ha sugerido la existencia de tres grupos: 1) Los desiertos de Sonora y Chihuahua, 2) La costa Sur de California y 3) La Península de Baja California. Siendo los individuos de Baja California los que emiten canciones con una frecuencia media más alta.

En la misma línea, un estudio molecular encontró diferencias genéticas, indicando la presencia de dos linajes dentro del complejo sugiriendo dos taxones: *C. affinis* endémica de la Península de Baja California y *C. brunneicapillus* nominal en el continente (Zink et al., 2001; Vázquez-Miranda, 2014;Vázquez-Miranda & Barker, 2021; Vázquez-Miranda et al., 2022). Por lo tanto, el debate taxonómico para la matraca del desierto aún está abierto y se necesitan estudios morfológicos y de comportamiento adicionales para aclarar los límites de las especies. Dichos estudios adicionales también deberían arrojar luz sobre nuestra comprensión delos factores ecológicos que promueven la divergencia en los rasgos morfológicos, de coloración y acústicos.

Aquí, estudiamos la variación geográfica en color, morfología y cantos en la matraca del desierto y su relación con los rasgos ecológicos. Caracterizamos la variación morfológica y acústica a lo largo de su distribución y analizamos la relación entre la variación morfológica y acústica con factores ambientales en el contexto de las reglas ecogeográficas de Gloger, Allen, Bergmann y la Hipótesis de Adaptación Acústica.

ECOLOGICAL FACTORS DRIVE THE DIVERGENCE OF MORPHOLOGICAL, COLOR AND BEHAVIORAL TRAITS IN THE CACTUS WREN (*CAMPYLORHYNCHUS BRUNNEICAPILLUS*).

Violeta Monserrath Andrade-González[1, 2], Hernán Vázquez-Miranda[1], Claudia Patricia Ornelas García[3], José Roberto Sosa-López[4]

[1]Colección Nacional de Aves, Departamento de Zoología, Instituto de Biología, Universidad Nacional Autónoma de México, Ciudad de México C.P. 04510, México.

[2]Posgrado en Ciencias Biológicas, Universidad Nacional Autónoma de México, Ciudad de México C.P. 04510, México.

[3]Colección Nacional de Peces, Departamento de Zoología, Instituto de Biología, Universidad Nacional Autónoma de México, Ciudad de México C.P. 04510, México.

[4]Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional Unidad Oaxaca (CIIDIR), Instituto Politécnico Nacional, Santa Cruz Xoxocotlán, Oaxaca, México.

Authors for correspondence: H. Vázquez-Miranda: hernan@ib.unam.mx; J.R. Sosa-López: jrsosa@ipn.mx

J.R. Sosa-López and H. Vázquez Miranda should be considered joint senior authors.

ORCID

V.M. Andrade-González: 0000-0002-8022-0470

H. Vázquez-Miranda: 0000-0002-4275-6979

C.P. Ornelas-García: 0000-0003-3163-448X

J.R. Sosa-López: 0000-0002-0120-0704

The study of ecological mechanisms influencing organisms’ phenotypic variation is a central subject of evolutionary biology. In this study, we characterised morphological, colour, and acoustic variation in cactus wrens *Campylorhynchus brunneicapillus* throughout its distribution. We assessed whether Gloger’s, Allen’s, and Bergmann’s ecogeographic rules, and the Acoustic Adaptation Hypothesis explain geographic trait variation. We analysed specimen colouration in belly and crown plumage, beak geometric morphometry, and structural song characteristics. We tested whether the subspecific classification or the peninsular/mainland groups mirrored the geographic variation in phenotypes and whether ecological factors explained patterns of trait variation. Our results suggest that morphological, colour and acoustic traits varied across the distribution range of the species complex, corresponding to two lineages described by genetics. The complex and simple versions of Gloger´s and Allen´s rules explained variations in colour traits and morphology between the peninsular/mainland groups. Conversely, Bergmann´s rule did not support patterns of phenotypic variation. The Acoustic Adaptation Hypothesis supported song divergence for frequency-related traits. Variation in phenotypic traits suggests the existence of two taxa (*C. affinis* in the Baja California peninsula and *C. brunneicapillus* in the mainland). The ecological factors explain phenotypic trait adaptations, suggesting that divergence between lineages could result from ecological selection.

Keywords: Ecological divergence, morphometrics, bioacoustics, speciation, songs, vocalization

1. Introduction

Understanding the role of ecological traits on speciation as a result of local adaptation to divergent environments is a fundamental question in biology [1,2] . According to Ecological Speciation, divergence occurs when disruptive selection acts on phenotypes that convey a fitness advantage in one environment but not another [3]. Thus, the ecological divergence between populations arises due to habitat structure, climate, resource differences, and the presence of both predators and competitors [2,4]. In birds, studies that shown divergent ecologies acting on colour, morphology, and song [5,6], refer to Gloger's, Bergmann's and Allen's rules for morphological traits or the acoustic adaptation hypothesis for vocalizations to explain geographical patterns of phenotypic variation [e.g. 7-11].

Gloger's rule explains spatial variation patterns in endothermic animals' coloration [12]. Particularly, it refers to patterns of achromatic variation (i.e., variation from pale to dark colour, commonly related to the deposition of melanin pigments) [12]. It predicts that the dark colour in endothermic animals arises in warm and humid regions, while paler colourations appear in animals that inhabit dry and warm areas [12], as seen in multiple mammals and birds [9,13-15]. Darker pelage and plumage in warm and humid environments should benefit from greater protection against feather-degrading bacteria due to increased melanin deposits [16]. Likewise, darker colouration might be favoured by camouflage in dense vegetation, which is also associated with warmer and humid conditions [17]. According to Gloger's rule, temperature and humidity must act together; however, either humidity or temperature can drive the pattern [12]. Rensch (1929) reformulated this rule suggesting a complex version to evaluate the climatic effects on the two types of melanin: eumelanin and pheomelanin. Eumelanin increases with humidity, decreases only at shallow temperatures and is responsible for black and white colours. While pheomelanin prevails in dry and warm regions, decreasing rapidly with low temperatures, it is responsible for reddish rusty colours [12]. The complex version of Gloger's rule has been tested in birds and mammals, showing similar patterns of phenotypic variation [9,14]. For instance, male populations of the variable antshrike (*Thamnophilus caerulescens*) with an underpart rufous colouration are found in warm regions, while males with white, black and grey are found in humid regions [9]. These studies support the notion of humidity and temperature as important factors driving phenotypic trait evolution in animals [9,11,18].

Bergmann's rule is based on the Heat Conservation Hypothesis, which assumes that endothermic animal volumes are a limiting factor for heat production, while surface area is a limiting factor for heat dissipation [7]. Under these assumptions, Bergmann (1847) proposed a relationship between body size and environmental conditions, where body size increases with latitude, with the smallest individuals typically found at lower latitudes where climates are generally warmer. Bergmann's rule has been shown in marine copepods, fishes, amphibians, mammals, and birds [19-22] Specifically, in birds, Bergmann´s rule has been evident in *Tyto* owls [23], the oriental magpie (*Pica serica*), and the oriental tit (*Parus minor*) [7]. However, some exceptions suggest changes in body size stem from genetic bases or physiological constraints and not in response to the environment or geography [11,24].

Allen's rule –an extension of Bergmann's rule– predicts that smaller appendages help to maintain constant temperatures in colder environments, while more prominent appendages favour heat loss in warmer climates [25]. Allen´s rule is evident in frogs, mammals and birds [7,26,27]. In birds, Allen's rule is often invoked to explain variations in beak size [28]. Beaks are insulated and well vascularized, serving as an essential thermal window for heat exchange [29]. Similarly, tarsus length has also been reported to influence heat dissipation [e.g. 7,30]. However, Allen's rule does not explain appendages size in all species [e.g. 11] because in multifunctional traits (e.g., beak length), other selective forces, such as feeding ecology, may be involved in morphological evolution [29,31].

The Acoustic Adaptation Hypothesis suggests that spectro-temporal characteristics of acoustic signals would adapt to local conditions to optimize signal transmission across the environment [32]. Overall, the density of the vegetation is expected to attenuate acoustic signals, with higher frequencies attenuating faster than lower frequencies, dense vegetation favouring signals with overall lower frequencies, and open areas favouring signals with higher frequencies [10]. Under this hypothesis, attenuation by absorption is expected to increase with temperature and reduce with increasing humidity [33]. This hypothesis has been used to explain variations in acoustic traits of anurans, mammals and birds [e.g. 10,34,35]. Many studies explain patterns of divergence in phenotypic traits using some of the ecological rules for morphology and colour or the Acoustic Adaptation Hypothesis for vocalizations [e.g.11,30]. However, to our knowledge, no study considers all these rules and hypotheses to explain variation simultaneously in multiple traits.

The cactus wren (*Campylorhynchus brunneicapillus*) is a highly vocal, insectivorous songbird that inhabits most deserts from the southern United States to central Mexico, showing marked geographic variation in morphology and behaviour [36,37]. Based on plumage colouration and size, some taxonomic authorities recognized seven to eight subspecies: *brunneicapillus*, anthonyi, *bryanti*, *affinis, guttatus*, *sandiegensis*, *seri* and *couesi* [38,39,40]. Others consider the complex as monotypic [41]. Based on vocal and colour traits there are differences between the populations of the Baja California peninsula and the continental populations [42,43,44]. Along those lines, molecular studies found fixed genetic differences indicating the presence of two lineages: one on the Baja California Peninsula and the other in the continent [45-48]. Thus, behavioural and morphological data could shed light on our understanding of ecological factors promoting divergence in phenotypic traits and assist in species limit clarification, helping to solve taxonomic controversies.

Here, we study the geographic variation in colour, morphology and songs in the cactus wren and their relationship to ecological traits. We characterised morphological and acoustic variation throughout its distribution and analysed the relationship between morphological and acoustic variation with environmental factors in the context of the ecogeographic rules of Gloger, Allen, Bergmann and the Acoustic Adaptation Hypothesis.

2. Methods

(a) Specimens and general photography procedure

We photographed 88 specimen skins (34 females and 54 males) for colouration analysis and 73 specimens (29 females and 44 males) for beak geometric morphometrics, discarding juvenile individuals of both sexes (for specimen information, electronic supplementary material Table S1 on Appendix A). Digital photography yields equivalent colour metrics to traditional spectrometric data [49]. We evaluated the colour and morphology from photographs using a Nikon D3500 camera with an 18-55 mm lens. For beak macro pictures, we mounted the lens on a 20mm extension tube (Neewer, 10037281@@001, UK). We took pictures using the maximum zoom, ISO400 and focal aperture *f*22 in NEF format. We photographed museum skins against a white background, with a ruler in mm and four lamps (Lumicon 3W full spectrum fluorescent bulbs; 5500K, >95CRI). We tested lighting environment uniformity with a colour calibration target X-Rite ColorChecker Passport (Calibrite CCPP2, USA).

We took pictures of the dorsal and ventral plumage for colour analyses. For morphological analyses, we photographed the beak's dorsal and lateral projections. We took specimen left and right-side pictures aligning the centre of the eye and the tip of the beak to a horizontal line. We found asymmetry for the dorsal view (see Results below), thus only considering the right lateral side.

(b) Colour variables

We calculated feather patch colour scores using the values of the long wavelength (red), medium wavelength (green), and short wavelength (blue) using ImageJ 1.53k [50]. Briefly, we selected a polygon in each plumage patch to calculate the mean values for Red-Green-Blue (RGB) using a scale of 0 to 350 randomly chosen pixels within the select polygon. We converted the RGB data into a visual system-independent opponent colour space that separated hue and luminance [49]. We converted RGB values into two new hue values: red-green channel (R-G)/(R+G) to represent the reddish colouration and green-blue channel (G-B)/(G+B) for dark colouration. We used R+G+B as the brightness variable.

(c) Morphological traits

We measured 10 linear traits [51]: tarsal length, tarsal diameter, wing length, beak length, beak length proximal and distal to nostril, beak depth proximal and distal to the nostril and beak width proximal and distal to nostril (definitions and error measurement in electronic supplementary material Appendix A).

We took linear measurements using a digital calliper (MITUTOYO, 500-196-30, USA) with a precision of 0.01 mm, following [51].

(d) Beak geometrics morphometric

We generated a TPS file for each beak projection using the software tpsUtil 1.78 [52]. Then, using the dorsal view of the beak, we set a landmark on the tip, a landmark in the middle of the beak close to the skull and two landmarks on the left and right side of the beak close to the lores. We included nine semi-landmarks on each side of the bill (electronic supplementary material Figure S1 on Appendix A,). For lateral views, we placed three landmarks on the upper mandible: one at the tip of the beak, the second near the forehead, and the third near the lore. We included nine semi-landmarks in each curve. Landmarks and semi-landmarks were set using the software tpsDig 2 ver. 2.31 [53]. To rotate, translate and scale the landmarks and semi-landmarks we used a Generalized Procrustes Analysis, with the function '*gpagen*' on geomorph [54]. We performed an allometric analysis computing the residuals from the regression of shape on centroid size to remove the influence of size on beak shape [55]. We also performed an object symmetry analysis on the beak dorsal view to separate symmetric and asymmetric components [56].

(e) Recording sampling and acoustic measurements

We obtained 108 recordings from sound repositories (electronic supplementary material Table S2 on Appendix A). We selected recordings of cactus wrens´ songs with a high signal-to-noise ratio. Female cactus wrens' songs are weak, higher pitched and uncommon [57]; thus, we assume that the songs included in the analyses came from males as they sing more often, and their songs are of higher intensity. We randomly chose up to five songs per male, obtaining a total of 89 songs. We extracted 12 structural song variables: number notes, rhythm, duration, minimum frequency, maximum frequency, bandwidth, centre frequency, aggregate entropy, and 5%, 25%, 75%, and 95% frequencies (definitions in electronic supplementary material Appendix A). We used the threshold method set at -20dB to extract frequency-related measurements (electronic supplementary material Appendix A) [58]. We used Raven Pro 1.6.1 [59] to generate spectrograms using a Fournier Fast Transformation (FFT) size = 512, 11.6 ms resolution, 80% overlap and Hanning window.

(f) Environmental and forest cover variables

We used georeferenced museum specimens' records and acoustic data to retrieve environmental variables from the WorldClim Global Climate database at a 30 arc-second resolution [60]. We extracted the information on annual mean temperature (bio1) and annual mean precipitation (bio12). We used the normalized differential vegetation index (NDVI) as forest cover, with large values indicating denser vegetation cover [61]. Using the recording year, we obtained NDVI maps from Climate (http://ClimateEngine.org) [62] from April-June, corresponding to the breeding season [40]. We extracted climatic and forest cover variables using QGIS 3.6.3 [63].

(g) Statistical analyses

We performed all statistical analyses in R 4.1.2 [64]. We tested whether sexes differ in colouration and morphology using a t-student test. For the geometric morphometric data, we performed a sexual dimorphism test using MANOVA procrustes with '*procD.lm'* function in geomorph [54]. As we found that sexes differ in size-related measurements (see Results below), subsequent analyses were conducted by sex on these variables.

We assessed whether patterns of phenotypic geographic variation mirrored subspecies classification or the two peninsular/mainland groups using general linear models (GLM) for normally distributed variables (darkness, redness, brightness, number notes, 5% and 25% frequencies, centre frequency and aggregate entropy) and Kruskal Wallis tests for variables that did not meet the normality assumption (morphometric variables, duration, rhythm, minimum frequency, maximum frequency, bandwidth, and 75% and 95% frequencies). We included colour values, morphometric and acoustic measurements as response variables, and the subspecies or the peninsular/mainland groups as fixed factors. We assessed whether ecogeographical rules explained colour and morphological variation using GLM. We included as response variables: redness, darkness, and brightness for Gloger´s; tarsus length for Allen´s rule; and wing length for Bergmann´s rule. We included as fixed factors temperature and precipitation. We tested the acoustic adaptation hypothesis using an identity-link Gaussian generalized linear model (GLIM) using as response variables the 12 vocal variables and the NDVI index, temperature and precipitation as fixed effects. For all GLMs and GLIMs, we conducted one analysis per independent variable using Bonferroni correction for pairwise comparisons. We used '*glm*' function in stats [65].

We tested whether the temperature is related to beak size with geometric morphometric data using GLIM. We included as response variable the log-centroid size and mean annual temperature as fixed effect. We conducted a permutation-based multivariant analysis of variance (PERMANOVA) for the shape analysis using '*adonis2*' function in vegan [66]. We included Procrustes coordinates as the response variable and temperature as the explicative variable.

3. RESULTS

(a) Sexual dimorphism

Males and females did not show sexual dimorphism in colour traits at the subspecies level or peninsular/mainland groups (electronic supplementary material Table S1, S2 on Appendix B). For morphological traits, we found that at the subspecies level, there was sexual dimorphism in some variables for some subspecies (electronic supplementary material Table S3 on Appendix B). Both peninsular/mainland groups, males and females varied in morphological traits in wing length, tarsus diameter and beak depth proximal and distal to the nostril. For the peninsular group, sexes varied in beak length and for the mainland group in tarsus length (electronic supplementary material Table S4 on Appendix B).

(b) Geographic variation

Our analyses showed that darkness and redness in ventral and crown areas significantly differed between subspecies and peninsular/mainland groups (electronic supplementary material Table S5 on Appendix B). Pairwise comparisons showed that ventral patch in *C. b. guttatus* was significantly darker than *C. b. bryanti*, while crown patch of *C. b. affinis* was significantly redder than *C. b. brunneicapillus* and *C. b. guttatus* (electronic supplementary material Figure S1 on Appendix B). All other subspecies were not significantly different in any of the colour variables. For peninsular/mainland groups, our results showed that ventral feather colouration was darker in the mainland, while the crown was darker, redder and brighter in the peninsula.

Between subspecies and peninsular/mainland groups tarsus length varied in both sexes (electronic supplementary material Figure S2 on Appendix B). Pairwise comparisons showed that tarsus length was longer in *C. b. affinis* than in *C. b. guttatus.* We found no significant differences in all other subspecies in any other morphometric variables. For peninsular/mainland groups, tarsus length showed significant differences between males and females, with the peninsular group having a longer tarsus. Only peninsular males had longer beaks than mainland males, while females had significant differences in beak proximal and distal-to-nostril length. Specifically, mainland females had deeper beak proximal and distal-to-nostril length (electronic supplementary material Table S6 on Appendix B).

Beak geometric morphometry showed directional asymmetry (difference between left and right sides; electronic supplementary material Table S7 on Appendix B), and the association between centroid size and shape of the beak was significant for both views analysed (electronic supplementary material Table S8 on Appendix B). This result implies that the allometric component influenced the beak shape; thus, the subsequent analyses were performed with residuals. The shape and centroid size did not show significant differences in subspecies or peninsular/mainland groups for the dorsal view. For the lateral view, the centroid size showed significant differences between peninsular/mainland groups and among *C. b. affinis*, *C. b. brunneicapillus* and *C. b. guttatus.* The beak shape for the lateral view was close to being significantly different among peninsular/mainland groups and was significant by subspecies, showing differences between *C. b. seri* and *C. b. affinis*, *C. b. guttatus* and *C. b. bryanti* (Table 1).

Cactus wren songs varied throughout their range (electronic supplementary material Figure S3 on Appendix B). Acoustic variables that differed significantly between subspecies were the number of notes, rhythm, lower frequency and bandwidth. Particularly, *C. b. affinis* had the lowest number of notes than *C. b. anthonyi* and *C. b. brunneicapillus*. The rhythm was lower in *C. b. bryanti* than *C. b. anthonyi* and was close to being significantly different from *C. b. brunneicapillus*. Songs of *C. b. affinis* had a lowest minimum frequency than *C. b. anthonyi* and *C. b. brunneicapillus*, whereas *C. b. affinis* had songs with greater bandwidth than *C. b. anthonyi* and *C. b. brunneicapillus*. Results did not show differences between subspecies considering the other variables. Peninsular and mainland groups' songs differed in the number of notes, duration, rhythm, and lower frequency. The peninsular group had lower number of notes, less duration, less rhythm, lower minimum frequency and greater bandwidth than mainland songs. We found no significant differences in the other acoustic variables (electronic supplementary material Table S9, S10 on Appendix B).

(c) Gloger´s rule

We assessed darkness, redness and brightness plumage colour variation in response to annual mean temperature and annual mean precipitation throughout the cactus wren distribution (Table 2; Figure 2). We found a negative correlation between darkness and redness colouration in the belly with temperature. We also found a positive association between darkness and redness with precipitation and a negative association with brightness. Thus, birds were darker in rainier areas. For the crown surface, we found a positive association between brightness and temperature but no association with precipitation. We found no relationship between darkness or redness and temperature or precipitation (electronic supplementary material Figure S4 on Appendix B).

(d) Allen´s rule

Our results showed a positive association between annual mean temperature and tarsus length for males and was close to being significantly in females, suggesting that male birds inhabiting warm areas have the longest tarsi (Table 3, Figure 3). For beak length, we found no association with temperature (electronic supplementary material Figure S5 on Appendix B). For the dorsal and lateral views of the bill, the size and shape were not associated with temperature (Table 4).

(e) Bergmann´s rule

We found no evidence for Bergmann's rule in both sexes. The wing length was not related to temperature or latitude (Table 3; electronic supplementary material Figure S6 on Appendix B).

(f) Acoustic Adaptation Hypothesis

We found a negative relationship between the minimum frequencies of the songs and temperature, suggesting that birds inhabiting places with higher temperatures sing songs with lower minimum frequencies (Table 5; Figure 4). Nevertheless, we found no association between the temperature, precipitation and NDVI index whit the maximum frequency, bandwidth, duration, rhythm and number of notes (electronic supplementary material Figure S7 on Appendix B).

4. Discussion

We found significant geographic variation in multiple traits in cactus wrens. In our subspecies analysis, most of the significant differences between subspecies corresponded to their geographic location, either in Baja California or the mainland in seven traits (colour traits: *C. b. affinis* vs *C. b. brunneicapillus*/*C. b. guttatus*; morphology: *C. b. affinis* vs *C. b. brunneicapillus*/*C. b. guttatus*; songs: *C. b.affinis* vs *C. b. anthonyi*/*C. b. brunneicapillus*). While in the comparison analysis between the peninsular and mainland groups, we found significant differences in 11 variables. Congruent variation in multiple traits has been shown in multiple species of dragonflies, butterflies, fishes, birds, lizards, snakes and bats suggesting that such variation is the result of isolation events [67-74]. For instance, a similar phylogeographic pattern has also been reported to occur in other birds, mammals, amphibians and reptiles across Baja California [45-48,75,76,77]. A possible explanation is that diversification might be the product of an expansion event throughout the peninsula and the subsequent formation of a mid-peninsular seaway that joined the Gulf of California and the Pacific Ocean in what is today the Vizcaino Desert one million years ago [45-48,75,78]. Our results correspond to findings in recent molecular studies suggesting two distinct evolutionary lineages within the complex (the mainland lineage: *brunneicapillus*, *anthonyi* and *guttatus*; and the peninsular lineage: *bryanti* and *affinis*) [45-48].

We found that the annual mean precipitation was negatively associated with the ventral brightness. These results are consistent with Gloger´s rule, which in its simple version indicates a negative correlation between brightness, temperature and precipitation [12]. The complex Gloger´s rule has been explored insufficiently in birds and mammals. The few studies to date support patterns of geographic colour variation in crimson rosella (*Platycercus elegan*)[15], the variable antshrike (*Thamnophilus caerulescens*)[9], Furnariidae [8], Meliphagidae and Acanthizidae [79], Strigiformes [80], and feral pigs (*Sus scrofa*) [14]. A possible explanation in birds is that in warm and wet environments, the dark feathers and pelage confer resistance to microbial degradation as melanin increases the abrasion resistance and keratin thickness [16,81]. Darker colours are also selected in low-light environments due to dense vegetation favouring crypsis [8,81,82]. While under the complex Gloger´s rule, reddish might be favoured in drier climates as redder soils tend to be more intense in this climate, also favoured by cryptosis [8]. Future studies should disentangle which mechanisms explain plumage colouration in cactus wrens.

According to Bergmann's rule, larger bodies are associated with colder environments [83]. We found no relationship between wing length and annual mean temperature or latitude, providing no support for this rule. Evidence for Bergmann´s rule is contradictory in birds, mammals and insects [e.g.

7,11,24,84-85]. A review study that considered the body geographic variation in 92 species of North American birds revealed that 42% of these species supported Bergmann´s rule, 12% provided weak or ambiguous support, and 16% showed no association between latitude or latitude. In contrast, 29% of the species contradicted the rule [86]. Furthermore, body size and temperature were associated in 3561 mammal species when the phylogenetic structure was considered, suggesting that the Bergmann's rule only applies to closely related species [87]. Thus, the available evidence suggest that thermoregulation does not explain overall patterns of variation in body size in birds, and other selective forces might govern the evolution of this trait. For instance, it is thought that body size could be restricted by evolutionary history, shaped by community ensembles through interspecific competition for recourses [88,89,90] or through sexual selection as this trait is related to aggressiveness in intrasexual competition (i.e., for territory defence) [91,92].

We found that the tarsus increases with temperature in males, but there is no significant relationship between temperature and beak size. According to Allen´s rule, shorter appendages are associated with colder environments [83]. Thus, our results suggest that thermoregulation might be a selective force determining leg length, as has been proposed for other birds (*Pica serica* and *Parus minor*) [7], frogs (*Rana temporaria*)[27], and rodents [26]. A possible explanation in birds is the vascular anatomy and physiological regulation of limb heat loss [25]. An experimental study found that heat loss can occur through the legs [25,94]. Research shows that tibial veins are strikingly more swollen during heat stress in herons and gulls, probably due to blood flow travelling backwards to the central arteries surrounded by veins, resulting in heat from the artery blood being able to be recovered by the veins surrounding the central arteries [25,93-95]. Similarly, other studies do not support Allen´s rule for explaining geographic variation in beak size [11,24]. Multiple selective pressures might determine the shape and size of the beak [96] because it is a multifunctional trait used to sing [97], in thermoregulation [25], and foraging [98], but also phylogenetic relationships [29]. In addition, diet has been associated with cranial morphology and beak shape or size [29,31], and it is likely that this may also play a role in determining beak shape or size in wrens as primarily insectivorous species [40,99].

We found a negative relationship between mean annual temperature and lower minimum frequency in songs. Our findings are in line with sound transmission's properties: higher frequencies are attenuated faster under higher atmospheric temperatures, producing refraction [33]. Signal refraction results in sound attenuation and occurs when warm ground heats the adjacent air layer, generating an air gradient with decreasing temperatures. Within this gradient, sound travels at different speeds (with the sound usually moving faster in warm layers), causing propagation sound trajectory to bend upwards [33,100]. Experimental playback studies could help elucidate whether refraction could explain divergent low frequencies.

We did not find a relationship between mean annual precipitation and NDVI and other structural characteristics of the song. A similar study in another *Campylorhynchus* wren showed no evidence of ecological variables affecting song structure and found that cultural drift explained variation in acoustic traits [72]. Other selective pressures could likely be acting on shaping cactus wren songs, such as sexual selection [101,102]. For instance, the lack of evidence for the acoustic adaptation hypothesis in other song traits could be attributed to morphological constrains: birds with larger beaks producing lower rhythm songs [103,104]. Our results indicate that males in the peninsula had the largest beaks and sang slower-paced songs, so this hypothesis is plausible. However, further analyses assessing beak's morphology and cactus wren songs are necessary to further explore this correlation.

5. Conclusion

Our results show a relationship between plumage colour, morphology, song and climatic factors with darker and brighter ventral patches associated with sites with higher precipitation and redder crowns in sites whit higher temperature (supporting Gloger´s rule), larger tarsus in sites with higher temperature (supporting Allen´s rule), and songs with lower frequencies in sites with higher temperatures (supporting the Acoustic Adaptation Hypothesis). These findings suggest that divergent environments play an essential role in the cactus wren's phenotypic differentiation [2,4,105]. We suggest that the divergence of these characters is the result of local adaptation to divergent environments promoting reproductive isolation [2,106]. Furthermore, our results based on colour, morphological, and acoustic data mirror previous genetic analysis, supporting the existence of two evolutionary lineages within the cactus wren complex that likely represent two separate species: *C. affinis* in Baja California peninsula and *C. brunneicapillus* on the mainland with significant implications for conservation in Mexico [44,45-48].

Data accessibility. Data have been deposited in https://figshare.com/s/5bdd1ffeece070f8566e FOR REVIEWING PURPOSES ONLY. PLEASE DO NOT DISTRIBUTE. Datasets supporting this article will be released upon acceptance.

Authors´ contributions. V.M.A.G., H.V.M. and J.R.S.L.: conceptualization, investigation, visualization, project administration, validation, writing—original draft, funding acquisition; V.M.A.G: data curation; V.M.A.G., C.P.O.G., H.V.M. and J.R.S.L: methodology, formal analysis writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Conflict of interest declaration. Authors declare no competing interests

Funding. V.M.A.G. was supported by a Graduate School Fellowship and a Research Assistantship from

UNAM (DGAPA-PAPIIT IA205422 #332622). H.V.M. was supported by UNAM (DGAPA-PAPIIT IA204220 & IA205422). J.R.S.L. was supported by IPN Research and Postgraduate Secretariat (SIP 20220208). Additional financial support was provided through a Frank M. Chapman Memorial Fund from the American Museum of Natural History research grant to V.M.A.G.

Acknowledgements. This paper serves as a fulfilment of VMAG for obtaining a M.Sc. degree in the Posgrado en Ciencias Biológicas UNAM. We thank P. Escalante from Instituto de Biología, Universidad Nacional Autónoma de México (UNAM) and A. Gordillo and A. Navarro from Facultad de Ciencias, UNAM for allowing us access to specimens and acoustic information in their care. Biblioteca de Sonidos de Aves de México, The Macaulay Library at Cornell Lab of Ornithology, The Borror Laboratory of bioacoustics, Florida Museum of Natural History and Xeno-Canto granted us access to song recordings. Frank M. Chapman Memorial Fund of the American Museum of Natural History for financial support.

References

1. Neto, J. M., Gordinho, L., Belda, E. J., Marín, M., Monrós, J. S., Fearon, P., & Crates, R. (2013). Phenotypic Divergence among West European Populations of Reed Bunting Emberiza schoeniclus: The Effects of Migratory and Foraging Behaviours. PLoS ONE, 8(5).

2. Rundle, H. D., & Nosil, P. (2005). Ecological speciation. Ecology Letters, 8(3), 336–352.

3. Shafer, A. B. A., & Wolf, J. B. W. (2013). Widespread evidence for incipient ecological speciation: A meta-analysis of isolation-by-ecology. Ecology Letters, 16(7), 940–950.

4. Schluter, D., & Conte, G. L. (2009). Genetics and ecological speciation. Proceedings of the National Academy of Sciences, 106, 9955-9962.

5. Fernández Gómez, R. A., Morales-Mávil, J. E., & Sosa-López, J. R. (2020). Geographic variation and divergence of songs in the Olive Sparrow species complex. Journal of Field Ornithology, 91(1), 77–91.

6. Simpson, R. K., & McGraw, K. J. (2018). Multiple signaling in a variable environment: expression of song and color traits as a function of ambient sound and light. Biotropica, 50(3), 531–540.

7. Fan, L., Cai, T., Xiong, Y., Song, G., & Lei, F. (2019). Bergmann's rule and Allen's rule in two passerine birds in China. Avian Research, 10(1), 1–11.

8. Marcondes, R. S., Nations, J. A., Seeholzer, G. F., & Brumfield, R. T. (2021). Rethinking Gloger's rule: Climate, light environments, and color in a large family of tropical birds (Furnariidae). The American Naturalist, 197(5), 592-606.

9. Marcondes, R. S., Stryjewski, K. F., & Brumfield, R. T. (2020). Testing the simple and complex versions of Gloger's rule in the Variable Antshrike (*Thamnophilus caerulescens*, Thamnophilidae). The Auk,

137(3), ukaa026.

10. Acero-Murcia, A. C., Raposo do Amaral, F., de Barros, F. C., da Silva Ribeiro, T., Miyaki, C. Y., & Maldonado-Coelho, M. (2021). Ecological and evolutionary drivers of geographic variation in songs of a Neotropical suboscine bird: The Drab-breasted Bamboo Tyrant (Hemitriccus diops, Rhynchocyclidae). Ornithology, 138(2).

11. Malpica, A., Mendoza-Cuenca, L., & González, C. (2022). Color and morphological differentiation in the Sinaloa Wren (Thryophilus sinaloa) in the tropical dry forests of Mexico: The role of environment and geographic isolation. Plos One, 17(6), e0269860.

12. Delhey, K. (2019). A review of Gloger's rule, an ecogeographical rule of colour: Definitions, interpretations and evidence. Biological Reviews, 94(4), 1294-1316.

13. Cerezer, F. O., Ribeiro, J. R. I., Graipel, M., & Cáceres, N. C. (2020). The dark side of coloration: Ecogeographical evidence supports Gloger's rule in American marsupials. Evolution, 74(9), 2046–2058.

14. Newell, C., Walker, H., & Caro, T. (2021). Pig pigmentation: testing Gloger's rule. Journal of Mammalogy, 102(6), 1525–1535.

15. Ribot, R. F. H., Berg, M. L., Schubert, E., Endler, J. A., & Bennett, A. T. D. (2019). Plumage coloration follows Gloger's rule in a ring species. Journal of Biogeography, 46(3), 584–596.

16. Burtt, J. E. H., & Ichida, J. M. (2004). Gloger 's rule, feather-degrading bacteria, and color variation among song sparrows. The Condor, 106, 681–686.

17. McNaught, M. K., & Owens, I. P. F. (2002). Interspecific variation in plumage colour among birds: Species recognition or light environment? Journal of Evolutionary Biology, 15(4), 505–514.

18. Delhey, K. (2018). Darker where cold and wet: Australian birds follow their own version of Gloger's rule. Ecography, 41(4), 673-683.

19. Campbell, M. D., Schoeman, D. S., Venables, W., Abu-Alhaija, R., Batten, S. D., Chiba, S., Coman, F., Davies, C. H., Edwards, M., Eriksen, R. S., Everett, J. D., Fukai, Y., Fukuchi, M., Esquivel Garrote, O., Hosie, G., Huggett, J. A., Johns, D. G., Kitchener, J. A., Koubbi, P. & Richardson, A. J. (2021). Testing Bergmann's rule in marine copepods. Ecography, 44(9), 1283–1295.

20. Fernández-Torres, F., Martínez, P. A., & Olalla-Tárraga, M. Á. (2018). Shallow water ray-finned marine fishes follow Bergmann's rule. Basic and Applied Ecology, 33(2017), 99–110.

21. Suzuki, T. A., Martins, F. M., Phifer-Rixey, M., & Nachman, M. W. (2020). The gut microbiota and Bergmann's rule in wild house mice. Molecular Ecology, 29(12), 2300–2311.

22. Yu, T. L., Wang, D. L., Busam, M., & Deng, Y. H. (2019). Altitudinal variation in body size in Bufo minshanicus supports Bergmann's rule. Evolutionary Ecology, 0123456789.

23. Romano, A., Séchaud, R., & Roulin, A. (2021). Generalized evidence for Bergmann's rule: body size variation in a cosmopolitan owl genus. Journal of Biogeography, 48(1), 51-63.

24. Lee, C. C., Fu, Y., Yeh, C. fen, Yeung, C. K. L., Hung, H. yi, Yao, C. J., Shaner, P. J. L., & Li, S. H. (2021). Morphological variations in a widely distributed Eastern Asian passerine cannot be consistently explained by ecogeographic rules. Ecology and Evolution, 11(21), 15249–15260.

25. Symonds, M. R. E., & Tattersall, G. J. (2010). Geographical variation in bill size across bird species provides evidence for Allen's rule. American Naturalist, 176(2), 188–197.

26. Alhajeri, B. H., Fourcade, Y., Upham, N. S., & Alhaddad, H. (2020). A global test of Allen's rule in rodents. Global Ecology and Biogeography, 29(12), 2248–2260.

27. Alho, J. S., Herczeg, G., Laugen, A. T., Räsänen, K., Laurila, A., & Merilä, J. (2011). Allen's rule revisited: Quantitative genetics of extremity length in the common frog along a latitudinal gradient. Journal of Evolutionary Biology, 24(1), 59–70.

28. Danner, R. M., & Greenberg, R. (2015). A critical season approach to Allen's rule: Bill size declines with winter temperature in a cold temperate environment. Journal of Biogeography, 42(1), 114–120.

29. Friedman, N. R., Miller, E. T., Ball, J. R., Kasuga, H., Remeš, V., & Economo, E. P. (2019). Evolution of a multifunctional trait: Shared effects of foraging ecology and thermoregulation on beak morphology, with consequences for song evolution. Proceedings of the Royal Society B: Biological Sciences, 286(1917).

30. VanderWerf, E. A. (2012). Ecogeographic patterns of morphological variation in elepaios (Chasiempis spp.): Bergmann's, Allen's, and Gloger's rules in a microcosm. Ornithological Monographs, 73(1), 1-34.

31. Olsen, A. M. (2017). Feeding ecology is the primary driver of beak shape diversification in waterfowl. Functional Ecology, 31(10), 1985–1995.

32. Morton, E. S. (1975). Ecological Sources of Selection on Avian Sounds. The American Naturalist, 109(965), 17–34.

33. Bradbury, J. W., & Vehrencamp, S. L. (2011). Principles of animal comunication. ed. Sunderland, Mass.: Sinauer.

34. Ey, E., & Fischer, J. (2009). The "acoustic adaptation hypothesis"—a review of the evidence from birds, anurans and mammals. Bioacoustics, 19(1-2), 21-48.

35. Sebastián-González, E., van Aardt, J., Sacca, K., Barbosa, J. M., Kelbe, D., & Hart, P. J. (2018). Testing

the acoustic adaptation hypothesis with native and introduced birds in Hawaiian forests. Journal of Ornithology, 159(3), 827–838.

36. Howell, S.N.G. & S. Webb. (1995). A guide to the birds of Mexico and Northern Central America. Oxford University Press. New York, EUA.

37. Brewer, D. & K. MacKay. (2001). Wrens, Dippers and Thrashers. Yale University Press. New Haven, Connecticut, EUA.

38. Rea, A., & Weaver, K. (1990). The Taxonomy, Distribution, and Status of Coastal California Cactus Wrens. Western Birds, 21(3), 81–126.

39. Gill F, D Donsker & P Rasmussen (Eds). 2021. IOC World Bird List (v11.1). doi : 10.14344/IOC.ML.11.1.

40. Hamilton, R. A., G. A. Proudfoot, D. A. Sherry, & S. L. Johnson (2020). Cactus Wren (*Campylorhynchus brunneicapillus*), version 1.0. In Birds of the World (A. F. Poole, Editor). Cornell Lab of Ornithology, Ithaca, NY, USA.

41. Chesser, R. T., S. M. Billerman, K. J. Burns, C. Cicero, J. L. Dunn, B. E. Hernández-Baños, R. A. Jiménez, A. W. Kratter, N. A. Mason, P. C. Rasmussen, J. V. Remsen, Jr., D. F. Stotz, & K. Winker. (2022). Check-list of North American Birds (online). American Ornithological Society. https://checklist.americanornithology.org/taxa/

42. Selander, R. K. (1964). Speciation in wrens of the genus Campylorhynchus. Univ. Calif. Pub. Zool., 74, 1-305.

43. Atwood, J. L., & Lerman, S. (2007). Geographic variation in cactus wren songs. Western Birds, 38, 29-46.

44. Navarro-Sigüenza, A. G., & Peterson, A. T. (2004). An alternative species taxonomy of the birds of Mexico. Biota Neotropica, 4(2), 1–32.

45. Zink, R. M., Kessen, A. E., Line, T. V., & Blackwell-Rago, R. C. (2001). Comparative phylogeography of some aridland bird species. Condor, 103(1), 1–10.

46. Vázquez-Miranda, H. (2014). Multilocus assessment of population diferentiation in Baja California birds: implications for community assembly and conservation. In Retrived from the University of Minnesota Digital Conservancy (Vol. 2014, Issue June).

47. Vázquez-Miranda, H., & Barker, F. K. (2021). Autosomal, sex-linked and mitochondrial loci resolve evolutionary relationships among wrens in the genus Campylorhynchus. Molecular Phylogenetics and Evolution, 163, 107242.

48. Vázquez-Miranda, H., Zink, R. M., & Pinto, B. J. (2022). Comparative phylogenomic patterns in the

Baja California avifauna, their conservation implications, and the stages in lineage divergence. Molecular Phylogenetics and Evolution, 171, 107466.

49. Mckay, B. D. (2013). The use of digital photography in systematics. Biological Journal of the Linnean Society, 110(1), 1–13.

50. Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. Nature Methods, 9(7), 671–675.

51. Eck S, Fiebig J, Fiedler W, Heynen BI, Nicolai T, Töpfer RV, Elzen D, Winkler R & Woog F (2011) Measuring birds. Vögelvermessen Deutsche Ornithologen, Wilhelmshaven

52. Rohlf, F.J. tpsUtil; Version 1.78 ;(2019). Department of Ecology and Evolution, Stony Brook University: New York, NY, USA.

53. Rohlf, F.J. tpsDig2; Version 2.31; (2017). Department of Ecology and Evolution, Stony Brook University: New York, NY, USA.

54. Adams, D. C., & Otárola-Castillo, E. (2013). Geomorph: An r package for the collection and analysis of geometric morphometric shape data. Methods in Ecology and Evolution, 4(4), 393–399.

55. Klingenberg, C. P. (2016). Size, shape, and form: concepts of allometry in geometric morphometrics. Development Genes and Evolution, 226(3), 113–137.

56. Klingenberg, C. P. (2015). Analyzing fluctuating asymmetry with geometric morphometrics: Concepts, methods, and applications. Symmetry, 7(2), 843–934.

57. Anderson, B. A. H., & Anderson, A. (1957). Life History of the Cactus Wren. Part I : Winter and Pre-Nesting Behavior. Cooper Ornithological Society Stable URL : http://www.jstor.org/stable/1364964 Accessed : 07-03-2016 13 : 54 UTC . 59(5), 274–296.

58. Ríos-Chelén, A. A., McDonald, A. N., Berger, A., Perry, A. C., Krakauer, A. H., & Patricelli, G. L. (2017). Do birds vocalize at higher pitch in noise, or is it a matter of measurement?. Behavioral Ecology and Sociobiology, 71(1), 1-12.

59. Cornell Lab of Ornithology. (2019). Raven Pro: Interactive Sound Analysis Software (Version 1.6.1) [Computer software]. Ithaca, NY: The Cornell Lab of Ornithology. Available from https://ravensoundsoftware.com/.

60. Fick, S.E. and R.J. Hijmans, 2017. WorldClim 2: new 1km spatial resolution climate surfaces for global land areas. International Journal of Climatology 37 (12): 4302-4315.

61. Sietse, O. L., Hall, F. G., Collatz, G., Meeson, B., Los, S., Brown de Colstoun, E., & Landis, D. (2010). ISLSCP II FASIR-adjusted NDVI, 1982-1998. ORNL DAAC.

62. Huntington, J. L., Hegewisch, K. C., Daudert, B., Morton, C. G., Abatzoglou, J. T., McEvoy, D. J., & Erickson, T. (2017). Climate engine: Cloud computing and visualization of climate and remote sensing data for advanced natural resource monitoring and process understanding. Bulletin of the American Meteorological Society, 98(11), 2397-2410.

63. QGIS.org, (2018). QGIS Geographic Information System. QGIS Association

64. R Core Team (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.URL https://www.R-project.org/

65. R Core Team (2022). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.URL https://www.R-project.org/

66. Anderson, M.J. (2001). A new method for non-parametric multivariate analysis of variance. Austral Ecology, 26: 32–46.

67. Busschau, T., Conradie, W., & Daniels, S. R. (2021). One species hides many: Molecular and morphological evidence for cryptic speciation in a thread snake (Leptotyphlopidae: Leptotyphlops sylvicolus Broadley & Wallach, 1997). Journal of Zoological Systematics and Evolutionary Research, 59(1), 195–221.

68. Camacho-Alpízar, A., Fuchs, E. J., & Barrantes, G. (2018). Effect of barriers and distance on song, genetic, and morphological divergence in the highland endemic Timberline Wren (Thryorchilus browni, Troglodytidae). PLoS ONE, 13(12), 1–17.

69. Ku-Peralta, W., Navarro-Sigüenza, A. G., Sandoval, L., & Roberto Sosa-López, J. (2020). Geographic variation in the duets of the Rufous-naped Wren (*Campylorhynchus rufinucha*) complex. Auk, 137(3), 1–14.

70. Piggott, M. P., Chao, N. L., & Beheregaray, L. B. (2011). Three fishes in one: Cryptic species in an Amazonian floodplain forest specialist. Biological Journal of the Linnean Society, 102(2), 391–403.

71. Rosser, N., Freitas, A. V. L., Huertas, B., Joron, M., Lamas, G., Mérot, C., Simpson, F., Willmott, K. R., Mallet, J., & Dasmahapatra, K. K. (2019). Cryptic speciation associated with geographic and ecological divergence in two Amazonian Heliconius butterflies. Zoological Journal of the Linnean Society, 186(1), 233–249.

72. Sosa-López, J. R., Mennill, D. J., & Navarro-Sigüenza, A. G. (2013). Geographic variation and the evolution of song in Mesoamerican rufous-naped wrens Campylorhynchus rufinucha. Journal of Avian Biology, 44(1), 027-038.

73. Vázquez-Miranda, H., Navarro-Sigüenza, A. G., & Omland, K. E. (2009). Phylogeography of the rufous-naped wren (Campylorhynchus rufinucha): Speciation and hybridization in Mesoamerica. Auk, 126(4),

765–778.

74. Vega-Sánchez, Y. M., Mendoza-Cuenca, L. F., & González-Rodríguez, A. (2019). Complex evolutionary history of the American Rubyspot damselfly, Hetaerina americana (Odonata): Evidence of cryptic speciation. Molecular phylogenetics and evolution, 139, 106536.

75. Riddle, B. R., Hafner, D. J., Alexander, L. F., & Jaeger, J. R. (2000). Cryptic vicariance in the historical assembly of a Baja California Peninsular Desert biota. Proceedings of the National Academy of Sciences of the United States of America, 97(26), 14438–14443.

76. Vázquez-Miranda, H., Griffin, J. A., Sheppard, J. M., Herman, J. M., Rojas-Soto, O., & Zink, R. M. (2017). Morphological and molecular evolution and their consequences for conservation and taxonomy in the Le Conte's thrasher Toxostoma lecontei. Journal of Avian Biology, 48(7), 941–954.

77. Upton, D. E., & Murphy, R. W. (1997). Phylogeny of the Side-Blotched Lizards (Phrynosomatidae:Uta) Based on mtDNA Sequences: Support for a Midpeninsular Seaway in Baja California. Molecular Phylogenetics and Evolution, 8(1), 104–113.

78. Dolby, G. A., Bennett, S. E. K., Lira-Noriega, A., Wilder, B. T., & Munguía-Vega, A. (2015). Assessing the Geological and Climatic Forcing of Biodiversity and Evolution Surrounding the Gulf of California. Journal of the Southwest, 57(2–3), 391–455.

79. Friedman, N. R., & Remeš, V. (2017). Ecogeographical gradients in plumage coloration among Australasian songbird clades. Global Ecology and Biogeography, 26(3), 261–274.

80. Passarotto, A., Rodríguez-Caballero, E., Cruz-Miralles, Á., & Avilés, J. M. (2022). Ecogeographical patterns in owl plumage colouration: Climate and vegetation cover predict global colour variation. Global Ecology and Biogeography, 31(3), 515–530.

81. Caro, T., & Mallarino, R. (2020). Coloration in Mammals. Trends in Ecology and Evolution, 35(4), 357–366.

82. Marcondes, R. S., & Brumfield, R. T. (2019). Fifty shades of brown: Macroevolution of plumage brightness in the Furnariida, a large clade of drab Neotropical passerines. Evolution, 73(4), 704–719.

83. Salewski, V., & Watt, C. (2017). Bergmann's rule: a biophysiological rule examined in birds. Oikos, 126(2).

84. Beerli, N., Bärtschi, F., Ballesteros-Mejia, L., Kitching, I. J., & Beck, J. (2019). How has the environment shaped geographical patterns of insect body sizes? A test of hypotheses using sphingid moths. Journal of Biogeography, 46(8), 1687–1698.

85. Youtz, J., Miller, K. D., Bowers, E. K., Rogers, S. L., Bulluck, L. P., Johnson, M., Peer, B. D., Percy, K. L., Johnson, E. I., Ames, E. M., Tonra, C. M., & Boves, T. J. (2020). Bergmann's rule is followed at multiple

stages of postembryonic development in a long-distance migratory songbird. Ecology and Evolution, 10(19), 10672–10686.

86. Zink, R. M., & Remsen, J. V. (1986). Evolutionary processes and patterns of geographic variation in birds. Current Ornithology, 4,1-69.

87. Clauss, M., Dittmann, M. T., Müller, D. W. H., Meloro, C., & Codron, D. (2013). Bergmann's rule in mammals: A cross-species interspecific pattern. Oikos, 122(10), 1465–1472.

88. Leyequién, E., De Boer, W. F., & Cleef, A. (2007). Influence of body size on coexistence of bird species. Ecological Research, 22(5), 735–741.

89. Olson, V. A., Davies, R. G., Orme, C. D. L., Thomas, G. H., Meiri, S., Blackburn, T. M., Gaston, K. J., Owens, I. P. F., & Bennett, P. M. (2009). Global biogeography and ecology of body size in birds. Ecology Letters, 12(3), 249–259.

90. Sato, E., Kusumoto, B., Şekercioğlu, Ç. H., Kubota, Y., & Murakami, M. (2020). The influence of ecological traits and environmental factors on the co-occurrence patterns of birds on islands worldwide. Ecological Research, 35(2), 394–404.

91. Price. (1984). The evolution of sexual size dimorphism in Darwin´s Finches. The American Naturalist, 41(4), 1–20.

92. Niederhauser, J. M., Slevin, M. C., Noonburg, E. G., & Anderson, R. C. (2021). Body size, habitat quality, and territory defense in Bachman's sparrow. Behaviour, 158(6), 479–502.

93. Steen, I., & Steen, J. B. (1965). The Importance of the Legs in the thermoregulation of Birds. Acta Physiologica Scandinavica, 63(3), 285–291.

94. Tattersall, G. J., Chaves, J. A., & Danner, R. M. (2017). Termoregulatory windows in Darwin´s Finches. Functional Ecology, 32(2), 358–368.

95. Rogalla, S., Shawkey, M. D., Vanthournout, B., & D'Alba, L. (2021). Thermoregulation and heat exchange in ospreys (Pandion haliaetus). Journal of Thermal Biology, 96, 102857.

96. Navalón, G., Bright, J. A., Marugán-Lobón, J., & Rayfield, E. J. (2019). The evolutionary relationship among beak shape, mechanical advantage, and feeding ecology in modern birds. Evolution, 73(3), 422–435.

97. Demery, A.-J. C., Burns, K. J., & Mason, N. A. (2021). Bill size, bill shape, and body size constrain bird song evolution on a macroevolutionary scale. Ornithology, 138(2), 1–11.

98. Van Oordt, F., Torres-Mura, J. C., & Hertel, F. (2018). Ecomorphology and foraging behaviour of Pacific boobies. Ibis, 160(2), 313–326.

99. Anderson, A. H., & Anderson, A. (1973). The cactus wren. University of Arizona Press

100. Pridmore-Brown, C. D., & Ingard, U. (1995). Sound propagation into the Shadow Zone in a Temperature-Stratified Atmosphere above a Plane Boundary. Journal of the Acoustical Society of America, 7(7), 36–42.

101. Podos, J., Huber, S. K., & Taft, B. (2004). Bird song: The interface of evolution and mechanism. Annual Review of Ecology, Evolution, and Systematics, 35, 55–87.

102. Podos, J., & Warren, P. S. (2007). The Evolution of Geographic Variation in Birdsong. Advances in the Study of Behavior, 37(07), 403–458.

103. Derryberry, E. P., Seddon, N., Claramunt, S., Tobias, J. A., Baker, A., Aleixo, A., & Brumfield, R. T. (2012). Correlated evolution of beak morphology and song in the neotropical woodcreeper radiation. Gavrilets 2004, 1–14.

104. Derryberry, E. P., Seddon, N., Derryberry, G. E., Claramunt, S., Seeholzer, G. F., Brumfield, R. T., & Tobias, J. A. (2018). Ecological drivers of song evolution in birds: disentangling the effects of habitat and morphology. Ecology and Evolution, 8(3), 1890-1905.

105. Nosil, P. (2012). Ecological Speciation. In P. H. Harvey, R. M. May, H. C. J. Godfray, & J. A. Dunne (Eds.), Oxford University Press.

106. Rundell, R. J., & Price, T. D. (2009). Adaptive radiation, nonadaptive radiation, ecological speciation and nonecological speciation. Trends in Ecology & Evolution, 24(7), 394-399.

TABLES

Table.1. Procrustes ANOVA results for differences between subspecies and peninsular/mainland groups in centroid size and shape of the beak in cactus wrens. Statistically significant results are highlighted in bold.

Beak view	Trait	df	SS	*F* value	*P*
Subspecies					
Dorsal view	Centroid size	5	0.001	1.83	0.1
	Shape	5	0.005	1.15	0.3
Lateral view	Centroid size	5	0.001	3.52	**0.01**
	Shape	5	0.01	2.22	**0.02**
Peninsular/mainland groups					
Dorsal view	Centroid size	1	1.56	0.05	0.8
	Shape	1	0.002	2.77	0.07
Lateral view	Centroid size	1	2.98E-06	8.17	**0.005**
	Shape	1	0.003	2.94	0.06

Table.2. GLM results for Gloger´s rule test across the entire cactus wren range. Statistically significant results are highlighted in bold.

Colour trait		Estimate	*t* value	*P*
Annual mean temperature				
Ventral	Darkness	-5.3e-03	-2.34	**0.021**
	Redness	-2.81e-03	-2.51	**0.013**
	Brightness	0.475	0.147	0.883
Crown	Darkness	0.006	0.591	0.556
	Redness	2.03e-03	0.476	0.635
	Brightness	2.307	2.065	**0.041**
Annual mean precipitation				
Ventral	Darkness	9.34e-05	3.199	**0.001**
	Redness	4.20e-05	2.938	**0.004**
	Brightness	-0.109	-2.649	**0.009**
Crown	Darkness	-0.00004	-0.339	0.736
	Redness	2.39e-05	0.438	0.066
	Brightness	-0.006	-0.421	0.674

Table 3. GLM results for Bergmann´s and Allen´s rule tests across the entire cactus wren range. Statistically significant results are highlighted in bold.

	Trait	Males			Females		
		Estimate	t value	P	Estimate	t value	P
Bergmann´s rule							
Temperature	Wing length	-0.13	-0.57	0.56	0.03	0.13	0.8
Latitude	Wing length	-0.05	-0.39	0.69	0.25	1.72	0.09
Allen´s rule							
Temperature	Tarsus length	0.19	2.22	0.03	0.18	1.97	**0.05**
	Beak length	-0.03	-0.32	0.74	-0.02	-0.4	0.6

Table 4. PERMANOVA results for Allen´s rule test whit geometric morphometric data across the entire cactus wren range. Statistically significant results are highlighted in bold.

View	Trait	df	SS	R^2	*F*	*P*
Dorsal	Centroid size	1	0.0039	-0.01	0.14	0.8
	Shape	1	-11066	-0.01	27.43	0.2
Lateral	Centroid size	1	0.00	0	0.54	0.5
	Shape	1	20.5	0.004	0.35	0.7

Table.5. GLIM results for the acoustic adaptation hypothesis test. Statistically significant results are highlighted in bold.

Vocal trait	Estimate	*t* value	*P*
Annual mean temperature			
Minimum Frequency	-15.40	-2.48	**0.01**
Maximum Frequency	1.87	0.57	0.5
Annual mean precipitation			
Minimum Frequency	0.20	1.31	0.1
Maximum Frequency	0.20	1.31	0.1
Normalized differential vegetation index (NDVI)			
Number Notes	-9.81	-1.25	0.2
Duration	-0.24	-0.47	0.6
Rhytm	-2.63	-1.02	0.3
Bandwidth	230.06	0.82	0.4
Minimum Frequency	-374.00	-1.60	0.1
Maximum Frequency	-124.84	-1.03	0.3

Figures

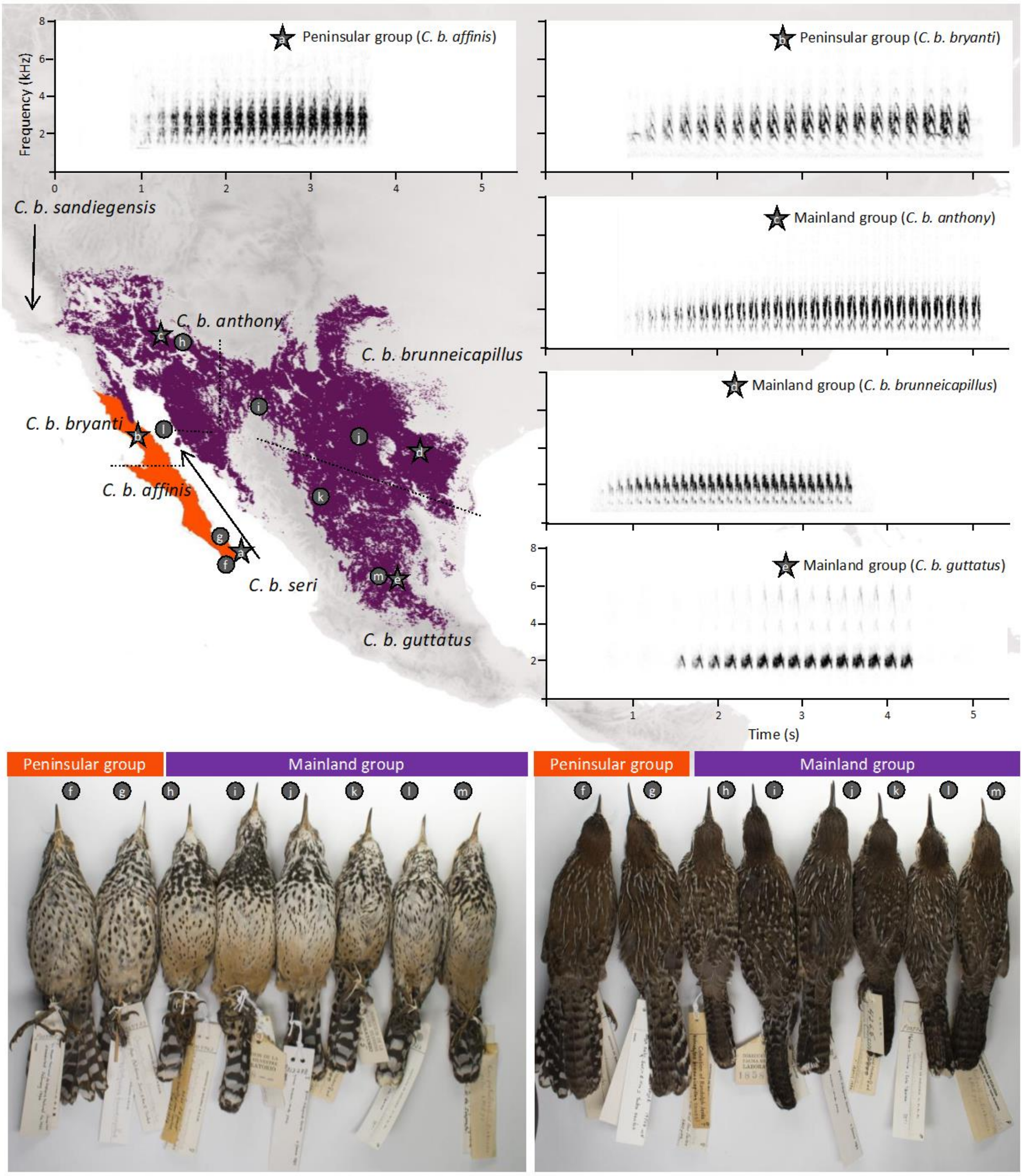

Figure 1. Map of North America showing the distribution ranges, photographs of dorsal and ventral view and spectrograms of *Campylorhynchus brunneicapillus*. Color represents two lineages (Vázquez-Miranda, 2021; Vázquez-Miranda et al., 2022), where orange= *Campylorhynchus affinis* and purple= *Campylorhynchus brunneicapillus*. Spectograms and specimens depict songs exemplars of the subspecies: *C. b. affinis*, *C. b. bryanti*, *C. b. anthonyi*, *C. b. brunneicapillus*, *C. b. seri* and *C. b. guttatus*. Dotted lines represent approximate limits of the distribution of the subspecies (Rea &Webber 1920). Letters inside the circles represents specimen localities and letters inside stars represents spectrogram localities including: a=Cabo Pulmo Baja California Sur, b=Cataviña Baja California, c=Organ Pipe Arizona,

d=Reynosa Tamaulipas, e=Zimapán Hidalgo , f= La Paz Baja California Sur, g= Todos los Santos Baja California, h= San Carlos Arizona, i= Gallego Chihuahua, j= Ramos Arizpe, Coahuila, k=Río Mezquital Durango, l= Isla Tiburón Sonora and m= Irapuato Guanajuato.

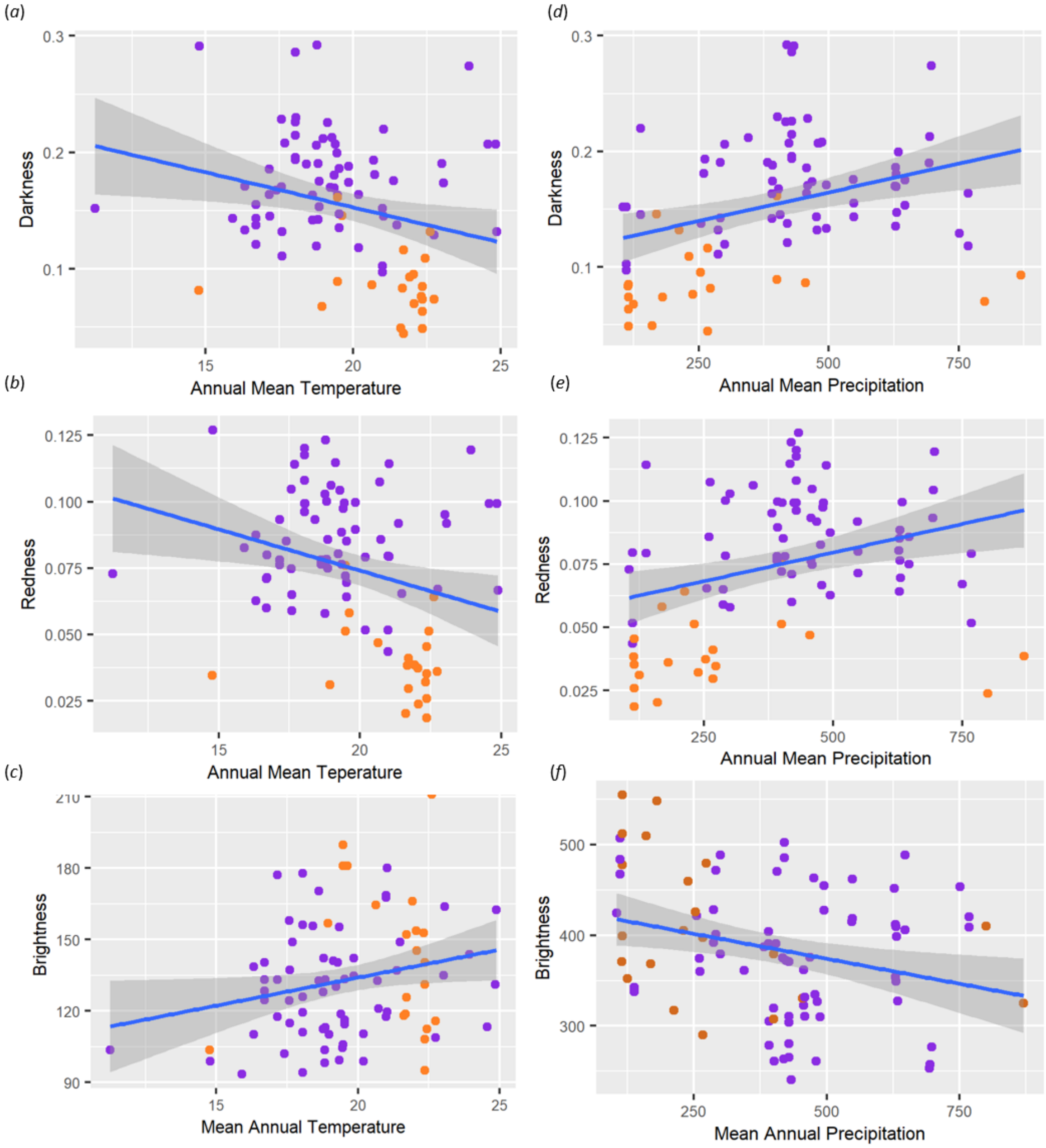

Figure 2. Linear regressions between colour traits and climatic variables. For ventral plumage, letters represent correlations between (*a*) darkness and (*b*) redness with annual mean temperature and (*d*) darkness, (*e*) redness and (*f*) brightness with mean annual precipitation. For crown plumage surface showed correlations between (*c*) brightness with mean annual temperature. Orange circles represent the peninsular group and purple circles represent the mainland group.

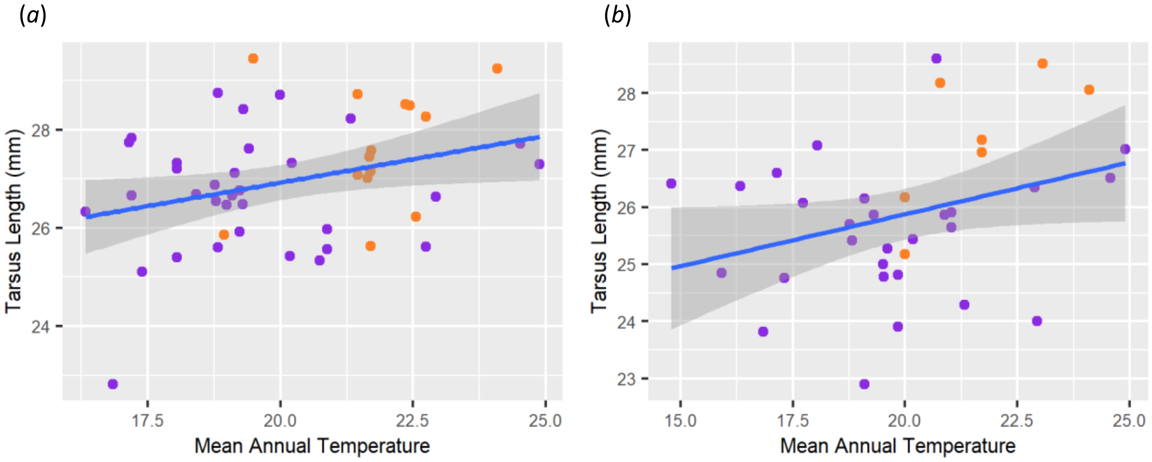

Figure 3. Linear regressions between tarsus length in (*a*) males and (*b*) females of cactus wrens across its range with annual mean temperature. Orange circles represent the peninsular group and purple circles represent the mainland group.

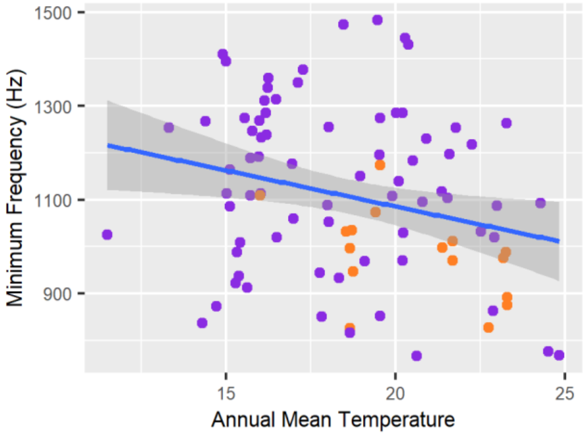

Figure 4. Linear regressions between cactus wren minimum frequency across its range with annual mean temperature. Orange circles represent the peninsular group and purple circles represent the mainland group.

Discusión general y conclusiones

Encontramos variación geográfica significativa en múltiples rasgos en la Matraca del Desierto. Nuestros resultados mostraron que, a nivel de subespecies, la mayoría de las diferencias significativas correspondían a diferencias entre las subespecies peninsulares y continentales en siete rasgos (rasgos de color: *C. b. affinis* vs *C. b. brunneicapillus*/*C. b. guttatus*; morfología: *C. b. affinis* vs *C. b. brunneicapillus*/*C. b. guttatus*; cantos: *C. b.affinis* vs *C. b. anthonyi*/*C. b. brunneicapillus*), mientras que en los grupos peninsulares encontramos diferencias significativas en 11 variables. Se ha demostrado variación en múltiples rasgos en especies de libélulas, mariposas, peces, pájaros, lagartijas, serpientes y murciélagos, lo que sugiere que dicha variación es el resultado de eventos vicariantes (Vázquez-Miranda et al., 2009; Piggott et al., 2011; Sosa-López et al., 2013; Camacho-Alpízar et al., 2018; Rosser et al., 2019; Vega-Sánchez et al., 2019; Ku-Peralta et al., 2020; Busschau et al., 2021). Porejemplo, también se ha informado que ocurre un patrón filogeográfico similar en otras aves, mamíferos, anfibios y reptiles (Upton & Murphy, 1997; Riddle et al., 2000; Zink et al., 2001; Vázquez-Miranda, 2014; Vázquez- Miranda et al., 2017, 2022; Vázquez-Miranda & Barker, 2021)a lo largo de la península de Baja California. Una posible explicación es que la diversificación seaproducto de un evento de expansión a lo largo de la península de Baja California y la posterior formación de una vía marítima mediapeninsular que unió el Golfo de California y el Océano Pacífico en lo que hoy es el Desierto Vizcaíno hace un millón de años (Riddle et al., 2000; Zink etal., 2001; Vázquez-Miranda, 2014; Dolby et al., 2015; Vázquez-Miranda & Barker, 2021; Vázquez-Miranda et al., 2022). Por lo tanto, nuestros resultados corresponden a hallazgos en estudios genéticos recientes que sugieren dos linajes evolutivos distintos dentro del complejo (el linaje continental: *brunneicapillus*, *anthonyi*, *seri* y *guttatus*; y el linaje peninsular: *bryanti* y *affinis*) (Zink et al., 2001; Navarro -Sigüenza & Peterson, 2004; Vázquez-Miranda, 2014; Vázquez- Miranda & Barker, 2021; Vázquez-Miranda et al., 2022).

Encontramos que la precipitación media anual se asoció negativamente con el brillo ventral, mientras que la temperatura media anual se asoció positivamente con la oscuridad y el color rojo y el brillo en la superficie de la corona. Estos resultados son consistentes con la regla de Gloger, que en su versión simple predice una correlación negativa entre brillo, temperatura y precipitación (Delhey & Delhey, 2019). La regla de Gloger compleja ha sido explorada insuficientemente en aves y mamíferos. Los pocos estudios hasta la fecha respaldan patrones de variación geográfica del color en el Perico Elegante (*Platycercus elegan*; Ribot et al., 2019) y la Choca Común (*Thamnophilus caerulescens*; Marcondes et al., 2020), en las familias Furnariidae (Marcondes, et al. ., 2020), Meliphagidae y Acanthizidae (Friedman & Remeš, 2017), y en Strigiformes (Passarotto et al., 2022). Para mamíferos en el jabalí (*Sus scrofa*; Newell et al., 2021). Una posible explicación es que en ambientes cálidos y húmedos las plumas o pelaje oscuro confieren resistencia a la degradación microbiana ya que la melanina aumenta la resistencia a la abrasión y el grosor de la queratina de las plumas o pelaje (Burtt & Ichida, 2004; Caro & Mallarino, 2020) . Los colores más oscuros también se seleccionan en ambientes con poca luz como resultado de una vegetación densa que favorece la criptosis (Marcondes & Brumfield, 2019; Caro & Mallarino, 2020; Marcondes, Nations, et al., 2020). Mientras que, bajo la regla compleja de Gloger, el tono rojizo podría verse favorecido en climas secos, ya que los suelos más rojos tienden a ser más intensos en este clima, también favorecidos por la criptosis (Marcondes et al., 2020). Futuras investigaciones deberían desentrañar cuál de estos mecanismos explica la coloración del plumaje en la matraca del desierto.

No encontramos relación entre la longitud del ala y la temperatura media anual o la latitud. Según la regla de Bergmann, los cuerpos más grandes se asocian a ambientes más fríos (Salewski Volker, 2017). Por lo tanto, nuestros resultados no respaldan esta regla ecogeográfica. La evidencia de la regla de Bergmann es contradictoria en aves, mamíferos e insectos (Nwaogu et al., 2018; Beerli et al., 2019; Fan et al., 2019; Stanchak & Santana, 2019; Romano et al., 2020b;

Youtz et al., 2020; Lee et al., 2021; DeVries et al., 2022; Malpica et al., 2022). Un estudio de revisión que consideró la variación geográfica del tamaño corporal en 92 especies de aves de América del Norte reveló que el 42% de estas especies apoyó la regla de Bergmann, el 12% proporcionó apoyo débil o ambiguo, y el 16% no mostró asociación entre altitud o latitud, mientras que el 29% de las especies contradijo la regla (Zink & Remsen, 1986). Para 3561 especies de mamíferos se demostró la regla de Bergmann cuando se consideró la estructura filogenética, sustentando que la regla solo se aplica a especies estrechamente relacionadas (Clauss et al., 2013). Por lo tanto, la termorregulación no explica los patrones generales de variación en el tamaño corporal de las aves y otras fuerzas selectivas podrían gobernar la evolución de este rasgo. Por ejemplo, se cree que el tamaño del cuerpo podría estar restringido por la historia evolutiva, formada por conjuntos comunitarios de aves a través de la competencia interespecífica por recursos (Leyequién et al., 2007; Olson et al., 2009; Sato et al., 2020) o selección sexual exhaustiva ya que este rasgo está relacionado con la agresividad en la competencia intrasexual (es decir, para la defensa del territorio u obtener un territorio de alta calidad) (Price, 1984; Cuadrado, 1995; Nota, 2003; Ippi et al., 2018; Niederhauser et al. , 2021).

Encontramos que en ambos sexos el tarso aumenta con la temperatura, pero encontramos una relación no significativa entre la temperatura y el tamaño del pico. Según la regla de Allen, los apéndices más cortos se asocian con ambientes más fríos (Salewski Volker, 2017). Por lo tanto, nuestros resultados sugieren que la termorregulación podría ser una fuerza selectiva que determina la longitud de las patas, como se ha propuesto que ocurre en otras aves como la Urraca Oriental y el Carbonero Chino (*Pica serica* y *Parus minor*; Fan et al., 2019) o en especies no aviares como la Rana Común (*Rana temporaria*; Alho et al., 2011). Además, la regla de Allen ha demostrado que para las colas de roedores existe una asociación positiva entre la longitud de la cola y la temperatura, siendo particularmente mayor en las especies del desierto (Alhajeri et al., 2020). Para las aves, una posible explicación podría estar relacionada con la anatomía vascular y la regulación fisiológica de la pérdida de calor de las extremidades (Symonds

& Tattersall, 2010). Un estudio experimental encontró que la pérdida de calor puede ocurrir a través de las piernas (Steen & Steen, 1965; Symonds & Tattersall, 2010). Este estudio mostró que las venas tibiales estaban notablemente más hinchadas durante el estrés por calor en garzas y gaviotas, probablemente debido a que el flujo de sangre viaja de regreso a las arterias centrales rodeadas de venas, lo que da como resultado que el calor de la sangre de la arteria pueda ser recuperado por las venas que rodean la arteria ó arterias centrales (Steen & Steen, 1965; Symonds & Tattersall, 2010). Este hallazgo está respaldado por otros estudios que utilizan imágenes térmicas (Tattersall et al., 2017; Rogalla et al., 2021). De manera similar a nuestros resultados, otros estudios no respaldan la regla de Allen para explicar la variación geográfica del tamaño del pico (Picoloro de Webb *Sinosuthora webbiana* y el Cucarachero de Sinaloa *Thryophilus sinaloa*; Lee et al. 2021; Malpica et al., 2022). En las aves, múltiples presiones selectivas podrían determinar la forma y el tamaño del pico (Friedman et al., 2019; Navalón et al., 2019), ya que es un rasgo multifuncional utilizado para cantar (Demery et al., 2021; Friis et al. al., 2022), en termorregulación (Symonds & Tattersall, 2010; van de Ven et al., 2019) y forrajeo (Van Oordt et al., 2018).

Sin embargo, otros factores también podrían influir en la variación del pico, como las relaciones filogenéticas (Friedman et al., 2019). Además, la dieta se ha asociado con la morfología craneal y la forma o el tamaño del pico (Olsen, 2017; Felice et al., 2019; Friedman et al., 2019; Laranjeiro et al., 2022), y es probable que esto también desempeña un papel en la determinación de la forma o el tamaño del pico en la Matraca del Desierto que es una especie principalmente insectívoras (Anderson & Anderson, 1973; Hamilton et al. 2020).

Encontramos una relación negativa entre la temperatura media anual y la menorfrecuencia mínima en los cantos. Nuestro hallazgo está asociados con la teoría de las propiedades de la transmisión del sonido, que sugiere que las frecuencias más altas se atenúanmás rápido que las frecuencias más bajas bajo una temperatura atmosférica más alta, lo que produce refracción (Bradbury & Vehrencamp, 2011). La refracción de la señal es un proceso queresulta en la atenuación del sonido y ocurre cuando el suelo cálido calienta la capa de aire inmediatamente adyacente. Se genera un gradiente de aire con temperatura decreciente, dentro de este gradiente el sonido viaja a diferentes velocidades (el sonido generalmente se mueve más rápido en cálidas) provocando que la trayectoria del sonido de propagación se doble hacia arriba y sea captado por el receptor (Pridmore-Brown & Ingard, 1995; Bradbury &Vehrencamp, 2011). Los estudios de reproducción experimental podrían ayudar a dilucidar si la refracción podría explicar las bajas frecuencias divergentes.

No encontramos relación entre la precipitación media anual, el índice NDVI y otras características estructurales finas del canto; por lo tanto, es probable que otras presiones selectivas puedan estar actuando sobre los cantos del reyezuelo, como la selección sexual, o presiones no selectivas, como la deriva cultural (Podos et al., 2004; Podos & Warren, 2007). Por ejemplo, la falta de evidencia para la hipótesis de la adaptación acústica en otros cantos de características acústicas podría atribuirse a la constricción morfológica, con aves con picos más grandes que producen cantos de menor ritmo (Derryberry et al., 2012, 2018). Nuestros resultados indican que los machos de la península tenían el pico más largo y cantaban con cantos más lentos, por lo que esta hipótesis es plausible. Sin embargo, son necesarios análisis más detallados que relacionen la morfología del pico con las características acústicas del canto.

Nuestros resultados, basados en datos de color, morfológicos y acústicos, reflejan análisis genéticos previos y respaldan la existencia de dos linajes evolutivos dentro del complejo de *Campylorhynchus brunneicapillus* que probablemente representan dos especies separadas: *C. affinis* en la península de Baja California y *C. brunneicapillus* en el continente (Zink et al., 2001;

Navarro-Sigüenza & Peterson, 2004; Vázquez-Miranda, 2014; Vázquez-Miranda & Barker, 2021; Vázquez-Miranda et al., 2022). Además, nuestros resultados muestran una relación para la coloración del plumaje entre la oscuridad, el brillo y la precipitación en los parches ventrales, y el enrojecimiento con la temperatura en la corona (respaldando la regla de Gloger), la longitud del tarso y la temperatura (respaldando la regla de Allen), y una menor frecuencia a mayor (respaldando la Hipótesis de la Adaptación Acústica), lo que sugiere que los ambientes divergentes juegan un papel importante en la diversificación de la Matraca del Desierto (Rundle & Nosil, 2005; Schluter & Conte, 2009; Nosil, 2012). Sugerimos que la divergencia de estos caracteres es el resultado de la adaptación local a ambientes divergentes que promueven el aislamiento reproductivo (Rundle & Nosil, 2005; Rundell & Price, 2009).

Referencias Bibliográficas

Acero-Murcia, A. C., Raposo do Amaral, F., de Barros, F. C., da Silva Ribeiro, T., Miyaki, C. Y., & Maldonado-Coelho, M. (2021). Ecological and evolutionary drivers of geographic variation in songs of a Neotropical suboscine bird: The Drab-breasted Bamboo Tyrant (Hemitriccus diops , Rhynchocyclidae) . *Ornithology*, *138*(2).

Adams, D. C., & Otárola-Castillo, E. (2013). Geomorph: An r package for the collection and analysis of geometric morphometric shape data. *Methods in Ecology and Evolution*, *4*(4), 393–399.

Alhajeri, B. H., Fourcade, Y., Upham, N. S., & Alhaddad, H. (2020). A global test of Allen's rule in rodents. *Global Ecology and Biogeography*, *29*(12), 2248–2260.

Alho, J. S., Herczeg, G., Laugen, A. T., Räsänen, K., Laurila, A., & Merilä, J. (2011). Allen's rule revisited: Quantitative genetics of extremity length in the common frog along a latitudinal gradient. *Journal of Evolutionary Biology*, *24*(1), 59–70.

Beerli, N., Bärtschi, F., Ballesteros-Mejia, L., Kitching, I. J., & Beck, J. (2019). How has the environment shaped geographical patterns of insect body sizes? A test of hypotheses using sphingid moths. *Journal of Biogeography*, *46*(8), 1687–1698.

Bradbury, J. W., & Vehrencamp, S. L. (2011). *Principles of Animal Communication*.

Burtt, J. E. H., & Ichida, J. M. (2004). GLOGER ' S RULE , FEATHER-DEGRADING BACTERIA , AND COLOR VARIATION AMONG SONG SPARROWS La Regla de Gloger , Bacterias Degradantes de Plumas y Variacio ´ n de Color en Melospiza melodia. *The Condor*, *106*, 681–686.

Busschau, T., Conradie, W., & Daniels, S. R. (2021). One species hides many: Molecular and morphological evidence for cryptic speciation in a thread snake (Leptotyphlopidae: Leptotyphlops sylvicolus Broadley & Wallach, 1997). *Journal of Zoological Systematics and Evolutionary Research*, *59*(1), 195–221.

Camacho-Alpízar, A., Fuchs, E. J., & Barrantes, G. (2018). Effect of barriers and distance on song, genetic, and morphological divergence in the highland endemic Timberline Wren (Thryorchilus browni, Troglodytidae). *PLoS ONE*, *13*(12), 1–17.

Campbell, M. D., Schoeman, D. S., Venables, W., Abu-Alhaija, R., Batten, S. D., Chiba, S., Coman, F., Davies, C. H., Edwards, M., Eriksen, R. S., Everett, J. D., Fukai, Y., Fukuchi, M., Esquivel Garrote, O., Hosie, G., Huggett, J. A., Johns, D. G., Kitchener, J. A., Koubbi, P., … Richardson, A. J. (2021). Testing Bergmann's rule in marine copepods. *Ecography*, *44*(9), 1283–1295.

Caro, T., & Mallarino, R. (2020). Coloration in Mammals. *Trends in Ecology and Evolution*, *35*(4), 357–366.

Cerezer, F. O., Ribeiro, J. R. I., Graipel, M., & Cáceres, N. C. (2020). The dark side of coloration: Ecogeographical evidence supports Gloger's rule in American marsupials. *Evolution*, *74*(9), 2046–2058.

Chávez-Hoffmeister, M. (2020). Bill disparity and feeding strategies among fossil and modern penguins. *Paleobiology*, *46*(2), 176–192.

Clauss, M., Dittmann, M. T., Müller, D. W. H., Meloro, C., & Codron, D. (2013). Bergmann's rule in mammals: A cross-species interspecific pattern. *Oikos*, *122*(10), 1465–1472.

Cuadrado, M. (1995). Winter territoriality in migrant black redstarts phoenicurus ochrurus in the mediterranean area. *Bird Study*, *42*(3), 232–239.

Danner, R. M., & Greenberg, R. (2015). A critical season approach to Allen's rule: Bill size declines with winter temperature in a cold temperate environment. *Journal of Biogeography*, *42*(1), 114–120.

Delhey, K. (2017). *Darker where cold and wet: Australian birds follow their own version of Gloger's rule*. 673–683.

Delhey, K., & Delhey, K. (2019). *A review of Gloger ' s rule , an ecogeographical rule of colour : definitions , interpretations and evidence*.

Demery, A.-J. C., Burns, K. J., & Mason, N. A. (2021). Bill size, bill shape, and body size constrain bird song evolution on a macroevolutionary scale. *Ornithology*, *138*(2), 1–11.

Derryberry, E. P., Claramunt, S., Tobias, J. A., Seddon, N., Derryberry, G. E., Brumfield, R. T., & Seeholzer, G. F. (2018). *Ecological drivers of song evolution in birds : Disentangling the effects of habitat and morphology*. *December 2017*, 1–16.

Derryberry, E. P., Seddon, N., Claramunt, S., Tobias, J. A., Baker, A., Aleixo, A., & Brumfield, R. T. (2012). *CORRELATED EVOLUTION OF BEAK MORPHOLOGY AND SONG IN THE NEOTROPICAL WOODCREEPER RADIATION*. *Gavrilets 2004*, 1–14.

DeVries, M. S., Waraczynski, M., Baldassarre, D. T., Slevin, M. C., Anderson, R., & Jawor, J. M. (2022). Geographic variation in morphology of Northern Cardinals: possible application of

Bergmann’s Rule? *Journal of Field Ornithology*, *93*(2).

Dolby, G. A., Bennett, S. E. K., Lira-Noriega, A., Wilder, B. T., & Munguía-Vega, A. (2015). Assessing the Geological and Climatic Forcing of Biodiversity and Evolution Surrounding the Gulf of California. *Journal of the Southwest*, *57*(2–3), 391–455.

Ey, E., & Fisher, J. (2009). Bioacoustics : The International Journal of Animal Sound and its Recording THE “ ACOUSTIC ADAPTATION HYPOTHESIS ”— A REVIEW OF THE EVIDENCE FROM BIRDS , ANURANS AND MAMMALS. *Journal of Bioacoustics*, *February 2013*, 21–48.

Fan, L., Cai, T., Xiong, Y., Song, G., & Lei, F. (2019). Bergmann’s rule and Allen’s rule in two passerine birds in China. *Avian Research*, *10*(1), 1–11.

Felice, R. N., Tobias, J. A., Pigot, A. L., & Goswami, A. (2019). Dietary niche and the evolution of cranial morphology in birds. *Proceedings of the Royal Society B: Biological Sciences*, *286*(1897).

Fernández-Torres, F., Martínez, P. A., & Olalla-Tárraga, M. Á. (2018). Shallow water ray-finned marine fishes follow Bergmann’s rule. *Basic and Applied Ecology*, *33*(2017), 99–110.

Fernández Gómez, R. A., Morales-Mávil, J. E., & Sosa-López, J. R. (2020). Geographic variation and divergence of songs in the Olive Sparrow species complex. *Journal of Field Ornithology*, *91*(1), 77–91.

Friedman, N. R., Miller, E. T., Ball, J. R., Kasuga, H., Remeš, V., & Economo, E. P. (2019). Evolution of a multifunctional trait: Shared effects of foraging ecology and thermoregulation on beak morphology, with consequences for song evolution. *Proceedings of the Royal Society B: Biological Sciences*, *286*(1917).

Friedman, N. R., & Remeš, V. (2017). Ecogeographical gradients in plumage coloration among Australasian songbird clades. *Global Ecology and Biogeography*, *26*(3), 261–274.

Friis, J. I., Sabino, J., Santos, P., Dabelsteen, T., & Cardoso, G. C. (2022). Ecological adaptation and birdsong: How body and bill sizes affect passerine sound frequencies. *Behavioral Ecology*, *33*(4), 798–806.

Gardner, J. L., Symonds, M. R. E., Joseph, L., Ikin, K., Stein, J., & Kruuk, L. E. B. (2016). Spatial variation in avian bill size is associated with humidity in summer among Australian passerines. *Climate Change Responses*, *3*(1), 1–11.

Hamilton, R. A., G. A. Proudfoot, D. A. Sherry, and S. L. Johnson (2020). Cactus Wren (*Campylorhynchus brunneicapillus*), version 1.0. In Birds of the World (A. F. Poole, Editor). Cornell Lab of Ornithology, Ithaca, NY, USA. https://doi.org/10.2173/bow.cacwre.01

Ippi, S., Cerón, G., Alvarez, L. M., Aráoz, R., & Blendinger, P. G. (2018). Relationships among territory size, body size, and food availability in a specialist river duck. *Emu*, *118*(3), 293–303.

Kirchman, J. J., & Schneider, K. J. (2014). Range expansion and the breakdown of bergmann's rule in red-bellied woodpeckers (Melanerpes carolinus). *Wilson Journal of Ornithology*, *126*(2), 236–248.

Klingenberg, C. P. (2015). Analyzing fluctuating asymmetry with geometric morphometrics: Concepts, methods, and applications. *Symmetry*, *7*(2), 843–934.

Klingenberg, C. P. (2016). Size, shape, and form: concepts of allometry in geometric morphometrics. *Development Genes and Evolution*, *226*(3), 113–137.

Ku-Peralta, W., Navarro-Sigüenza, A. G., Sandoval, L., & Roberto Sosa-López, J. (2020). Geographic variation in the duets of the Rufous-naped Wren (Campylorhynchus rufinucha) complex. *Auk*, *137*(3), 1–14.

Laiolo, P., & Rolando, A. (2001). Ecogeographic correlates of morphometric variation in the red-billed Chough Pyrrhocorax pyrrhocorax and the Alpine Chough Pyrrhocorax graculus. *Ibis*, *143*(3), 602–616.

Laranjeiro, M. I., Farré, M., Phillips, R. A., Quillfeldt, P., Bonadonna, F., Gémard, C., Daigre, M., Suazo, C. G., Barbraud, C., & Navarro, J. (2022). Variation among species and populations in bill shape and size in three planktivorous petrels. *Marine Biology*, *169*(2), 1–12.

Lee, C. C., Fu, Y., Yeh, C. fen, Yeung, C. K. L., Hung, H. yi, Yao, C. J., Shaner, P. J. L., & Li, S. H. (2021). Morphological variations in a widely distributed Eastern Asian passerine cannot be consistently explained by ecogeographic rules. *Ecology and Evolution*, *11*(21), 15249–15260.

Leyequién, E., De Boer, W. F., & Cleef, A. (2007). Influence of body size on coexistence of bird species. *Ecological Research*, *22*(5), 735–741.

Malpica, A., Mendoza-Cuenca, L., & González, C. (2022). Color and morphological differentiation in the Sinaloa Wren (Thryophilus sinaloa) in the tropical dry forests of Mexico: The role of environment and geographic isolation. *Plos One*, *17*(6), e0269860.

Marcondes, R. S., & Brumfield, R. T. (2019). Fifty shades of brown: Macroevolution of plumage brightness in the Furnariida, a large clade of drab Neotropical passerines. *Evolution*, *73*(4), 704–719.

Marcondes, R. S., Nations, J. A., Seeholzer, G. F., & Brum, R. T. (2020). *Rethinking Gloger ' s Rule : Climate , Light Environments , and Color in a Large Family of Tropical Birds (Furnariidae)*. *197*(5).

Marcondes, R. S., Stryjewski, K. F., & Brumfield, R. T. (2020). *Testing the simple and complex versions of Gloger's rule in the Variable Antshrike (*. *137*, 1–13.

Mckay, B. D. (2013). The use of digital photography in systematics. *Biological Journal of the Linnean Society*, *110*(1), 1–13.

McNaught, M. K., & Owens, I. P. F. (2002). Interspecific variation in plumage colour among birds: Species recognition or light environment? *Journal of Evolutionary Biology*, *15*(4), 505–514.

Morton, E. S. (1975). Ecological Sources of Selection on Avian Sounds. *The American Naturalist*, *109*(965), 17–34.

Navalón, G., Bright, J. A., Marugán-Lobón, J., & Rayfield, E. J. (2019). The evolutionary relationship among beak shape, mechanical advantage, and feeding ecology in modern birds*. *Evolution*, *73*(3), 422–435.

Navarro-Sigüenza, A. G., & Peterson, A. T. (2004). An alternative species taxonomy of the birds of Mexico. *Biota Neotropica*, *4*(2), 1–32.

Neto, J. M., Gordinho, L., Belda, E. J., Marín, M., Monrós, J. S., Fearon, P., & Crates, R. (2013). Phenotypic Divergence among West European Populations of Reed Bunting Emberiza schoeniclus: The Effects of Migratory and Foraging Behaviours. *PLoS ONE*, *8*(5).

Newell, C., Walker, H., & Caro, T. (2021). Pig pigmentation: testing Gloger's rule. *Journal of Mammalogy*, *102*(6), 1525–1535.

Niederhauser, J. M., Slevin, M. C., Noonburg, E. G., & Anderson, R. C. (2021). Body size, habitat quality, and territory defense in Bachman's sparrow. *Behaviour*, *158*(6), 479–502.

Nosil, P. (2012). Ecological Speciation. In P. H. Harvey, R. M. May, H. C. J. Godfray, & J. A. Dunne (Eds.), *Oxford University Press*.

Nota, Y. (2003). Effects of body size and sex on foraging territoriality of the little egret (Egretta garzetta) in Japan. *Auk*, *120*(3), 791–798.

Nwaogu, C. J., Tieleman, B. I., Bitrus, K., & Cresswell, W. (2018). Temperature and aridity determine body size conformity to Bergmann's rule independent of latitudinal differences in a tropical environment. *Journal of Ornithology*, *159*(4), 1053–1062.

Olsen, A. M. (2017). Feeding ecology is the primary driver of beak shape diversification in waterfowl. *Functional Ecology*, *31*(10), 1985–1995.

Olson, V. A., Davies, R. G., Orme, C. D. L., Thomas, G. H., Meiri, S., Blackburn, T. M., Gaston, K. J., Owens, I. P. F., & Bennett, P. M. (2009). Global biogeography and ecology of body size in birds. *Ecology Letters*, *12*(3), 249–259.

Passarotto, A., Rodríguez-Caballero, E., Cruz-Miralles, Á., & Avilés, J. M. (2022). Ecogeographical patterns in owl plumage colouration: Climate and vegetation cover predict global colour variation. *Global Ecology and Biogeography*, *31*(3), 515–530.

Piggott, M. P., Chao, N. L., & Beheregaray, L. B. (2011). Three fishes in one: Cryptic species in an Amazonian floodplain forest specialist. *Biological Journal of the Linnean Society*, *102*(2), 391–403.

Podos, J., Huber, S. K., & Taft, B. (2004). Bird song: The interface of evolution and mechanism. *Annual Review of Ecology, Evolution, and Systematics*, *35*, 55–87.

Podos, J., & Warren, P. S. (2007). The Evolution of Geographic Variation in Birdsong. *Advances in the Study of Behavior*, *37*(07), 403–458.

Price. (1984). The evolution of sexual size dimorphism in Darwin´s Finches. *The American Naturalist*, *41*(4), 1–20.

Pridmore-Brown, C. D., & Ingard, U. (1995). Sound propagation into the Shadow Zone in a Temperature-Stratified Atmosphere above a Plane Boundary. *Journal of the Acoustical Society of America*, *7*(7), 36–42.

R Development Core Team (2021). R: A language and environment for statistical computing. : R Foundation for Statistical Computing. Vienna, Austria. Retrieved from http://www.r-project.org

Rea, A., & Weaver, K. (1990). The Taxonomy, Distribution, and Status of Coastal California Cactus Wrens. *Western Birds*, *21*(3), 81–126.

Ribot, R. F. H., Berg, M. L., Schubert, E., Endler, J. A., & Bennett, A. T. D. (2019). Plumage coloration follows Gloger's rule in a ring species. *Journal of Biogeography*, *46*(3), 584–596.

Riddle, B. R., Hafner, D. J., Alexander, L. F., & Jaeger, J. R. (2000). Cryptic vicariance in the historical assembly of a Baja California Peninsular Desert biota. *Proceedings of the National Academy of Sciences of the United States of America*, *97*(26), 14438–14443.

Rogalla, S., Shawkey, M. D., Vanthournout, B., & D’Alba, L. (2021). Thermoregulation and heat exchange in ospreys (Pandion haliaetus). *Journal of Thermal Biology*, *96*(January), 102857.

Romano, A., Séchaud, R., & Roulin, A. (2020a). Generalized evidence for Bergmann ’ s rule : body size variation in a cosmopolitan owl genus. *Journal of Biogeography*, *48*(August), 1–13.

Romano, A., Séchaud, R., & Roulin, A. (2020b). Geographical variation in bill size provides evidence for Allen’s rule in a cosmopolitan raptor. *Global Ecology and Biogeography*, *29*(1), 65–75.

Rosser, N., Freitas, A. V. L., Huertas, B., Joron, M., Lamas, G., Mérot, C., Simpson, F., Willmott, K. R., Mallet, J., & Dasmahapatra, K. K. (2019). Cryptic speciation associated with geographic and ecological divergence in two Amazonian Heliconius butterflies. *Zoological Journal of the Linnean Society*, *186*(1), 233–249.

Rundell, R. J., & Price, T. D. (2009). *Adaptive radiation , nonadaptive radiation , ecological speciation and nonecological speciation*. *May*.

Rundle, H. D., & Nosil, P. (2005). Ecological speciation. *Ecology Letters*, *8*(3), 336–352.

Ryeland, J., Weston, M. A., & Symonds, M. R. E. (2019). Leg length and temperature determine the use of unipedal roosting in birds. *Journal of Avian Biology*, *50*(5), 1–9.

Salewski, V., Hochachka, W. M., & Fiedler, W. (2010). Global warming and Bergmann’s rule: Do central European passerines adjust their body size to rising temperatures? *Oecologia*, *162*(1), 247–260.

Salewski Volker, W. C. (2017). Accepted Ar tic le dynamics Accepted Ar tic le. *Oikos*, *126*(Accepted Author Manuscript. doi:10.1111/jav.00934).

Sato, E., Kusumoto, B., Şekercioğlu, Ç. H., Kubota, Y., & Murakami, M. (2020). The influence of ecological traits and environmental factors on the co-occurrence patterns of birds on islands worldwide. *Ecological Research*, *35*(2), 394–404.

Schluter, D., & Conte, G. L. (2009). Genetics and ecological speciation. *In the Light of Evolution*, *3*, 47–64.

Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*, *9*(7), 671–675.

Sebastián-González, E., van Aardt, J., Sacca, K., Barbosa, J. M., Kelbe, D., & Hart, P. J. (2018). Testing the acoustic adaptation hypothesis with native and introduced birds in Hawaiian forests. *Journal of Ornithology*, *159*(3), 827–838.

Shafer, A. B. A., & Wolf, J. B. W. (2013). Widespread evidence for incipient ecological speciation: A meta-analysis of isolation-by-ecology. *Ecology Letters*, *16*(7), 940–950.

Simpson, R. K., & McGraw, K. J. (2018). Multiple signaling in a variable environment: expression of song and color traits as a function of ambient sound and light. *Biotropica*, *50*(3), 531–540.

Sosa-López, J. R., González, C., & Navarro-Sigüenza, A. G. (2013). Vocal geographic variation in mesoamerican Common Bush Tanagers (Chlorospingus ophthalmicus). *Wilson Journal of Ornithology*, *125*(1), 24–33.

Stanchak, K. E., & Santana, S. E. (2019). Do ecogeographical rules explain morphological variation in a diverse, Holarctic genus of small mammals? *Journal of Biogeography*, *46*(1), 110–122.

Steen, I., & Steen, J. B. (1965). The Importance of the Legs in the Thermoregulation of Birds. *Acta Physiologica Scandinavica*, *63*(3), 285–291.

Suzuki, T. A., Martins, F. M., Phifer-Rixey, M., & Nachman, M. W. (2020). The gut microbiota and Bergmann’s rule in wild house mice. *Molecular Ecology*, *29*(12), 2300–2311.

Symonds, M. R. E., & Tattersall, G. J. (2010). Geographical variation in bill size across bird species provides evidence for Allen’s rule. *American Naturalist*, *176*(2), 188–197.

Tattersall, G. J., Chaves, J. A., & Danner, R. M. (2017). Termoregulatory windows in Darwin´s Finches. *Functional Ecology*, *32*(2), 358–368.

Upton, D. E., & Murphy, R. W. (1997). Phylogeny of the Side-Blotched Lizards (Phrynosomatidae:Uta) Based on mtDNA Sequences: Support for a Midpeninsular Seaway in Baja California. *Molecular Phylogenetics and Evolution*, *8*(1), 104–113.

van de Ven, T. M. F. N., McKechnie, A. E., & Cunningham, S. J. (2019). The costs of keeping cool : behavioural trade - offs between foraging and thermoregulation are associated with significant mass losses in an arid - zone bird. *Oecologia*, *191*(1), 205–215.

Van Oordt, F., Torres-Mura, J. C., & Hertel, F. (2018). Ecomorphology and foraging behaviour of Pacific boobies. *Ibis*, *160*(2), 313–326.

VanderWerf, E. A. (2012). Ecogeographic patterns of morphological variation in elepaios (Chasiempis SPP.): Bergmann's, Allen's, and Gloger's rules in a microcosm. In *Ornithological Monographs* (Vol. 73, Issue 73).

Vázquez-Miranda, H. (2014). Multilocus assessment of population diferentiation in Baja California birds: implications for community assembly and conservation. In *Retrived from the University of Minnesota Digital Conservancy* (Vol. 2014, Issue June).

Vázquez-Miranda, H., & Barker, F. K. (2021). Autosomal, sex-linked and mitochondrial loci resolve evolutionary relationships among wrens in the genus Campylorhynchus. *Molecular Phylogenetics and Evolution*, *163*(August 2020).

Vázquez-Miranda, H., Griffin, J. A., Sheppard, J. M., Herman, J. M., Rojas-Soto, O., & Zink, R. M. (2017). Morphological and molecular evolution and their consequences for conservation and taxonomy in the Le Conte's thrasher Toxostoma lecontei. *Journal of Avian Biology*, *48*(7), 941–954.

Vázquez-Miranda, H., Navarro-Sigüenza, A. G., & Omland, K. E. (2009). Phylogeography of the rufous-naped wren (Campylorhynchus Rufinucha): Speciation and hybridization in Mesoamerica. *Auk*, *126*(4), 765–778.

Vázquez-Miranda, H., Zink, R. M., & Pinto, B. J. (2022). Comparative phylogenomic patterns in the Baja California avifauna, their conservation implications, and the stages in lineage divergence. *Molecular Phylogenetics and Evolution*, *171*(January).

Vega-Sánchez, Y. M., Mendoza-Cuenca, L. F., & González-Rodríguez, A. (2019). Complex evolutionary history of the American Rubyspot damselfly, Hetaerina americana (Odonata): Evidence of cryptic speciation. *Molecular Phylogenetics and Evolution*, *139*(March), 106536.

Youtz, J., Miller, K. D., Bowers, E. K., Rogers, S. L., Bulluck, L. P., Johnson, M., Peer, B. D., Percy, K. L., Johnson, E. I., Ames, E. M., Tonra, C. M., & Boves, T. J. (2020). Bergmann's rule is followed at multiple stages of postembryonic development in a long-distance migratory songbird. *Ecology and Evolution*, *10*(19), 10672–10686.

Yu, T. L., Wang, D. L., Busam, M., & Deng, Y. H. (2019). Altitudinal variation in body size in Bufo minshanicus supports Bergmann's rule. *Evolutionary Ecology*, *0123456789*.

Zink, R. M., Kessen, A. E., Line, T. V., & Blackwell-Rago, R. C. (2001). Comparative phylogeography of some aridland bird species. *Condor*, *103*(1), 1–10.

Zink, R. M., & Remsen, J. V. (1986). Evolutionary processes and patterns of geographic variation in birds. *Current Ornithology*, *4*(January 1986), 1–69.

Apéndices

ECOLOGICAL FACTORS DRIVE THE DIVERGENCE OF MORPHOLOGICAL, COLOR, AND BEHAVIORAL TRAITS IN THE CACTUS WREN (*CAMPYLORHYNCHUS BRUNNEICAPILLUS*)

Violeta Monserrath Andrade González[1. 2]. Hernán Vázquez-Miranda[1]. Claudia Patricia Ornelas García[3]. José Roberto Sosa-López[4]

[1]Colección Nacional de Aves. Departamento de Zoología. Instituto de Biología. Universidad Nacional Autónoma de México. Ciudad de México C.P. 04510. México.
[2]Posgrado en Ciencias Biológicas. Universidad Nacional Autónoma de México. Ciudad de México C.P. 04510. México.
[3]Colección Nacional de Peces. Departamento de Zoología. Instituto de Biología. Universidad Nacional Autónoma de México. Ciudad de México C.P. 04510. México.
[4]Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional Unidad Oaxaca (CIIDIR). Instituto Politécnico Nacional. Santa Cruz Xoxocotlán. Oaxaca. México.

Appendix A: Extended Methods

Specimen information

We obtained specimens from Colección Nacional de Aves. Instituto de Biología and Museo de Zoología "Alfonso L. Herrera". Both scientific collections at the Universidad Nacional Autónoma de México.

Definitions of morphological variables

Following Eck at al. (2011):

- Tarsal length: The measurement from the joint between tarsus and toes to the intertarsal joint.
- Tarsal diameter: The width in the middle of the tarsus.
- Wing length: The distance between the carpal joint and the primaries plumages
- Beak length: Distance at the skull to the tip of the bill
- Beak length proximal to nostril: Distance from the proximal edge of the nostrils to the tip of the bill
- Beak length distal to nostril: Distance from the distal edge of the nostrils to the tip of the bill
- Beak depth proximal to nostril: Height at the proximal edge of the nostrils
- Beak depth distal to nostril: Height at the distal edge of the nostrils
- Beak width proximal to nostril: Width at the proximal edge of the nostrils
- Beak width distal to nostril: Width at the distal edge of the nostrils

These characters were chosen because they are considered to be good indicators of body size and less susceptible to deformations of the taxidermy process (Zink and Remsen 1986).

Measurement error

To minimize measurement error. we measured a series of 30 specimens during three sessions on consecutive days and a Pearson product-moment correlation between the sessions was calculated. We repeated the process until the correlations of all the variables obtained significant positive values (following Benítez-Díaz 1993).

Acoustic information

We obtained recordings from six natural sound libraries. including the Macaulay Library (Cornell Lab of Ornithology. Ithaca. NY). The Ohio State Borror Laboratory of Bioacoustics. Florida Museum of Natural History. Biblioteca de Sonidos de Aves de México. Biblioteca de Sonidos Naturales Alfonso L. Herrera and xeno-canto (https://xeno-canto.org/).

Acoustic variable definitions

We extracted 12 fine structural variables to describe the frequency and temporal characteristics of the songs following Charif at al. (2010) and Catchpole and Slater (2008):

- Number notes: Number of elements in the song
- Rhythm: Number notes divided by the length of the song
- Duration: The difference between being time and end time of the song.
- Minimum frequency: The frequency at which the minimum power occurs within the selection.
- Maximum frequency: The frequency at which the maximum power occurs within the selection.
- Bandwidth: The difference between minimum and maximum frequency.
- Centre frequency: The frequency that divides the selection into two frequency intervals of equal energy.
- Aggregate entropy: Entropy average of each frame in the selection.
- Frequency 5%: The frequency that divides the selection into two frequency intervals containing 5% and 95% of the energy in the selection.

- Frequency 25%: The frequency that divides the selection into two frequency intervals containing 25% and 75% of the energy in the selection.
- Frequency 75%: The frequency that divides the selection into two frequency intervals containing 75% and 25% of the energy in the selection.
- Frequency 95 %: The frequency that divides the selection into two frequency intervals containing 95% and 5% of the energy in the selection.

Threshold method for acoustic analysis

Measurements were taken using the threshold method. which involves the use of power spectrum to measure frequency-related traits (following Ríos-Chelén et al. 2017). Frequency variables were calculated using the power spectrum and a threshold setting of -20dB related to the song´s peak amplitude. including all the peaks that exceeded the threshold.

Statistical analyses

We conducted multiple general linear models and generalized linear models to test the ecogeographical rules and the acoustic adaptation hypothesis. The linear models were used when assumptions of normality. homogeneity. linearity and independence were meet and generalized linear model were used when the assumption of normality is not meet.

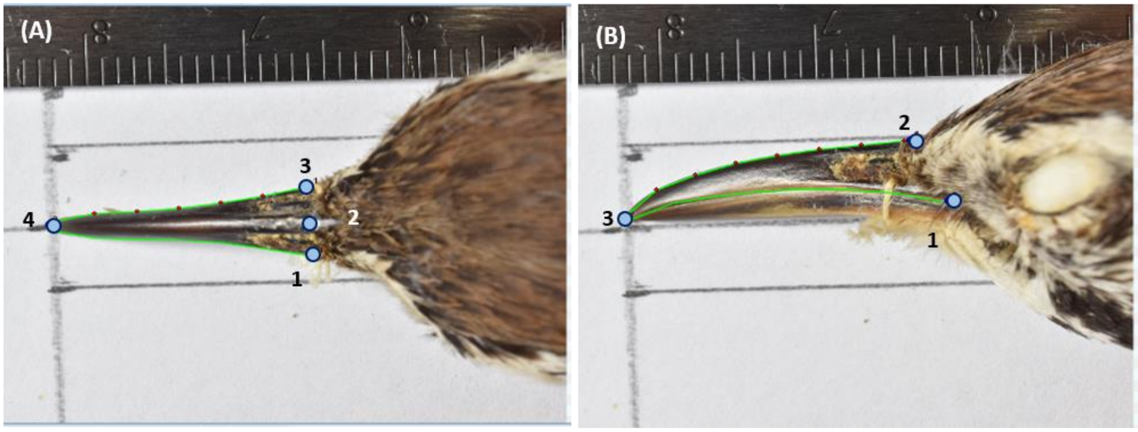

Figure S1. Image of a cactus wrens bill with semiladmarks shown. (a) Dorsal view and (b) lateral view. Blue points are landmarks. red points are semilandmarks digitized along the curves (green lines).

Table S1. List of cactus wrens skins used in the study and information of their corresponding collection, state or province, country, coordinates, date collection and sex.

Collection Number	Collection	State/Province	Country	Latitude	Longitude	Collection Date	Sex
P019370	CNAV	Aguascalientes	Mexico	22.33333	-102.23333	21/10/1940	M
P004963	CNAV	Arizona	United States	32.95	-112.724722	23/02/2023	F
P004964	CNAV	Arizona	United States	32.95	-112.724722	27/02/1936	M
P018495	CNAV	Baja California	Mexico	27.301111	-112.893889	03/11/1946	M
P004969	CNAV	Baja California	Mexico	28.835	-114.21	25/05/1975	M
P0024723	CNAV	Baja California	Mexico	27.301111	-112.893889	08/06/1996	M
P024724	CNAV	Baja California	Mexico	27.301111	-112.893889	08/06/1996	F
P002425	CNAV	Baja California	Mexico	27.28	-112.9	09/06/1996	M
P024726	CNAV	Baja California	Mexico	25.261667	-111.774444	08/06/1996	M
P004967	CNAV	Baja California Sur	Mexico	25.66	-110.78	30/10/1930	F
P018497	CNAV	Baja California Sur	Mexico	24.71666	-111.53166	10/05/1973	M
P004973	CNAV	Baja California Sur	Mexico	23.41527	-110.20666	19/02/1978	M
P004970	CNAV	Baja California Sur	Mexico	24.15	-110.25	11/09/1977	F
P004971	CNAV	Baja California Sur	Mexico	23.73638	-109.87055	11/09/1977	M
P004972	CNAV	Baja California Sur	Mexico	23.73638	-109.87055	12/09/1977	M
P018485	CNAV	Baja California Sur	Mexico	23.7667	-110.3167	28/09/1974	M
P019375	CNAV	Baja California Sur	Mexico	27.338889	-112.266944	19/05/1973	F
P019360	CNAV	California	United States	34.3	-118.38	25/10/2018	F
P018487	CNAV	Chihuahua	Mexico	29.25	-106.91666	14/11/1968	F
P018501	CNAV	Chihuahua	Mexico	26.89833	-105.6	31/10/1971	F
P019367	CNAV	Chihuahua	Mexico	26.89833	-105.6	30/10/1971	M
1187	CNAV	Chihuahua	Mexico	27.65	-105.16	20/06/1949	F
P004974	CNAV	Coahuila	Mexico	25.76666	-101.48333	17/06/1984	F
P004975	CNAV	Coahuila	Mexico	25.76666	-101.48333	17/06/1984	M
P012916	CNAV	Coahuila	Mexico	25.61666	-101.05	23/10/1987	M
P013288	CNAV	Coahuila	Mexico	25.61666	-101.05	06/02/1989	F
P019361	CNAV	Durango	Mexico	24.78	-104.46	28/02/1946	F
P019366	CNAV	Durango	Mexico	23.42874	-104.39537	02/04/1972	M
P019374	CNAV	Durango	Mexico	23.42874	-104.39537	01/04/1972	F
25754	CNAV	Durango	Mexico	26.685	-103.741	08/06/1999	M
P019369	CNAV	Guanajuato	Mexico	20.765	-101.36	19/04/1945	M
P019371	CNAV	Guanajuato	Mexico	20.71833	-101.43	24/02/1942	F
P30801	CNAV	Guanajuato	Mexico	21.4626	-100.99472	16/11/2012	F
P004980	CNAV	Guanajuato	Mexico	20.79722	-101.35972	05/03/1983	F
P004981	CNAV	Guanajuato	Mexico	20.79722	-101.35972	06/03/1983	M
P004938	CNAV	Guanajuato	Mexico	20.79722	-101.35972	13/03/1983	F
P018496	CNAV	Guanajuato	Mexico	20.765	-101.36	17/08/1942	M
P004984	CNAV	Hidalgo	Mexico	20.26	-98.92	27/06/1934	F
P004985	CNAV	Hidalgo	Mexico	20.26	-98.92	28/06/1934	M
P016560	CNAV	Hidalgo	Mexico	20.40833	-99.345	30/01/1981	M

P018499	CNAV	Hidalgo	Mexico	20.54666	-99.30666	10/05/1936	M
P004979	CNAV	Jalisco	Mexico	20.26666	-102.56666	31/05/1964	M
P019372	CNAV	Jalisco	Mexico	20.26666	-102.56666	31/05/1964	F
P013287	CNAV	Nuevo Leon	Mexico	24.90666	-99.50333	26/02/1989	M
P018510	CNAV	San Luis Potosí	Mexico	21.78333	-100.63333	-	M
P018493	CNAV	Sinaloa	Mexico	25.30833	-108.52333	07/03/1937	F
P005727	CNAV	Sonora	Mexico	29	-112.5	15/05/1985	M
P005728	CNAV	Sonora	Mexico	29	-112.5	15/05/1985	F
P005730	CNAV	Sonora	Mexico	29	-112.5	12/08/1985	M
P018494	CNAV	Sonora	Mexico	29	-109.57666	05/03/1948	M
P018511	CNAV	Sonora	Mexico	29.01	-110.14	01/09/1964	F
P004976	CNAV	Tamaulipas	Mexico	27.41	-99.66	11/10/1936	F
12982	MZFC	Aguascalientes	Mexico	22.33333	-102.23333	-	M
12488	MZFC	Baja California	Mexico	32.60556	-115.77444	10/11/1994	M
16024	MZFC	Baja California	Mexico	31.836944	-115.4416	08/04/2011	M
19651	MZFC	Baja California Sur	Mexico	23.75333	-109.975	06/08/2005	F
6801	MZFC	Baja California Sur	Mexico	23.55861	-109.86556	27/04/1988	M
21987	MZFC	Baja California Sur	Mexico	23.28792	-110.12931	06/05/2007	M
6831	MZFC	Baja California Sur	Mexico	23.05	-109.68333	04/05/1988	M
27512	MZFC	Baja California Sur	Mexico	26.68333	-111.75	05/06/2014	M
24601	MZFC	Baja California Sur	Mexico	26.68333	-111.75	03/09/2005	F
16045	MZFC	Baja California Sur	Mexico	25.93111	-111.62083	17/04/2001	F
16310	MZFC	Baja California Sur	Mexico	25.93111	-111.62083	18/04/2001	M
20159	MZFC	Coahuila	Mexico	26.04294	-101.20881	26/02/2007	F
20160	MZFC	Coahuila	Mexico	25.89503	-101.10721	28/02/2007	M
12491	MZFC	Durango	Mexico	24.83	-104.54	23/11/1994	M
12466	MZFC	Durango	Mexico	24.01	-104.855	23/11/1994	F
5435	MZFC	Guanajuato	Mexico	20.22	-101.04667	28/06/1986	M
4654	MZFC	Queretaro	Mexico	20.74	-99.94	18/05/1986	M
4653	MZFC	Queretaro	Mexico	20.74	-99.94	19/05/1986	F
9652	MZFC	Queretaro	Mexico	20.7	-99.91667	25/03/1990	F
9648	MZFC	Queretaro	Mexico	20.7	-99.91667	26/03/1990	M
13218	MZFC	Queretaro	Mexico	20.86103	-99.78712	10/03/1997	F
13215	MZFC	Queretaro	Mexico	20.86103	-99.78712	11/03/1997	M
13233	MZFC	Queretaro	Mexico	20.86103	-99.78712	05/03/1997	M
13390	MZFC	Queretaro	Mexico	20.86103	-99.78712	18/05/1997	-
13407	MZFC	Queretaro	Mexico	20.91764	-99.80467	06/06/1997	M
13065	MZFC	Queretaro	Mexico	20.60667	-99.60833	29/08/1996	F
13228	MZFC	Queretaro	Mexico	21.41626	-99.73613	05/03/1997	M
13242	MZFC	Queretaro	Mexico	20.86103	-99.78712	06/03/1997	M
13056	MZFC	Queretaro	Mexico	20.60667	-99.60833	27/08/1996	F
12481	MZFC	San Luis Potosí	Mexico	21.87	-99.97333	28/11/1994	F
22938	MZFC	Sinaloa	Mexico	26.305	-108.80833	08/08/2009	M
19650	MZFC	Sinaloa	Mexico	26.275	-108.795	27/07/2005	F
20703	MZFC	Sinaloa	Mexico	26.30333	-108.69833	03/08/2006	M

14993	MZFC	Sonora	Mexico	29.01528	-112.2933	23/02/2000	F
14990	MZFC	Sonora	Mexico	29.01528	-112.29333	23/02/2000	F
14992	MZFC	Sonora	Mexico	28.96944	-112.21444	18/02/2000	-
14991	MZFC	Sonora	Mexico	28.96944	-112.21444	16/02/2000	M
14994	MZFC	Sonora	Mexico	29.01528	-112.29333	20/02/2000	F
23536	MZFC	Tamaulipas	Mexico	23.575	-99.755	02/03/2004	-
23535	MZFC	Tamaulipas	Mexico	23.575	-99.755	03/03/2004	F
15831	MZFC	Zacatecas	Mexico	21.63211	-103.07579	19/11/2000	M
15830	MZFC	Zacatecas	Mexico	21.63211	-103.07579	20/11/2000	-

[a] CNAV: Colección Nacional de Aves Instituto de Biología and MZFC:Museo de Zoología Alfonso L. Herrara de la Facultad de Ciencias. UNAM.
For sex: F = females and M = males

Table S2. List of cactus wrens sound recordings used in the study and information of their corresponding sound library, coordinates, state or province and country.

Sound file	Library	Latitude	Longitude	Province	Country
BLB16972	BLB	33.5975	-112.2711	Arizona	United States
BLB7097	BLB	32	-109.15	Arizona	United States
BLB9587	BLB	32.3333	-110.7	Arizona	United States
BLB9609	BLB	32.18051	-110.73708	Arizona	United States
BLB9631	BLB	32.25	-110.73	Arizona	United States
BLB9683	BLB	32.2692	-110.8758	Arizona	United States
BLB10322	BLB	26.08	-98.14	Texas	United States
BLB10360	BLB	26.2167	-97.3486	Texas	United States
BLB16231	BLB	37.1	-113.95	Utah	United States
BLB25732	BLB	37.1042	-113.5833	Utah	United States
103225	ML	31.954	-112.80095	Arizona	United States
109086	ML	31.84916	-109.07583	Arizona	United States
109088	ML	31.845	-109.132	Arizona	United States
118619	ML	32.0875	-112.90661	Arizona	United States
197865	ML	32.4983248	-110.92741	Arizona	United States
20826	ML	31.9155	-109.09166	Arizona	United States
45064	ML	32.226066	-111.06145	Arizona	United States
56860	ML	32.32	-110.81	Arizona	United States
63211	ML	32.353	-111.16805	Arizona	United States
63212	ML	32.3	-111.17	Arizona	United States
63216	ML	32.243889	-111.16806	Arizona	United States
44345	ML	31.88	-109.05194	Arizona	United States
118614	ML	31.69296	-110.3714	Arizona	United States
1502	ML	29.7141	-114.7363	Baja California	México
179895	ML	31.4041	-115.732	Baja california	México
74293	ML	23.151383	-109.76389	Baja California Sur	México
118663	ML	35.6408333	-118.24917	California	United States
169542	ML	33.573152	-115.56765	California	United States
20938	ML	35.6005556	-117.80861	California	United States
120407	ML	35.856592	-119.30944	California	United States
189284	ML	35.12	-115.52	California	United States
140304	BISAM	26.88972	-103.5471	Durango	México
140307	BISAM	26.4125	-103.5471	Durango	México
8805	ML	20.7425	-99.383889	Hidalgo	México
44343	ML	31.8388	-109.11172	New Mexico	United States
63217	ML	32.5881	-107.97425	New Mexico	United States
200408	ML	25.6022	-109.0525	Sinaloa	México

105580	ML	26.5797	-99.13722	Texas	United States
146797	ML	30.6005	-103.92944	Texas	United States
FLMNH00851	FLMNH	34.13	-116.32	California	United States
FLMNH00852	FLMNH	33.87	-115.9	California	United States
FLMNH24678	FLMNH	25.44	-100.97	Coahuila	México
FLMNH19847	FLMNH	20.55	-99.31	Hidalgo	México
FLMNH21355	FLMNH	20.05	-99.34	Mexico	México
FLMNH19843	FLMNH	28.02	-111.05	Sonora	México
FLMNH24677	FLMNH	28.831	-111.803	Sonora	México
FLMNH10258	FLMNH	24.06	-98.38	Tamaulipas	México
FLMNH19846	FLMNH	26.05	-98.3	Tamaulipas	México
FLMNH25555	FLMNH	29.1377778	-103.2425	Texas	United States
FLMNH28267	FLMNH	26.07	-98.15	Texas	United States
FLMNH19842	FLMNH	23.791	-103.773	ZACATECAS	México
FLMNH24676	FLMNH	22.75	-102.58	Zacatecas	México
FLMNH00854	FLMNH	23.203	-102.392	ZACATECAS	México
44756	MZFC	32.27	-111.15	Arizona	United States
44683	MZFC	32.243889	-111.17	Arizona	United States
44684	MZFC	32.24	-111.17	Arizona	United States
44768	MZFC	31.9125	-109.14139	Arizona	United States
1501	MZFC	29.7152	-114.7302	Baja California	México
1503	MZFC	29.74	-114.7275	Baja California	México
1534	MZFC	29.608	-115.01634	Baja California	México
1536	MZFC	29.5986	-115.0061	Baja California	México
1432	MZFC	26.5875	-111.6766	Baja California Sur	México
1459	MZFC	27.24527	-112.95861	Baja California Sur	México
1409	MZFC	25.8397	-111.5533	Baja California Sur	México
1439	MZFC	26.5875	-111.6766	Baja California Sur	México
1196	MZFC	23.3488	-109.4294	Baja California Sur	México
1197	MZFC	23.34027	-109.43083	Baja California Sur	México
1208	MZFC	23.3613	-109.4283	Baja California Sur	México
1422	MZFC	26.38277	-111.67833	Baja California Sur	México
1468	MZFC	27.2452	-112.9586	Baja California Sur	México
167833	MZFC	35.00833	-115.39	California	United States
140307	BISAM	26.8911	-103.935	Durango	México
140306	BISAM	26.4125	-103.5471	Durango	México
WREN_Cactus_s 6x_c Portal 1400m 030910 0742	Xeno-Canto	31.913	-109.07194	Arizona	United States

WREN_Cactus 12x Rodeo NM 1200m 052409 0709	Xeno-Canto	31.89	-109.08472	Arizona	United States
WREN_Cactus 2x Round V 1300m 051809 0656	Xeno-Canto	31.8644	-109.05972	Arizona	United States
WREN_Cactus 3x Portal 1400m 051309 0503	Xeno-Canto	31.8661	-109.0758	Arizona	United States
WREN_Cactus 4x Portal 1400m 052309 0623	Xeno-Canto	31.90027	-109.136	Arizona	United States
WREN_Cactus 4x Rodeo NM 1200m 051709 0706	Xeno-Canto	31.92	-109.13	Arizona	United States
WREN_Cactus 4x Round V 1300m 050509 0709	Xeno-Canto	31.92805	-109.123	Arizona	United States
WREN_Cactus 5x 2nd Portal 1400m 020306 1200	Xeno-Canto	31.9263	-109.095	Arizona	United States
WREN_Cactus 5x Portal 1400m 020306 1200	Xeno-Canto	31.89	-109.24	Arizona	United States
WREN_Cactus 5x Rodeo NM 1200m 051309 0734	Xeno-Canto	31.9172	-109.14083	Arizona	United States
WREN_Cactus 6x Portal 1400m 020406 0815	Xeno-Canto	31.87638	-109.11	Arizona	United States
WREN_Cactus 6x Rodeo NM 1200m 050609 0719	Xeno-Canto	31.9372	-109.17055	Arizona	United States
WREN_Cactus 7x Portal 1400m 051415 0541	Xeno-Canto	31.91	-109.1294	Arizona	United States
WREN_Cactus_s 8th 4x Portal 1400m 040312 1226	Xeno-Canto	31.9155	-109.1425	Arizona	United States
WREN_Cactus_s_c_interact at nest Portal 1400m 022513 0932	Xeno-Canto	31.93	-109.12472	Arizona	United States
CCWRs-c_gd_MojaveNP_SBE_1-21-16_1149fltr	Xeno-Canto	35.357	-115.579	California	United States

[a] BLB: The Borror Laboratory of Bioacoustics; ML: The Macaulay Library at Cornell Lab of Ornithology; FMNH: Florida Museum of Natural History ; MZFC: Biblioteca de Sonidos de Aves de México at Museo de Zoología de la Facultad de Ciencias. UNAM; BISAM: Biblioteca de Sonidos de Aves de México. INECOL A.C.; and Xeno-Canto: https://xeno-canto.org.

Appendix B: Extended Results

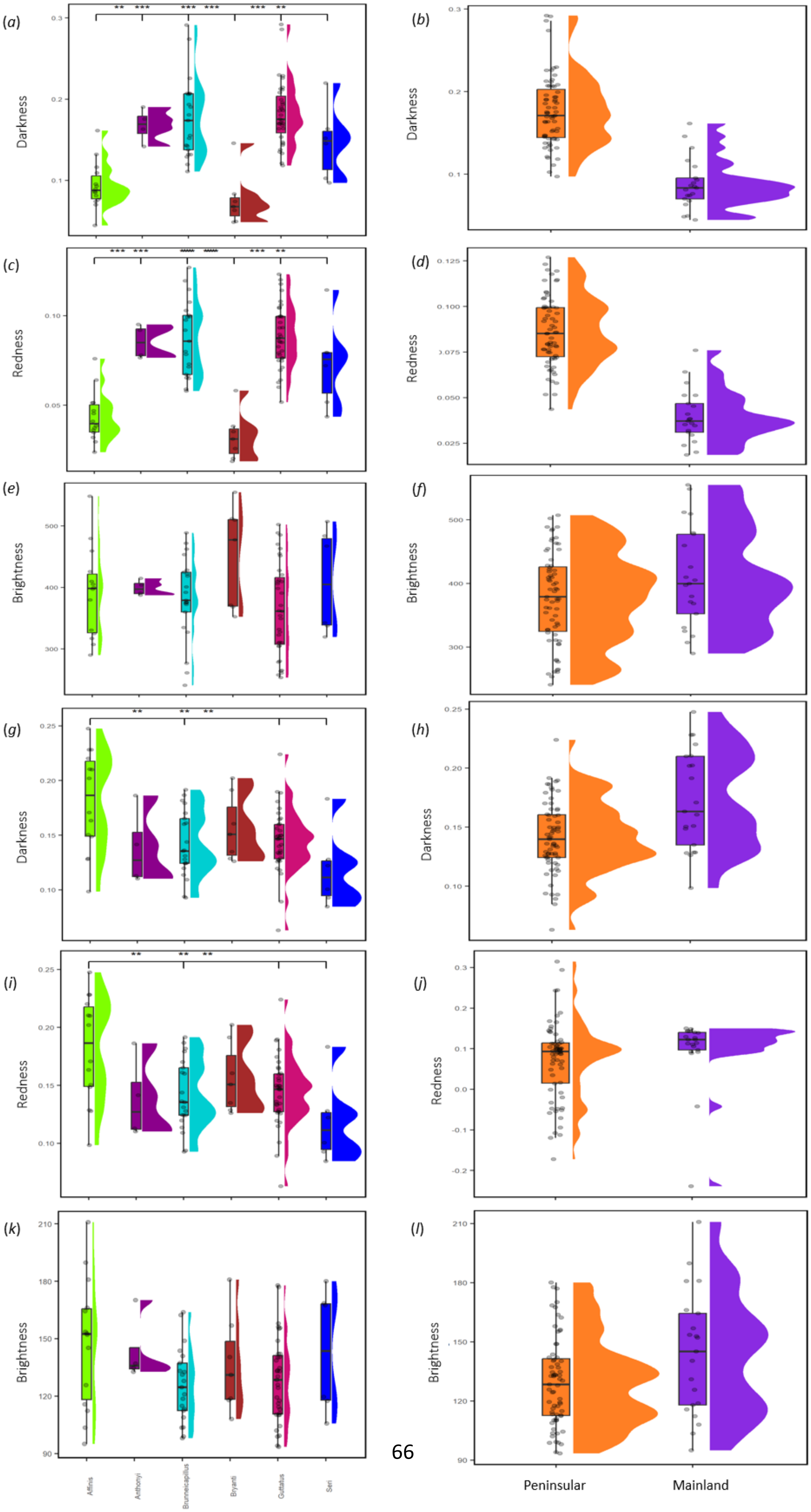
(a)
Darkness
0.1
0.2
0.3
** *** *** *** *** **
(b)
Darkness
0.1
0.2
0.3
(c)
Redness
0.05
0.10
*** *** ***** ***** *** **
(d)
Redness
0.025
0.050
0.075
0.100
0.125
(e)
Brightness
300
400
500
(f)
Brightness
300
400
500
(g)
Darkness
0.10
0.15
0.20
0.25
** ** **
(h)
Darkness
0.10
0.15
0.20
0.25
(i)
Redness
0.10
0.15
0.20
0.25
** ** **
(j)
Redness
-0.2
-0.1
0.0
0.1
0.2
0.3
(k)
Brightness
90
120
150
180
210
Affinis
Anthonyi
Brunneicapillus
Bryanti
Guttatus
Seri
(l)
Brightness
90
120
150
180
210
Peninsular
Mainland

Figure S1 Boxplots showing the pairwise comparisons in cactus wren (*a* - *f*) ventral and (*g* - *l*) crown colour traits between subspecies (left) and peninsular/mainland groups (right). Significant (*a*) darkness and (*c*) redness ventral differences are shown for *C. b. bryanti* with *C. b. anthonyi*. *C. b. brunneicapillus*. *C. b. guttatus* and *C. b. seri*; also for *C. b. affinis* whit *C. b. anthonyi*. *C. b. brunneicapillus* and *C. b. guttatus*. (*g*) Darkness crown significant differences are shown for *C. b.affinis* with *C. b. anthonyi*. *C. b. guttatus* and *C. b. seri* with *C. b. brunneicapillus*. *C. b. bryanti* and *C. b. guttatus*. (*i*) Crown redness significant differences are shown for *C. b. affinis* with *C. b. seri*. Box plots show the percentiles of 25%. 50% (median) and 75% upper and lower whisker show quartiles of 25%. Significant differences between subspecies or between peninsular/ mainland groups are shown with asterisks (*) where 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’

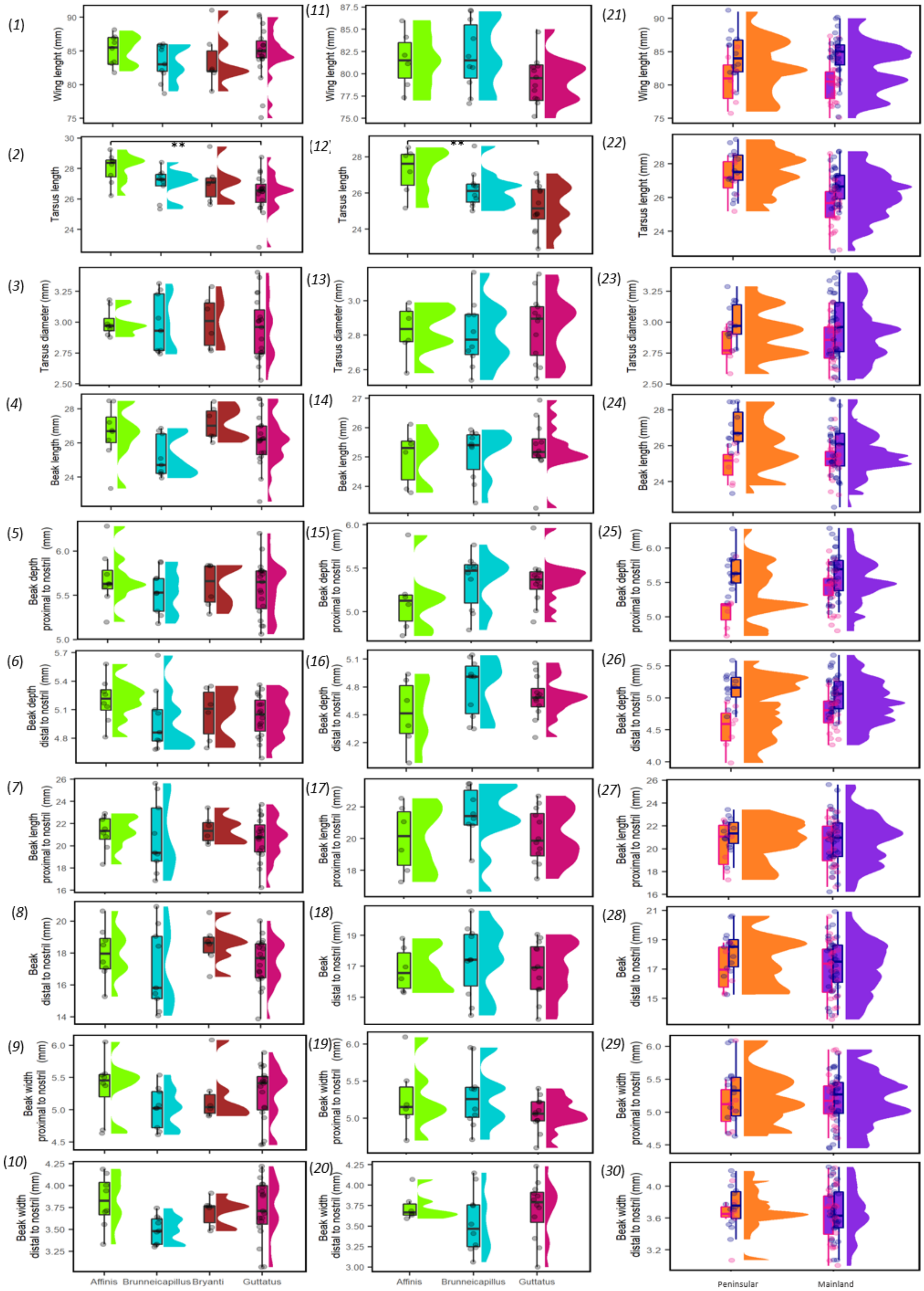
(1)
Wing lenght (mm)
(2)
Tarsus length
**
(3)
Tarsus diameter (mm)
(4)
Beak length (mm)
(5)
Beak depth proximal to nostril (mm)
(6)
Beak depth distal to nostril (mm)
(7)
Beak length proximal to nostril (mm)
(8)
Beak distal to nostril (mm)
(9)
Beak width proximal to nostril (mm)
(10)
Beak width distal to nostril (mm)
Affinis
Brunneicapillus
Bryanti
Guttatus
(11)
Wing lenght (mm)
(12)
Tarsus length
**
(13)
Tarsus diameter (mm)
(14)
Beak length (mm)
(15)
Beak depth proximal to nostril (mm)
(16)
Beak depth distal to nostril (mm)
(17)
Beak length proximal to nostril (mm)
(18)
Beak distal to nostril (mm)
(19)
Beak width proximal to nostril (mm)
(20)
Beak width distal to nostril (mm)
Affinis
Brunneicapillus
Guttatus
(21)
Wing lenght (mm)
(22)
Tarsus lenght (mm)
(23)
Tarsus diameter (mm)
(24)
Beak length (mm)
(25)
Beak depth proximal to nostril (mm)
(26)
Beak depth distal to nostril (mm)
(27)
Beak length proximal to nostril (mm)
(28)
Beak distal to nostril (mm)
(29)
Beak width proximal to nostril (mm)
(30)
Beak width distal to nostril (mm)
Peninsular
Mainland

Figure S2. Boxplots showing pairwise comparisons for cactus wren males (*1-10*) and females (*11-20*) between subspecies (*1-20*) and peninsular/mainland groups (*21-30*). (*2*, *12*) Tarsus length significant differences are shown for *C. b. affinis* and *C. b guttatus.* Males and females between peninsular/mainland groups shown significant differences in tarsus length (*23*). Beak length (*24*) and only for females in beak length proximal (*27*) and distal to nostril (*28*). Box plots show the percentiles of 25%. 50% (median) and 75% upper and lower whisker show quartiles of 25%. Significant differences among subspecies or variables that were significantly different between peninsular/mainland groups are shown with asterisks (*).

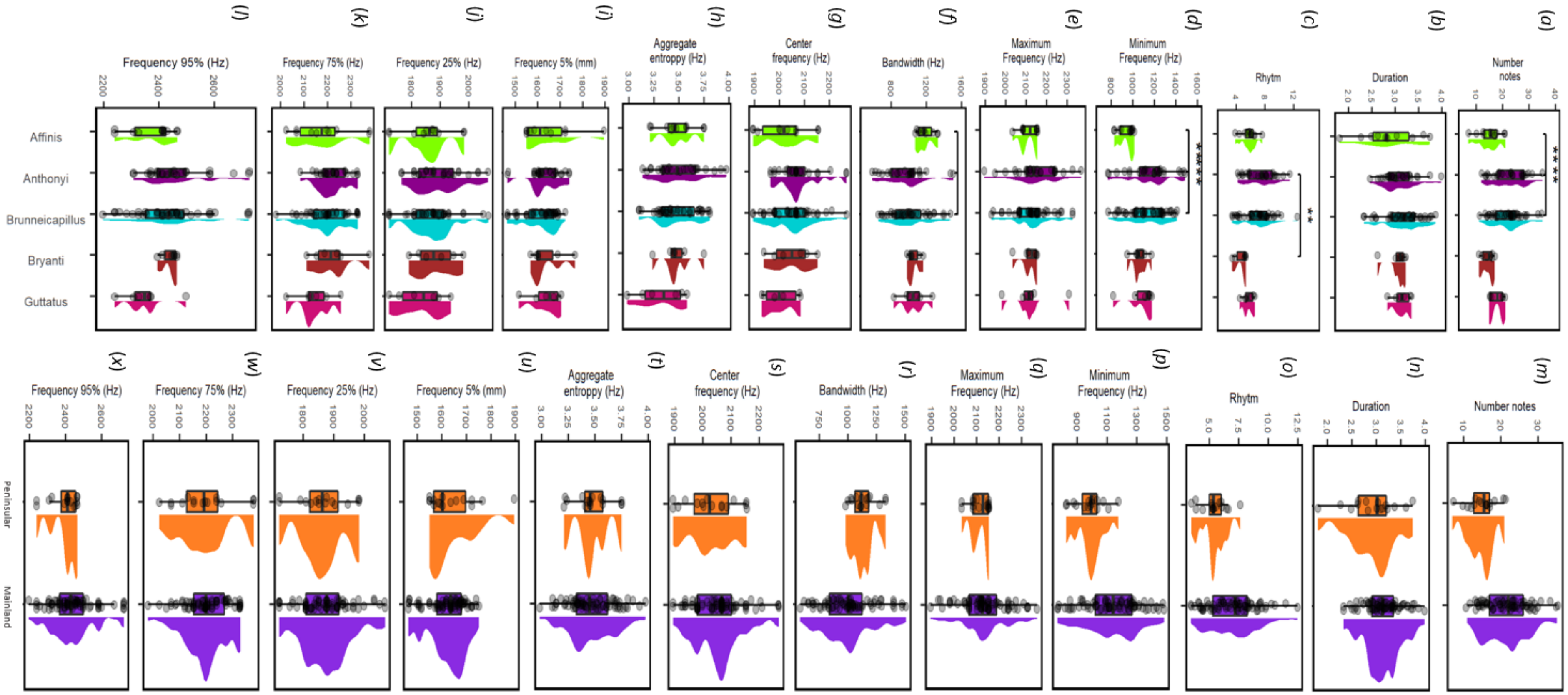
(a) Number notes
(b) Duration
(c) Rhytm
(d) Minimum Frequency (Hz)
(e) Maximum Frequency (Hz)
(f) Bandwidth (Hz)
(g) Center frequency (Hz)
(h) Aggregate entroppy (Hz)
(i) Frequency 5% (mm)
(j) Frequency 25% (Hz)
(k) Frequency 75% (Hz)
(l) Frequency 95% (Hz)
Affinis
Anthonyi
Brunneicapillus
Bryanti
Guttatus
****
**
****
(m) Number notes
(n) Duration
(o) Rhytm
(p) Minimum Frequency (Hz)
(q) Maximum Frequency (Hz)
(r) Bandwidth (Hz)
(s) Center frequency (Hz)
(t) Aggregate entroppy (Hz)
(u) Frequency 5% (mm)
(v) Frequency 25% (Hz)
(w) Frequency 75% (Hz)
(x) Frequency 95% (Hz)
Peninsular
Mainland

Figure S3. Boxplots showing the pairwise results in cactus wren (*a-l*) subspecies and (*m-x*) peninsular/mainland groups. Number notes (*a*), lower frequency (*d*) and bandwidth (*f*) differences are shown for *C. b affinis* with *C. b anthonyi* and *C. b brunneicapillus*. In rhythm (*c*) differences are shown for *C. b bryanti* and *C. b anthonyi.* Peninsular/mainland groups are shown significant differences innumber notes (*m*), duration (*n*), rhythm (*o*), low frequency (*p*) and bandwidth (*r*). Box plots show the percentiles of 25%. 50% (median) and 75% upper and lower whisker show quartiles of 25%. Significant differences among subspecies or variables that were significantly different between peninsular/mainland groups are shown with asterisks (*) where 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' .

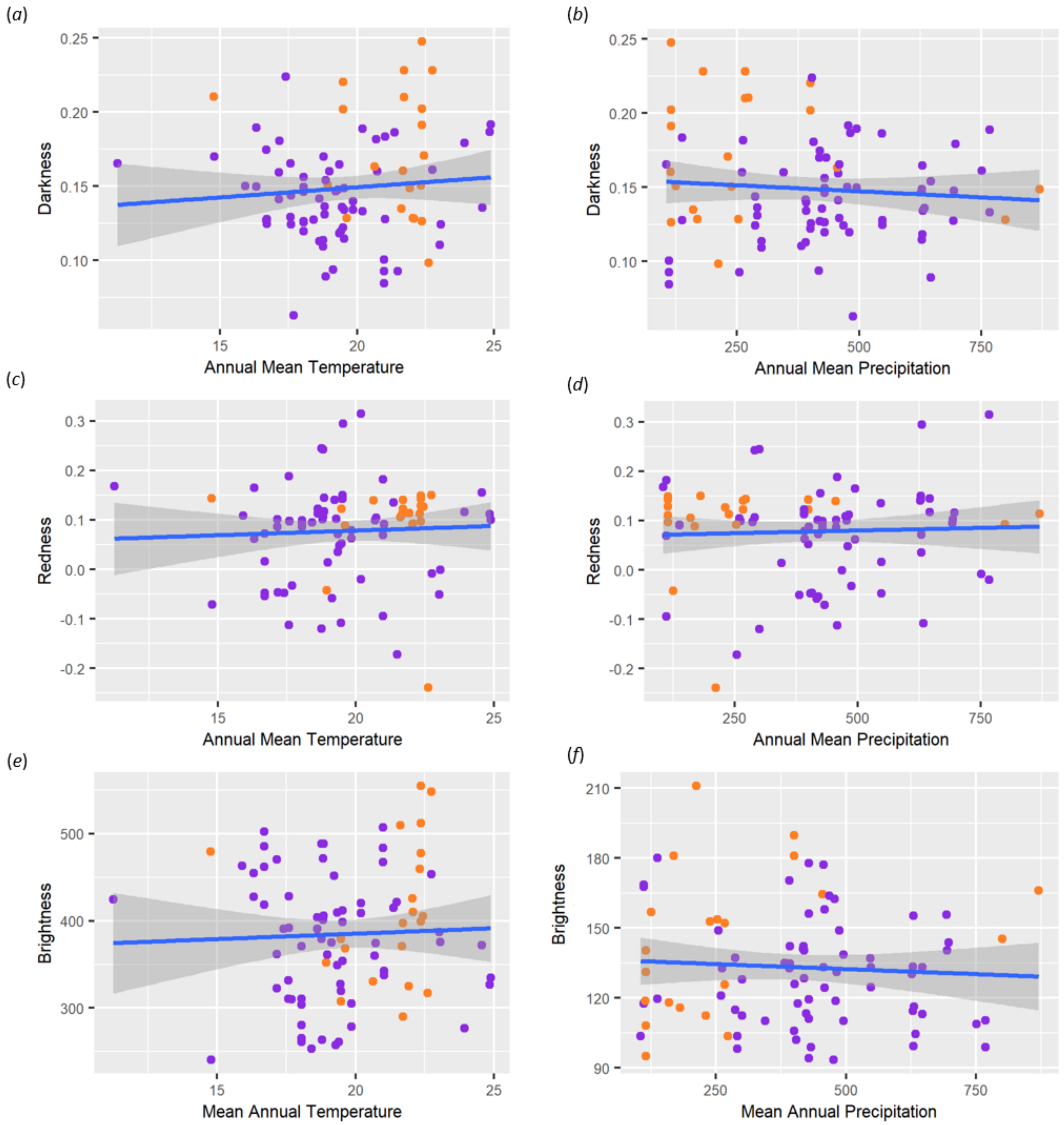

Figure S4 Linear regressions whit not significant results between cactus wren crown colorimetric variables (*a.c*) and (*e*) ventral plumage surface across its range with Annual Mean Temperature and Mean Annual Precipitation with crown surface (*b,d,f*). Orange circles represent the peninsular group and purple circles represent the mainland group.

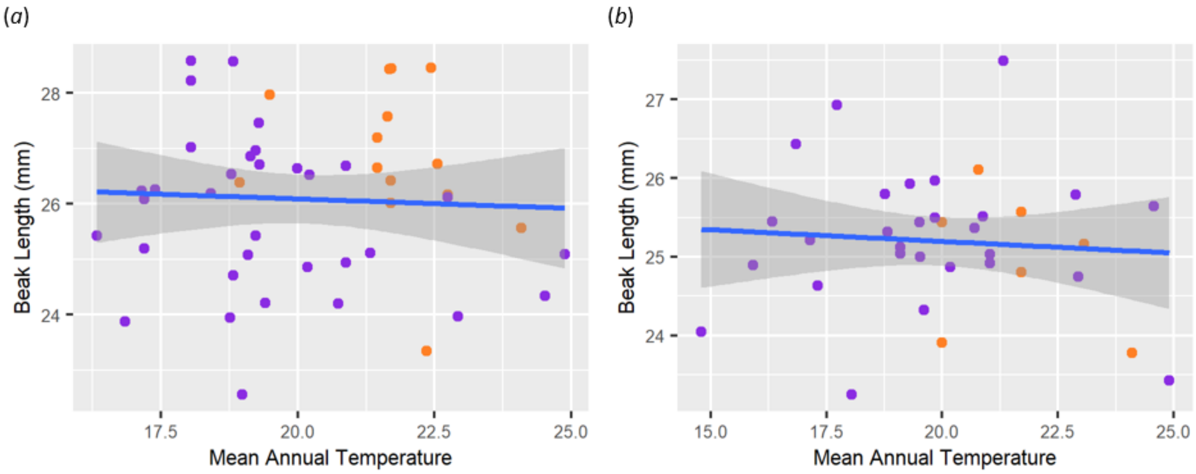

Figure S5 Linear regressions between cactus wren beak length in males (*a*) and females (*b*) across its range with Mean Annual Temperature and Mean Annual Precipitation. Orange circles represent the peninsular group and purple circles represent the mainland group.

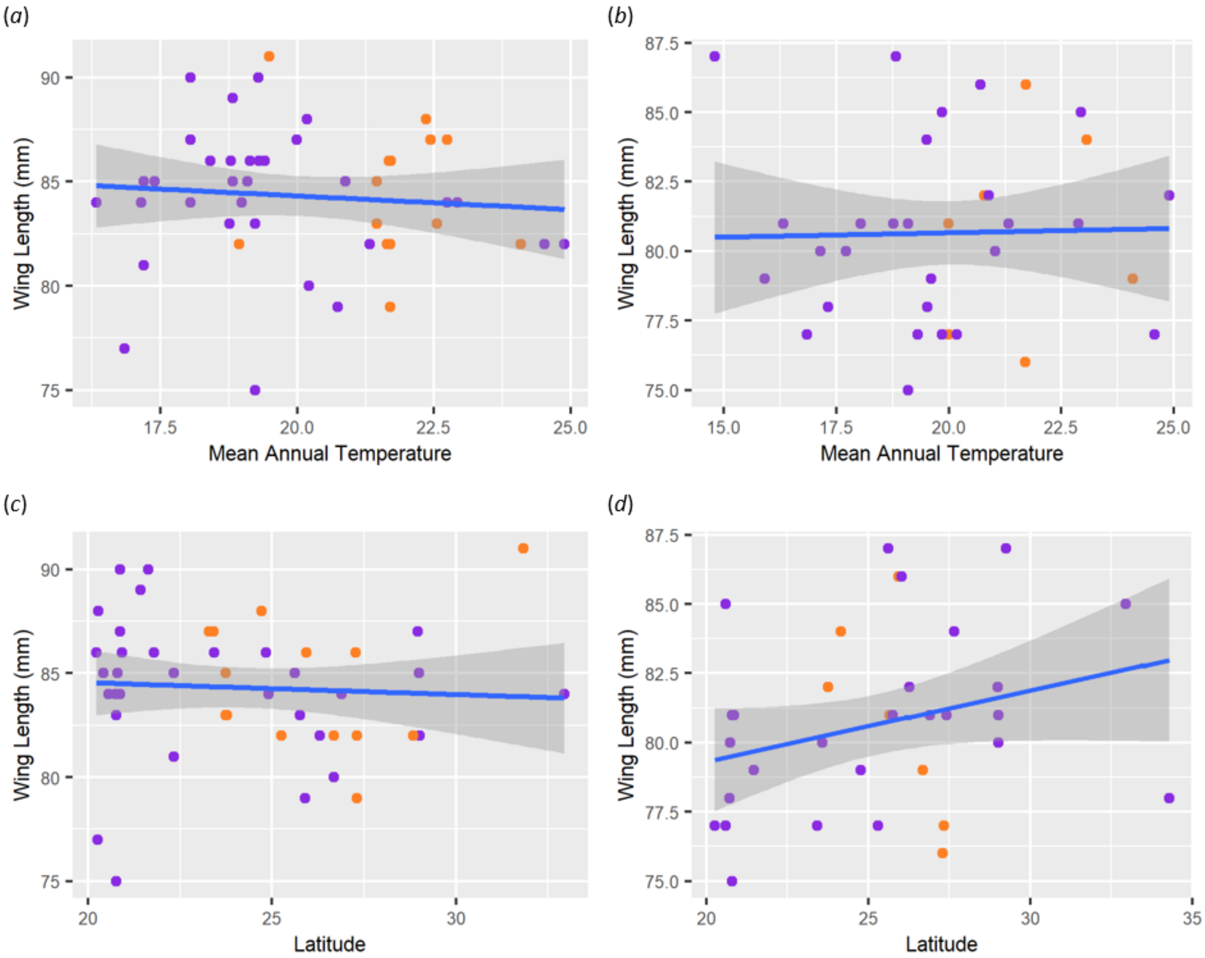

Figure S6. Linear regressions between cactus wren wing length of across its range with Mean Annual Temperature (*a*,*b*) and Latitude (*c*,*d*). Orange circles represent the peninsular group and purple circles represent the mainland group.

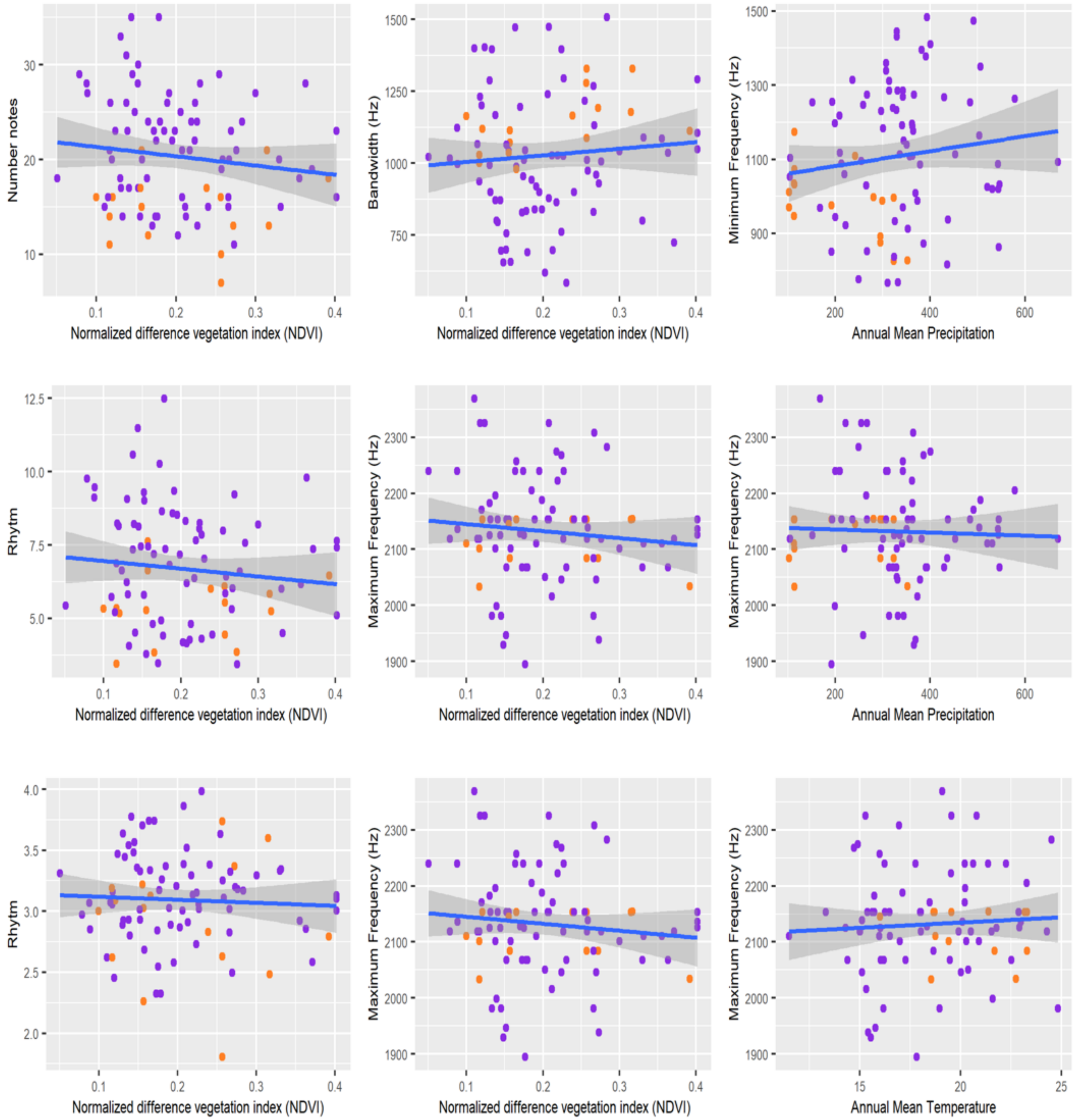

Figure S7. Linear regressions between cactus wren acoustic variables across its range with Normalized Difference Vegetation Index (NDVI). Mean Annual Temperature and Mean Annual Precipitation. Orange circles represent the peninsular group and purple circles represent the mainland group.

Table S1. Sexual dimorphism between cactus wren subspecies in colour traits on ventral and crown plumage surfaces. Mean values and standard deviation are shown in both sexes for each colour trait.

		Males	Females	*W*	*P*
C. b. affinis	Ventral				
	Darkness	0.086±0.023	0.112±0.035	30	0.187
	Redness	0.041±0.012	0.047±0.019	23	0.733
	Brightness	407.17±64.92	350.34±87.29	10	0.18
	Crown				
	Darkness	0.174±0.049	0.197±0.034	25	0.539
	Redness	0.090±0.117	0.125±0.127	20	1
	Brightness	149.16±35.20	144.03±35.69	20	1
C. b. brunneicapillus					
	Ventral				
	Darkness	0.172±0.049	0.182±0.050	63	0.553
	Redness	0.084±0.021	0.090±0.020	63	0.553
	Brightness	379.92±63.02	386.54±75.83	54	1
	Crown				
	Darkness	0.136±0.031	0.148±0.027	68	0.345
	Redness	0.021±0.100	0.107±0.108	79	0.08
	Brightness	128.91±18.31	121.35±20.76	40	0.34
C. b. guttatus					
	Ventral				
	Darkness	0.187±0.046	0.174±0.027	134	0.677
	Redness	0.090±0.020	0.085±0.013	129	0.56
	Brightness	360.77±76.12	370.70±76.25	161	0.65
	Crown				
	Darkness	0.151±0.31	0.135±0.021	84	0.34
	Redness	0.077±0.080	0.081±0.112	167	0.51
	Brightness	127.74±21.47	129.04±23.95	150	0.93
C. b. seri					
	Ventral				
	Darkness	0.139±0.032	0.153±0.061	4	1
	Redness	0.067±0.014	0.079±0.035	5	1
	Brightness	431.21±98.91	387.89±82.94	4	1
	Crown				
	Darkness	0.099±0.019	0.137±0.042	8	0.2
	Redness	0.046±0.138	0.083±0.012	6	0.7
	Brightness	130.21±32.83	156.03±32.14	8	0.2

Table S2. Sexual dimorphism between cactus wren peninsular/mainland groups in colour traits on ventral and crown plumage surfaces. Mean values and standard deviation are shown in both sexes for each colour trait.

		Males	Females	*t* Value	*P*
Peninsular group					
	Ventral				
	Darkness	0.083±0.027	0.103±0.037	1.085	0.323
	Redness	0.038±0.013	0.043±0.019	0.489	0.644
	Brightness	419.027±72.43	382.649±104.563	-0.725	0.49
	Crown				
	Darkness	0.169±0.042	0.183±0.043	6.622	0.564
	Redness	0.090±0.0991	0.119±0.016	1.153	0.264
	Brightness	144.64±32.21	141.43±31.45	-0.19	0.84
Mainland group					
	Ventral				
	Darkness	0.177±0.046	0.173±0.038	-0.421	0.67
	Redness	0.086±020	0.085±0.017	-0.184	0.854
	Brightness	374.21±72.43	381.89±71.11	0.434	0.665
	Crown				
	Darkness	0.141±0.033	0.141±0.0264	-0.016	0.986
	Redness	0.054±0.092	0.094±0.097	1.669	0.1
	Brightness	128.624±20.23	130.58±25.10	0.343	0.732

Table S3 Sexual dimorphism among subspecies of cactus wren morphological traits. All measurements in millimetres. Mean values and standard deviation are shown in both sexes for each morphological measurement within subspecies. Statistically significant differences between sexes within subspecies are highlighted in bold.

	Trait	Males	Females	*W* Value	*P*
C. b. affinis					
	Wing length	85.12±2.23	81.5±3.27	8	**0.04**
	Tarsus length	28.01±0.98	27.21±1.31	14	0.22
	Tarsus diameter	2.99±0.11	2.82±0.15	10	0.08
	Beak length	26.56±1.65	24.99±0.94	7.5	**0.03**
	Beak depth distal to nostril	5.20±0.23	4.51±0.37	2	**0.002**
	Beak width proximal to nostril	5.34±0.47	5.26±0.47	20.5	0.69
	Beak distal to nostril	17.97±1.66	16.79±1.46	15	0.28
	Beak length proximal to nostril	21.20±1.54	20.00±2.15	15	0.28
	Beak depth proximal to nostril	5.68±0.31	5.14±0.40	6.5	**0.02**
	Beak width distal to nostril	3.82±0.29	3.73±0.17	18	0.49
C. b. brunneicapillus					
	Wing length	83.2±2.68	82.1±3.81	38	0.5
	Tarsus length	27.02±0.98	26.21±1.04	23	0.07
	Tarsus diameter	2.97±0.23	2.80±0.18	23	0.07
	Beak length	25.17±1.19	25.10±0.86	46	0.9
	Beak depth distal to nostril	4.99±0.32	4.78±0.30	34	0.39
	Beak width proximal to nostril	5.02±0.32	5.28±0.41	61	0.2
	Beak distal to nostril	17.00±2.56	17.34±2.28	47	0.9
	Beak length proximal to nostril	20.76±3.24	21.23±2.19	50	0.71
	Beak depth proximal to nostril	5.55±0.25	5.35±0.31	29	0.2
	Beak width distal to nostril	3.49±0.15	3.54±0.37	44	0.96
C. b. guttatus					
	Wing length	84.57±3.84	79.25±2.66	31	**<0.001**
	Tarsus length	26.40±1.25	25.23±1.27	53	**0.01**
	Tarsus diameter	2.84±0.25	2.85±0.19	87.5	0.29
	Beak length	26.14±1.53	25.30±0.91	68	0.06
	Beak depth distal to nostril	5.02±0.21	4.68±0.22	30	**<0.001**
	Beak width proximal to nostril	5.24±0.43	5.05±0.22	72	0.09
	Beak distal to nostril	17.44±1.50	16.80±1.80	91.5	0.37
	Beak length proximal to nostril	20.65±1.92	20.15±1.69	94.5	0.44
	Beak depth proximal to nostril	5.58±0.31	5.35±0.27	65.5	**0.05**
	Beak width distal to nostril	3.73±0.34	3.70±0.35	110.5	0.9
C. b. seri					
	Wing length	85.6±1.15	80.6±1.15	0	0.07
	Tarsus length	26.7±1.71	25.8±0.13	3	0.7
	Tarsus diameter	3.1±0.13	2.9±0.11	0	0.1
	Beak length	26±0.99	25.15±0.31	2	0.4
	Beak depth distal to nostril	5.4±0.18	5.1±0.21	1	0.2

	Beak width proximal to nostril	5.3±0.311	5.5±0.28	7	0.3
	Beak distal to nostril	17.64±1.55	16±1.80	2	0.4
	Beak length proximal to nostril	21.27±1.40	19±1.53	1	0.2
	Beak depth proximal to nostril	5.95±0.23	5.7±0.08	2	0.4
	Beak width distal to nostril	3.97±0.36	3.8±0.19	3	0.7

Table S4. Table. Sexual dimorphism among peninsular/mainland groups of cactus wren morphological traits. Mean values and standard deviation are shown in both sexes for each morphological measurement. Statistically significant differences are highlighted in bold.

	Trait	Males	Females	*t* Value	*P*
Peninsular group					
	Wing length	84.5±3.180	80.714±3.638	-2.341	**0.039**
	Tarsus length	27.616±1.210	27.177±1.202	-0.787	0.446
	Tarsus diameter	3.007±0.156	2.810±0.144	-2.771	**0.015**
	Beak length	26.810±1.389	24.967±0.864	-3.727	**0.001**
	Beak depth distal to nostril	5.140±0.252	4.525±0.340	-4.2342	**0.001**
	Beak width proximal to nostril	5.286±0.449	5.182±0.491	-0.468	0.648
	Beak distal to nostril	18.220±1.507	17.078±1.535	-1.6154	0.132
	Beak length proximal to nostril	21.312±1.381	20.317±2.135	-1.121	0.292
	Beak depth proximal to nostril	5.654±0.280	5.151±0.371	-3.158	**0.01**
	Beak width distal to nostril	3.768±0.249	3.641±0.299	-0.966	0.355
Mainland group					
	Wing length	84.212±3.304	80.642±3.234	-4.252	**<0.001**
	Tarsus length	26.668±1.212	25.549±1.190	-3.626	**<0.001**
	Tarsus diameter	2.959±0.248	2.840±0.171	-2.19	**0.0325**
	Beak length	25.776±1.428	25.252±0.902	-1.735	0.088
	Beak depth distal to nostril	5.062±0.277	4.783±0.272	-3.937	**<0.001**
	Beak width proximal to nostril	5.187±0.387	5.210±0.353	0.243	0.808
	Beak distal to nostril	17.320±1.769	17.024±1.888	-0.627	0.533
	Beak length proximal to nostril	20.776±2.215	20.615±1.926	-0.303	0.762
	Beak depth proximal to nostril	5.636±0.319	5.415±0.288	-2.836	**0.006**
	Beak width distal to nostril	3.681±0.316	3.651±0.330	-0.357	0.722

Table S5. Results of the General Lineal Models for differences between cactus wren subspecies and peninsular/mainland groups in colour traits. Statistically significant differences are highlighted in bold. *These variables were log-transformed

Subspecie		df	SS	MS	F value	P
	Ventral					
	Darkness	5	0.133	0.026	16.51	**<0.001**
	Redness	5	0.036	0.007	22.62	**<0.001**
	Brightness	5	48243	9649	1.815	0.119
	Crown					
	Darkness	5	0.022	0.004	4.063	**0.002**
	Redness	5	0.022	0.004	4.033	**0.002**
	Brightness	5	0.287	0.057	1.732	0.137*
Peninsular/mainland groups						
	Ventral					
	Darkness	1	0.124	0.12	76.16	**<0.001**
	Redness	1	0.034	0.034	105.8	**<0.001**
	Brightness	1	17229	17229	3.192	0.077
	Crown					
	Darkness	1	0.617	0.6168	11.33	**0.001***
	Redness	1	0.649	0.6489	11.68	**<0.001***
	Brightness	1	0.151	0.151	4.568	**0.035***

Table S6. Results of Kruskal-Wallis for differences between cactus wren subspecies and peninsular/mainland groups in morphological traits. Statistically significant differences are highlighted in bold.

		males			females		
		df	*X^2 value*	*P*	df	*X^2 value*	*P*
Subspecie	Wing length	3	2.83	0.41	2	4.12	0.12
	Tarsus length	3	8.78	**0.03**	2	7.16	**0.02**
	Tarsus diameter	3	0.81	0.84	2	0.3	0.85
	Beak length	3	6.47	0.09	2	0.09	0.95
	Beak length proximal to nostril	3	1.21	0.74	2	2.08	0.35
	Beak length distal to nostril	3	3.07	0.38	2	0.77	0.67
	Beak depth proximal to nostril	3	3.93	0.26	2	2.38	0.3
	Beak depth distal to nostril	3	0.66	0.88	2	2.51	0.28
	Beak width proximal to nostril	3	3.23	0.35	2	1.87	0.39
	Beak width distal to nostril	3	6.97	0.07	2	1.57	0.45
Peninsular/mainland groups	Wing length	1	0.002	0.96	1	0.003	0.95
	Tarsus length	1	4.98	**0.02**	1	7.1	**0.007**
	Tarsus diameter	1	0.48	0.48	1	0.17	0.67
	Beak length	1	4.78	**0.02**	1	0.3	0.57
	Beak length proximal to nostril	1	0.97	0.32	1	0.08	0.77
	Beak length distal to nostril	1	2.76	0.09	1	0	1
	Beak depth proximal to nostril	1	0.01	0.91	1	3.7	**0.05**
	Beak depth distal to nostril	1	1.12	0.28	1	3.22	0.07
	Beak width proximal to nostril	1	0.26	0.6	1	0.09	0.75
	Beak width distal to nostril	1	1.09	0.29	1	0.02	0.86

Table S7 Results of Procrustes ANOVA on deviations from beak dorsal view mean landmarks configuration. Statistically significant differences are highlighted in bold.

	df	SS	*F*	*P*
Individual	145	0.3	8.69	0.91
Side	1	0.002	12.4	**<0.001**
Individual x side	145	0.03	0	0.5
Error	0	0		
Total	291	0.34		

Table S8. Results of Procrustes ANOVA for allometry analysis for beak dorsal and lateral views. Statistically significant differences are highlighted in bold.

View	Trait	df	SS	*F*	*P*
Dorsal	Log (Centroid size)	1	0.004	4.74	**0.02**
Lateral	Log (Centroid size)	1	0.01	10.24	**<0.001**

Table S9. Results of Kruskal-Wallis for differences between cactus wren subspecies and peninsular/mainland groups in acoustic traits. Statistically significant differences are highlighted in bold.

		df	X^2	*P*
Subspecies	Duration	4	4.52	0.33
	Rhytm	4	14.27	**0.006**
	Minimum frequency	4	13.43	**0.009**
	Maximum frequency	4	1.31	0.85
	Bandwidth	4	11.78	**0.01**
	Frequency 75%	4	4.74	0.31
	Frequency 95%	4	6.45	0.16
Peninsular/mainland groups	Duration	1	2.367	0.123
	Rhytm	1	8.82	**0.002**
	Minimum frequency	1	10.34	**<0.001**
	Maximum frequency	1	0.84	0.35
	Bandwidth	1	7.71	**0.005**
	Frequency 75%	1	0.24	0.61
	Frequency 95%	1	0.92	0.33

Table S10. Results of General Lineal Model for differences between cactus wren subspecies and peninsular/mainland groups in acoustic traits. Statistically significant differences are highlighted in bold.

		df	SS	MS	*F*	*P*
Subspecies	Number notes	4	714,3	178,59	6.23	**<0.001**
	Frequency 25%	4	27241	6810	1.01	0.4
	Frequency 5%	4	13579	3395	0.56	0.68
	Center frequency	4	40741	10185	1.41	0.23
	Aggregate entropy	4	0,161	0,04	0.91	0.46
Peninsular/mainland groups	Number notes	1	590,5	590,5	20.14	**<0.001**
	Frequency 25%	1	940	940	0.14	0.7
	Frequency 5%	1	4907	4907	0.79	0.37
	Center frequency	1	9363	9363	1.28	0.26
	Aggregate entropy	1	0,001	0,001	0.03	0.86