



**UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO**  
**POSGRADO EN CIENCIAS BIOLÓGICAS**  
FACULTAD DE CIENCIAS

**DIVERSIFICACIÓN DE *GLOSSOPHAGA SORICINA* EN MESOAMÉRICA**

**TESIS**

QUE PARA OPTAR POR EL GRADO DE:

**DOCTORA EN CIENCIAS**

PRESENTA:

**ADRIANA CALAHORRA OLIART**

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CIUDAD UNIVERSITARIA, CD. MX.

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**2022**

COORDINACIÓN DEL POSGRADO EN CIENCIAS BIOLÓGICAS  
FACULTAD DE CIENCIAS  
DIVISIÓN ACADÉMICA DE INVESTIGACIÓN Y POSGRADO  
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**M. en C. Ivonne Ramírez Wence**  
**Directora General de Administración Escolar, UNAM**  
**Presente**

Me permito informar a usted que en la reunión del Comité Académico del Posgrado en Ciencias Biológicas, celebrada el día **14 de febrero de 2022** se aprobó el siguiente jurado para el examen de grado de **DOCTORA EN CIENCIAS** del estudiante **CALAHORRA OLIART ADRIANA** con número de cuenta **408038324** con la tesis titulada: **“DIVERSIFICACIÓN DE GLOSSOPHAGA SORICINA EN MESOAMÉRICA”**, realizada bajo la dirección del (la) **DRA. LIVIA SOCORRO LEÓN PANIAGUA**:

Presidente: DR. ADOLFO GERARDO NAVARRO SIGÜENZA  
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Secretario: DR. DANIEL IGNACIO PIÑERO DALMAU

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

**ATENTAMENTE**  
**“POR MI RAZA HABLARÁ EL ESPÍRITU”**  
Ciudad Universitaria, Cd. Mx., a 12 de mayo de 2022

**COORDINADOR DEL PROGRAMA**



**DR. ADOLFO GERARDO NAVARRO SIGÜENZA**



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A la Dra. Eréndira Álvarez Pérez

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## RESUMEN

Los complejos de especies crípticas están presentes en todos los grupos de seres vivos. La mayoría de las especies descritas actualmente son a partir de la detección de estos complejos, y ha ido de la mano con los avances en técnicas moleculares. Originalmente descrita como una sola especie de amplia distribución, *Glossophaga soricina* demostró tener muy altos niveles de variación molecular, lo cual levantó sospechas de que pudiese tratarse de un complejo de especies crípticas. En este estudio, se utilizó el análisis de morfometría geométrica para estudiar la variación en forma y tamaño de de cráneos y mandíbulas, y el modelado de nicho ecológico para caracterizar los requerimientos ambientales de estos taxa. Al integrar datos de diferentes tipos de caracteres, se obtiene información independiente sobre las características que soportan la ubicación taxonómica de los linajes bajo estudio y se puede analizar su historia evolutiva. La variación en tamaño y forma apoyó los principales linajes moleculares reportados previamente, y su análisis permitió describir las características morfológicas de los taxa. Se encontraron convergencias en tamaño y forma entre las especies de distribución restringida, por lo que se exploró su posible correlación con variables abióticas. Las especies continentales tuvieron los valores más altos de solapamiento de nicho a pesar de su diferenciación morfológica, mientras que los grupos insulares resultaron ecológicamente distintos. Así, las convergencias y diferencias en la forma de estos grupos no se debe a los factores ambientales considerados en este estudio, sino posiblemente a factores relacionados con sus nichos eltonianos. Las principales regiones de no afinidad ecológica de estas especies corresponden con las regiones más elevadas de los Andes y explican la barrera al flujo génico que podría estar detrás de la divergencia de los grupos y aportan evidencia de que se trata de linajes que están evolucionando separadamente. A partir de la evidencia presentada en este trabajo, se elevan cuatro de las cinco subespecies al nivel taxonómico de especie. Los nombres correctos de las especies en el complejo taxonómico analizado son: *G. soricina* (Pallas 1766), *G. mutica* Merriam 1898, *G. antillarum* Rehn 1902, y *G. valens* Miller 1913.

## ABSTRACT

Cryptic species complexes are widespread in all groups of living beings. Their discovery represents the majority of the newly described species in recent years and is related with the advances in molecular techniques. Originally described as a single widespread species, *Glossophaga soricina* was found to have high levels of molecular variation within it, suggesting that it could be a cryptic species complex. In the present study, we used geometric morphometrics analyses to study size and shape variation of skulls and mandibles, and ecological niche modeling to characterize the ecological requirements of these taxa. Integrating data from different types of characters provides independent information of the characteristics that support the taxonomic positions of the studied lineages. and allows to study their evolutionary history. Variation in shape and size of skull and mandible supports the main molecular lineages reported in previous studies, and its study allowed morphological descriptions of the taxa to be made. Convergences in size and shape between the species with restricted distributions were found, so their correlation with abiotic variables was explored. Mainland species presented the highest niche overlap values regardless of their morphological differentiation, while the insular groups proved to be ecologically distinct. Therefore, the convergence and dissimilarities in the shape of these groups must be driven by factors not considered in this study, most likely related to their Eltonian ecological niches. The non-suitability regions that separate the distributions of the continental species explain the barrier to the genetic flux that could be behind their divergence and add evidence of them being separately evolving lineages. Based on the evidence we present in this work we elevate four out of the five subspecies to the taxonomic level of species. The correct names for the species of the analyzed complex are *G. soricina* (Pallas 1766), *G. mutica* Merriam 1898, *G. antillarum* Rehn 1902, and *G. valens* Miller 1913.

## INTRODUCCIÓN GENERAL

A lo largo del desarrollo de las ciencias naturales, las formas biológicas han sido descritas y clasificadas predominantemente con base en sus características externas, seguramente debido a que el sentido de la vista es el que da la mayor información sensorial al ser humano (Bickford et al. 2007). La observación de similitudes y diferencias físicas ha sido el primer criterio que ha llevado a clasificar a los seres vivos y, en el proceso, a detectar discontinuidades taxonómicas (MacLeod 2002; Cardini y Elton 2008; Adams et al. 2013), así como a cuestionarse sobre sus relaciones de parentesco y su historia evolutiva (Darwin 1859). Este proceso de clasificación de especies, al estar basado principalmente en la información morfológica percibida, es propenso a imprecisiones y a errores (Beheregaray y Caccone 2007), puesto que dichas semejanzas y diferencias externas muchas veces no son un reflejo fidedigno de las relaciones filogenéticas entre los ejemplares. Esto ha dado lugar a que la historia de la taxonomía ha estado llena de procesos de reclasificación que se han usado para corregir esto, sobre todo a la luz de los nuevos descubrimientos moleculares, como se detallará más adelante.

Las especies crípticas se definen como aquellas que resultan morfológicamente indistinguibles ante el observador humano y que, por lo tanto, son agrupadas juntas bajo la misma unidad taxonómica a pesar de estar filogenéticamente separadas en mayor o menor medida (Jones 1997). La prevalencia de complejos de especies crípticas es frecuente incluso en la actualidad: la mayoría de las especies descritas, incluso dentro de grupos bien estudiados, corresponde a ellos, los cuales son hallados en mucha mayor medida que el descubrimiento de especies novedosas o de aquellas que se creían extintas (por ejemplo, Castella et al. 2001; Mayer y von Helversen 2001; Evin et al. 2008; Clare et al. 2013; Morales et al. 2016; Molinari et al. 2017). Aunque la mayoría de los estudios de este tipo se concentran en animales (Bickford et al. 2007), las especies crípticas se encuentran en todos los grupos de organismos y regiones biogeográficas (Pfenninger y Schwenk 2007): tanto en especies terrestres como en marinas (Knowlton et al. 1993; Vrijenhoek et al. 1994), en hongos (Bidochka et al. 2001), en insectos (Herbert et al. 2004; Janzen et al. 2017), y en vertebrados pequeños o tan grandes como jirafas (Fennessy et al. 2016). Su estudio resulta crucial para tener un mejor entendimiento de los procesos evolutivos. Por ejemplo, lleva a plantear preguntas acerca de las causas de la convergencia o estasis morfológica, aporta argumentos

a la discusión acerca del umbral necesario para considerar a las especies como distintas, y enriquece la discusión sobre la utilidad de los diferentes conceptos de especie para la delimitación de las mismas. Además, su detección es importante para tener un correcto estimado de la biodiversidad y para la toma de decisiones informadas basadas en información correcta, por ejemplo, respecto a programas de conservación y manejo adecuado de especies de importancia médica y comercial (Bickford et al. 2007; Tsang et al. 2016).

Las causas de la existencia de complejos de especies crípticas obedecen a factores tanto metodológicos como biológicos. Respecto a las razones metodológicas, las técnicas de descripción de las especies, el concepto de especie elegido y las herramientas taxonómicas utilizadas determinan si se consigue o no separar un grupo taxonómico. Asimismo, la variación morfológica existente puede ser tan sutil que pase desapercibida al observador humano. A nivel biológico, existen procesos evolutivos tanto bióticos como abióticos que favorecen la estasis morfológica o las convergencias (Mayer y von Helversen 2001; Smith et al. 2011; Losos et al. 2011; Friedman et al. 2016; Zelditch et al. 2017). De igual manera, es importante considerar que la divergencia en otro tipo de caracteres (como pueden ser moleculares, químicos, de comunicación, entre otros) no necesariamente irá acompañada de cambios externos apreciables (de Queiroz 2007). Es así como el término “especie críptica” no hace referencia a una propiedad biológica como tal de los grupos en cuestión, sino simplemente al hecho de que han sido clasificadas bajo el mismo nombre por su aparente similitud morfológica.

Otro factor fundamental a tomar en cuenta para el estudio de los complejos de especies crípticas, y para la la biología evolutiva en general, es que la especiación es un proceso heterogéneo, a lo largo del cual los linajes divergen en características particulares en distintos momentos, a raíz de diferentes fuerzas evolutivas, y en distinto orden (de Queiroz 2007), con lo cual las historias evolutivas de las especies no se reflejan de igual manera en todos sus caracteres (i.e., Ruedi y Mayer 2001; Evin et al. 2008). Por lo tanto, basarse en un solo tipo de datos podría llevar a conclusiones incompletas o engañosas acerca de sus relaciones filogenéticas. Es por esto que la mejor estrategia cuando se busca hacer inferencias filogenéticas y delimitar especies es echar mano de la integración de datos derivados de distintas disciplinas y metodologías, buscando aportar información independiente sobre las características que soportan la ubicación taxonómica de los organismos en estudio

(Raxworthy et al. 2007; Rissler y Apodaca 2007; Gager et al. 2016). Este es el objetivo de la taxonomía integrativa, la cual consiste en utilizar múltiples perspectivas complementarias para identificar y describir los taxa, por ejemplo, ecología, filogeografía, morfología, acústica, etología, entre otros (Dayrat 2005).

Dentro de la variedad de caracteres que se pueden analizar al estudiar la historia evolutiva de las especies, los marcadores moleculares suelen considerarse los más objetivos, ya que contienen cinco estados de carácter fácilmente identificables y mutuamente excluyentes (aunque no están exentos de presentar homoplasia) y son lo suficientemente numerosos e independientes entre ellos como para que su coincidencia asegure homología, por lo que las divergencias resultan más informativas (Gould 1986; Grauer y Li 2000; Wiens 2004; Suárez-Díaz y Anaya-Muñoz 2008 ) haciéndolos, en la actualidad, la base de las reconstrucciones filogenéticas. Resalta su papel, sobre todo, en el descubrimiento de especies crípticas, donde el hallazgo de divergencias moleculares suele dar la primera pista de la existencia de más de un linaje (i.e. Mayer y von Helversen 2001; Clare 2011; Gager et al. 2016).

Sin embargo, a pesar del consenso general sobre la importancia de utilizar datos moleculares, es deseable complementar la evidencia taxonómica hallada con datos fenotípicos. No sólo por buscar un enfoque integrativo que implique los beneficios mencionados anteriormente, sino porque la utilización de datos moleculares también presenta limitantes. Por ejemplo, en casos de atracción de ramas largas, en especies recientemente derivadas donde las diferencias genéticas fuesen insuficientes para ser detectadas, o en casos de divergencia con flujo génico y procesos selectivos donde los loci estudiados no pudieran reflejar estos procesos. Esto compromete su poder para detectar los límites de las especies y reflejar adecuadamente los procesos de divergencia, por lo cual se recomienda utilizar datos genéticos a la par con datos fenotípicos. Los datos moleculares ayudan a dar la certidumbre de si los caracteres fenotípicos son homologías u homoplasias, condición indispensable para los estudios evolutivos. Por su parte, los caracteres fenotípicos son indispensables para describir las especies detectadas a partir de divergencias moleculares e identificar sus caracteres diagnósticos, requisito indispensable para el quehacer taxonómico (Wiens 2004; Mayer y von Helversen 2001; Solis-Lemus et al. 2014; Herdina et al. 2014).

El orden Chiroptera es particularmente propenso a tener especies pasadas por alto dentro de su taxonomía, dada su naturaleza esquiva al ser nocturnos, voladores, y a tener diferencias morfológicas muy sutiles incluso entre especies lejanamente emparentadas (Jones 1997; Mayer y von Helversen, 2001; Clare et al. 2013). Esta situación presenta interesantes retos taxonómicos. Es sabido que en murciélagos existe incongruencia filogenética entre árboles contruidos a partir de distintos tipos de caracteres (Dávalos et al. 2012), demostrando que el proceso de especiación no se ve reflejado de igual manera en todos ellos. Debido a esto, se estima que el número de especies en este orden podría estar muy subestimada: hasta 42% mayor de lo que sugieren las cifras actuales (Clare 2011), o hasta del doble de lo reconocido por morfometría clásica (Francis et al. 2010). Tal es el caso de *Glossophaga soricina*, una especie nominal del Neotrópico perteneciente a la familia Phyllostomidae. A lo largo de su amplia distribución, *G. soricina* ha sido dividida en cinco subespecies con distribuciones no simpátricas: *G. s. antillarum* (Rehn, 1902) en Jamaica, *G. s. handleyi* (Webster y Jones, 1980) en México, Centroamérica y norte de Colombia, *G. s. mutica* (Merriam, 1898) en las Islas Mariás de México, *G. s. soricina* (Pallas, 1766) en Sudamérica al este de los Andes y *G. s. valens* (Miller, 1913) en Sudamérica al oeste de los Andes. Estudios moleculares previos han coincidido al reportar alta variación molecular al interior de esta supuesta especie. Específicamente, estos hallazgos han demostrado la existencia de dos clados monofiléticos bien soportados y con elevada distancia molecular entre ellos: uno formado por las poblaciones del este de los Andes, correspondientes a la subespecie *G. s. soricina*, y otro linaje que incluye al resto de las subespecies, las cuales a su vez también forman al interior de éste clados monofiléticos bien soportados, aunque separados por una menor distancia genética (Dias et al. 2017; Hoffmann et al. 2019).

En este proyecto, se utilizó taxonomía integrativa para sumar evidencias que aporten las pruebas necesarias para discernir la situación taxonómica de *G. soricina*, con base en los análisis moleculares previos que indican que podría ser un complejo de especies crípticas (Dias et al. 2017; Hoffmann et al. 2019). Específicamente, se analizó con morfometría geométrica el tamaño y la forma de cráneos y mandíbulas de las subespecies que conforman el complejo *G. soricina*, y mediante modelado de nicho ecológico se analizó el espacio ambiental que cada una habita a partir de la idoneidad climática de cada subespecie.

La morfometría geométrica es una rama de la morfometría que comprende una serie de métodos que describen matemáticamente las formas biológicas a partir de definiciones geométricas de su tamaño y forma (Savriama 2018). Se diferencia de la tradicional en que, en vez de utilizar medidas lineales, registra las estructuras anatómicas utilizando configuraciones de marcas con coordenadas cartesianas (Mitteroecker y Gunz 2009, Webster y Sheets 2010). Estas constelaciones de marcas se utilizan como las variables de la forma. Así, es posible analizar estadísticamente las estructuras biológicas y generar representaciones gráficas de la misma. Además, es particularmente apta para detectar variación morfológica sutil, lo cual la vuelve útil para los estudios taxonómicos y filogenéticos en murciélagos, donde su poder descriptivo y estadístico para separar taxa crípticos y variación a nivel de especie se ha demostrado (Mayer y von Helversen 2001; Evin et al. 2008). Los análisis de modelado de nicho ecológico, por su parte, aportan información acerca los requerimientos ambientales de las especies estableciendo una asociación entre la zona que habita una especie y las condiciones ambientales de esa región, combinando los datos de ocurrencia de las especies con capas de información de sistemas de información geográfica (Warren y Seifert 2011; Alvarado-Serrano y Knowles 2014). Esto permite describir las características ambientales del espacio que ocupan y clasificar las celdas que componen el área de estudio según el grado al cual cada una es adecuada para la especie, esto es, qué tanto se asemejan a las condiciones del área de distribución conocida de ésta (Hirzel et al. 2002). Estos análisis aportan también pruebas de su separación y de las posibles causas detrás de los eventos que originaron su divergencia. Todo esto permite arrojar luz sobre los factores que afectan los límites geográficos de los linajes y sobre los posibles motores detrás de su divergencia (Graham et al. 2004b; Rissler y Apodaca 2007; Tocchio et al. 2015).

Al intergrar distintos tipos de datos, es posible comprender los procesos que explican los patrones de variación morfológica y molecular que muestran las especies a lo largo del paisaje que ocupan (Alvarado-Serrano y Knowles 2014). Se ha demostrado que requerimientos ambientales, estructura genética y fenotipo están íntimamente relacionados en los procesos de divergencia de los organismos (Zamudio et al. 2016; Morales et al. 2018). Además, la cantidad cada vez mayor de bases de datos con información climática y georreferencias de especímenes, los avances en técnicas moleculares junto con herramientas informáticas y de sistemática molecular y genómica, y la disposición de ejemplares en



colecciones de museos permiten acceder a estos tres tipos de datos fácilmente (Morales et al. 2016). Al analizarlos conjuntamente se puede explorar cómo el ambiente moldea la variación genética y morfológica de una especie a lo largo de su rango de distribución. Esto permite responder preguntas sobre la validez taxonómica de los grupos, identificar y ubicar en el tiempo las posibles fuerzas que dieron origen a su divergencia, y tener un mejor entendimiento de su historia evolutiva. Los estudios que incorporan datos derivados de estas tres aproximaciones no son poco frecuentes y se han comprobado los beneficios de hacer uso de un enfoque integrativo para inferir historias evolutivas e identificar especies tanto en murciélagos (Sztencel-Jablonka et al. 2009; Morales et al. 2016; Ospina-Garcés y de Luna 2017; Hernández-Canchola y León-Paniagua 2017; Taylor et al. 2013; Srinivasulu et al. 2019) como en otros grupos (Pavlova et al. 2014; Solis-Lemus et al. 2014; Ruane 2015; Ravinet et al 2016; Grinarg et al. 2019; Nascimento et al. 2021).

El objetivo general de esta tesis fue explorar la existencia de más de un taxón agrupado dentro de la especie nominal *G. soricina* a partir de los resultados reportados por análisis moleculares previos, utilizando nuevas líneas de evidencia derivadas de una aproximación integrativa, específicamente, morfometría geométrica y modelado de nicho ecológico. La tesis fue dividida de la siguiente manera: el primer capítulo consiste en un artículo de revisión sobre la utilidad de los análisis de morfometría geométrica en murciélagos, el capítulo 2 es un artículo publicado que analiza la morfometría geométrica del complejo y el 3 sus requerimientos ambientales y su posible asociación con la variación en las formas craneales, además de una introducción y discusión generales. Gracias a los resultados obtenidos fue posible elevar a nivel de especie cuatro de las cinco subespecies que conformaban al complejo *G. soricina*, las cuales fueron descritas en términos de su morfometría craneal y mandibular, y de sus requerimientos ambientales. El estudio de las especies crípticas ofrece una excelente oportunidad para estudiar de manera práctica la heterogeneidad de los procesos y patrones de divergencia antes mencionados, mientras que obliga a la integración de datos derivados de distintas disciplinas (Fišer et al. 2018).

## EL COMPLEJO *GLOSSOPHAGA SORICINA*

*Glossophaga soricina* (Pallas 1766; Glossophaginae) es una especie nominal de murciélago nectarívoro de la familia Phyllostomidae. Es endémica del Neotrópico, donde se distribuye desde el norte de México hasta el sur de Paraguay y Brasil y norte de Argentina, así como en las Islas Marías y Jamaica. Se caracteriza por ser un murciélago de tamaño pequeño (Largo total:  $\bar{x}$  = 62.86 mm, mínimo = 50 mm máximo = 90 mm; cola:  $\bar{x}$  = 7.25 mm, min. = 4 mm, máx. = 13 mm, pata  $\bar{x}$  = 10.63, min. = 6, máx. = 17; oreja  $\bar{x}$  = 14 mm, min. = 8 mm, máx. = 17 mm ) y pelaje oscuro, rostro medianamente alargado y lengua larga. A simple vista, es casi imposible de diferenciar de otras especies del género a menos de que se recurra a caracteres dentales y craneales. Entre ellos, los principales caracteres diagnósticos son la presencia de alas pterigoidales, los incisivos internos procumbentes y notablemente más grandes que los exteriores, y el arco zigomático completo, además de una cola corta, la cual alcanza menos de la mitad del uropatagio (Miller 1913). Fue dividida en cinco subespecies correspondientes a las distintas regiones geográficas donde se distribuyen sin sobrelaparse: *G. s. antillarum* (Rehn 1902) en Jamaica, *G. s. handleyi* (Webster y Jones 1980) en México, Centroamérica y norte de Colombia, *G. s. mutica* (Merriam 1898) en las Islas Marías, México, *G. s. soricina* (Pallas 1766) en Sudamérica al este de los Andes y *G. s. valens* (Miller 1913) en Sudamérica al oeste de los Andes.

Respecto a su ecología, se trata de un murciélago generalista y ampliamente distribuido, que no presenta asociación con ningún tipo de vegetación en particular dentro del rango de elevación en el cual se distribuye (<3000 msnm; Álvarez et al. 1991; Clare et al. 2014). Pertenece a una subfamilia de especies típicamente nectarívoras (Glossophaginae), sin embargo, numerosos estudios reportan que su dieta es más bien generalista (Sánchez-Casas and Álvarez 2000; Clare et al. 2014; Pellón et al. 2021). Esto coincide con su morfología craneal, la cual retiene rasgos de sus ancestros insectívoros tales como tres molares con patrón ectolofo, incisivos bien desarrollados, y rostro menos elongado respecto a otras especies de su género (Howell y Hodgkin 1976; Calahorra-Oliart et al. 2021). Este tipo de dieta podría estarle permitiendo explotar un mayor número de recursos, por ejemplo en áreas perturbadas por actividad humana (Kruszynski et al. 2016; Pellón et al. 2021), o dándole ventajas respecto a sus competidores (Howell 1974; Louzada and Pessôa 2013) en regiones de alta densidad de especies, como lo es Sudamérica (Villalobos and Arita 2010).

Análisis moleculares han dado indicios consistentes de que *G. soricina* podría ser un complejo que englobara más de una especie (Hoffmann y Baker 2001; Clare 2011; Dias et al. 2017; Hoffmann et al. 2019). Los estudios más concluyentes revelan, mediante el uso de marcadores mitocondriales, que dentro de esta especie nominal existen dos linajes monofiléticos principales con máximo soporte (Dias et al. 2017; Hoffmann et al. 2019): uno formado por las poblaciones del Este de los Andes, correspondientes a la distribución de la subespecie *G. s. soricina*, y otro linaje que incluye a las demás subespecies formando clados monofiléticos bien soportados, aunque separados por una menor distancia genética. Específicamente, la distancia genética que separa a la subespecie *G. s. soricina* del clado formado por el resto de las subespecies es de más del doble de la distancia hallada entre estas últimas, e incluso, más alto que la distancia promedio observada entre especies hermanas dentro de los filostómidos. *G. s. handleyi* a su vez está dividida en dos linajes, uno formado por poblaciones del sur de Centroamérica, y otro que incluye poblaciones del resto de la distribución en México y norte de Centroamérica, así como a la subespecie *G. s. mutica* (Hoffmann et al. 2019).

Esta división molecular concuerda geográficamente con las poblaciones separadas por los Andes (Dias et al. 2017; Hoffmann et al. 2019). Concuerda también con la discontinuidad morfológica más notoria, según la descripción de Webster (1983), quien basándose en medidas externas y craneales encontró que, entre todas las subespecies, *G. s. soricina* era la más pequeña y fácilmente distinguible por algunas características entre las cuales resaltan su pelaje más oscuro y un rostro más corto y angosto, si bien sus poblaciones presentan variación geográfica externa (Louzada y Pessoa 2013). Así, estas dos evidencias apuntan a que *G. soricina* podría estar compuesto por, al menos, dos linajes, es decir, tratarse de un complejo de especies crípticas, situación que requiere resolución taxonómica.

Es posible que un evento de vicarianza haya sido el responsable de la divergencia de los dos linajes principales, los cuales habrían divergido entre 2.4 y 5 millones de años atrás con el levantamiento de los Andes. El último alzamiento en la formación de esta cordillera impuso una barrera al flujo génico que antes no existía, al separar las poblaciones a uno y otro lado. *G. soricina* es una especie generalista y tolerante; la limitante principal a su distribución es la altitud, pues no se encuentra en ambientes mayores a 3000 msnm. Así, se cree que fueron los eventos orográficos y no los climáticos los que estructuraron sus poblaciones, en contraste

con otras especies de murciélagos neotropicales, cuyo tamaño y estructura poblacional se deben a las fluctuaciones climáticas del pleistoceno (Dias et al. 2017).

Por su parte, las poblaciones de Jamaica (*G. s. antillarum*) habrían llegado a la isla desde la península de Yucatán entre 0.9 y 2.2 millones de años atrás, durante un periodo en el cual el nivel del mar era hasta 80 metros menor (Baker y Genoways 1978; Bintanja y Wall 2011). La ocupación de las Islas Mariás por *G. s. mutica* se atribuye a un evento de dispersión bastante más reciente, el cual se ve reflejado en la nula separación molecular con las poblaciones continentales de *G. s. handleyi* (Hoffmann et al. 2019).

Los conocimientos que se tienen sobre la filogeografía de este grupo sirven como punto de partida para realizar una revisión de su situación taxonómica. Numerosos autores (Webster 1983; Hoffmann y Baker 2001; Louzada y Pessôa 2013; Dias et al. 2017) han coincidido en la necesidad de realizar más estudios sobre la variación y taxonomía de esta especie, así como sobre los mecanismos responsables de esta variación. Para complementar la información molecular que se tiene de este grupo y resolver su situación taxonómica, es deseable la integración de datos derivados de distintas aproximaciones: contrastar los resultados de distintos tipos de análisis aportará información independiente sobre las características que soportan la ubicación taxonómica de los organismos en estudio, lo cual ayudará a tener un cuerpo de evidencia más robusto que permita describir la variación que en él se encuentra, y permitirá resolver la existencia de especies crípticas (Raxworthy et al. 2007; Rissler y Apodaca 2007; Padial et al. 2010; Gager et al. 2016). Numerosos estudios con murciélagos se han beneficiado de utilizar este tipo de aproximación para llegar a conclusiones taxonómicas y filogenéticas (i.e. Ruedi y Mayer 2001; Evin et al. 2008; Gager et al. 2016; Morales et al. 2016; Taylor et al. 2018; Srinivasulu et al. 2019).

## ELECCIÓN DEL CONCEPTO DE ESPECIE Y SU IMPORTANCIA PARA LA DELIMITACIÓN TAXONÓMICA

Delimitar especies requiere de la detección de evidencias que prueben su independencia (de Queiroz 2007). Esto implica seleccionar los caracteres de interés, cuantificar las similitudes y diferencias que guardan entre linajes, y delimitar umbrales pertinentes que permitan clasificarlos como especies distintas. Pero antes, resulta necesario establecer una definición de especie. Durante años ha existido un debate en la comunidad científica en torno a este concepto que es imprescindible resolver, pues la manera en la cual se definen las especies afecta cómo y cuántas se delimitan, así como la forma en que se comprenden los procesos que dan lugar a la especiación (Johnson et al. 2004; Rissler y Apodaca 2007).

El esfuerzo por conceptualizar las especies ha estado lleno de controversias y ha sido marcado, según la época, por tendencias epistemológicas relacionadas con los avances en las técnicas de análisis. Las distintas ramas de la biología han acuñado conceptos de especie que funcionan en su campo, pero que no son del todo compatibles con las definiciones de especie que han acuñado otras áreas. Principalmente, estos distintos conceptos difieren en las características que consideran como requerimiento para diagnosticarlas (de Queiroz 2007). Por ejemplo, el bien conocido concepto biológico de especie marca como límite entre dos linajes el aislamiento reproductivo (Mayr 1942; Dobzhansky 1951); el ecológico requiere que todos sus componentes compartan la misma zona adaptativa o nicho (Van Valen 1976; Andersson 1990); el fenético requiere que hayan adquirido diferenciación fenotípica cuantitativa (Michener 1970; Sneath y Sokal 1973; Sokal y Crovello 1970); el genotípico requiere distinción genotípica sin intermediarios genéticos entre otros grupos (Mallet 1995); y el monofilético que el grupo esté conformado por un ancestro y todos sus descendientes (Rosen 1979; Donoghue 1985; Mishler 1985).

La explicación biológica de este conflicto teórico es que cada historia evolutiva es única: la especiación es un proceso heterogéneo, durante el cual los linajes nacientes adquieren características particulares en distintos momentos, a raíz de diferentes fuerzas evolutivas, y en distinto orden. Mientras tanto, dentro de la taxonomía, el sistema para delimitar a las especies se hacen de manera discreta y binaria (especie: ¿sí, o no?), lo cual implica necesariamente contratiempos al momento de lidiar con los límites difusos que se pueden

encontrar a lo largo de la continuidad del proceso evolutivo (Zachos 2018). Si bien puede existir consenso sobre que se trata de una sola especie antes de que ocurra el proceso de divergencia, y sobre que al final de éste son ya dos o más especies, entre ambos momentos existe un momento de irresolución durante el cual las características acumuladas no son las suficientes como para que la especiación sea obvia. Dado que cada una de las diferentes definiciones de especie determina como requisito que exista divergencia en caracteres que pueden o no haberse diferenciado hasta dicho momento, habrá diferencias en el número linajes que logrará delimitar cada una de ellas (de Queiroz 2007; Padial et al. 2010).

Más allá del componente filosófico detrás del debate sobre los conceptos de especie, resulta necesario acordar una definición así sea para fines prácticos, como pueden ser la definición de los objetos de estudio y la toma de decisiones (Zachos 2017). Para separar el proceso de delimitación de especies del problema de su definición, de Queiroz (2007) propone crear un concepto unificador de especie. La propuesta de este concepto es detectar aquella característica en la que todos los conceptos de especie convergen, y asignarla como única propiedad imprescindible para definir a las especies. Así, de Queiroz define entonces a las especies como “segmentos de linajes de meta-poblaciones que están evolucionando separadamente”. Cualquier otra propiedad además de esta, como podría ser la distinción fenética o la divergencia ecológica, resulta secundaria, contingente u opcional, y funciona para aportar evidencias complementarias para respaldar o rechazar la hipótesis de la separación de los linajes pero sin ser determinante para ello, además de ayudar a caracterizar dichos linajes. La congruencia entre caracteres estudiados por más líneas de evidencia será asociada a un mayor grado de corroboración del estado de la especie, mientras que ausencia de alguna de estas propiedades secundarias no supone una contradicción de dicha hipótesis, sino que simplemente pone en evidencia la mencionada heterogeneidad del proceso de especiación, donde la divergencia no se refleja de igual manera en todos los caracteres bajo estudio. Sería necesaria la ausencia de todas las propiedades para considerar que las poblaciones bajo estudio no son especies separadas (de Queiroz 2007).

Para la delimitación de especies, existen desacuerdos sobre qué tanta congruencia se debe exigir de los distintos datos. Por un lado, se puede esperar coherencia en todos los caracteres como indicador de separación de los linajes, situación que es poco probable que ocurra por casualidad y que, por lo tanto, se puede afirmar una historia evolutiva aislada. Este marco

teórico es nombrado por Padial et al. (2010) como “integración por congruencia”. Por otro lado, la nombrada “integración por acumulación” considera que hallar divergencia en cualquiera de los atributos bajo estudio supone evidencia de la especie, sin esperar congruencia entre todos, puesto que el proceso evolutivo no produce necesariamente cambios a todos los niveles ni a las mismas tasas en todos los caracteres. La ventaja de la primera aproximación radica en que da mayor certidumbre. Sin embargo, al ser tan estricto el criterio puede subestimar el número de especies. La segunda, en cambio, resulta más sensible para detectar linajes más recientes y permite interpretar las discordancias como evidencias del proceso evolutivo detrás del grupo en cuestión. El riesgo de sobre estimar especies podrá ser sobrepasado si la línea de evidencia se utiliza con suficiente criterio o se usa más de una (Padial et al. 2010).

Es por todo esto que la mejor estrategia cuando se busca hacer inferencias filogenéticas y delimitar especies es echar mano de la integración datos derivados de distintas disciplinas y metodologías, los cuales aportan información independiente sobre las características que soportan la ubicación taxonómica de los organismos en estudio (Raxworthy et al. 2007; Rissler y Apodaca, 2007; Gager et al. 2016). Dado que las historias evolutivas de las especies no se reflejan de igual manera en todos sus caracteres (i.e., Ruedi y Mayer 2001; Evin et al. 2008), basarse en un solo tipo de datos podría llevar a conclusiones incompletas o engañosas acerca de sus relaciones filogenéticas.

Las especies crípticas, tan comunes en murciélagos (i.e. Barlow et al. 1997; Clare et al. 2013; Burneo-Nuñez and Camacho-Monge 2017; Morales-Martínez et al. 2021), son un buen ejemplo de esta heterogeneidad evolutiva que se manifiesta como contradicciones entre los caracteres taxonómicos. Es importante recordar que los procesos evolutivos que están promoviendo falta de divergencia morfológica, sea por ausencia de cambio o por convergencia, son los mismos que producen la diversificación de especies claramente distinguibles, mucho más estudiadas y comprendidas, posiblemente porque resulte más intuitivo asociar el proceso evolutivo con cambio que con estasis. Las incongruencias entre datos pueden dar lugar a hipótesis taxonómicas tan robustas como aquellas apoyadas por congruencia, y ambas arrojan luz sobre los procesos evolutivos detrás de las especies (Fišer et al. 2018).

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## CAPÍTULO 1

Geometric morphometrics as a key tool of integrative taxonomy for the resolution of cryptic species in bats

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## ABSTRACT

Cryptic species complexes are very common in bats, causing an important underestimation of bat species diversity, imposing taxonomic challenges and affecting our knowledge of their evolutionary history and the decisions derived from it. The descriptive power of geometric morphometrics has proved to be an excellent tool for answering evolutionary questions and to be particularly useful for exploring morphology at lower taxonomic levels, which is the case of cryptic species complexes. Geometric morphometrics allows the detection of subtle morphological variations that, along with molecular divergences, could help disentangle bat taxonomy. It also provides the tools for describing diagnostic taxonomical characters in newly described species, as well as for exploring the morphological covariation with other interesting variables that provide insights into the evolution of this fascinating order. Chiropteran taxonomy and evolutionary biology can only benefit of incrementing the use of geometric morphometrics in these studies.

Key words: bat diversity, bat evolution, Chiroptera, phenotypic characters, species delimitation.

## RESUMEN

Los complejos de especies crípticas son muy comunes en murciélagos, causando una importante sub-estimación de la diversidad de especies en murciélagos, imponiendo retos taxonómicos y afectando nuestro conocimiento de su historia evolutiva y las decisiones derivadas de éste. Se ha demostrado que el poder descriptivo de la morfometría geométrica es una excelente herramienta para responder preguntas evolutivas y que es particularmente útil para detectar variación morfológica a niveles taxonómicos menores, como es el caso de los complejos de especies crípticas. La morfometría geométrica permite la detección de variación morfológica sutil que, junto con hallazgos de divergencias moleculares, podría permitir resolver la taxonomía de los murciélagos. Además de esto, brinda herramientas para describir los caracteres taxonómicos de las nuevas especies descritas, y para explorar la covariación de la morfología con otras variables de interés que arrojen luz sobre la evolución



de este fascinante orden. La taxonomía y la biología evolutiva de quirópteros no puede sino beneficiarse de incrementar el uso de la morfometría geométrica en estos estudios.

Palabras clave: caracteres fenotípicos, Chiroptera, delimitación de especies, diversidad de murciélagos, evolución de murciélagos.

The Chiroptera are an order of mammals that is exceptionally interesting when it comes to evolutionary matters. Their extraordinary adaptive radiations make them one of the most morphologically and ecologically diverse groups (Smith 1976; Rossoni et al. 2017). They are the second most diverse mammal order after Rodentia (over 1400 species of bats versus over 2050 in rodents), and the high species richness within them is well known: more 1400 species distributed on all continents except Antarctica, occupying a heterogeneity of environments (Burgin et al. 2018). However, it is believed that these numbers could be highly underestimated (Ibáñez et al. 2006; Clare et al 2011; Francis et al 2010; Srinivasulu et al. 2019), mainly because this group is particularly prone to having overlooked species within its taxonomy, given their elusive nature since they are nocturnal, flying, small mammals, and that they often have very subtle morphological differences even between species that are not closely related (Mayer and von Helversen 2001; Clare et al. 2013), a situation that supposes interesting taxonomic challenges.

It is believed that the hidden diversity in this order corresponds mostly to cryptic species complexes: groups of independent lineages that have been classified as a single species given their external similarity. These complexes represent the majority of the newly described species in recent years, even in well studied groups, outnumbering newly discovered species or findings of species that were thought to be extinct (i.e., Castella et al. 2001; Mayer y von Helversen 2001; Evin et al. 2008; Clare et al. 2013; Morales et al. 2016; Molinari et al. 2017). Most of the works with cryptic species are conducted in animals (Bickford et al. 2007); nonetheless, cryptic species are found in all groups of living beings and in every biogeographic region (Pfenninger and Schwenk 2007): both in terrestrial and marine species

(Knowlton 1993; Vrijenhoek et al. 1994), within the fungi kingdom (Bidochka et al. 2001); in insects (Herbert et al. 2004; Janzen et al. 2017) and in vertebrates as small as shrews (Taylor et al. 2013) or as big as giraffes (Fennessy et al. 2016). Their study is crucial for the correct estimation of biodiversity and for addressing several relevant questions in evolutionary biology, such as the causes behind the morphological convergences or stasis, the divergence threshold for considering lineages as independent species, and the usefulness of the different species concepts for their delimitation, as well as for making informed decisions based on correct information, for example, regarding conservation programs and the management of medical and economical important species (Johnson et al. 2004; de Queiroz 2007; Rissler and Apodaca 2007; Bickford et al. 2007; Tsang et al. 2016).

The causes behind said morphological resemblance between distinct species are diverse and not mutually exclusive. Sometimes, cryptic complexes are conformed by sister species who share a recent common ancestor, and the little amount of time since their divergence can account for the little or inexistent accumulation of perceptible external differences. In many cases, the morphology of diverging lineages can be subject to selective processes, but the divergence is so subtle that remains imperceptible to the human observer. Another possibility is that the complex comprises lineages whose morphology is under morphological stasis: stabilizing selection could restrict the morphological changes while divergence does occur in other types of characters, like pheromones, ecology, communication and other types of behavior. Another quite frequent situation are the cases of morphological convergences or homoplasies, that is, independent lineages that present similar characters because they have gone through similar selective pressures (Bickford et al. 2007; Fišer et al. 2018). Special caution needs to be observed in these cases since, as it will be detailed hereunder, undetected homoplasies could lead to fallacious conclusions about the phylogenetic relationships between the taxa. In bats, there is incongruence between trees estimated using different types of characters (Dávalos et al. 2012), which reflects the different rates at which the characters within a species are evolving.

It is important to consider that, traditionally, our understanding of taxonomical classifications has been based mostly on the visual observation of similarities and discontinuities (Evin 2008), probably because the sense of sight is the one which provides the most information to

the human brain (Bickford et al. 2007). This clearly has led to misclassifications, for physical traits of organisms are shaped by evolutionary forces in ways that do not always reflect their phylogenetic relationships, giving rise to homoplasies, convergences, and even physical changes shaped by the life history of the organism. With the rise of molecular techniques, findings where molecular and phenotypic data do not match coherently have become quite common (i.e. Ruedi and Mayer 2001; Wiens and Penkrot 2002; Evin et al. 2008; Dávalos et al. 2012), demonstrating that morphologically similar organisms are not necessarily closely related phylogenetically. In order to understand the phenomenon of cryptic species, it is important to consider that throughout the diversification process each type of character is subject to distinct selective forces, which may cause differences to appear in different order, timing, or to simply do not occur (de Queiroz 2007).

Usually, the suspicions of the existence of more than one independent lineage inside a single nominal species start with clues derived from molecular studies, which find more variation and genetic distances than would be expected if it was an actual single species (i.e., Hoffmann and Baker 2001; Oliver et al. 2009; Trontelj et al. 2009; Timm et al. 2019). The desirable approach in these cases is to use integrative taxonomy, which means gathering different types of data on more than one of their characters and analyzing the evolutionary history that they tell altogether to get independent sources of information that support the taxonomic positions of the organisms under study (Raxworthy et al. 2007; Rissler and Apodaca 2007; Solís-Lemus et al. 2015; Gager et al. 2016). Given that the evolutionary histories of the species are not reflected equally in all their characters (i.e., Ruedi and Mayer, 2001; Evin et al. 2008), being based in only one type of data could lead to incomplete or spurious conclusions about their phylogenetic relationships. Thus, initial molecular clues can then be complemented with other types of data, such as morphology, acoustics, ecological requirements or behavior, aiming to gather evidence that allows to confirm or dismiss that we are dealing with more than a single species (Bickford et al. 2007). Amongst all these options, geometric morphometric stands out. Given that cryptic species misclassifications are due to incorrect morphological interpretations, having a tool that allows to study shape in a formal, mathematical way, represents an excellent approach to address this problematic.

The quantitative study of biological shapes and their variation is called morphometrics (Bookstein 1997), and it includes not only the quantification of biological shape but also the exploration of the possible causes behind them. The increasingly rigorous analysis of shapes has allowed to describe and compare the morphological variation of living beings, its correlation with other variables, and to conduct phylogenetic inferences based on them (Adams et al. 2013; Morales et al. 2018). Its best virtue is that it introduces formality to the visualization, description and comparison of shapes and identification of the causes of their variation, so it benefits every branch of biology that makes use of comparative morphology, such as systematics, evolutionary biology, and developmental biology (Webster and Sheets 2010; Adams et al. 2004). Nowadays, two branches are distinguished in morphometrics: linear or traditional morphometrics, which collects morphological data as linear measurements such as length and width, angles and radius, and geometric morphometrics, which uses cartesian coordinates of landmarks that describe anatomic structures (Mitteroecker and Gunz 2009).

Numerous authors agree that geometric morphometrics has powerful advantages when compared to linear morphometrics. Its methods allow to analyze shape “in its pure form”, defined as “all the geometric information that remains when the effects of position, rotation and size are removed from an object” (Dryden and Mardia 1998). Shape is captured as landmark coordinates which are set on specific points of digitized images of anatomic structures in either two or three dimensions (Fornel and Cordeiro-Estrela 2012). These constellations of landmarks are interpreted as shape variables that replace the traditional distance measures. Throughout all the procedure of the acquisition, processing and analysis, these shape variables preserve the totality of the geometric information contained in original data, and changes in the positions relative to each other can be quantified and analyzed statistically (Slice 2005). The obtainment of said shape is accomplished by removing all non-descriptive information, which is achieved by translating, rotating and rescaling each configuration, a method called Generalized Procrustes Analysis (Rohlf 1990; Goodall 1991). At the same time, size can be analyzed independently from shape: this measure is called Centroid Size, it’s calculated as the square root of the sum of the squared distances of all landmarks from their centroid (Rohlf and Slice 1990; Bookstein 1997) and is specific to

geometric morphometrics analyses. For a detailed description of the methods, see Adams et al. (2013) and Zelditch et al. (2012).

These shape variables have a number of possible applications in evolutionary studies. They can be used as taxonomic characters (Zelditch et al. 2004; Ospina-Garcés et al. 2016), their differences and similarities are useful for delimiting species and for inferring phylogenetic relationships when included in cladograms, using the consensus shape of each species as characters (Catalano et al. 2010), shape evolution can be visualized by mapping characters on a reference tree inferred with molecular markers (Catalano et al. 2010), and ancestral morphological states can be reconstructed and visualized by creating hypothetical nodal landmark configurations from mean shapes of the taxa (Adams et al. 2013; Klingenberg and Gidaszewski 2010).

An important advantage of geometric morphometrics is that graphic representations of said landmark configurations can be made, which allows to immediately visualize the changes in an intuitive way, and they can furthermore be exaggerated such as their detection is visually easier (Zelditch et al. 2012; Schmeider et al. 2015). These changes are interpreted from displacement of the landmarks, which illustrate which regions of the analyzed structures are changing and how so. When it comes to describing species, these graphic representations can be very useful to identify the exact structures which have distinctive taxonomical characters useful for species identification, which is particularly useful for museum and field identifications (Grinang 2019; Carpaneto et al. 2020; Calahorra-Oliart et al. 2021). This allows the phenotypical description of newly described species that have been differentiated based on molecular data (Lalis et al. 2009; Huang et al. 2014; Ruane 2015). This is very important, because if species are delimited based solely on their molecular differentiation, they will lack the phenotypic characterization so much needed for taxonomical practices. Besides, it's been demonstrated that molecular data alone, despite its popularity, has limitations when detecting species limits, for some divergence processes will not be effectively reflected on these markers, for example, when they have derived recently, when there is gene flow or when speciation has been driven by reproductive isolation. Considering multiple types of data by incorporating phenotypic characters allows for a more accurate estimate of species boundaries, aiming to reflect the heterogeneity of speciation processes (Solís-Lemus et al. 2015).

In addition to all of this, the detailed way in which geometric morphometrics captures shape makes it particularly well suited to finding differences that would have gone otherwise undetected with traditional morphometrics methods (Cordeiro-Estrela et al. 2006; Evin et al. 2008; Fontoura and Morais 2011), for example, when working at lower taxonomic levels, where morphological divergence can be so subtle that traditional methods are insufficient to detect it. (Loy 1996; Schmieder et al. 2015). This makes it useful for taxonomic and phylogenetic studies in cryptic species complexes, in which its descriptive and statistical power to separate cryptic taxa and intraspecific variation has been demonstrated (Francuski et al. 2009; Fontoura and Morais 2011; Angulo-Bedoya et al. 2019; Chatpiyaphat et al. 2021). In bats, it has been proven that it's the best tool to detect morphological differences (Schmieder et al. 2015; Mayer and von Helversen 2001; Evin et al. 2008).

For geometric morphometrics studies in bats, and in vertebrates in general, the most used region as source of morphological characters is the skull, usually along with the mandible. These complex structures are an excellent model to test evolutionary hypotheses given that they are target of important selective pressures, since they host important structures related to the senses, the brain, breathing, and the feeding (Marroig and Cheverud 2004; Caumul and Polly 2005; Ospina-Garcés et al. 2016; Rossoni et al. 2017). Furthermore, in scientific collections the skulls and mandibles are usually the best-preserved parts of the skeleton, which makes their study easier (Morales et al. 2018). It has been found that the ventral view of the skull might be the region that holds the most phylogenetic information (Caumul and Polly 2005; Calahorra-Oliart et al. 2021). This means that, while other structures might be more prone to having their morphology shaped by other forces, such as life histories, plasticity and genetic drift, the differences found in the ventral region would be more reliable of phylogenetic relationships; therefore, its analysis should be paramount when it comes to detecting species. Lastly, the morphological study of the skull is relevant for taxonomic practices, for the particular characteristics of this structure are especially relevant when it comes to species identification. The analysis of cranial and mandible shapes has allowed to propose explanations on the forces behind the divergences in Chiroptera (Evin et al. 2008; Ospina-Garcés et al. 2016; Rossoni et al. 2017), which contributes to a better delimitation of the species. Moreover, the existent variation along the geographic distribution can be used to

identify recent biogeographic breaks or environmental transitions that are not always evident with other types of data (Padiál et al. 2010).

Some applications of geometric morphometrics in studies with bats include the exploration of the relation between shape and diet (Nogueira et al. 2009; Ospina-Garcés et al. 2016; Bubadué et al. 2021), adaptations to echolocation (Giacomini et al. 2021), the characterization of phenotypic variability (Caumul and Polly 2005; Morales et al. 2018; Mutumi et al. 2021), the behavior of size and shape in islands (Evin et al. 2011) shape and environment (Budinski et al. 2015), and even fossil identification (Jansky et al. 2016). It has also been used to address intra-specific questions, such as sexual dimorphism (Calahorra-Oliart et al. 2021; Maucieri et al. 2021), ontogenetic development (Monteiro 2000; Watanabe and Slice 2014) and variation between breeds (Drake and Klingenberg 2010).

Possibly, the most important critique that has been made to using geometric morphometrics on evolutionary studies is concerned with the reliability of shape characters as indicators of phylogenetic relationships. This controversial topic has been widely discussed, given the plasticity of anatomical features and the possibility of convergences (Bookstein 1997; Webster and Sheets 2010; Adams et al. 2004; Caumul and Polly 2005; Klingenberg and Gidaszewski 2010; Catalano and Torres 2017). Just as morphological stasis and convergences demonstrate, not every character that shows similarity between lineages is a reliable source of phylogenetic information: only those characters that are similar because they are inherited from a common ancestor, called homologous, will be useful for this (Martínez-Castilla 2007). However, geometric morphometrics have methods aimed to guarantee the identification between homologies and homoplasies. On one hand, shape characters can be mapped on trees inferred with molecular characters, which are, in general, considered to be more reliable than morphological ones (Cardini and Elton 2008; Suárez-Díaz and Anaya Muñoz 2008), even though they are not exempt from having convergences as well (Grauer and Li 2000). By doing this, it is possible to explore if the observed variation actually reflects phylogenetic relationships and is not just product of other types of other misleading sources of morphological change (Adams et al. 2004; Klingenberg and Gidaszewski 2010; Catalano and Torres 2017), meaning that they are homologous. For their part, homoplastic characters contain no phylogenetic information because do not imply

relatedness, but they can however provide other types of relevant data for discerning the evolutionary history of the lineages under study, for instance, relative to the similar evolutionary processes that produce these convergent adaptations in non-related lineages (Wake 1991). This is why it is important to carry tests that allow to discern between one type and the other: it is crucial to assure the validity of the conclusions derived from these analyses.

A good example of this is the integrative study with bats of the genus *Myotis* by Ospina-Garcés and De Luna (2017). Here, they used molecular markers and geometric morphometrics to estimate a phylogeny combining both types of characters. As mentioned earlier, the integration of these two types of data allows to prove with certainty the homology of the studied characters, demonstrating that they can contain phylogenetic information. Plotting the shape changes onto a molecular tree allowed to find how morphological changes are related with diet evolution on this group, specifically, with insectivorous, facultative piscivorous and truly piscivorous diets. The use of landmark data made it possible to describe in which structures these changes were occurring and to interpret them as synapomorphies, while some homoplasies were also found.

Another characteristic that allows to distinguish phylogenetically relevant information and makes geometric morphometrics particularly fit for the specific case of evolutionary studies relies in the way it captures the shape information: it assures that the landmark arrangements that are being analyzed are homologous between individuals, since the landmarks are placed on anatomic structures and not randomly, as could happen in linear morphometrics (Webster and Sheets 2010; Adams et al 2004): landmarks depict anatomical loci, and they must be placed at the same anatomical point on every individual of the sample (Zelditch et al. 2012). For example, on the intersection between cranial sutures, the angle of a foramen or the articulation between the phalanx of the finger. By doing this, we do not capture only the distance between points, as would be the case with traditional morphometrics, but a constellation of marks that capture the shape of the structure of interest.

There are some wonderful examples of the benefits of using geometric morphometrics for answering evolutionary questions in bats. A remarkable one is the study by Evin and colleagues (2008) with a group of species and subspecies of the genus *Myotis*, who were



justifiably suspected to be a cryptic species complex after molecular divergences were found between them (Ruedi et al. 1990; Castella et al. 2001). These molecular divergences, however, were not coherent with the phenotypic differentiation previously reported with traditional morphometric methods, which found only two different clades (Benda and Horacek 1995b). When re-analyzed using geometric morphometrics, the differences were congruent with most of the taxonomic assignments based on molecular differences. They found which taxa differed, which taxa did not (and so their species category should be abandoned) and found morphological convergences between species which were not closely related. These tools also allowed to make specific phenotypic descriptions of the differences between their cranial shapes, and to propose their feeding ecology as the possible driver behind their morphological variation.

A similar use was given by Huang et al. (2014) in their study with bamboo bats of the genus *Tylonycteris*. They found they had distinct karyotype numbers which indicated the existence of cryptic species in the *T. pachypus* complex. Their similar external features, product of convergent evolution, made them difficult to distinguish visually, but geometric morphometrics analyses made possible the detection of shape differences between these two lineages. In addition, given the specific regions of the skull in which they differed (the zygomatic arch, the tympanic bullae and the jaws), they were able to suggest that their foraging ecology had shaped said differences.

Morales et al (2018) used geometric morphometrics to delve on previous findings based on linear measures that indicated that widespread bat *Tadarida brasiliensis* lacked morphological differentiation (and genetic structuring) between its subspecies, and hence that this classification should be abandoned. Their geometric morphometric results contrasted slightly with those derived from traditional morphometrics: not only did they find that morphological variation existed between insular subspecies, but they also allowed them to describe how and which regions of their skull and mandible differed between one another and to make taxonomic proposals based on this variation. Multivariate analyses were used to identify the extrinsic factors shaping skull variation, finding a correlation between shape and spatial factors, biogeographical breaks and biome transitions. They attribute these differences in skull and mandible shape to local adaptations to types of foraging and prey availability,

which are linked to the different environmental conditions this species encounters throughout its wide distribution.

While many studies have demonstrated the utility of geometric morphometrics for addressing bat taxonomy and evolution, not enough of them have incorporated this approach, considering how prevalent cryptic species are in Chiroptera. The prevalence of these complexes is a great example of the evolutionary heterogeneity that is manifested as contradictions between taxonomic characters. It is important to remember that evolutionary processes that are promoting the lack of morphological differences are the same that produce the divergences in easily distinguishable species, which are much more studied and better understood, possibly because it is more intuitive to associate the evolutionary process with change than with stasis. Incongruences between data can give rise to taxonomic hypotheses as robust as those supported with congruence, and both shed light on the evolutionary process behind species (Fišer et al. 2018). Aiming for a correct estimation of the diversity and richness within this group is crucial for understanding the evolutionary processes that have shaped this very interesting order and for making informed decisions based on accurate information, for example, regarding conservation programs (Tsang et al. 2016). The use of molecular characters together with phenotypic characters is crucial for distinguishing which characters are homologous and which are homoplasies and making correct evolutionary conclusions (Rohlf 1998; Martínez-Castilla 2007; Evin et al. 2008).

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## CAPÍTULO 2

Cryptic species in *Glossophaga soricina*: do morphological and ecological data support molecular evidence?

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## Cryptic species in *Glossophaga soricina* (Chiroptera: Phyllostomidae): do morphological data support molecular evidence?

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Cryptic species, defined as those that are morphologically indistinguishable but phylogenetically distinct, are common in bats and correspond to the majority of newly described species. Such seems to be the case of *Glossophaga soricina*, a generalist, glossophagine bat that is broadly distributed throughout the Neotropics. Several studies have found high levels of molecular variation within *G. soricina*, suggesting that it could be a cryptic species complex. Here, we explore data derived from two-dimensional geometric morphometric analyses of cranial characters and their covariation with environmental variables, aiming to test the existence of more than one species grouped within it and to contribute to the knowledge of its variation and evolutionary history. Variation in shape and size of skull and mandible supports the two main mitochondrial lineages reported in previous studies, one corresponding to the east side of the Andes (subspecies *G. s. soricina*) and the other one corresponding to populations from Mesoamerica and the west side of the Andes, in turn composed of at least three monophyletic and morphologically differentiated taxa. Environmental variables correlate with shape variation and might be responsible for convergences in shape and size between the species with the smallest distributions. Based on the evidence we present in this work we elevate four subspecies to the taxonomic level of species. The correct names for the species of the analyzed complex are: *G. soricina* (Pallas 1766), *G. mutica* Merriam 1898, *G. antillarum* Rehn 1902, and *G. valens* Miller 1913.

Key words: geometric morphometrics, Glossophaginae, integrative taxonomy, nectarivorous bat, Neotropics, species delimitation

Las especies crípticas, definidas como aquellas morfológicamente indistinguibles pero filogenéticamente separadas, son particularmente comunes en murciélagos y corresponden a la mayoría de las especies descritas recientemente. Tal parece ser el caso de *Glossophaga soricina* (Chiroptera: Phyllostomidae), un murciélago glosófago generalista de amplia distribución en el Neotrópico. Numerosos estudios han encontrado altos niveles de variación molecular en *G. soricina*, lo cual sugiere que se podría tratar de un complejo de especies crípticas. Exploramos datos derivados de análisis de morfometría geométrica en dos dimensiones de caracteres craneales y su covariación con variables ambientales, con el objetivo de explorar la existencia de más de una especie agrupada dentro de *G. soricina*, y contribuir al conocimiento de su variación e historia evolutiva. La variación en tamaño y forma apoya los principales linajes mitocondriales reportados previamente, uno correspondiente al Este de los Andes (subespecie *G. s. soricina*), y el otro correspondiente a las poblaciones mesoamericanas y del Oeste de los Andes, el cual a su vez se compone de al menos tres taxones monofiléticos y morfológicamente diferenciados. Las variables ambientales se correlacionan con la variación morfológica, y podrían ser responsables de las convergencias en tamaño y forma en las especies de distribución más reducida. Sugerimos que la evidencia presentada es suficiente para someter a *G. soricina* a una revisión taxonómica y elevar cuatro grupos a nivel de especie. Los nombres correctos de las especies en el complejo taxonómico analizado son: *G. soricina* (Pallas 1766), *G. mutica* Merriam 1898, *G. antillarum* Rehn 1902, y *G. valens* Miller 1913.

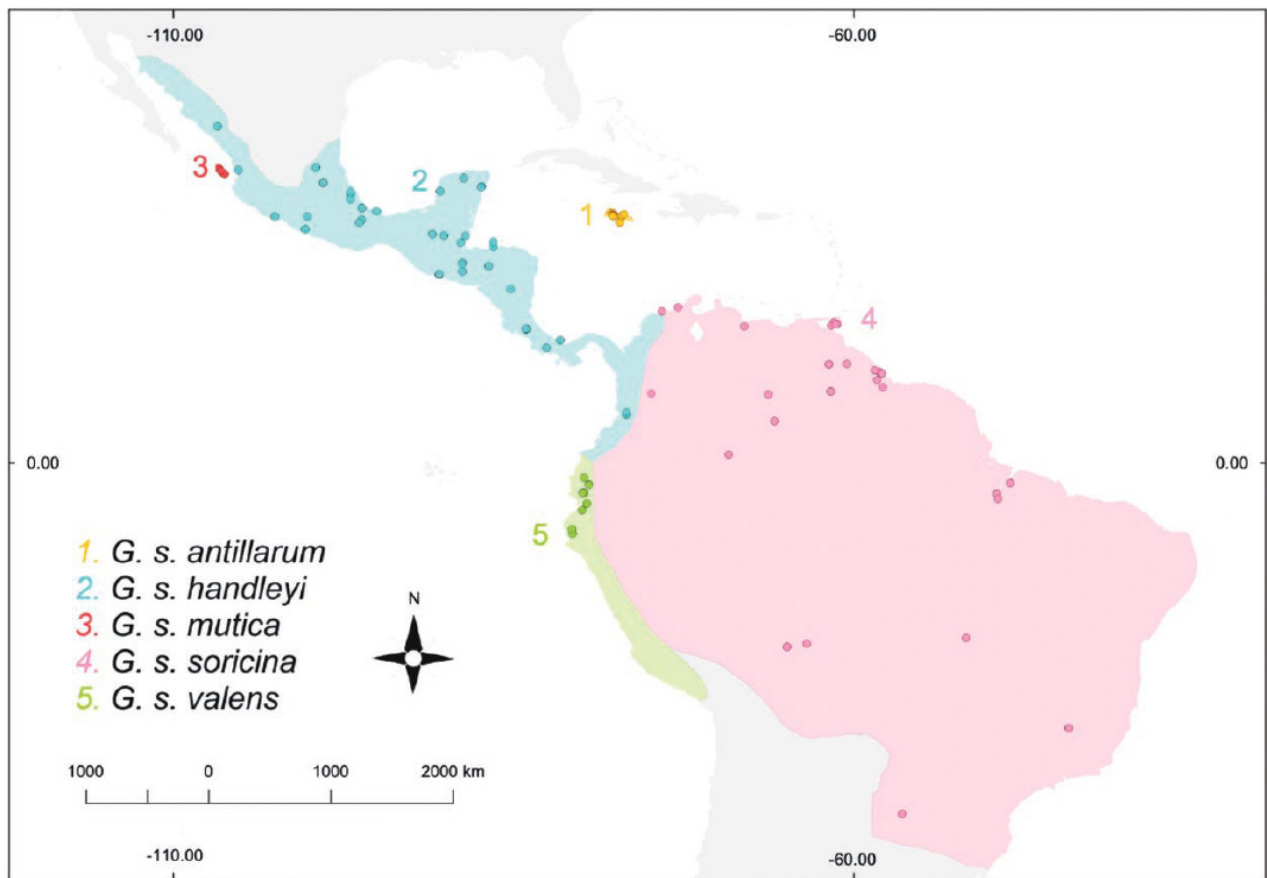
Palabras clave: delimitación de especies, Glossophaginae, morfometría geométrica, murciélago nectarívoro, taxonomía integrativa

Historically, morphological features have been the first criterion under which biological entities are classified based in their resemblances, differences, and discontinuities (Evin et al. 2008), and these features have driven the questions about the processes behind such diversity (Darwin 1859). This proclivity of use has, however, also led to misclassifications, because phenotypic characters are prone to convergence (Cole et al. 2002; Cardini and Elton 2008) and subtle morphological differences easily can go unnoticed even after extensive examination, resulting in the phenomenon that phylogenetically distinct individuals that seem indistinguishable morphologically to the human observer are grouped together as the same taxonomic unit, giving origin to cryptic species complexes. The development of molecular techniques and integrative analyses have revealed phylogenetic patterns that are in discordance with the original taxonomic classifications (Ruedi and Mayer 2001; Bickford et al. 2007; Dávalos et al. 2012; Velazco et al. 2018), making morphologically cryptic species the majority of newly described species within bats, as well as mammals in general (i.e., Taylor et al. 2012; Molinari et al. 2017; Morales and Carstens 2018).

Bats are a notably diverse, speciose, and well-studied, order. Nonetheless, they also constitute a group that is particularly prone to the existence of overlooked species given their

elusive nature: they are nocturnal, relatively small, flying animals whose morphological differences can be very subtle even between phylogenetically separated groups (Jones 1997; von Helversen et al. 2001; Mayer and von Helversen 2001; Dávalos et al. 2012; Clare et al. 2013; Morales and Carstens 2018).

There is evidence to consider that such is the case in the nominal species *Glossophaga soricina* (Pallas, 1766). However, its taxonomic situation has yet to be revisited in this respect. The species has been described as a widely distributed omnivorous phyllostomid bat that can be found in a variety of ecosystems, having no particular association with any of them (Álvarez et al. 1991; Clare et al. 2014), and whose only known dispersal restrictions are elevations above 3,000 m above sea level (Webster 1983). *Glossophaga soricina* has been divided into five subspecies corresponding to geographic distribution (Webster and Jones 1980; Webster 1983; Fig. 1): *G. s. antillarum* (Rehn 1902) in Jamaica; *G. s. handleyi* (Webster and Jones 1980; synonymized with *G. mutica* by Gardner 2008) from northern Mexico to Central America and western Colombia; *G. s. mutica* (Merriam 1898; synonymized with *G. leachii* by Hall 1981) in Tres Mariás Islands of Mexico; *G. s. soricina* (Pallas 1766) in South America at the east side of the Andes; and *G. s. valens* (Miller 1913) in the west side of the Andes in Ecuador and Peru.



**Fig. 1.**—Map showing sites sampled for morphometric data within the distribution of the five subspecies of *Glossophaga soricina*: 1. *G. s. antillarum*; 2. *G. s. handleyi*; 3. *G. s. mutica*; 4. *G. s. soricina*; 5. *G. s. valens*. The dots indicate the sampling localities from which morphological data were obtained.

High levels of intraspecific molecular variation within *G. soricina* suggest that it might comprise at least two cryptic species: one corresponding to *G. s. soricina* and the other corresponding to the remaining subspecies. These two clades are monophyletic and the genetic distance between them is as large as those found between sister species (Ditchfield 2000; Hoffmann and Baker 2001; Clare 2011; Lim and Arcila-Hernández 2016; Dias et al. 2017; Hoffmann et al. 2019). This divergence is attributed to a vicariant event caused by the most recent Andes uplift, which would have imposed a physical barrier to their gene flow (Dias et al. 2017). When it comes to external differences, aside from the insular populations *G. s. mutica* and *G. s. antillarum* along with *G. s. valens* all being larger in size, and *G. s. soricina* being the smallest form and having a darker pelage, no evident phenotypic differences are remarked upon in their taxonomic description (Webster 1983; Miller 1913). Authors who have worked with this group (Hoffmann and Baker 2001; Clare 2011; Dias et al. 2017; Hoffmann et al. 2019) agree that, to solve the taxonomic status of the taxa within this complex, there is a need for more studies using larger sample sizes and more molecular markers, as well as the analysis of morphological characters.

Integrating information obtained from the analyses of different traits is useful for inferring the evolution of a species because each of them will provide independent information about the taxonomic support of the groups (de Queiroz 2007; Raxworthy et al. 2007; Rissler and Apodaca 2007; Gager et al. 2016). During the evolutionary process, traits of an organism are subject to different rates of diversification, so that they will not necessarily reflect the evolutionary history of the species in a congruent fashion (i.e., Ruedi and Mayer 2001; Evin et al. 2008). In fact, phyllostomid bats have shown incongruence in their morphologically and genetically derived phylogenetic hypotheses (Dávalos et al. 2012). The integration of distinct types of data therefore will decrease the chance of coming to incomplete or spurious conclusions about the phylogeny of a group of organisms. In particular, in the face of a potential instance of cryptic species being present, it is desirable to collate data that will sum evidence to the molecular findings (Clare 2011).

Geometric morphometrics are considered better suited than linear morphometric analyses for the study of subtle differences in the shapes of biological structures, and their power to separate cryptic taxa and variation in species level has been demonstrated in certain cases (i.e., Mayer and von Helversen 2001; Evin et al. 2008). Moreover, the use of environmental data can give an ecological context to evolutionary explanations (Rissler and Apodaca 2007; Hernández-Canchola and León-Paniagua 2017). It is known that environmental variables can influence bats' morphology as a response to the different selective pressures that a species might encounter throughout its distribution (Evin et al. 2008; Richards et al. 2012; Hernández-Canchola and León-Paniagua 2017; Morales et al. 2018).

Our goal in this study is to explore the patterns of morphological variation to provide new data to test the hypothesis that *G. soricina* is a single, species level, taxonomic entity. A two-dimensional geometric morphometrics protocol was

implemented to describe cranial shape and size variation, quantify morphological differences, and estimate morphological characters in the putative groups forming the *G. soricina* complex. We also evaluated whether climatic variables correlate with said morphological variation.

## MATERIALS AND METHODS

**Group delimitation.**—To delimit the groups for which morphological and environmental variation was explored, we used the subspecies classification within the nominal species *G. soricina*, which corresponds to the five recognized subspecies (Webster 1983) based on molecular evidence and geographic separation: *G. s. antillarum*, *G. s. handleyi*, *G. s. mutica*, *G. s. soricina*, and *G. s. valens* (Fig. 1). The South American *G. s. soricina* initially was divided into two groups based on molecular findings (Hoffmann and Baker 2001; Dias et al. 2017; Hoffmann et al. 2019) that revealed it contains two separate clades: one in the north formed by populations in Trinidad, Venezuela, Suriname, and Guyana, and one in the south formed by populations from Brazil, Paraguay, Bolivia, and eastern Peru. However, our morphometric analyses showed that there are no significant differences in shape or size between the two genetic clades ( $P > 0.05$ ), so *G. s. soricina* was therefore analyzed as a single unit.

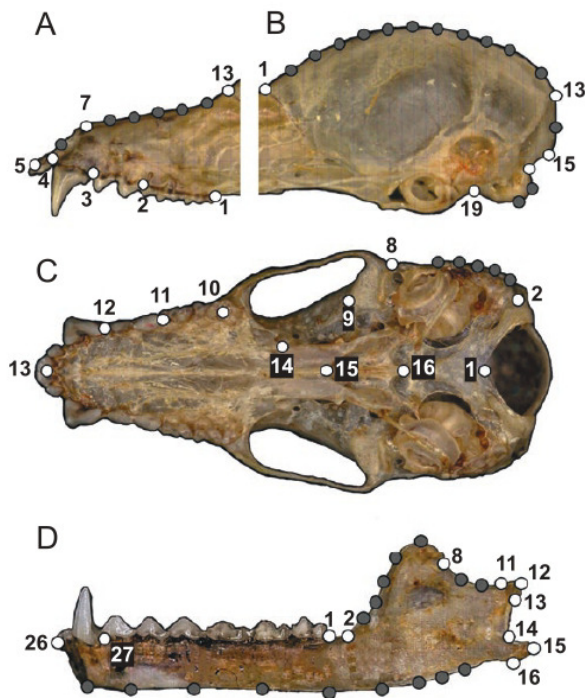
**Geometric morphometrics.**—A total of 231 specimens were photographed (Appendix I) from three mammal collections: Museo de Zoología “Alfonso. L. Herrera” at the Universidad Nacional Autónoma de México (Mexico City, Mexico); the National Museum of Natural History Mammal Collection (Washington, DC, United States); and the American Museum of Natural History (New York City, New York, United States). Our sampling contained individuals from the five subspecies throughout their distribution range (Fig. 1). Sample sizes are uneven, reflecting the differences in distribution areas among subspecies. However, we aimed to have a minimum of 10 specimens of each sex per subspecies, with an even number of males and females.

Digital photographs of lateral and ventral views of skulls, and lateral views of mandibles, were acquired using a tripod mounted Nikon D3200 reflex camera (Nikon Corporation, Tokyo, Japan) with an AF-S Micro Nikkor 60 mm lens (Nikon Corporation, Tokyo, Japan), keeping the skulls and mandibles always in the same position and at the same distance from the camera lens. Two-dimensional landmark and semilandmark coordinates from images were marked on the digital images using the program TpsDig 2.16 (Rohlf 2010). Semilandmarks were placed using guides drawn with the program Make Fan (Sheets 2014). Skull and mandible shapes were registered in four landmark configurations. The lateral view of the skulls was divided in two modules corresponding to separate developmental regions based on studies that have demonstrated separate rostral and basicranial modules for all mammals including bats (Marroig et al. 2009; Porto et al. 2009). One module corresponds to the fronto-maxillary region and was registered with a configuration of seven landmarks and six semilandmarks

(Fig. 2A). The other skull module corresponds to the parieto-occipital region and was registered with a configuration of five landmarks and 14 semilandmarks (Fig. 2B). The ventral view of the skull was registered with a configuration of 11 landmarks and five semilandmarks (Fig. 2C). The lateral view of the mandible was unable to be divided in modules due to the lack of homologous marks to define such modules; therefore, the lateral view of the mandible was registered as a whole with a configuration of 11 landmarks and 16 semilandmarks (Fig. 2D). For a detailed description on the location of the landmarks and semilandmarks, see [Supplementary Data SD1](#).

After each shape character was registered on the image, each was aligned separately and shape variables obtained via a Generalized Procrustes Analysis, which translates each configuration to the origin, scales, and rotates it using a least squares distance criterion to obtain the shape information once all other variance factors have been removed (Rohlf 1990).

Semilandmarks were declared using a protocol and aligned along the contour to minimize the bending energy necessary to produce the change in the target outline to the consensus shape (Mitteroecker et al. 2013). We obtained shape variables as coordinates, and a size estimator called centroid size (CS) as the square root of the sum of distances from each landmark to the centroid (Bookstein 1997). CS values were log-transformed to procure the normality of their distribution (Mitteroecker et al. 2013). The geometric morphometric procedures were carried



**Fig. 2.**—Four configurations of landmarks registering the shape of two lateral skull modules (A: fronto-maxillary region; B: parieto-occipital region); one ventral module (C); and one lateral mandible module (D). Landmark positions are shown by circles with white filling; semilandmarks are depicted by circles with gray filling. See [Supplementary Data SD1](#) for description of landmarks.

out in the package Geomorph 3.1.2 (Adams et al. 2020) in R version 3.6.0 (R Core Team 2020). In addition, two linear inter-landmark distances were measured to compare linear size descriptors between described species: greatest length of skull (GLS), measured from the anteriormost alveolar border of the first incisor to the posteriormost border of the foramen magnum in the lateral view of the skull, and the braincase breadth (BCB), measured at the greatest breadth of the globular part of the braincase in the ventral view. Each specimen was measured two times using the same scale, in order to decrease measurement error.

To evaluate the module partitioning in the lateral view of the skull, the correlation between the two modules was tested with a Partial Least Squares (PLS) analysis, which calculated the correlations between the configuration matrices by the first PLS vectors from each matrix. Permutation tests with 1,000 random repetitions of specimen order were conducted to test the significance of the correlations (Zelditch et al. 2004) using package Geomorph 3.1.2 (Adams et al. 2020).

*Statistical analyses of size and shape variation.*—Analyses were carried out to test for differences in CS and shape variables between sexes (sexual dimorphism) and also between subspecies, for each shape character. Differences in CS inside subspecies were explored with paired sex comparisons using a *t*-test on the log-transformed CS. The mean CS values for each subspecies were compared using an ANOVA model and the significance of pairwise comparisons among subspecies was assessed with the Tukey Honest Significant Differences Method (Miller 1981). These analyses were undertaken with the R package stats (R Core Team 2020).

A Procrustes ANOVA model was applied to test the effect of CS, subspecies, and sex nested in subspecies, on shape variance, which was calculated as the Procrustes distance variance respect to the mean shape of each factor (Adams and Otáloro-Castillo 2013). Considering that we did not have equal sample sizes between levels of factors and groups, and the loss of independence between shape data (Klingenberg and Monteiro 2005), the significance of the *F*-statistic to each factor and variable was tested using a resample test with 1,000 replicates on the residuals of the model in the R package RRPP version 0.4.2 (Collyer and Adams 2019). Differences in shape between sexes in each subspecies were explored using paired comparisons between group means, and their significance was tested by permutation testing, comparing the observed Procrustes distance (Pdis) to those obtained from random assignment of observation to groups in the R package Morpho (Schlager 2017).

Differences in shape among subspecies were explored using ordination methods. Differences between mean shape configurations of subspecies were evaluated with Canonical Variate Analysis (CVA) on a previous Principal Components Analyses. We selected the first five PCs as shape variables to avoid bias caused by differences in sample size among subspecies. For each of the four shape characters (fronto-maxillary region, parieto-occipital region, ventral view of skull, and lateral view of mandibles), we obtained the Mahalanobis distances between mean shapes for each of the subspecies, as well as their

*P*-value, assessed by a permutation test on the original data matrix, with 1,000 replicates. Deformation grids and vectors for each consensus configuration were obtained to examine shape variation graphically among subspecies. Because changes were small and therefore difficult to appreciate visually, deformation grids were exaggerated by a magnitude of three in order for them to be more evident. This was done with the R package Morpho version 2.7 (Schlager 2017).

Allometry analyses were undertaken to evaluate differences in the effect of CS on shape variation (allometric vectors) among subspecies. First, multivariate regressions of shape variables on the CS values were carried out within subspecies. We then tested for differences in the direction of the allometric trajectories among subspecies, using the angle between vectors as the comparison parameter and a resample of residuals of the allometric model (shape ~ CS \* subspecies) with 1,000 replicates in the package RRPP version 0.4.2 (Collyer and Adams 2019). Differences in allometric vectors were visualized for each subspecies, using the predicted shape for the first PC, for each CS with the previously described allometric model.

*Association between shape and environmental variables.*—We explored whether environmental factors are associated with shape variation in the skull and mandible of *G. soricina*. To select the environmental variables, we obtained environmental data from the WorldClim database (Fick and Hijmans 2017) at a resolution of 1 km for current conditions, for an occurrence database of *G. soricina* obtained from VertNet that was visually inspected, depurated of dubious specimens, and spatially thinned, leaving only registers at least 10 km apart using the spThin Package in R (Aiello-Lammens et al. 2014). A Spearman correlation test was carried out to detect and exclude highly correlated variables ( $r \geq 0.7$ ). The selected variables were BIO2 (Mean Diurnal Range), BIO3 (Isothermality), BIO8 (Mean Temperature of Wettest Quarter), BIO9 (Mean Temperature of Driest Quarter), BIO13 (Precipitation of Wettest Month), BIO14 (Precipitation of Driest Month), BIO15 (Precipitation Seasonality), BIO18 (Precipitation of Warmest Quarter), and BIO19 (Precipitation of Coldest Quarter; Supplementary Data SD2). The degree of association of environmental variation with skull and mandible shape variables was assessed with a Two-block partial least squares analysis for Procrustes shape variables using the R package Geomorph version 3.1.2 (Adams et al. 2020). Finally, we extracted said environmental variables from the coordinates of the localities from which the examined specimens were collected.

*Comparison between molecular and morphological evidence.*—To analyze the congruence between molecular and morphological evidence, we compared the position of the subspecies in the topology of the most recently published molecular phylogeny and the most parsimonious morphological tree, which was obtained by morphometric phylogenetic analyses of the four geometric morphometric characters. The aligned coordinates of each subspecies mean shape for each structure were analyzed under parsimony following Catalano et al. (2010) in the program TNT 1.5 (Goloboff and Catalano 2016). This analysis was executed initially approximating landmark positions

with a  $6 \times 6$  grid, nesting Sankoff two times, using iterative-pass for landmark optimizations and realigning during Tree Bisection and Reconnection (TBR; Goloboff and Catalano 2016); we carried out 1,000 replicates, each replicate starting from a Wagner tree.

## RESULTS

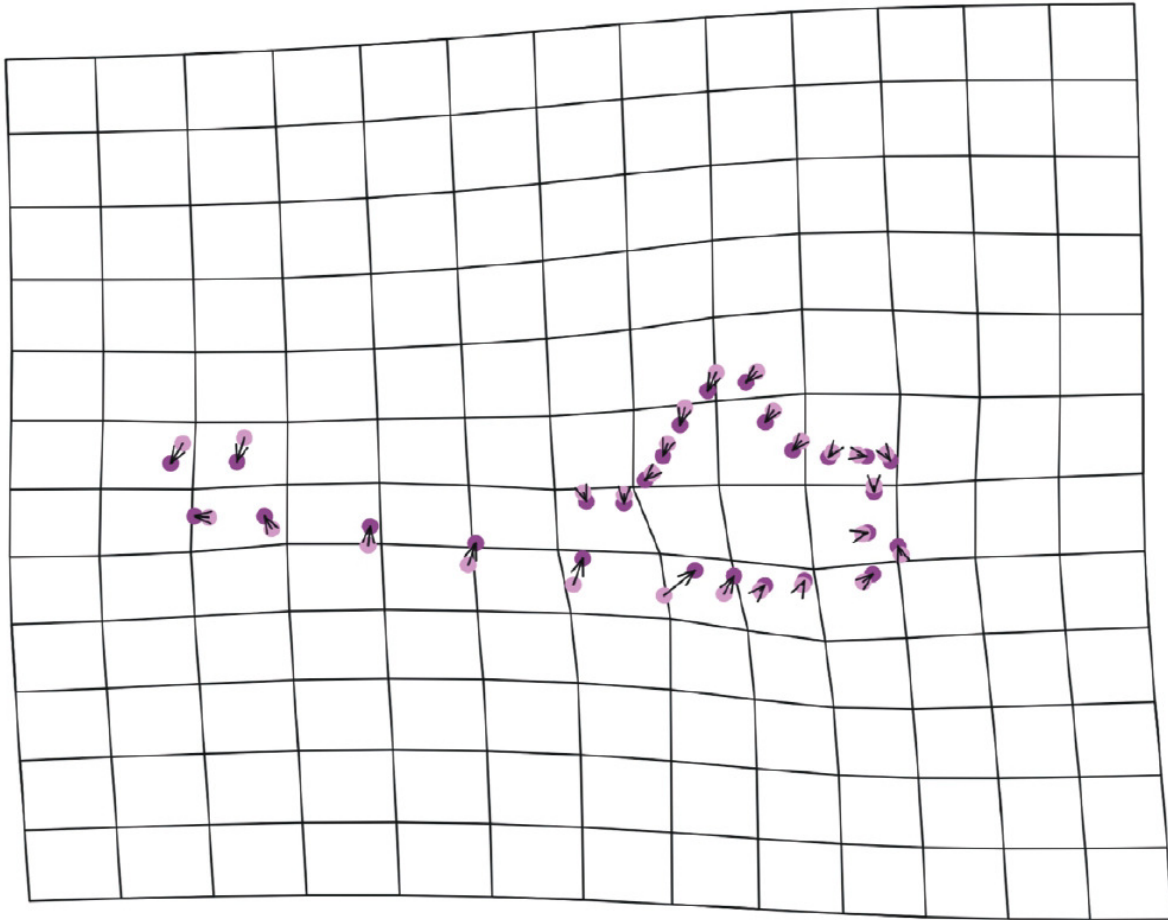
*Modularity.*—The correlation between the two modules in which the lateral view of the skull was divided (parieto-occipital region and fronto-maxillary region) was significant ( $r = 0.686$ ;  $P = 0.001$ ), meaning there is a degree of integration with which these two modules covary (Adams 2016), as revealed by the Bootstrap analyses of the data with 1,000 replicates. We decided to keep module partition in spite of this, given the separate ontogenetic origins of each (Marroig et al. 2009; Porto et al. 2009).

*Sexual dimorphism.*—The mandible was the only one of the four modules where sexual dimorphism was present, where all the subspecies except for *G. s. valens* ( $P = 0.086$ ) showed significant differences in shape. The largest distance between mean shapes of male and females was found in *G. s. mutica* (dist = 0.034;  $P = 0.003$ ), while the smallest difference was found in *G. s. soricina* (dist = 0.018;  $P = 0.001$ ). The average female shape for the mandible is narrower in vertical height than that of the male, as shown by the deformation grids (Fig. 3) where the largest vectors of displacement with respect to male shape are more evident in the dentary bone in the alveolar region of the mandible. For this reason, posterior analyses of mandible shape were carried out separately for males and females. As for CS, only the subspecies *G. s. antillarum* differed between male and female size ( $t = -2.316$ ;  $P = 0.036$ ), with males being larger than females (mean males = 3.209; mean females = 3.188). In the ventral region, a small but significant difference in shape was found only for subspecies *G. s. handleyi* (dist = 0.008;  $P = 0.008$ ), but not in remaining subspecies. No differences in CS were present between sexes in the rest of the studied modules.

*Size variation among subspecies.*—All four shape configurations exhibited the same pattern, in which insular subspecies (*G. s. antillarum*, *G. s. mutica*) along with continental subspecies *G. s. valens*, do not differ in size between one another ( $P > 0.05$ ), while continental subspecies *G. s. soricina* and *G. s. handleyi* differ with them and also between each other ( $P < 0.001$ ; Fig. 4). *Glossophaga s. soricina* is the smallest of the groups, *G. s. handleyi* is intermediate, and the other three subspecies form a cluster with the highest CS values.

*Shape variance in relation to size, sex, and subspecies.*—Procrustes ANOVAs show a significant effect of CS and subspecies on morphological variation, but that effect is not significant when sex was nested in the model, except for the case of the mandible, where the interaction between subspecies and sex did show a significant effect on shape variation (Table 1).

*Differences in shape between subspecies.*—Significant differences between subspecies were found in all of the studied shape characters. *Glossophaga s. soricina* was the only subspecies



**Fig. 3.**—Deformation grid showing vector displacements of the landmarks of the configuration of the mandible from the male mean shape to the female mean shape in the subspecies with the largest distance between mean shapes: *Glossophaga soricina mutica*. Deformation grids were exaggerated by a factor of 2 to improve visualization.

that differed from remaining groups at all times. It displayed the largest differences with the insular groups and the smallest ones with *G. s. handleyi*. The largest differences among mean shapes were found in the ventral region of the skull ( $P_{dis} < 3.754$ ) and in the mandible ( $P_{dis} < 3.576$ ). In the ventral view, the subspecies that most differed were *G. s. antillarum* and *G. s. soricina* ( $P_{dis} = 3.754$ ), while in the mandible the two subspecies that differed the most are *G. s. antillarum* with both *G. s. soricina* and *G. s. valens* (Table 2).

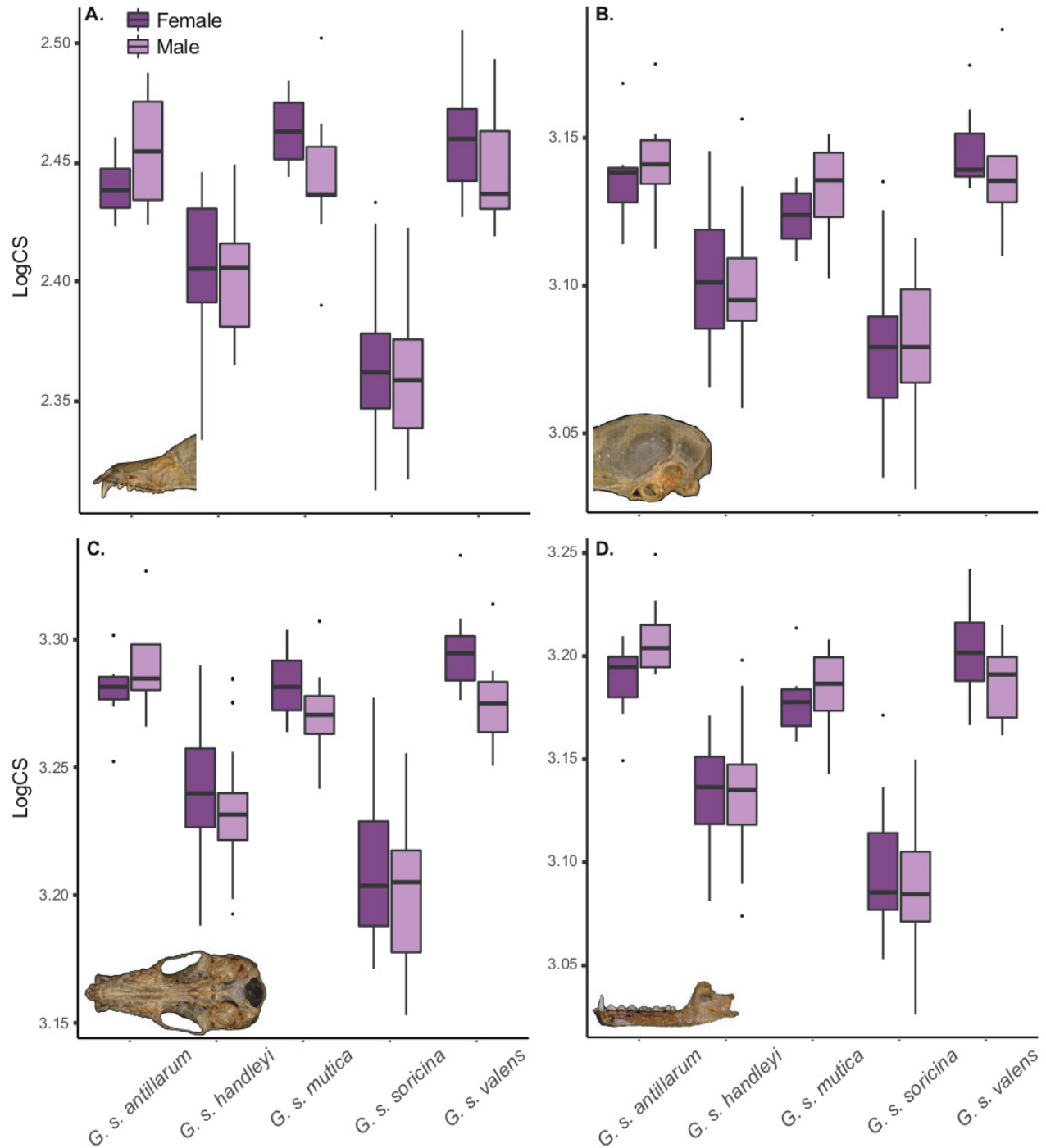
*Glossophaga s. soricina* and *G. s. antillarum* were the groups that differed the most, because they had the largest distances between their mean shapes for the three skull modules, and the second largest in the mandible (dist = 3.539) after *G. s. antillarum* and *G. s. valens* (dist = 3.576). This pattern can be observed in the CVA ordination plots, where these two groups show a tendency to occupy the most distant regions in morphospace (Fig. 5). The most similar groups were *G. s. handleyi* and *G. s. valens*, with no significant differences between the mean shapes of their mandibles and their fronto-maxillary shape. The ventral region of the skull was the only configuration in which all the subspecies differed significantly

among each other (Table 2), suggesting this might be the region of the skull that holds the most phylogenetic information.

**Allometry.**—Fitted linear models found a significant effect of size on shape variation in all the shape characters. The highest morphological variation explained by size was found in the parieto-occipital region (22.13%) and lowest for the ventral region of the skull (18.58%), while for the fronto-maxillary region it was 20.84%. For the mandible, the effect of the size on shape was similar between males (19.89%) and females (19.66%). The pairwise comparisons of directions of allometric vectors indicate significant differences only between *G. s. antillarum* and *G. s. mutica* in the parieto-occipital region (angle =  $129^\circ$ ,  $P = 0.036$ ); these subspecies showed opposite trends of shape variation with the increment of CS (Supplementary Data SD3).

**Environmental effect on shape variation.**—There is a significant effect of environmental variables upon shape variation. The module with the highest adjustment was the ventral view of the skull ( $r^2 < 0.417$ ,  $P = 0.001$ ), followed by the fronto-maxillary region ( $r^2 < 0.405$ ,  $P = 0.001$ ). The lowest correlation between shape and environmental variation





**Fig. 4.**—Boxplot showing average size and standard deviations for males and females in the five subspecies of *Glossophaga soricina* for (A) anterior-maxillary region; (B) parieto-occipital region; (C) ventral region; and (D) mandible.

was in the mandible ( $r^2 < 0.314$ ,  $P = 0.012$ ). Subspecies *G. s. antillarum*, *G. s. mutica*, and *G. s. valens* cluster in the same area of the PLS plot, indicating that they share the same morphospace and environmental region, which differs to that displayed by subspecies *G. s. handleyi* and *G. s. soricina*.

*Comparison between molecular and morphological evidence.*—Our morphological assessment of relationships matches the most recent published molecular phylogeny in most of its topology (Supplementary Data SD4). It coincides in placing *G. s. soricina* basal to remaining subspecies, and in obtaining *G. s. handleyi* as sister to *G. s. valens*. The difference

**Table 1.**—Shape variance components for the Procrustes ANOVA for each of the four studied modules. CS = centroid size.

		<i>d.f.</i>	SS	MS	<i>R</i> <sup>2</sup>	<i>F</i>	<i>Z</i>	Pr(>SS)
Fronto-maxillary	CS	1	0.051	0.051	0.11	26.191	5.076	0.001**
	Subspecies	4	0.035	0.009	0.076	4.537	4.501	0.001**
	Subsp.:Sex	5	0.012	0.002	0.027	1.266	1.024	0.149
	Residuals	188	0.363	0.002	0.788			
	Total	198	0.461					
Parieto-occipital	CS	1	0.018	0.018	0.074	18.162	4.347	0.001**
	Subspecies	4	0.034	0.008	0.126	8.391	6.111	0.001**
	Subsp.:Sex	5	0.006	0.001	0.025	1.234	0.865	0.189
	Residuals	188	1.19	0.001	0.765			
	Total	189	0.248					
Ventral	CS	1	0.01	0.01	0.085	19.805	6.117	0.001**
	Subspecies	4	0.01	0.0020	0.082	5.8	6.357	0.001**
	Subsp.:Sex	5	0.002	0.0004	0.02	0.915	-0.202	0.582
	Residuals	190	0.098	0.0005	0.813			
	Total	200	0.12					
Mandible	CS	1	0.017	0.017	0.057	13.538	5.185	0.001**
	Subspecies	4	0.022	0.006	0.075	4.504	5.929	0.001**
	Subsp.:Sex	5	0.028	0.005	0.084	3.987	6.035	0.001**
	Residuals	187	0.232	0.001	0.784			
	Total	197	0.296					

\*\*Significant probabilities.

**Table 2.**—Distance values from mean comparisons between mean shapes of each subspecies. Only significant distances ( $P < 0.05$ ) are shown.

		<i>G. s. antillarum</i>	<i>G. s. handleyi</i>	<i>G. s. mutica</i>	<i>G. s. valens</i>
Fronto-maxillary	<i>G. s. handleyi</i>	1.673			
	<i>G. s. mutica</i>	1.537	1.608		
	<i>G. s. valens</i>	1.556		1.544	
	<i>G. s. soricina</i>	2.498	1.804	3.168	2.060
Parieto-occipital	<i>G. s. handleyi</i>	1.157			
	<i>G. s. mutica</i>				
	<i>G. s. valens</i>	2.381	1.815	1.548	
	<i>G. s. soricina</i>	2.542	1.794	2.749	2.935
Ventral	<i>G. s. handleyi</i>	2.495			
	<i>G. s. mutica</i>	1.747	1.905		
	<i>G. s. valens</i>	2.123	2.122	1.376	
	<i>G. s. soricina</i>	3.754	1.585	2.706	2.974
Mandible	<i>G. s. handleyi</i>	3.159			
	<i>G. s. mutica</i>	1.735	1.482		
	<i>G. s. valens</i>	3.576		1.876	
	<i>G. s. soricina</i>	3.539	1.100	1.852	1.083

is that our morphological phylogeny groups *G. s. antillarum* and *G. s. mutica* as sister taxa recently split groups, in contrast with the molecular phylogeny which groups *G. s. mutica* and *G. s. handleyi* in the same clade (Hoffman et al. 2019).

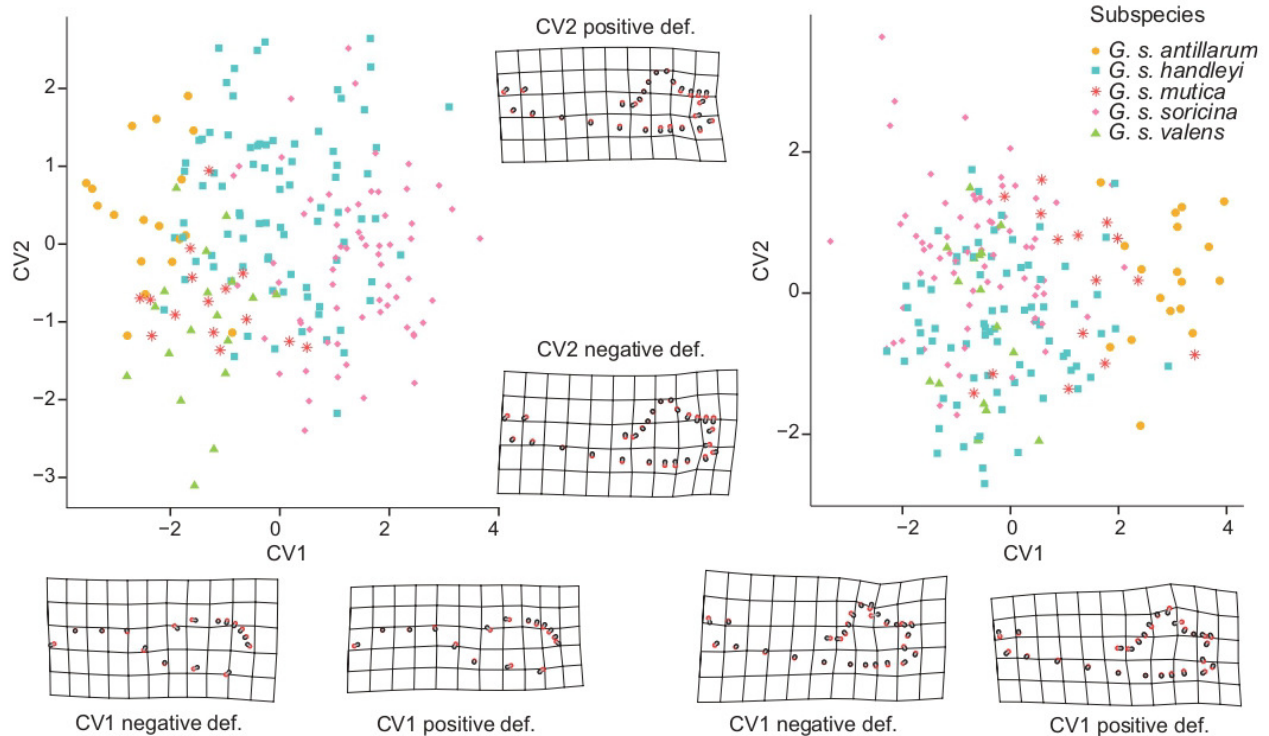
*Glossophaga s. antillarum* has the largest amount of change on its branch when it comes to morphology. *Glossophaga s. antillarum* represents a clade with maximal support divergent in nuclear and mitochondrial markers (Hoffmann et al. 2019).

## DISCUSSION

Morphologically cryptic species complexes are common among all mammal groups, and bats are particularly prone to this kind of misclassification given their small size, elusive habits, morphological convergences, and subtle differences (Jones 1997; Mayer and von Helversen 2001; Clare et al. 2013). Given that morphological characteristics traditionally are the first criterion under

which species are classified and described, it is common that groups with very subtle external differences are grouped together despite being phylogenetically distinct. In this study, we found that the subspecies currently contained within *G. soricina* differ in cranial and mandibular shapes. Together with their known molecular differentiation and nonsympatric distributions, this constitutes enough evidence to state that *G. soricina* is a complex of at least four taxa that are following independent evolutionary trajectories. We recommend that, moving forward, these taxa to be considered independent species, and their trinomen subspecies name be conserved, as follows: *G. antillarum*, *G. mutica*, *G. valens*, and *G. soricina*. More detailed description of distinguishing characters is provided below.

The taxa analyzed herein presented the same patterns of sexual dimorphism, in which sexes differ in mandible shape, but not in skull shape. While there are no differences in size, the body of females' mandibles is significantly thinner than



**Fig. 5.**—Ordination plots for the first two canonical axes from the ventral region of the skull and the mandible, with deformation grids derived from each canonical axis.

males' mandibles. This likely is due to pregnancy and lactation: for other bat species, it has been reported that during these periods, fetal and neonate bats obtain their calcium requirements from the mother's skeletal reserves, which leads to smaller volume of adult females' bones, including the dentary region (Kwieceński et al. 1987; Booher and Hood 2010). The lack of differences in CS between sexes (except for *G. antillarum* mandible) contrasts with previous descriptions of sexual dimorphism in this species (Webster 1983; Louzada and Pessoa 2013), because the estimation of CS values is different than that of linear measures (Bookstein 1997). A similar pattern has been reported previously for *Myotis* species (Ospina-Garcés et al. 2016), where differences between sexes were found when analyzing shape variables but not with CS.

Shape variation best is predicted by different factors, depending on the module, as shown by the Procrustes ANOVA, but in all cases the adjustment was low ( $R^2 < 0.126$ ), suggesting that shape variation could be explained better by other factors not taken into account by this model. The significant effect of size on shape variation is equal in all of the groups analyzed, which do not differ in allometric trends although they do differ in size. Subspecies affiliation also has a significant effect in shape variation in all shape characters, suggesting phylogenetic divergence is reflected in shape differentiation.

*Glossophaga soricina* (Pallas 1766) is the most distinct taxon, among the taxa examined herein, because it is the only one that in all cases presented significant differences with respect to all the studied groups, in both size and shape, for the

four shape modules. According to previous molecular studies, *G. soricina* represents a monophyletic clade whose genetic distance to the other groups is more than twice the distance found between the remaining subspecies, and as large as that found between sister species (Hoffmann and Baker 2001; Dias et al. 2017; Hoffmann et al. 2019). Similarly, in our morphological phylogeny, *G. soricina* is sister to remaining taxa in this complex, reflecting the fact that this species is differentiated both molecularly and morphologically.

*Glossophaga soricina* has the smallest CS values of the groups within this complex, which agrees with earlier reports (Webster 1983; Miller 1913). In terms of shape, we present statistical evidence that supports previous descriptions of this taxon having a domed braincase (rather than square, as in the other taxa of the species group), and short rostrum (Supplementary Data SD5). We did not, however, find evidence of the rostrum being narrower and with a more moderate rostral slope (Webster 1983); deformation grids showed that the rostrum is taller on the lateral view and that the slope actually is the most pronounced among the five taxa examined. We conclude that the diagnostic characteristics for this group would be a smaller size (GLS:  $\bar{x} = 20.5$  mm, range, 19.5–21.7 mm; BCB:  $\bar{x} = 9.2$  mm, 8.7–9.5 mm), a domed, round shape for the braincase, and a shorter rostrum, along with the previously reported darker pelage (Webster 1983). This species is widely distributed on the eastern side of the Andes, from Colombia to Paraguay and northern Argentina.

Another taxon that showed important differentiation was *G. antillarum*, which displayed the largest distances when compared to remaining taxa (Table 2). Specifically, the distance between the mandible shape of *G. antillarum* and that of *G. valens* is the largest we found (dist = 3.576,  $P = 0.001$ ), where the largest vector displacements are the ones corresponding to the landmarks of the condyloid process, which is notably larger in *G. antillarum* than in remaining taxa examined (Supplementary Data SD5). Recent findings (Hoffmann et al. 2019) place *G. antillarum* in a monophyletic clade with very high support, based on genetic differentiation in the molecular markers *Cytb* and *Fgb*. We consider these factors, combined with its distribution restricted to Jamaica and the aforementioned morphological particularities, to be sufficient to conclude that *G. antillarum* is an independent species.

*Glossophaga antillarum* Rehn 1902 is characterized by its large size (GLS:  $\bar{x} = 22.1$  mm, 21.6–22.7 mm; BCB:  $\bar{x} = 9.5$  mm, 9.1–9.7 mm), slender and flattened cranium, and noticeably large condyloid process (Supplementary Data SD5). Deformation grids showed that this species also has the largest occipital bone, as shown by the displacement on the landmark set on the cranial suture between the occipital and temporal bones (landmarks 12–15). This species' distribution is restricted to Jamaica and is not known to occur on any other island in the Caribbean (Webster 1983).

Molecular evidence indicates that what currently is recognized as *G. s. handleyi* is a paraphyletic group, which should be treated in its own right as a species complex that warrants further attention (Hoffman and Baker 2001; Dias et al. 2017; Hoffman et al. 2019). Individuals from Mexico and northern populations of Central America (Guatemala and El Salvador) form a monophyletic clade that includes *G. s. mutica*, with which there is no molecular separation. The taxonomic description of *G. s. mutica* (Merriam 1898) precedes that of *G. s. handleyi* (Webster and Jones 1980), so this taxon should henceforth be named *G. mutica*. Populations from the remainder of Central America (Panama and Nicaragua) seem to form another independent lineage, whose relation to the rest of the species and taxonomic situation should be addressed in future studies.

*Glossophaga mutica* Merriam 1898 continental specimens are intermediate in size and shape between *G. soricina* and the insular groups (GLS:  $\bar{x} = 20.1$  mm, range 19.9–21.9 mm; BCB:  $\bar{x} = 9.4$ , 9.1–9.9 mm). This is evident in the CVA plots, where *G. mutica* (originally *G. s. handleyi*) occupies an intermediate region of the morphospace for all studied characters, with the insular species always clustering to one side and *G. soricina* to the other. *Glossophaga mutica* differs from the insular species and *G. valens* in having a rounder braincase and a wider rostrum with a moderate slope, but not as round and wide as those presented in *G. soricina* (Supplementary Data SD5). It is identical to *G. valens* in the mandible and the fronto-maxillary shape, but can be distinguished from *G. valens* by being smaller in cranial size. *Glossophaga mutica* is a broadly distributed taxon that occupies a variety of environments and displays high variance in size and shape, as well as at a molecular level. It already has been suggested that it could be composed by more than one lineage (Dias et al. 2017; Hoffmann et al. 2019).

Insular specimens of *G. mutica* from Tres Marias islands are clearly recognizable from its continental counterparts by their larger size (GLS:  $\bar{x} = 21.9$  mm, 21.2–22.4 mm; BCB:  $\bar{x} = 9.5$  mm, 9.3–9.7 mm), long rostrum, flattened braincase, and larger condyloid processes in the mandible. This morphological differentiation that insular *G. mutica* presents when compared to its continental counterparts likely is a product of the occupation of the islands, hence the morphological convergence with *G. antillarum*, with whom differences are visually imperceptible both to the naked eye and when visually comparing their mean shapes with the deformation grids, even when shown to be statistically different. We consider that this insular subspecies requires a new trinomial name.

*Glossophaga valens* Miller 1913 is a large species (GLS:  $\bar{x} = 22.2$  mm, 21.5–22.8 mm; BCB:  $\bar{x} = 9.7$  mm, 9.3–10.1 mm) whose cranial and mandibular shape resembles continental *G. mutica*, but they can be distinguished easily by size. Although some phylogenetic studies have found introgression with *G. soricina* in some molecular markers (Clare 2011; Hoffmann et al. 2019), this species is geographically isolated by the Andes (Dias et al. 2017). The geographic isolation, molecular divergence, and morphological differentiation, in concert indicate that *G. valens* likewise should be elevated to species level.

The most phylogenetically informative of the analyzed shape characters was the ventral region of the skull, as previously has been reported for other groups, which is attributed to its structural, functional, and developmental complexity (Caumul and Polly 2005). The module that showed the lowest variation was the parieto-occipital region, because it houses organs of great importance such as the brain and all the structures related to sight and hearing; thus, in this region strong selective pressures allow scant variation to happen (Marroig and Cheverud 2004; Caumul and Polly 2005; Monteiro and Nogueira 2011; Ospina-Garcés et al. 2016; Rossoni et al. 2017). In contrast, the anterior-maxillary region of the skull and the mandible proved to be more malleable structures (Freeman 2000; Drake and Klingenberg 2010) that can undergo rapid evolutionary changes related to individual life stories and recent ecological adaptations and hence could be useful to infer responses to dietary pressures (Freeman 2000; Caumul and Polly 2005). A good example of this are continental *G. mutica* and *G. valens*, differing significantly in the shape of their parieto-occipital region and in the ventral view of their skull and more phylogenetically informative (Caumul and Polly 2005), while being identical in their mandible and fronto-maxillary shapes, which might reflect that the two groups are using the same dietary niches throughout their continental distribution (Monteiro and Nogueira 2011).

In fact, our results suggest that environment plays a significant role on this shape variation. It is evident that similar environments are producing similar shapes on separate species: in the PLS plot, *G. antillarum*, *G. valens*, and insular *G. mutica* cluster in the same area not only in terms of environmental components but also in terms of shape, while continental *G. mutica* (former *G. s. handleyi*) and *G. soricina* occupy a different region (Fig. 6). This convergent pattern also is observed in CS measures: *G. antillarum*, insular *G. mutica*, and *G. valens* are

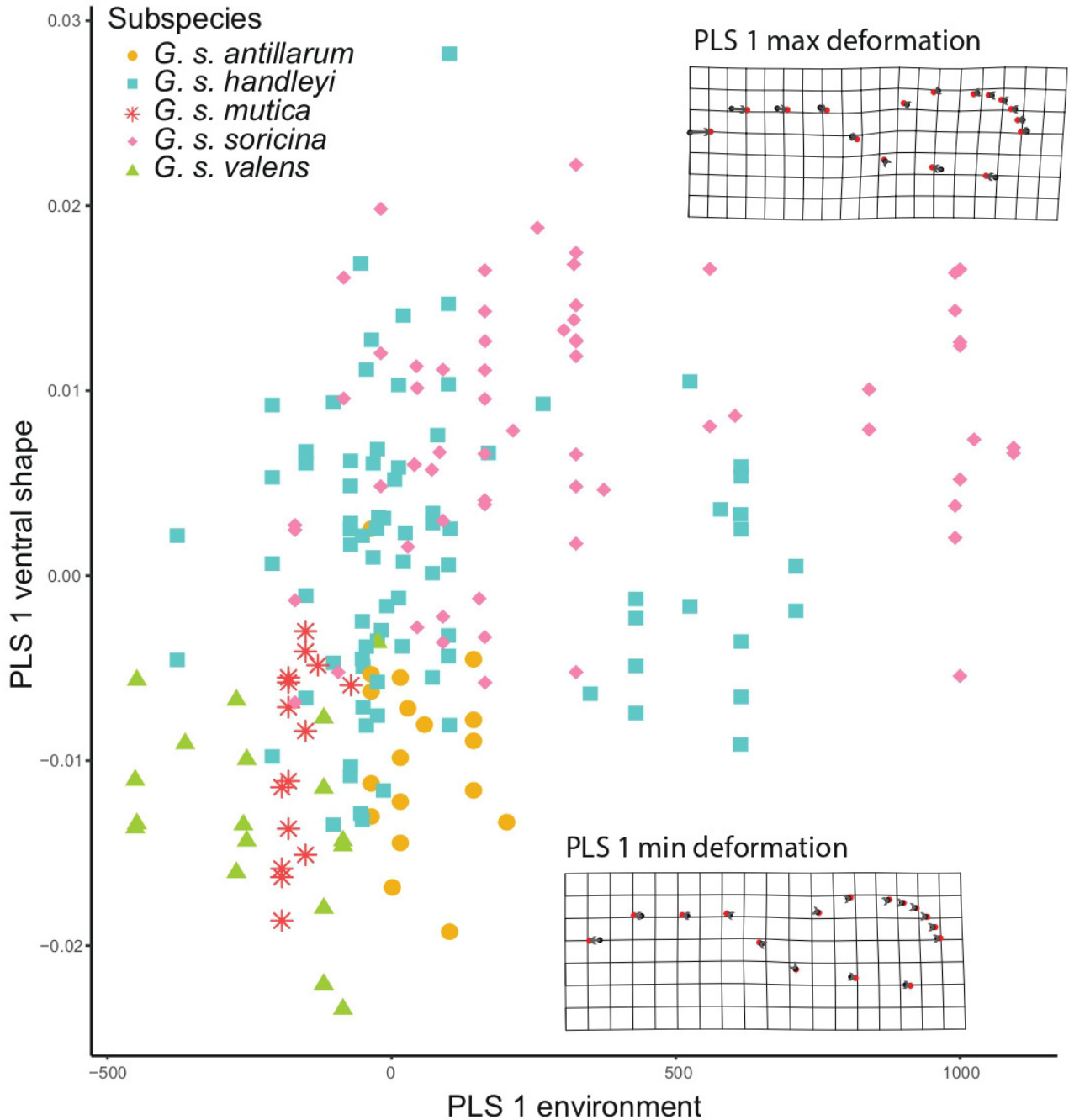


Fig. 6.—Partial Least Squares (PLS) plot for the ventral view of the skull, with deformation grids derived from each canonical axis.

not different in size, as opposed to continental groups *G. mutica* and *G. soricina* that differ significantly between each other and also with the insular subspecies. This agrees with Miller (1913) who, in his revision of this group, stated that insular specimens are easily distinguished when compared to the continental ones, but not between one another. Webster (1983) also found that insular species and *G. valens* cluster together in a multivariate analysis of cranial and postcranial measurements, pelage color, and qualitative cranial characters. These three groups are not

closely related, nor interbreeding with each other given their separate geographic ranges, so this similarity is an evolutionary convergence that can be explained by the “Island Rule” (Foster 1964), which states that mammals that generally are small in the mainland tend to evolve toward a larger size after invading islands because of the change in the selective pressures ensuing the insular invasion, such as enemy and competitive release, that enables the species to undergo rapid evolutionary change as a response to drastic changes in the environment (Lomolino

2005; Millien 2006). *Glossophaga valens* is the largest continental species, which could indicate that it has been released from some competitive pressures in an analog manner to those of taxa occupying islands. This adds up to the evidence that *G. valens* and the insular groups share a morphological convergence driven by facing similar selective pressures.

The small size of *G. soricina* also could be explained by its environment. Previous studies of this species found that bats in regions with high biodiversity have a smaller size than those from arid regions (Louzada and Pessôa 2013). Because of resource competition, smaller sizes are favored in regions of high species richness such as the one this species encounters throughout its distribution range (Heaney 1978; Aguirre et al. 2002). Moreover, the distinctive shape of this species also could be reflecting the strong competition it encounters. The elongation of the mandible and rostrum is a feature that makes the nectar-feeding tribe Glossophaginae (Baker et al. 1989) stand out from the rest of the phyllostomid bats (Monteiro and Nogueira 2011), and is associated with a better support of the tongue at the expense of a weaker bite force (Aguirre et al. 2002; Monteiro and Nogueira 2011). However, *G. soricina* is the least specialized of the glossophagines: it has an omnivorous diet (Sánchez-Casas and Álvarez 2000) and displays a retention of morphological features related to insectivory, such as having three molars with an ectoloph pattern and well-developed incisors (Howell 1976), and the rostrum is the least elongated of the genus. These omnivorous traits allow them to explore a broader diversity of feeding niches, which could have the advantage of reducing resource competition (Howell 1974).

We find patterns of morphological differentiation are consistent with the previously documented molecular divergence (Dias et al. 2017; Hoffmann et al. 2019) and also suggest convergence in size and shape of insular populations. These previously unnoticed morphological differences have now been explored by virtue of the capacity of geometric morphometric techniques to detect subtle morphological differences. We therefore conclude that there is enough morphological evidence that, along with the molecular findings, allows *G. soricina* to be separated in at least four species, three of which correspond to previously recognized subspecies (*G. s. antillarum*, *G. s. valens*, and *G. s. soricina*). *Glossophaga handleyi* appears to be a species complex that includes at least two lineages: a monophyletic one of populations from Mexico to north Central America, including specimens from Tres Marías islands, for which we propose the name *G. mutica*, and another one in the southern populations that requires further attention.

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#### SUPPLEMENTARY DATA

Supplementary data are available at *Journal of Mammalogy* online.

**Supplementary Data SD1.**—Description of landmark and semilandmark positions for each of the four studied shape configurations.

**Supplementary Data SD2.**—Spearman correlation matrix showing the degree of association between 19 environmental variables for *Glossophaga soricina* presence records.

**Supplementary Data SD3.**—Allometric trend plots (shape regression score on CS) for each of the four studied shape characters.

**Supplementary Data SD4.**—Topologies for most the recent molecular tree as published by Hoffmann et al. (2019; A) and for the tree we built based on morphological characters (B). The numbers on the nodes represent the amount of morphological change on each branch from an ancestral shape configuration reconstructed using parsimony. The dotted line represents the position of *G. s. mutica* and *G. s. handleyi*, which have no molecular distinction between each other.

**Supplementary Data SD5.**—Deformation grids illustrating the changes in the fronto-maxillary (A) and parieto-occipital (B) regions of the skull between the mean shapes of the three most distinctive species: *G. s. antillarum* (now *G. antillarum*), *G. s. handleyi* (now *G. mutica*), and *G. s. soricina* (now *G. soricina*). Light dots represent the landmarks and semilandmarks corresponding to the target mean shape of the subspecies in Y; dark dots represent the landmarks and semilandmarks corresponding to the mean shape of the subspecies in X. The arrows show the magnitude and direction of the displacement vectors from subspecies in X to target (subspecies in Y), while the deformation in the grid shows the bending energy. Deformation grids were exaggerated by a factor of 3 to improve visualization.

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*Glossophaga soricina mutica*.—USNM: 512268, 512269, 512270, 512271, México, Nayarit, María Cleofas; 89271, 512255, 512256, 512258, 512259, 512261, México, Nayarit, María Madre; 512262, 512263, 512266, 512267, México, Nayarit, María Magdalena; 512272, México, Nayarit, San Juanito.

*Glossophaga soricina soricina*.—AMNH: M-209357, Bolivia, Beni, Itenez; M-209354, M-209355, M-209356, Bolivia, Beni; M-97230, M-97233, M-97244, M-97246, M-97248, Brazil, Para, Cameta; M-97263, Brazil, Para, Mocajuba; M-75236, Colombia, La Guajira, Riohacha; M-15157, M-15158, M-15159, Colombia, Magdalena, Santa Marta; M-207927, Colombia, Tolima, Mariquita; M-207315, Guyana, Demerara; M-207316, M-48251, Guyana, Demerara, Georgetown; M-182722, M-182723, Guyana, West Demara, Leguan Island; M-42615, M-42617, M-42621, M-42623, M-42627, M-42629, M-42632, M-42634, M-42635, M-42636, M-42637, M-42638, Guyana, Mahaica-Berbice Region, Canje River; M-217528, M-217530, M-217532, M-217533, Paraguay, Guaira, Villarica; M-175816, Trinidad and Tobago, Trinidad, Caroni County; M-175806, M-207064, M-183857, Trinidad and Tobago, Trinidad, Saint Andrew County; M-175812, Trinidad and Tobago, Trinidad, Victoria County; M-30682, M-30685, M-30686, M-30687, M-30689, M-30690, M-30691, M-30692, M-30693, M-30694, Venezuela, Bolivar, El Callao; M-130657, M-130658, M-130660, M-130661, Venezuela, Bolivar, Gran Sabana; M-31502, Venezuela, Carabobo, Puerto Cabello. USNM: 562721, 562722, Brazil, Amazonas, Laurete; 393653, 393654, Brazil, Mato Grosso, Serra Do Roncador; 391040, 391041, 391042, 391043, Brazil, Minas Gerais, Sete Lagoas; 361545, 361546, 361547, 361552, Brazil, Para, Utinga; 582297, 582298, Guyana, Barima-Waini, Baramita; 582296, Guyana, Upper Demerara-Berbice, Dubulay Ranch; 407705, 407707, Venezuela, Amazonas, San Juan; 545336, 545338, Venezuela, Amazonas, Rio Orinoco.

*Glossophaga soricina valens*.—AMNH: M-61426, M-61427, M-61428, Ecuador, El Oro, Santa Rosa; M-62943, M-62944, M-62945, Ecuador, Guayas, Duran; M-62105, Ecuador, Guayas, Guayaquil; M-64560, M-64562, M-64563, Ecuador, Guayas, Santa Elena; M-36276, Ecuador, Pichincha, Duale River.

USNM: 498834, 498836, 498837, 498839, Ecuador, Guayas, Balao; 498845, 498846, 498847, 498848, Ecuador, Los Ríos, Pueblo Viejo; 531243, Perú, Piura, Rio Chira.

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## APPENDIX I

*Specimens examined*.—List of 231 specimens in alphabetical order by subspecies grouping. Museum names were abbreviated as follows: AMNH = American Museum of Natural History, New York, United States; MZFC = Museo de Zoología “Alfonso L. Herrera,” Facultad de Ciencias, UNAM, México; USNM = National Museum of Natural History, Smithsonian Institute, Washington, DC, United States.

*Glossophaga soricina antillarum*.—AMNH: M-271585, Jamaica, Clarendon, Portland Cottage; M-271587, Jamaica, Saint Ann, Mosely Hall Cave; M-214129, M-271588, Jamaica, Saint James, Montego Bay; M-271586, Jamaica, Saint Mary, Port Maria. USNM: 511236, 511237, 511238, 511239, Jamaica, Clarendon, Mahoe Gardens; 545155, 545156, 545157, 545158, Jamaica, Trelawny, Good Hope Estate; 545150, 545151, 545152, 545153, 511235, Jamaica, Trelawny, Quick Step.

*Glossophaga soricina handleyi*.—AMNH: M-269457, Colombia, Valle del Cauca, Dagua; M-136108, M-136109, M-136127, M-136129, M-136130, M-136134, Costa Rica, San José, San José; M-144704, M-144706, M-144717, M-144718, M-144720, Guatemala, Petén, La Libertad; M-126470, M-126477, M-126481, Honduras, Francisco Morazán, Guaimaca; M-203607, M-203608, M-203609, M-203613, M-203614, M-203615, M-203616, México, Veracruz, Veracruz; M-254616, México, Veracruz, Coatepec; M-213217, Nicaragua. MZFC: 6839, 6863, 6864, 7113, 7197, México, Chiapas, Ocosingo; 9324, 9333, 9336, 9339, México, Campeche, Tenabo; 961, 962, 963, México, Guerrero, Atoyac de Álvarez; 10558, 10570, 10573, México, Guerrero, Arcelia; 5799, 5800, 5806, 5808, 5812, México, Hidalgo, El Cardonal; 10170, 10172, 10173, 10174, México, Michoacán, Arteaga; 13835, 13836; México, Nayarit, San Blás; 4999, 5214, 5215, México, Oaxaca, San Juan Bautista Valle Nacional; 6415, México, Oaxaca, San José Chiltepec; 9761, 9762, 9763, México, Quintana Roo, Solidaridad; 11990, 11991, 12084, 12088, México, San Luis

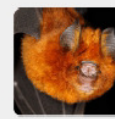
### CAPÍTULO 3

Cranial morphological convergences are independent from abiotic ecological requirements in Neotropical glossophaginae bat species

Adriana Calahorra-Oliart, Andrés Lira-Noriega, y Livia León-Paniagua.

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**En revisión**



# Cranial morphological convergences are independent from abiotic ecological requirements in Neotropical glossophaginae bat species

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**Type:**

Original paper

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**Abstract:**

Recent molecular and morphometric studies have found that what was originally described as a single species, *Glossophaga soricina* (Chiroptera: Phyllostomidae), was indeed a cryptic species complex conformed by at least four different lineages with non-overlapping geographic distributions and molecular differentiation. Morphological differences have been reported between continental species *G. mutica* and *G. soricina*, while insular *G. mutica* and *G. antillarum* and the continental species *G. valens* present convergences in shape and size. In the present study, we conducted ecological niche model analyses to characterize the ecological requirements of these recently recognized species, and to assess if niche conservatism could be a factor behind the morphological convergences between them and behind their divergence. Mainland species presented the highest niche overlap values regardless of their morphological differentiation, while the insular groups proved to be ecologically distinct. Therefore, the convergence and dissimilarities in the shape of these groups must be driven by factors not considered in this study most likely related to their Eltonian rather than their Grinnellian ecological niches. The non-suitability regions that separate the distributions of the continental species explain the barrier to the genetic flux that could be behind their divergence and add evidence of them being separately evolving lineages.

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**Keywords:**

ecological niche modeling, Geometric Morphometrics, *Glossophaga*, integrative taxonomy, morphological convergences, niche conservatism

## ABSTRACT

Recent molecular and morphometric studies have found that what was originally described as a single species, *Glossophaga soricina* (Chiroptera: Phyllostomidae), is indeed a cryptic species complex formed from at least four different lineages with non-overlapping geographic distributions and molecular differentiation. Morphological differences have been reported between continental species *G. mutica* and *G. soricina*, while insular *G. mutica* and *G. antillarum* and the continental species *G. valens* present convergences in shape and size. In the present study, we conducted ecological niche model analyses to characterize the ecological requirements of these recently recognized species, and to assess whether niche conservatism could be a factor behind the morphological convergences between them and behind their divergence. Mainland species presented the highest niche overlap values regardless of their morphological differentiation, while the insular groups proved to be ecologically distinct. Therefore, the convergence and dissimilarities in the shape of these groups must be driven by factors not considered in this study most likely related to their Eltonian rather than their Grinnellian ecological niches. The non-suitability regions that separate the distributions of the continental species explain the barrier to the genetic flux that could be behind their divergence and add evidence of them being separately evolving lineages.

Keywords: Ecological niche modeling, geometric morphometrics, *Glossophaga*, integrative taxonomy, morphological convergences, niche conservatism.

## INTRODUCTION

The ecological niche of a species is part of its phenotype and, as such, is shaped by evolutionary forces (Peterson et al. 2011). The interaction between a species' ecological requirements and the landscapes that it encounters throughout its distribution can be a major force of diversification: selective pressures, such as resource partition or the incapacity of populations to adapt to a different environment, can impose a barrier to genetic flux, resulting in the divergence from an ancestral population and its consequent differentiation, which may be reflected differently on each of its characters (Wiens 2004; de Queiroz, 2007; Nosil 2012; Fišer et al. 2018).

During the speciation process, the diverging lineages acquire their distinctive characteristics in different moments, as a result of different evolutionary forces and in a different order. Some of these characteristics may not even diverge at any point (de Queiroz 2007). In terms of their ecological requirements, sister species might maintain similar niche characteristics related to their phylogenetic proximity (niche conservatism) or might differentiate in their niche early in their speciation process (niche divergence).

Originally considered as a single species, *Glossophaga soricina* was described as a Neotropical phyllostomid bat with a wide distribution from the north of Mexico to the north of Argentina (Miller 1993; Webster 1983), where it occurs in a variety of ecosystems. It belongs to the Glossophaginae subfamily, characterized by having a predominantly nectivorous diet which implies a series of morphological specializations like elongated rostrums and tongues, and a reduction in teeth (Baker et al. 1989; Datzmann et al. 2010), that are associated with a better support of the tongue at the expense of a weaker bite force (Aguirre et al. 2002; Monteiro and Nogueira 2011). However, *G. soricina* least specialized diet amongst glossophagines (Sánchez-Casas and Álvarez 2000), using also fruits, insects, leaves and flower parts as part of its diet (Álvarez et al. 1991; Clare et al 2014; Pellón et al. 2021). Diet is known to play an important role on the evolution of cranial shapes (Dumont et al. 2012; Ospina-Garcés and De-Luna 2017), and the omnivorous diet of *G. soricina* is reflected on its craniodental morphology, which presents a retention of features related to insectivory, such as having the least elongated rostrum of the genus and having three molars with an ectoloph pattern and well-developed incisors (Howell 1976). These omnivorous traits allow

them to explore a broader diversity of feeding niches, which could give the advantage of reducing resource competition, especially in the regions where it encounters higher bat species richness (Heaney 1978; Aguirre et al. 2002).

Previous analyses have revealed the existence of consistent molecular (Hoffmann and Baker 2001; Clare et al. 2011; Dias et al. 2017; Hoffmann et al. 2019) and morphological (Calahorra-Oliart et al. 2021) differences within the subspecies, suggesting that *G. soricina* is composed from at least four species with non-overlapping geographic distributions: *G. antillarum* (Rehn 1902) in Jamaica, *G. mutica* (conformed by two former subspecies: *G. s. handleyi* Webster and Jones 1980; synonymized with *G. s. mutica* by Gardner 2008 from the north of Mexico to the north of Colombia, and *G. s. mutica* Merriam 1898; synonymized with *G. leachii* by Hall 1981 in Tres Mariás Islands, Mexico), *G. soricina* (Pallas 1766) in south America east of the Andes, and *G. valens* (Miller 1913) in south America west of the Andes (Dias et al. 2017; Hoffmann et al. 2019; Calahorra-Oliart et al. 2021; Fig. 1).

The deepest molecular divergence is found between the groups situated at the east of the Andes, corresponding to *G. soricina*, and the clade composed by the rest of the groups of this complex: *G. mutica*, *G. soricina*, and *G. valens*. It is estimated that the latest Andes uplift (between ~2.7 and 5 Ma) formed a barrier that originated this speciation event (Dias et al. 2017). Within the clade composed by the rest of the species, each of them forms monophyletic clades (Dias et al. 2017; Hoffmann et al. 2019).

A previous study (Calahorra-Oliart et al. 2021) suggested that the abiotic environment could be related cranial shape variation in these lineages: species which are geographically and phylogenetically separated converge in mandible and skull size and shape, which could be explained by them occupying similar environments. On insular groups (*G. antillarum* and *G. mutica* populations from Tres Mariás Islands) and the continental species with the narrowest distribution *G. valens*, individuals show larger sizes, larger mandibular processes, a longer rostrum and a flattened braincase. When comparing mean shapes, distance values were smallest when comparing insular *G. mutica* with *G. antillarum* and *G. valens*, and no significant differences were found in terms of size. On the other hand, the widely distributed continental species *G. mutica* and *G. soricina* are smaller, have shorter rostrums and domed braincases. These features are more accentuated in *G. soricina*, which makes it the most

easily distinguishable lineage, having also a darker pelage, smallest size and the flattest rostrum, while continental *G. mutica* is intermediate in size and shape. They present significant differences in size (Calahorra-Oliart et al. 2021). While these two widely distributed species are expected to occupy a more heterogeneous variety of environments given their extended distributions, the insular groups and *G. valens* could be occupying more restricted niches with the respective environmental conditions potentially accounting for their morphological convergences.

Ecological niche modeling sheds light upon the factors that affect geographic limits of lineages, and on the possible drivers of species divergence (Graham et al. 2004b; Rissler y Apodaca 2007; Tocchio et al. 2015). Ecological niche analysis can provide insight into a species history and therefore contribute to a better understanding and delimitation of the species (Padiál et al. 2010). Based on previous morphological evidence (Calahorra-Oliart et al. 2021), in the present study we conducted ecological niche studies aiming to compare the niches of the four species of the former *G. soricina* complex to explore in depth how the respectively inhabited ecological space could account for the convergence in their skull and mandible shape. Specifically, we ask whether the morphological convergences can be explained environmentally. We expect that clades with similar cranial shapes will be occupying similar environmental conditions. In addition, the analyses of their ecological niches will allow to describe for the first time the requirements of these newly elevated species, and to have a new line of evidence that supports the existence of separate lineages.

## MATERIALS AND METHODS

*Study area.* - Study area was determined based on the distribution of the species that formerly constituted the *G. soricina* complex. These four species present allopatric distributions, which go as follows: *G. antillarum* (Rehn 1902) in Jamaica, *G. mutica* in Mexico and Central America including Tres Mariás Islands, *G. soricina* (Pallas 1766) in South America at east of the Andes, and *G. valens* (Miller 1913) in South America west of the Andes, in Ecuador and Peru.

*Group delimitation.* - The compared groups were delimited following the molecular (Hoffmann et al. 2019) and morphological (Calahorra-Oliart et al. 2021) findings, which divide the former nominal species *G. soricina* in four independent lineages: *G. antillarum*, *G. mutica* (composed by former *G. s. handleyi* and *G. s. mutica*), *G. soricina*, and *G. valens* (Fig. 1). *Glossophaga mutica* (Merriam 1989) was divided between the mainland populations (Mexico, Central America and north of Colombia) and those in the Tres Mariás Islands because of their disjunct distribution and morphological differences, in order to test the existence of possible environmental divergences.

*Occurrence and environmental data.* - Occurrence data were obtained from online databases VertNet (<http://vertnet.org/>) and GBIF (<https://www.gbif.org/>). Occurrences were visually inspected using QGIS 3.4.8 (<http://qgis.osgeo.org/>) to eliminate incorrect and dubious records, as well as those prior to year 1970. To avoid sampling biases, occurrence databases were thinned with package spThin in R (Aiello-Lammens et al. 2015), leaving only one record per 10 km. Environmental and elevation variables were obtained from the WorldClim database version 2.1 (Fick and Hijmans 2017) at a resolution of 2.5 minutes for current conditions. Because spatial autocorrelation in environmental predictors can lead to incorrect predictions, a Spearman correlation test was performed to detect and exclude highly correlated variables ( $r \leq 0.7$ ). The selected variables were Bio2 (Mean Diurnal Range), Bio3 (Isothermality), Bio8 (Mean Temperature of Wettest Quarter), Bio9 (Mean Temperature of Driest Quarter), Bio13 (Precipitation of Wettest Month), Bio14 (Precipitation of Driest Month), Bio15 (Precipitation Seasonality), Bio18 (Precipitation of Warmest Quarter), and Bio19 (Precipitation of Coldest Quarter).

*Shape assessment.* - Shape data of the ventral view of the skull were registered via two-dimensional geometric morphometrics. A total of 201 specimens were photographed (Appendix 1) from three mammal collections: Museo de Zoología “Alfonso. L. Herrera” at the Universidad Nacional Autónoma de México (Mexico City, Mexico); the National Museum of Natural History Mammal Collection (Washington, DC, USA); and the American Museum of Natural History Mammal Collection (New York, NY, USA). Our sampling contained individuals from the four species throughout their distribution range (Fig. 1). Sample sizes are uneven, reflecting the differences in distribution areas among subspecies. However, we aimed to have a minimum of 10 specimens of each sex per subspecies, with an



even number of males and females, which were pooled given there are no sex-specific differences in shape between them (Calahorra-Oliart et al. 2021). Digital photographs of the ventral view of skulls were acquired using a tripod mounted Nikon D3200 reflex camera (Nikon Corporation, Tokyo, Japan) with an AF-S Micro Nikkor 60 mm lens (Nikon Corporation, Tokyo, Japan), keeping the skulls always in the same position and at the same distance from the camera lens. We chose the ventral view of the skull since it has proved to be the more phylogenetically informative in *Glossophaga* as well as in other species (Caumul and Polly 2005; Calahorra-Oliart et al. 2021). Two-dimensional landmark and semilandmark coordinates were marked on the digital images using the program TpsDig 2.16 (Rohlf 2010). The ventral view of the skull was registered with a configuration of 11 landmarks and 5 semilandmarks. For a detailed description on the location of the landmarks and semilandmarks, see Appendix 1. After shape variables were extracted from the images, a Generalized Procrustes Analysis was performed to align and obtain the shape, by translating each configuration to the origin, scaling and rotating it using a least-squares distance criterion to obtain the shape information once all other variance factors, such as size and position, have been removed (Rohlf 1990). Semilandmarks were aligned along the contour to minimize the bending energy necessary to produce the change in the target outline to the consensus shape (Mitteroecker et al. 2013). We obtained shape variables as coordinates (Bookstein 1997). The geometric morphometric procedures were performed in the package Geomorph 3.1.2 (Adams et al. 2019) in R version 3.6.0 (R Core Team, 2019).

We conducted an exploratory PCA to visually inspect the morphospace in order to identify the two individuals that were occupying the two extremes along the morphometric PC1. These were a female *G. antillarum* and a male *G. soricina* from Venezuela. These two species have the largest distance values of all when comparing their mean shapes (Calahorra-Oliart et al. 2021). The shape configurations of these individuals were plotted, and a Thin Plate Spline Analysis (TPS) was performed to visualize shape differences between one and the other, represented by landmark displacement and deformation on the grid (Mitteroecker and Gunz 2009). Deformation grids were exaggerated by a magnitude of three so displacements were more evident, with the R package Morpho version 2.7 (Schlager 2017).

A between-group Principal Component Analysis (PCA) was performed on shape variables using R package Morpho (Schlager et al. 2017). This type of PCA analysis accounts for differences in sample sizes, particularly when the number of shape variables (coordinates) is close to the number of individuals in some samples, which was the case for our insular groups. The values derived from the between-group PCA were later used as input data for creating the plot with projections of the morphospace using NicheROVER (see below; Swanson et al. 2015). This method was chosen as it is the one used for ecological space, so the plots were comparable. We projected the morphospace for each group as ellipse plots, density plots and two-dimensional scatterplots, in order to visually examine geometry and overlap patterns.

*Bioclimatic variables comparisons.* - For the 19 bioclimatic variables obtained from the WorldClim database version 2.1 (Fick and Hihmans 2017) for current conditions at a resolution of 2.5 minutes, values were extracted for each occurrence point's coordinates. Using these values, differences between groups were assessed using an ANOVA model and the significance of pairwise comparisons among subspecies was assessed with the Tukey Honest Significant Differences Method (Miller 1981) with the R package stats (R Core Team 2020). Each group's values were presented with boxplots for each variable.

*Ecological niche modeling.* - We generated ecological niche models with the R package NicheToolBox, which uses minimum volume ellipsoids to estimate species' niches (Osorio-Olvera, et al. 2020). According to best modeling practices (Warren and Seifert et al. 2011), we first conducted best model selection based on omission rate (0.5) and statistical significance (Partial ROC) using *a priori* spatially filtered occurrences using a 20 km distance threshold as our calibration dataset and the rest of the occurrences as evaluation points, with the exception of insular species for which we used Mobility Oriented Parity (MOP; Owens et al. 2013) in NicheToolBox (Osorio-Olvera, et al. 2020). This spatial filtering has been demonstrated to be useful in eliminating model overfitting (Veloz 2009; Kramer-Schadt et al. 20013; Boria et al 2014). Model selection was based on least correlated bioclimatic variables ( $r < 0.7$ ) for each species, selecting among all combinations of three, six and nine variables. Then, the first five best model parameterizations were used to get the final estimates of species' potential distributions in geographic space according to the median of

these suitability maps, which were then converted to a binary prediction using the 10<sup>th</sup> percentile threshold to give a final suitability map. To provide an adequate biogeographic context to each model prediction, we calibrated and projected these in an *a priori* defined accessibility area (M) for each species following Barve et al. (2011). These areas were defined as the polygon where all terrestrial ecoregions intersect with each species' occurrences plus a 0.166 degree (~20 km) buffer surrounding them.

*Island-continent environmental background similarity.* - Because insular groups had less occurrence records and in general their environmental background is overall smaller compared to the continental groups, we wanted to assess the amount of environmental similarity between these two types of land masses independently of species' occurrence information and niche models. Thus, environmental similarity between each island and the continental region were carried out using the Mobility Oriented Parity (MOP) metric (Owens et al. 2013) in NicheToolBox based on the first two principal components of the island's M regions onto the continental region. Overall, this can be a useful tool not only for evaluating environmental similarity (i.e., whether two territories share similar environments) but also to assess strict model extrapolation.

*Climatic niche overlap.* - We used NicheROVER (Swanson et al. 2015) to perform paired comparisons of environmental overlap between species. This approach defines the niche space as the joint probability density function of the multidimensional niche at a probability that we defined as 95%. We used as niche dimensions the first five principal components of the 9 previously selected variables, which accounted for 95% of the ecological variance. This method provides directional estimates of niche overlap in multidimensional space and allows to plot unique bivariate projections of the niche region as ellipsoids, thus facilitating the visual examination of their geometry and overlap patterns. The overlap metric is calculated with paired comparisons in both directions, meaning that we obtain the probability that an individual from species A could be found in the niche of species B, and also the reverse option, i. e. the probability that an individual from species B could be found in the niche of species A. The directional estimates of niche overlap from this method are considered to be more robust for niche characterization than the other traditional methods that use percentages to measure overlap because this method takes data uncertainty into account with a Bayesian

framework, does not assume uniform distribution of individuals within the niche region, and is insensitive to sample size, which prevents spurious variations in niche region and overlap (Swanson et al. 2015; Luna-Arangur  et al. 2019).

## RESULTS

In terms of cranial shape, we found that insular *G. mutica*, *G. antillarum* and *G. valens* occupied mostly the same morphospace region, while *G. soricina* had the most differentiated shape, and continental *G. mutica* occupied an intermediate region (Fig. 2B). The first four PCs of morphological variables explained 100% of the variance. Visual inspection of the deformation grid derived from comparing the shapes of the two more distant specimens in the morphospace (a female *G. antillarum* and a male *G. soricina* from Venezuela; Fig. 3) confirmed that there were displacements in the landmarks which corresponded to the differences in shape previously described for these species (Miller 1913; Webster 1983; Calahorra-Oliart et al. 2021), where *G. soricina* presented an evidently shorter rostrum and a more domed braincase than *G. antillarum*.

The first five PCs of the environmental variables explained 91% of the variance. The first component (PC1; 48%) had a homogeneous contribution of most of the included variables; it was influenced negatively mainly by precipitation of the driest month (Bio13), mean temperature of the driest quarter (Bio9) and precipitation of coldest quarter (Bio19), and positively by mean diurnal range (Bio2). Variation in second component (PC2; 17.6%) was mostly influenced negatively by precipitation seasonality (Bio15) and positively by precipitation of the driest month (Bio14). For the complete list of the loadings of each variable to each PC, see supplementary table 1.

Bioclimatic boxplots of the continental species showed a higher variability that is coherent with the extension of their distribution range, while insular groups presented a much narrower range of conditions which also relates to their smaller distribution. Remarkably, insular *G. mutica* and *G. antillarum* had contrasting patterns: for the variables in which one of them had high values, the other one had low values and vice versa (Fig. 4); however, when tested for

significance, paired comparisons only found significant differences in six out of 19 variables (supplementary table 2).

*G. valens* and insular *G. mutica* are the groups that tolerate the driest environments, inhabiting regions with the lowest precipitation values and dry seasons (Bio12 – Bio19), which were significantly different from the other 3 groups. Continental *G. mutica* and *G. soricina* presented several outliers with the lowest values for temperature variables, indicating that some of their populations tolerate notably lower temperatures than the other groups. They also presented several outliers in precipitation-related variables, indicating that they have populations located in regions of precipitation higher than average.

The most differing variable between groups was precipitation seasonality (Bio15), which refers to the variation in precipitation levels throughout the months of the year. *G. valens* and insular *G. mutica* occupy the regions with the highest seasonal variation, *G. antillarum* and *G. soricina* inhabit regions with low variation, and continental *G. mutica* presented an intermediate value. The only variable for which none of the groups differed was precipitation of warmest quarter (Bio18).

The posterior probability niche overlap comparisons showed ecological differences for the groups. Through visual inspection of the ellipsoid models of the niche regions we identified a nested pattern, in which the niche of *G. valens* contained most of the areas of the niche spaces of the other groups. Insular groups occupied narrow climatic spaces which did not overlap between one another, in contrast with the ecological space of the continental ones, which were wider and with larger overlap areas. The ecological space of the insular groups fell inside of that of the continental ones. All of them clearly differed in their centroid values and their breadth. The continental groups occupied the most heterogeneous conditions as shown by their wider ellipsoid volumes (Fig. 2A). Values of niche overlap ranged from 0% to almost 100% depending on the species (Table 1). The two ecological niches that overlapped the most when taking both directions of the overlap metric into account were those between *G. soricina* and continental *G. mutica*. *G. soricina* had nearly 80% probability of finding suitable regions within the ecological space of continental *G. mutica*, while continental *G. mutica* had 61% of overlap within *G. soricina*'s niche. The niche of continental

*G. mutica* where more groups could find environmental suitability. *G. antillarum* and insular *G. soricina* both showed a great probability for finding suitable regions within the ecological niche of the continental groups, except for the case of the comparison between *G. antillarum* and *G. valens* (15% and 0% probability, respectively). In contrast, continental species barely found any suitable regions within the niche of insular groups (<2% probability). Except for insular *G. mutica*, most species would not find suitable regions within the ecological niche of *G. valens*, though overlap values were not as low as when compared to the insular groups (Table 1).

The predicted ranges for the three continental groups for present conditions were consistent with their actual distributions, while also having areas of potential distribution outside of their known range (Fig. 5). All three of them showed very low suitability scores in areas with higher elevations, corresponding to the Andes, the Mexican Plateau, and the oriental and occidental Sierra Madre. They also coincide in having low suitability values in humid regions such as some parts of the south American tropical rainforest region, and humid forests in the central America. Interestingly, they all seem to have the potential to colonize regions where none of the species of this clade are present, such as the rest of the Antilles islands (besides Jamaica), and the east southern coasts of the United States. These regions correspond to tropical climates with seasonal changes, alternating between humid summers and dry winters. Particularly, *G. soricina* was the species with the largest suitability areas outside of its known distribution (Fig. 5).

The visualization of the projections of insular *G. mutica* and *G. antillarum* niches onto the study area G showed different patterns. *G. antillarum* had large extrapolation regions in south America, west side of the Andes, south of México, the coasts of central America and some of the Antillean islands. Insular *G. mutica* presented only small regions of extrapolation in the rest of our study area, which corresponded to dry areas in Mexico and South America (Fig. 6).

## DISCUSSION

The species forming the former *G. soricina* complex have ecological niche characteristics that provide relevant information about their evolutionary history and more evidence of the separation of these lineages: using ecological niche modeling and exploring the bioclimatic variables ranges that these groups occupy, we characterized their particular abiotic requirements, while the suitable and non-suitable regions in the continental groups shed light on the reasons behind their allopatric speciation process, as ecological barriers are coherent with the disjunct geographic distributions of each of them (Dias et al. 2017).

Contrary to our expectation, we did not find evidence that morphologically similar species (*G. antillarum*, insular *G. mutica* and *G. valens*) occupy similar ecological niches. In fact, their niches were the least overlapping ones, while the highest levels of niche overlap were presented between the continental species, which are not the most similar morphologically nor the closest phylogenetically (Dias et al. 2017; Hoffmann et al. 2019; Calahorra-Oliart et al. 2021). Moreover, the two species that represent the most radically different morphologies, *G. antillarum* and *G. soricina* (Fig. 3), proved to have an important degree of niche similarities. We thus conclude that the morphological variation (convergences in insular groups and differentiation in continental species) does not seem to be driven by the abiotic factors considered in this study, and instead could be more likely related to their Eltonian rather than their Grinnellian ecological niches. It is common in this type of studies to find that, while the environment seems to have influence upon the evolution of the shape of their skull, there is always a percentage of the variance that is not explained by the considered environmental variables (i.e. Cardini et al. 2007; Cardini and Elton 2009; Soberón and Martínez-Gordillo 2012; Morales et al. 2018). This implies that other factors are playing a role on cranial shape variation, and they should be taken into consideration to lead following studies. Most likely, diet could be a major force on the evolution of the cranial features, given the importance that cranial morphology has in the obtainment and processing of food, but precise information on the feeding habits of a species is not always available (Evin et al 2008; Dumont et al. 2012; Ospina-Garcés et al. 2016). An alternative is to use biotic variables as a proxy of the food that animals could have access to, especially those related to precipitation as they can be used to estimate habitat productivity (Cardini et al. 2007), but more studies

that address specifically the diet of these animals are needed to make reliable comparisons and to analyze non-scenopoetic variables in general (Soberón and Martínez-Gordillo 2012).

Visual inspection of the plots demonstrated that insular groups present narrower niche conditions and, interestingly, their niche ellipsoids do not overlap with each other. In addition, their overlap probability values were extremely low (Table 1). However, when comparing between morphologically similar groups, the niche of insular *G. mutica* has more similarities with that of *G. valens*, as shown by the posterior probability's comparisons, the niche space and the boxplot comparisons. These two groups occupy drier regions characterized by low precipitation during the dry months and pronounced seasonality in precipitation (Fig. 4) and have the same cranial shape and size (Calahorra-Oliart et al. 2021). *G. antillarum*, on the other hand, does not seem to be following this ecological pattern, as shown by its evident differences with the abiotic conditions that insular *G. mutica* and *G. valens* inhabit, despite its morphological similarities with these two groups. This result adds up to the evidence that *G. antillarum* is a clearly differentiated species, not only phylogenetically and morphologically (Dias et al. 2017; Hoffmann et al. 2019; Calahorra-Oliart et al. 2021) but also in its ecological requirements. It is estimated that *G. antillarum* colonized Jamaica during a period with significant lower sea level (about 80 m lower than the current sea level; Bintanja and Wall 2011), with a divergence time dating back between ~0.9 and 2.2 Ma (Dias et al. 2017). Since then, local evolutionary forces have probably shaped its ecological tolerances. Morphological convergences in bats have been found in different scenarios: in species of the African bat genus *Otomops* (Richards et al. 2012), those who occupy semi-arid environments converge in larger sizes, in a similar way as *G. valens* and insular *G. mutica*. Size convergences have also been found between groups that do not inhabit the same abiotic conditions nor have gene flow between them, as in the case of the widespread bat, *Tadarida brasiliensis*, whose populations from the east side of the Andes in South America present the same type of cranial phenotype as those in the Antilles, including Jamaica (Morales et al. 2018), which is similar to what we found that is happening with *G. antillarum*.

Morphological changes after new habitat invasion are well documented (Losos et al. 1998; Orr and Smith 1998), such as after island colonization (Foster 1964; Heaney 1978; Aguirre



et al. 2020) or related to changes in competitor density (Louzada and Pessôa 2013). For instance, *Glossophaga soricina* inhabits a region with the highest species richness of other phyllostomid bats (Villalobos and Arita 2009), which may imply a higher level of competition and hence the selection for a smaller body size (Louzada and Pessôa 2013). Moreover, the generalist diet of this species, which is reflected in its cranial morphology, is likely to be adaptive, as it offers an advantage over more specialized nectarivore competitors and allows exploring a variety of feeding options even in urban and disturbed areas (Pellón et al. 2021). A very different case can be expected after island colonization, in which the species could experience a reduced number of competitors which might favor other morphologies, such as an increase in size (Foster 1964; Lomolino 2005; Millien 2006). However, the majority of the studies that explore these morphological changes use linear measures that account for size, while geometric morphometric studies that analyze changes in shape instead of (or in addition to) size are scarcer. Contrary to cranial size, shape could be more resistant to change and therefore a better proxy for phylogeny (Cardini and Elton 2009). Our study demonstrates that cranial shape is, nonetheless, also prone to morphological convergence, as seen in our geographically isolated taxa, so phylogenetic conclusions derived from shape comparisons would be misleading.

Niche convergence and the identification of regions of low climatic suitability provide insights into the speciation process between the continental species. Continental groups presented considerable degrees of niche overlap, suggesting that they retained to some degree their ancestral ecological requirements after the speciation process, implying ecological niche conservatism. While suitable regions are observed throughout the continent for all three species, which suggests they could potentially colonize each other's range, they are coherent in that their suitability areas are interrupted by a non-suitable region which corresponds to the highest elevations of the Andes. This is coherent with the hypothesis that the divergence occurred as a result of the barrier imposed by the latest Andes uplift (Dias et al. 2017), and sheds light on the ecological aspects of said barrier: the highest elevations of the Andes (>2000 m) imply environmental conditions that do not allow the establishments of populations from both species, and also make the use of these regions as corridors rather unlikely. Allopatric speciation, often considered the most common geographic type of speciation (Kozak and Wiens 2006), may happen when populations are isolated by a

geographic barrier with suboptimal environmental conditions. In such cases, ecological niche conservatism can explain why said barrier was unsurmountable, since it can limit the adaption to the drastically different environmental conditions at the barrier. This is often the case of lowland species that are separated by mountains with less favorable climate (Wiens and Graham 2005; Kozak and Wiens 2006).

When dealing with cryptic species or recently diverged lineages, ecological niche modeling may provide evidence of that the lineages are evolving separately by demonstrating a break on the genetic flow either by ecological divergences or niche conservatism paired with non-suitable regions (Wiens and Graham 2005; Raxworthy et al. 2007). In the case of the insular groups, it is very evident that they were separated by the oceans from their continental sister species. In the case of the continental species, several non-suitable regions, such as the Andes, provide evidence that distributions most likely isolated from each another. This argues that these species follow separate evolutionary trajectories, which further supports their status as separate species. For instance, although *G. valens* has developed particular ecological tolerances, the climatic conditions associated with the highlands of the Andes are nonetheless outside its niche envelope, separating it from the other two continental species. *G. valens* seems to be quite differentiated in its ecological niche, as the response curves show that it occupies the most extreme PC values and also had a low overlap with the ecological niches of the rest of the species (Fig. 2). It seems that this species occupies a region with environmental conditions that is not accessible to the other continental species, which adds to the argument that it is a separately evolving lineage (Kozak and Wiens 2006).

For defining species, the best strategy is to integrate data derived from different approaches (Raxworthy et al. 2007; Rissler y Apodaca 2007; Gager et al. 2016; Morales et al. 2016). In bats, it has been demonstrated that environmental and spatial variables influence their morphological variation and genetic structure (Evin et al. 2008; Richards et al. 2012; Hernández-Canchola and León-Paniagua 2017; Morales et al. 2018). Our hypothesis that morphological convergence in the species that once formed the *G. soricina* complex could be driven by ecological similarities was not supported. While the tendency for animals to reach larger sizes when colonizing islands is well known (Foster 1964; Lomolino 2005; Millien 2006) and convergence in shape using linear measures has been reported (Losos et

al. 1998), the exploration of morphological convergence by geometric morphometrics is an area yet to be explored, especially in bats. Comparing niche and morphological evolution is not an easy task, and while some studies have found evidence that suggests that ecological requirements could be an important force shaping the morphological diversity, they also conclude that cranial shape could better be explained if other types of factors were taken into account, such as biological interactions and genetics (i.e. Soberón and Martínez-Gordillo 2012; Morales et al. 2018). We expect new studies approaching different types of data could shed light on the reasons behind the morphological convergences and differences of these Neotropical bats.

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TABLES

Table 1. Estimates of niche overlap probability in percentage, with an alpha value of 95%. The values represent the probability of the niche of the species in rows within the niche of the species in the columns.

	<i>G. antillarum</i>	<i>G. mutica (c)</i>	<i>G. mutica (i)</i>	<i>G. soricina</i>	<i>G. valens</i>
<i>G. antillarum</i>		97.33	0	98.26	15.2
<i>G. mutica (c)</i>	0.31		0	60.99	8.48
<i>G. mutica (i)</i>	0	99.92		93.81	54.57
<i>G. soricina</i>	1.56	78.67	0		8.23
<i>G. valens</i>	0.2	55.9	0	29.9	

## FIGURES

Figure 1. Map showing the distribution of the five compared groups: 1. *G. antillarum*; 2. Continental *G. mutica*; 3. Insular *G. mutica*; 4. *G. soricina*; 5. *G. valens*. Presence localities from which environmental was obtained are represented by small circles; localities from which morphological data was obtained are represented by large rhombus.

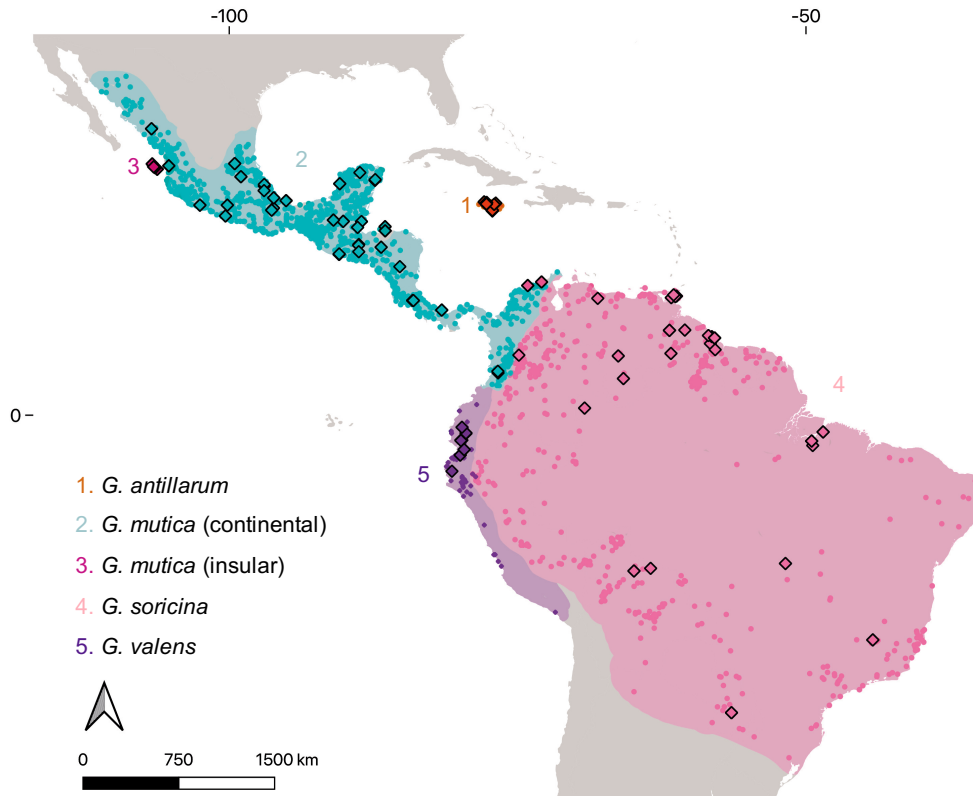


Figure 2. Response curves, ellipsoid models and scatterplots derived from NicheROVER models of ecological niches (A) and morphospace (B) for the five groups: *G. antillarum*, continental *G. mutica*, insular *G. mutica*, *G. soricina* and *G. valens*. Environmental conditions were summarized using the first five principal components of nine selected variables: Bio2 (Mean Diurnal Range), Bio3 (Isothermality), Bio8 (Mean Temperature of Wettest Quarter), Bio9 (Mean Temperature of Driest Quarter), Bio13 (Precipitation of Wettest Month), Bio14 (Precipitation of Driest Month), Bio15 (Precipitation Seasonality), Bio18 (Precipitation of Warmest Quarter) and Bio19 (Precipitation of Coldest Quarter). Morphometric data is derived from constellations of landmarks and semilandmarks in the ventral view of the skull.

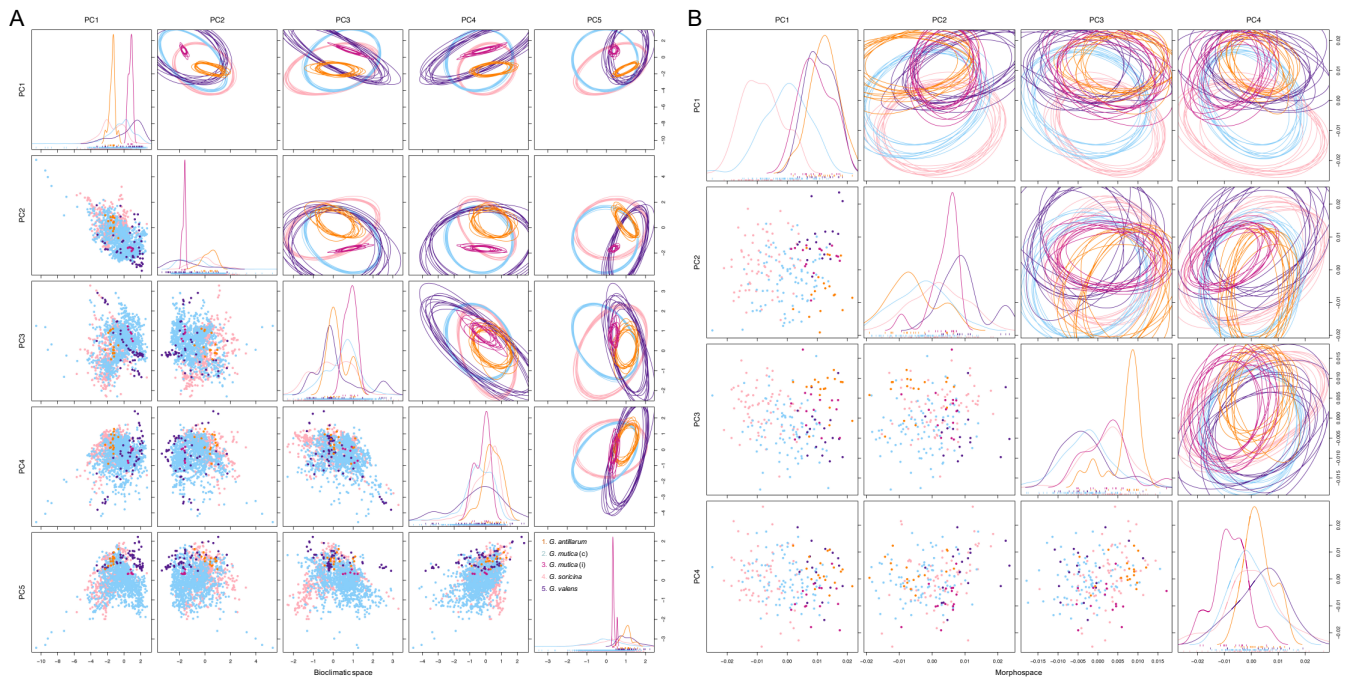


Figure 3. Deformation grid showing vector displacements of the landmarks of the configuration of an individual of *G. antillarum* (orange) and an individual of *G. soricina* (blue). The compared individuals were occupying each extreme along the first PC of the morphospace. Deformation grids were exaggerated by a factor of 3 to improve visualization.

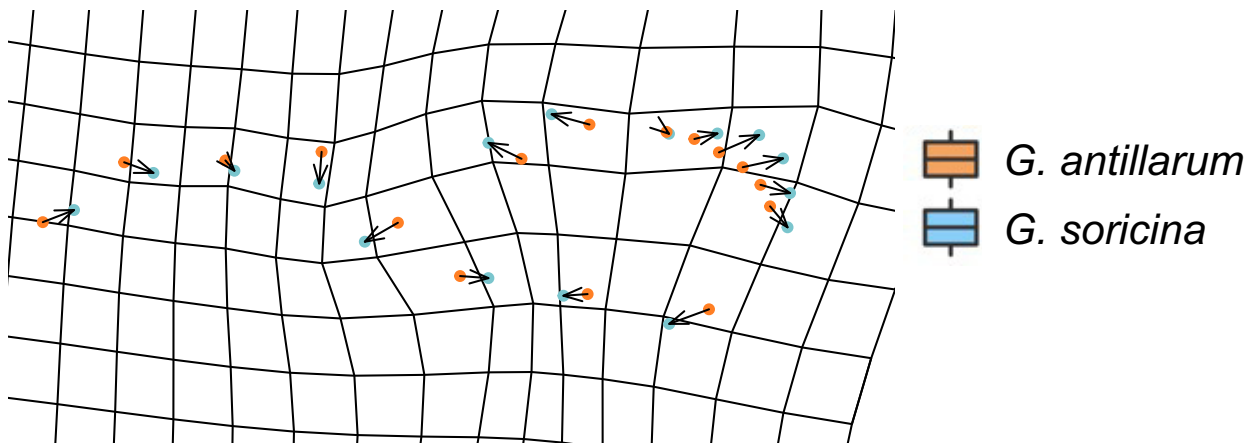


Figure 4. Boxplots for 19 bioclimatic variables for the five studied groups *G. antillarum*, continental *G. mutica*, insular *G. mutica*, *G. soricina* and *G. valens*.

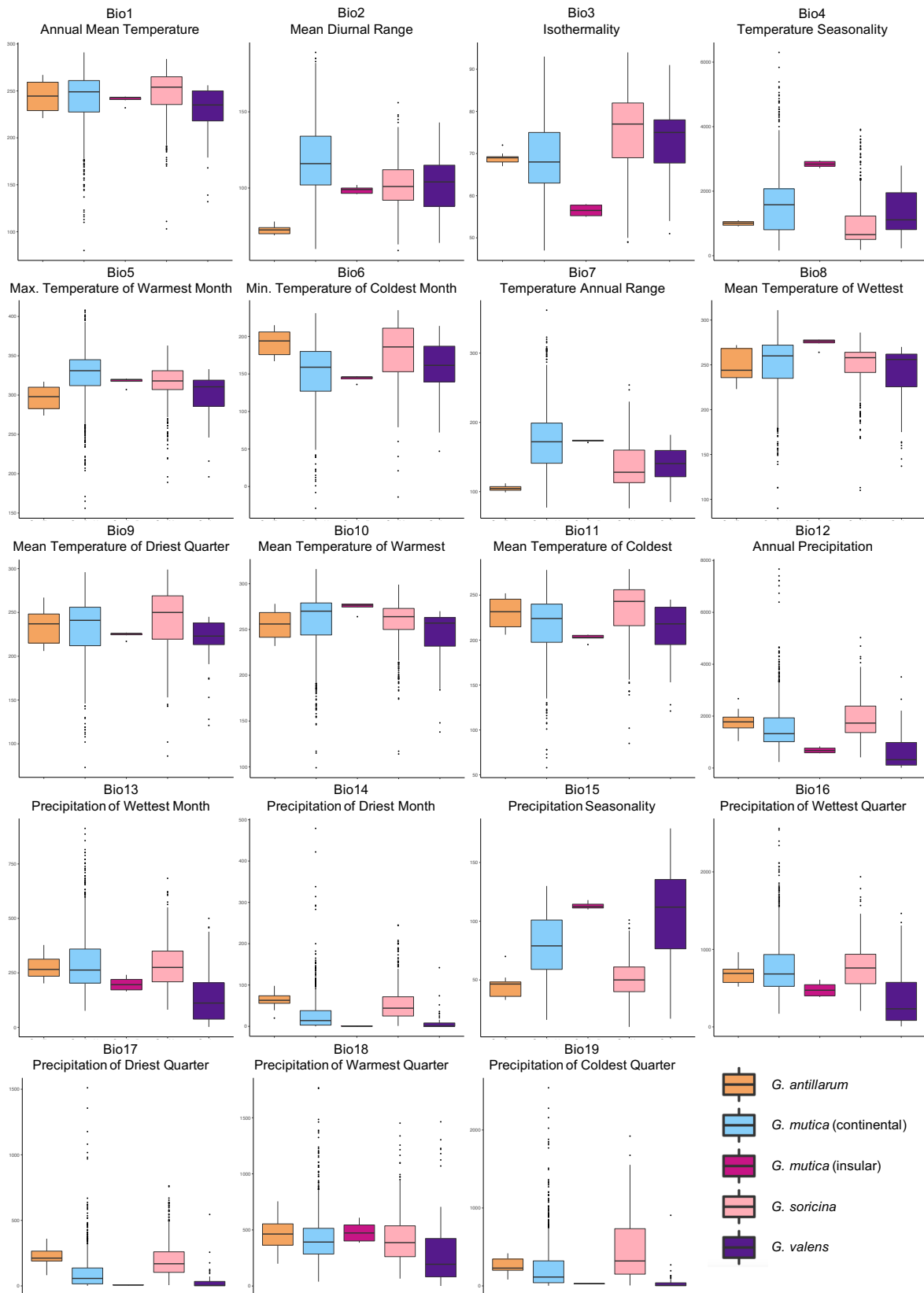


Figure 5. Ecological niche models for current potential distributions for the three continental species *G. mutica* (A), *G. soricina* (B) and *G. valens* (C). Stronger shades of blue colors indicate higher suitability values, while paler tones represent lower suitability values. Presence points are indicated as dark purple dots. Binary predictions using the 10<sup>th</sup> percentile threshold are indicated with bold black lines.

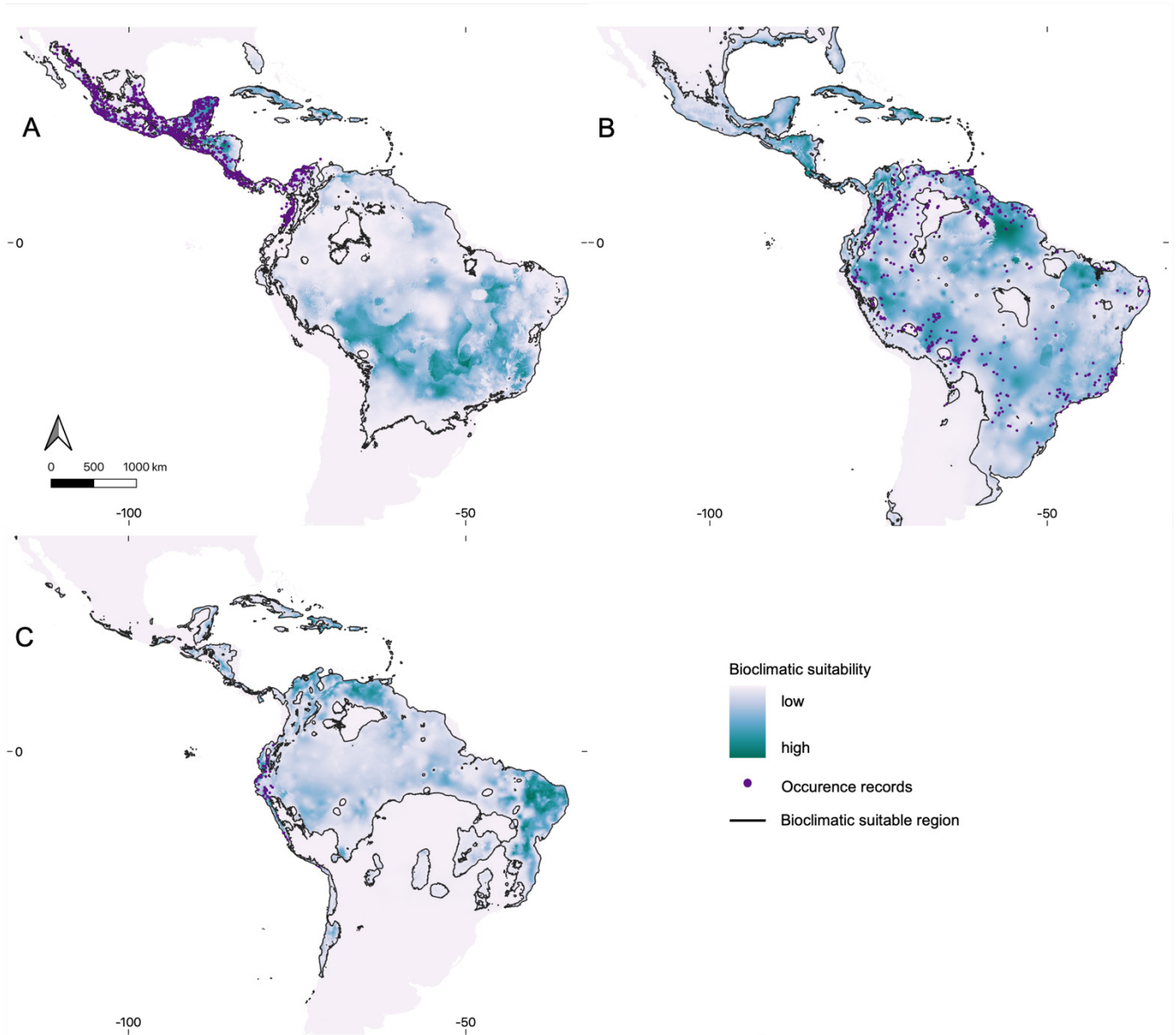
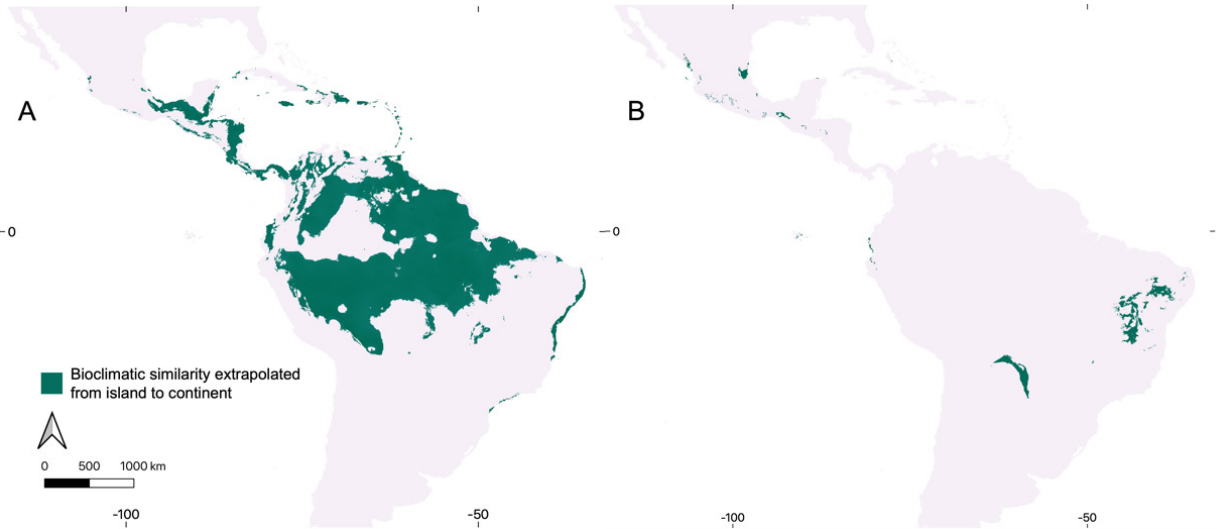


Figure 6. MOP derived maps for *G. antillarum* (A) and insular *G. mutica* (B). Potential extrapolation regions are indicated in dark green.





SUPPLEMENTARY DATA

Supplementary table 1. Loadings of bioclimatic variables to each PC. The names of the bioclimatic variables are: Bio2 = mean diurnal range; Bio3 = Isothermality; Bio8 = mean temperature of wettest quarter; Bio9 = mean temperature of driest quarter; Bio13 = precipitation of wettest month; Bio14 = precipitation of driest month; Bio15 = precipitation seasonality; Bio18 = precipitation of warmest quarter; Bio19 = precipitation of coldest quarter.

Bioclimatic Variables	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9
Bio2	0.381	-0.041	0.178	-0.226	-0.604	0.516	0.368	-0.052	0.028
Bio3	-0.334	-0.272	-0.261	-0.377	0.381	0.575	0.082	0.349	-0.021
Bio8	-0.252	-0.310	0.584	0.449	-0.171	0.198	-0.291	0.371	0.087
Bio9	-0.382	-0.349	0.076	0.272	0.086	0.108	0.397	-0.662	-0.194
Bio13	-0.410	-0.192	-0.051	-0.271	-0.313	-0.389	0.258	0.087	0.629
Bio14	-0.335	0.484	0.016	-0.095	-0.107	0.381	-0.459	-0.419	0.318
Bio15	0.210	-0.628	-0.065	-0.348	-0.119	-0.096	-0.568	-0.295	-0.056
Bio18	-0.279	0.195	0.559	-0.557	0.000	-0.212	0.033	0.015	-0.464
Bio19	-0.362	0.048	-0.485	0.133	-0.574	-0.043	-0.126	0.169	-0.488
Explained variance (%)	47.967	17.570	11.125	9.104	5.117	4.331	1.992	1.810	0.984
Cumulative proportion	47.967	65.537	76.662	85.766	90.883	95.214	97.206	99.016	100.000

Supplementary table 2. Bioclimatic variables that showed significant values when testing for differences in paired comparisons between groups. The names of the bioclimatic variables are: Bio1= annual mean temperature; Bio2 = mean diurnal range; Bio3 = Isothermality; Bio4 = temperature seasonality; Bio5 = maximum temperature of warmest month; Bio6 = minimum temperature of coldest month; Bio7 = temperature annual range; Bio8 = mean temperature of wettest quarter; Bio9 = mean temperature of driest quarter; Bio10 = mean temperature of warmest quarter; Bio11 = mean temperature of coldest quarter; Bio12 = annual precipitation; Bio13 = precipitation of wettest month; Bio14 = precipitation of driest month; Bio15 = precipitation seasonality; Bio16 = precipitation of wettest quarter; Bio17 = precipitation of driest quarter; Bio18 = precipitation of warmest quarter; Bio19 = precipitation of coldest quarter

	<i>G. antillarum</i>	<i>G. mutica (c)</i>	<i>G. mutica (i)</i>	<i>G. valens</i>
<i>G. mutica (c)</i>	Bio2, Bio5, Bio6, Bio7, Bio14, Bio15, Bio17			
<i>G. mutica (i)</i>	Bio3, Bio4, Bio7, Bio14, Bio15, Bio17	Bio3, Bio4, Bio15		
<i>G. valens</i>	Bio2, Bio6, Bio12, Bio13, Bio14, Bio15, Bio16, Bio17	Bio1, Bio2, Bio3, Bio4, Bio5, Bio7, Bio8, Bio9, Bio10, Bio12, Bio13, Bio14, Bio15, Bio16, Bio17, Bio19	Bio3, Bio4, Bio8, Bio15	
<i>G. soricina</i>	Bio2, Bio3, Bio7	Bio1, Bio2, Bio3, Bio4, Bio5, Bio6, Bio7, Bio9, Bio11, Bio12, Bio14, Bio15, Bio17, Bio19	Bio3, Bio4, Bio12, Bio14, Bio17, Bio19	Bio1, Bio5, Bio6, Bio8, Bio9, Bio10, Bio11, Bio12, Bio13, Bio14, Bio15, Bio16, Bio17, Bio19

#### DATA ACCESSIBILITY STATEMENT

All data generated or analyzed during this study are included in these published articles and its supplementary information files.

#### CONFLICT OF INTEREST AND COMPETING INTERESTS STATEMENT

The authors declare that they have no conflicts of interest or competing interest.

## DISCUSIÓN Y CONCLUSIONES GENERALES

El esclarecimiento de los complejos de especies crípticas es fundamental para lograr una estimación más correcta de la biodiversidad, para comprender los procesos evolutivos detrás de la especiación y, sobre la marcha, probar los alcances y limitantes de las herramientas que permiten este quehacer, así como para tomar decisiones concernientes a especies amenazadas y de importancia sanitaria y comercial (Bickford et al. 2007; Tsang et al. 2016). Para una adecuada resolución taxonómica, dos aspectos resultan de suma importancia. Por un lado, contar con un concepto de especie que permita establecer un umbral para delimitarlas (Johnson et al. 2004; Rissler y Apodaca 2007). Por otro lado, elegir herramientas que ayuden a detectar suficiente evidencia que permita probar la independencia evolutiva de los linajes en cuestión. Para esto último, la estrategia deseable es hacer uso de una aproximación integrativa, pues esto permitirá obtener y contrastar información proveniente de varios tipos de caracteres y tener así un panorama más completo acerca de la historia evolutiva y posición taxonómica de los grupos bajo estudio (de Queiroz 2007; Clare 2011; Srinivasulu et al. 2019).

Los murciélagos son un grupo de mamíferos con peculiaridades que hacen muy interesante su estudio en términos evolutivos, donde las especies crípticas son abundantes y cuya taxonomía es constantemente objeto de revisiones (Jones 1997; Mayer y von Helversen, 2001; Dávalos et al. 2012; Clare et al. 2013). Se estima, incluso, que la cantidad de especies dentro de este grupo podría ser hasta el doble de las reconocidas actualmente (Francis et al. 2010; Clare 2011). Es importante atender los retos taxonómicos que presenta este orden realizando un estudio cada vez más integral sobre sus relaciones e historia, con el fin de aproximarse de una manera más cada vez más completa a la realidad de su compleja historia evolutiva, y de estimar con mayor certidumbre el número de especies que componen este orden.

La taxonomía integrativa busca delimitar y describir especies utilizando múltiples perspectivas complementarias (Dayrat 2005). Dentro de esta variedad de aproximaciones, los análisis de caracteres morfológicos y ambientales han demostrado su utilidad para complementar los hallazgos moleculares en la resolución de complejos crípticos (i.e., Ruedi y Mayer, 2001; Evin et al. 2008; Sztencel-Jablonka et al. 2009; Pavlova et al. 2014; Morales

et al. 2016; Srinivasulu et al. 2019). En este trabajo, se encontró correspondencia entre las principales separaciones moleculares y los caracteres morfológicos de dichas poblaciones. Se describió por primera vez la forma de cráneo y mandíbula de estas especies, y se determinaron características útiles para la identificación de cada una de ellas. Esto se logró gracias al poder descriptivo de la morfometría geométrica, la cual tiene características que la hace particularmente útil para trabajar con grupos donde la divergencia morfológica sea tan sutil que los métodos de morfometría tradicional resulten insuficientes para detectarla, lo cual la vuelve útil para los estudios taxonómicos y filogenéticos en complejos crípticos, donde su poder descriptivo y estadístico para separar taxa crípticos y variación a nivel de especie se ha demostrado (Mayer y von Helversen 2001; Evin et al. 2008).

Por su parte, los análisis de modelado de nicho ecológico permitieron conocer los requerimientos ambientales de cada una de las especies. Se comprobó que las zonas de alta elevación, sobre todo la cordillera de los Andes, representan una barrera climática al flujo para estas especies lo cual podría dar cuenta de las razones detrás de su especiación, pues corresponde con los datos filogeográficos reportados (Dias et al. 2017). Muy posiblemente, la orografía de México y Centroamérica podría estar dando pie a la estructuración genética entre poblaciones continentales de *G. mutica* separadas por sistemas montañosos en la región en la que se distribuyen, y se reporta que dentro de éstas podrían encontrarse por lo menos dos linajes separados (Hoffmann et al. 2019). Esta especie, además, comprende una alta heterogeneidad ambiental y morfológica, por lo que serían interesantes análisis que explorasen más a fondo su variación en caracteres fenotípicos y si coincide con los clados reportados por análisis moleculares, con el objetivo de discernir si pudiera, a su vez, tratarse de más de una especie.

Resalta el caso de los grupos de las islas, que son regiones con muchos endemismos y de biodiversidad muy subestimada (Srinivasulu et al. 2019). Se concluyó que las poblaciones insulares de *G. mutica*, a pesar de su diferenciación en tamaño, forma y nicho ecológico, presentan las distancias genéticas más pequeñas al compararse con las otras especies del complejo, sin separarse del clado formado por las poblaciones continentales de esta especie, por lo cual no puede considerarse como una especie separada de ésta (Hoffmann et al. 2019). Caso contrario es el de *G. antillarum*, que presentó una diferenciación coherente en los caracteres estudiados y pudo ser elevada a nivel de especie, contribuyendo así al

conocimiento de la riqueza de especies de las Antillas. En general, al ser toda el área de estudio una región de alta riqueza de especies, que además comprende islas (León-Paniagua et al. 2007; Villalobos y Arita 2010; Srinivasulu et al. 2019), se puede esperar con certidumbre que la biodiversidad se encuentre muy subestimada. Es crucial resolver esto no sólo por interés taxonómico, sino también para poder tener una correcta estimación y poder hacer planes de manejo en esta región de tanto impacto antropogénico (Bickford et al. 2007).

Finalmente, el uso conjunto de estos tres tipos de datos permitió explorar si existe una correlación entre forma craneal y factores abióticos que pudiera estar dando cuenta de las convergencias y diferencias entre estas especies. A pesar de que los primeros análisis indicaron que los grupos insulares y *G. valens* pudiesen estar habitando regiones ambientalmente similares cuyas condiciones podrían explicar la convergencia en cráneos de mayor tamaño y más alargados, los análisis de solapamiento de nicho encontraron que las especies con convergencia morfológica presentaron menor solapamiento de nicho. Por su parte, las especies continentales, que están diferenciadas morfológicamente, presentaron los niveles más altos para esta métrica. Esto puede deberse, por un lado, a que los niveles de solapamiento están influenciados por el tamaño de las distribuciones de las especies, donde las especies continentales, al tener una distribución más extensa con mayor heterogeneidad ambiental, tienen mayores probabilidades de habitar regiones similares, mientras que en las especies de distribución restringida se daría la situación opuesta. Por otro lado, es muy probable que la forma de los cráneos en estas especies esté influenciada por factores no considerados en este análisis, como podría ser la dieta y la posición trófica que ocupan en sus comunidades. De hecho, se ha reportado que habitar regiones con mayor riqueza de especies con dietas similares están relacionadas con un menor tamaño en murciélagos (Louzada y Pessôa 2013), así como características morfológicas que les permitan explorar una mayor variedad de nichos dietéticos (Howell 1974). Tal podría ser el caso de *G. soricina*, el cual ocupa una región de alta biodiversidad y tiene el menor tamaño. El efecto en las islas es el contrario, donde el relajamiento en presiones selectivas relacionados a depredadores y competidores permite que se seleccionen tamaños más grandes (Foster 1964, Lomolino 2005, Millien 2006). Resultaría de interés explorar en el futuro si factores bióticos, como la dieta y las interacciones con otras especies, pudieran arrojar luz sobre el patrón morfológico observado en estas especies.

Los datos moleculares de análisis previos y los análisis de morfometría geométrica y de nicho ecológico llevados a cabo en este proyecto se complementaron para comprender la biología, posición taxonómica e historia evolutiva de estas especies. Se encontró que se trata de un caso de integración por acumulación (Padial et al. 2010) en la cual no hubo divergencia coherente en todos los caracteres medidos, pero que, sin embargo, fue suficiente para delimitar estas especies y llevar a una mejor comprensión de sus requerimientos abióticos y su historia evolutiva. Estas discordancias son también evidencias del proceso evolutivo detrás de este grupo: las convergencias y la estasis son productos asimismo de los mismos procesos que gobiernan la especiación divergente y las radiaciones. La ausencia (o aparente ausencia) de diferencias es también un resultado evolutivo que se puede estudiar con las mismas herramientas (Fišer et al. 2018).

La mayoría de las especies crípticas son detectadas a partir de hallazgos moleculares que encuentran divergencias importantes a nivel genético, de modo que el descubrimiento de éstas se ha potenciado con los avances en técnicas moleculares (aunque estas reclasificaciones se han hecho desde mucho antes de que se contara con estas técnicas; Derham 1718; Winker 2005). En el contexto actual, se puede esperar que, en tanto se sigan realizando este tipo de estudios, se seguirán encontrando más de estos complejos. Sin embargo, la aproximación molecular no es suficiente. Como se demostró en este trabajo, es importante sumar a estos datos moleculares evidencias que permitan probar con certidumbre la separación de los taxa, así como caracterizarlos. Aunado a esto, resulta de suma importancia nombrar y describir las especies halladas a partir de diferencias genéticas. La taxonomía integrativa permite acumular distintos tipos de datos sobre ellas, y sólo nombrándolas será posible que todos estos datos puedan ser agrupados y asociados bajo una unidad taxonómica explícita (Wiens 2004; Herdina et al. 2014; Pante et al. 2015).

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