

# Morphological and ecological data confirm *Reithrodontomys cherrii* as a distinct species from *Reithrodontomys mexicanus*

DAILY MARTÍNEZ-BORREGO<sup>1\*</sup>, ELIZABETH ARELLANO<sup>1</sup>, DARYL D. CRUZ<sup>1</sup>, FRANCISCO X. GONZÁLEZ-CÓZATL<sup>1</sup>, ELIZABETH NAVA-GARCÍA<sup>2</sup>, AND DUKE S. ROGERS<sup>3</sup>

<sup>1</sup> Centro de Investigación en Biodiversidad y Conservación, Universidad Autónoma del Estado de Morelos. Avenida Universidad 1001, C. P. 62209, Cuernavaca. Morelos, México. Email: [daily.marbo@gmail.com](mailto:daily.marbo@gmail.com) (DM-B), [elisabet@uaem.mx](mailto:elisabet@uaem.mx) (EA), [daryldavidcf@gmail.com](mailto:daryldavidcf@gmail.com) (DDC), [xavier@uaem.mx](mailto:xavier@uaem.mx) (FXG-C).

<sup>2</sup> Facultad de Ciencias Biológicas, Universidad Autónoma del Estado de Morelos. Avenida Universidad 1001, C. P. 62209, Cuernavaca. Morelos, México. Email: [elizabeth.nava@uaem.mx](mailto:elizabeth.nava@uaem.mx) (EN-G).

<sup>3</sup> M. L. Bean Life Science Museum and Department of Biology, Brigham Young University 84602, Provo. Utah, USA. Email: [duke\\_rogers@byu.edu](mailto:duke_rogers@byu.edu) (DSR).

\*Corresponding author

The integrative taxonomy approach has recently been widely suggested in systematic studies. Lines of evidence such as the geometric morphometrics and ecological analyses have been useful for discriminating between genetically well-differentiated species. Within the genus *Reithrodontomys*, *R. mexicanus* is one of the more taxonomically complex species, being considered a cryptic species complex. *R. cherrii* was considered a subspecies of *R. mexicanus*, until molecular evidence raised it to the species-level. Herein, we evaluate these two forms using morphological and ecological data based on the premise that they constitute genetically differentiated species. We carried out geometric morphometric analyses on dorsal and ventral views of the skull. Landmark and semi-landmark configurations for both views of the skull were selected based on previous studies of cricetid rodents. We tested the presence of sexual dimorphism, and the skull shape and size differences between species on both cranial views. Additionally, we characterized the environmental space of each species habitat using bioclimatic variables, elevation, and the Normalized Difference Vegetation Index (NDVI). Females and males of *R. mexicanus* and *R. cherrii* did not show sexual dimorphism in shape or size of both skull views. We found significant differences between the two species in both shape and size of the skull. Cranial structures of the ventral view were more useful to differentiate both species. *R. mexicanus* exhibited a broader environmental space than *R. cherrii*, with relatively similar values of temperature and elevation, but not of precipitation. The pairwise comparison showed significant differences in the majority of the environmental variables analyzed. Although for each view, we found statistical differences in the skull shape of *R. cherrii* and *R. mexicanus*, the ventral side showed major resolutive power differentiating both species. Our findings suggest that *R. cherrii* tends to have a larger skull than *R. mexicanus*. However, the morphological and pelage coloration similarity between these species reported in the past, could explain the previous inclusion of *R. cherrii* as a subspecies of *R. mexicanus*. *R. mexicanus* occurs in a variety of vegetation-types coinciding with the broader environmental space that it occupies compared to that of *R. cherrii*. The natural areas where both species are distributed were associated with high NDVI values. Our results complement the molecular evidence and, under an integrative taxonomy approach, support *R. cherrii* as a different species from *R. mexicanus*.

Recientemente, el enfoque de taxonomía integrativa ha sido ampliamente sugerido en estudios sistemáticos. Líneas de evidencia como la morfometría geométrica y los análisis ecológicos han sido útiles para discriminar entre especies genéticamente bien diferenciadas. Dentro del género *Reithrodontomys*, *R. mexicanus* es una de las especies más complejas taxonómicamente, siendo considerada un complejo de especies crípticas. *R. cherrii* se consideró una subespecie de *R. mexicanus*, hasta que la evidencia molecular la elevó al nivel de especie. En este estudio, evaluamos las diferencias entre estas dos formas utilizando datos morfológicos y ecológicos, basados en la premisa de que constituyen especies genéticamente diferenciadas. Se realizaron análisis de morfometría geométrica para las vistas dorsal y ventral del cráneo. Las configuraciones de marcas y semimarcas para ambas vistas fueron seleccionadas utilizando estudios previos en roedores cricétidos. La presencia de dimorfismo sexual y las diferencias en la forma y tamaño del cráneo entre las especies se evaluaron en ambas vistas del cráneo. Asimismo, se caracterizó y comparó el espacio ambiental que cada especie ocupa utilizando variables bioclimáticas, la elevación y el Índice de Vegetación de Diferencia Normalizada (NDVI). Hembras y machos de *R. mexicanus* y *R. cherrii* no mostraron dimorfismo sexual para la forma y el tamaño en ambas vistas del cráneo. Se encontraron diferencias significativas entre las especies para la forma y tamaño del cráneo. Las estructuras craneales de la vista ventral resultaron más útiles para diferenciar ambas especies. *R. mexicanus* exhibió un espacio ambiental más amplio que *R. cherrii*, con valores relativamente similares de temperatura y elevación, pero no de precipitación. La comparación por pares mostró diferencias significativas en la mayoría de las variables ambientales. Aunque para cada vista, encontramos diferencias estadísticas en la forma del cráneo de *R. cherrii* y *R. mexicanus*, el lado ventral mostró mayor poder resolutive diferenciando ambas especies. Nuestros resultados sugieren que *R. cherrii* tiende a tener el cráneo de mayor tamaño que *R. mexicanus*. No obstante, la gran similitud morfológica y de coloración del pelaje reportada entre estas especies en el pasado, pudiera explicar la previa inclusión de *R. cherrii* como una subespecie de *R. mexicanus*. *R. mexicanus* ocurre en distintos tipos de vegetación, coincidiendo con el espacio ambiental más amplio que ocupa en comparación con el de *R. cherrii*. Las áreas naturales donde se distribuyen ambas especies mostraron asociación con altos valores de NDVI. Nuestros resultados complementan la evidencia molecular y, con un enfoque de taxonomía integrativa, confirman a *R. cherrii* como una especie diferente de *R. mexicanus*.

**Keywords:** Cricetidae; cryptic species; environmental space; harvest mice; integrative taxonomy; skull morphometry.

## Introduction

The morphological species concept has been used historically to describe new taxa (Mayden 1997; Mayr 2000). In fact, the majority of species that we know today were delimited and/or assigned to different taxonomic categories based on their morphological characteristics (Seifert 2014). However, establishing species boundaries in some instances is problematic (Mayr 2000). For example, for cryptic species, which are genetically different entities that do not show distinctive morphological characteristics (Struck et al. 2018). In these cases, species concepts with different criteria have been proposed, most of them based on the use of a single operational criterion to describe new species (Sites and Marshall 2003, 2004). In contrast, the General Lineage concept (de Queiroz 1998, 2007) employs multiple lines of evidence (genetic, morphological, ecological, ethological, etc.) to propose species as a separately evolving metapopulation lineage. This approach implements the use of an integrative taxonomy, which has been widely suggested in systematic studies (Dayrat 2005; Alström et al. 2008; Sangster 2018).

Recently, Leaché et al. (2009) argued that the use of morphological and ecological data, in addition to molecular data, allows better discrimination among species. Geometric morphometric analysis is a robust tool to highlight interspecific variation in zoological groups, such as mammals, corroborating the phylogenetic relationships within them (e. g., Bogdanowicz et al. 2005; Camul and Polly 2005; Pavan and Marroig 2016). In addition, ecological niche studies are being increasingly used for these same purposes (e. g., Rissler and Apodaca 2007; Rivera et al. 2018; Zhao et al. 2019), and for making inferences related to evolutionary questions of both historical distributions and speciation processes (Graham et al. 2004).

The genus *Reithrodontomys* (Cricetidae, Rodentia) constitutes a taxon in which species were originally described based on pelage coloration (Allen 1895), and morphological characteristics of the skull and dentition (Merriam 1901; Hooper 1952). However, variation in these characters overlap among populations of different species (Hooper 1952), making taxonomic recognition difficult and leading to misidentifications in many cases.

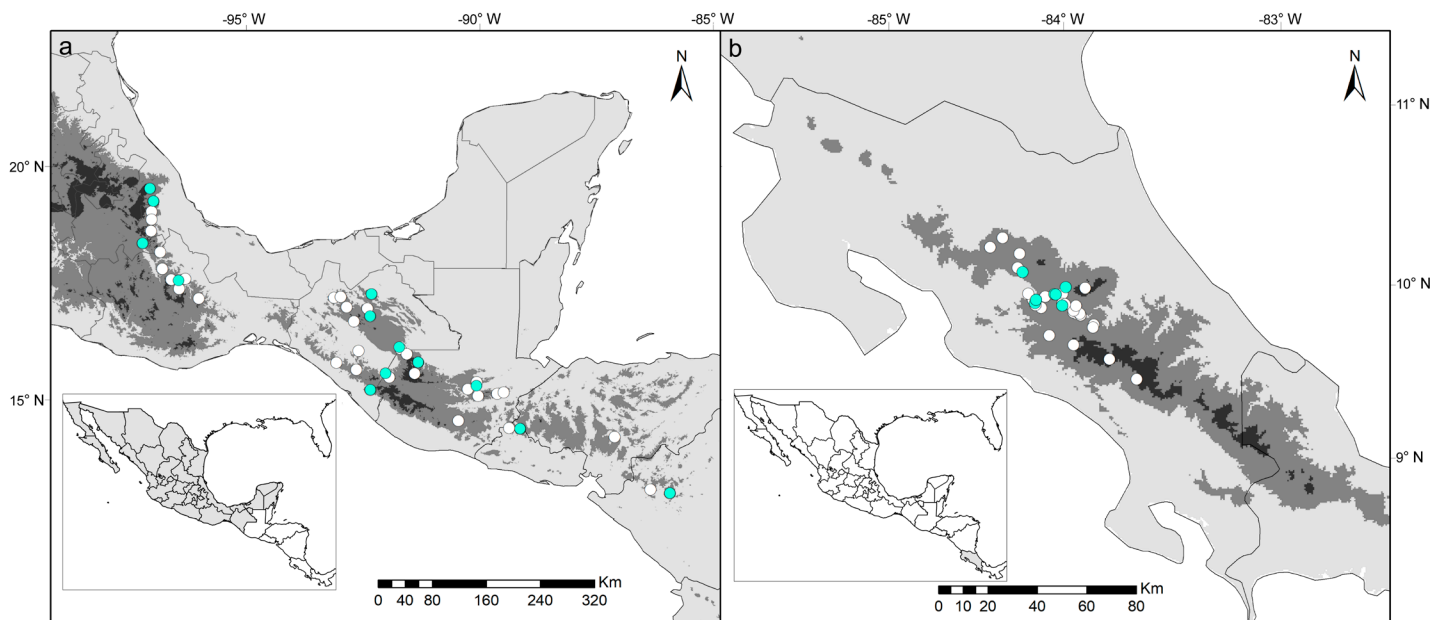
*Reithrodontomys mexicanus* (Saussure, 1860) is one of the most taxonomically complex species within the genus, nowadays considered as a complex of cryptic species (Arellano et al. 2003, 2005; Miller and Engstrom 2008). Hooper (1952, 1959) recognized 13 subspecies, but currently only 10 remain valid (Bradley 2017). One of the species that has been subject to taxonomic changes is *R. cherrii* (Allen, 1891), restricted to some highland localities in central Costa Rica (Hooper 1952; Hall 1981). Howell (1914) relegated *R. cherrii* as a subspecies of *R. mexicanus*, without a clear justification for this nomenclatural change. Almost a century later, Arellano et al. (2005) raised *R. cherrii* back to the species level based on a molecular phylogenetic analysis of the mitochondrial cytochrome b gene.

Specimens of *R. cherrii* were grouped into a genetically well-differentiated clade from *R. mexicanus* (recognized herein as *R. mexicanus* "classic" clade), with a genetic distance value (using the K2P evolutionary model) greater than 12 % (Arellano et al. 2006). Also, the species status of *R. cherrii* was recognized by Gardner and Carleton (2009) using traditional morphometrics and pelage coloration. However, it is desirable to complement the taxonomic distinction between *R. cherrii* and *R. mexicanus* with a larger sampling and under an integrative taxonomy approach employing alternative data sources such as geometric morphometrics and ecological attributes. Therefore, the goal of this study is to compare both species using cranial features and environmental characteristics of their habitats, based on the premise that they constitute genetically well-differentiated species.

## Materials and methods

**Geometric morphometrics data.** We examined 47 skulls of adult individuals (M3 erupted, age classes following Arellano et al. 2012) from different museum collections (Appendix 1), and each specimen was photographed in the dorsal and ventral views of the skull. We based the selection of specimens; *R. mexicanus* ( $n = 28$ ) and *R. cherrii* ( $n = 19$ ); and their localities (Figure 1; Appendix 1), on the *R. mexicanus* "classic" and *R. cherrii* clades obtained in the phylogeny reported by Arellano et al. (2005). The specimens used in Arellano et al. (2005), but not available for morphometric analysis, were replaced by individuals from the same localities or within a radius less than 60 km. Digital images of skulls were taken using an Olympus DP73 Digital Camera coupled to an optical microscope, and to the computer through the CellSens program. The skulls were positioned on a black background, always keeping the same distance from the camera lens, and using a millimeter rule as a scale bar.

Landmark and semi-landmark configurations for both views were selected based on previous studies of cricetid rodents (e. g., Martínez and Di Cola 2011). Configurations were digitized assuming positional homology among individuals (Zelditch et al. 2004), with the TPSdig 2.31 program (Rohlf 2015). We used 17 landmarks, 13 semi-landmarks for the ventral view and 12 landmarks, 23 semi-landmarks for the dorsal view (Figure 2; Appendix 2). We aligned, rotated, and scaled all landmarks and semi-landmarks configurations using a Generalized Procrustes analysis (GPA; Rohlf and Slice 1990) implemented in the package geomorph 3.3.1 (Adams and Otárola-Castillo 2013) from R library (R Core Team 2018). During this processing, semi-landmarks position on the curved structures were allowed to slide along their tangent vectors until reaching the minimum point of bending energy (Bookstein 1997; Zelditch et al. 2004). Shape variables (Procrustes distances and Procrustes coordinates) and centroid size (CZ) were obtained from the GPA. The CZ is related to skull size and computed as the square root of the sum of the squared distances between each landmark and the configuration centroid (Bookstein 1991).



**Figure 1.** Map showing localities of the specimens used in this study. (a) *Reithrodontomys mexicanus* from Mexico and Central America and (b) *R. cherrii* from Costa Rica. Localities for geometric morphometric analysis are highlight in blue dots. Gray tones depict elevation gradient: light gray < 1,000 m, gray 1,000 to 2,500 m; and dark gray > 2,500 m.

**Sexual dimorphism and allometry.** We evaluated the sexual dimorphism in shape and size of the skull within species for each view. A Procrustes ANOVA (Goodall 1991; Anderson 2001) was performed for skull shape using the function ProcD.lm, and a factorial design with shape as the dependent variable, sex as the main factor, and CZ as a covariate. Significant differences ( $P \leq 0.05$ ) in skull size between sex were tested using the function lm.rpp in the R package RRPP (Collyer and Adams 2018). The allometric component (possible effect of the skull size on the shape variation) was analyzed using a Procrustes ANOVA and the same factorial design previously declared. Analyses of sexual dimorphism (shape) and allometry were carried out in geomorph 3.3.1.

**Skull shape and size differences.** Principal component analysis (PCA) on the Procrustes coordinates were performed for each view of the skull in geomorph 3.3.1. Then, we used the first two components to visualize the ordering of the data according to the skull morphometric variation, and the extreme variation (minimum and maximum) of the shape along component 1 were represented using deformation grids.

We determined shape differences between species for each skull view using a Procrustes ANOVA in geomorph 3.3.1. A factorial design was used with shape as the dependent variable, species as the main factor, and CZ as a covariate. The mean skull shape differences between species were quantified (Procrustes distances) and tested for its statistical significance ( $P \leq 0.05$ ; resampling = 1000), using a pairwise permutation test (function permudist) in the R package Morpho 2.4 (Schlager 2016).

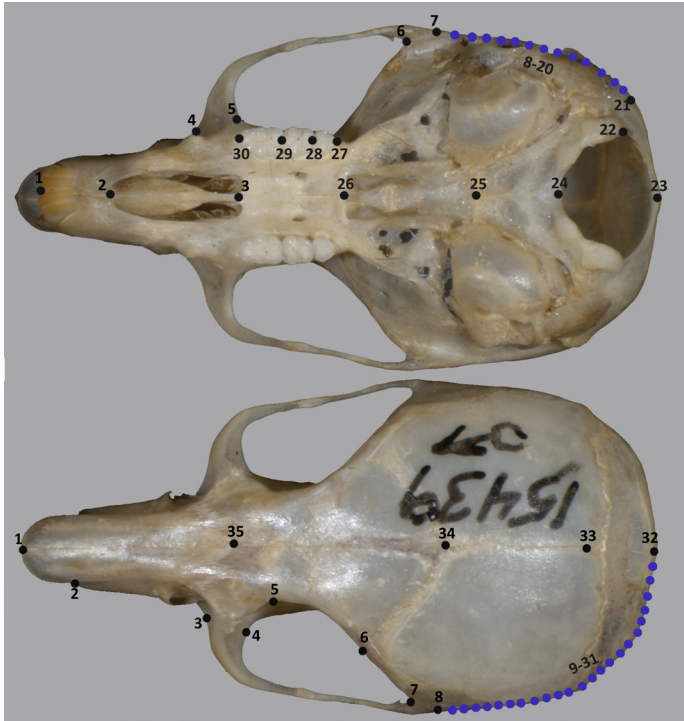
We employed a similar approach to compare skull shape between species for skull size. This analysis was developed for each view with the function lm.rpp of the RRPP pack-

age. The factorial design consisted of CZ as the dependent variable, species as the main factor, and shape as a covariate. We visualized the results of these analyses using the shiny application Extended Boxplot Graphics (Ramirez-Arrieta et al. 2020).

**Ecological niche data.** For the ecological analysis, we included the occurrences localities for the specimens used in the morphometric analysis. Additional localities were incorporated to represent the largest number of sites reported for both species (Figure 1; Appendix 3). Occurrence records were obtained from museums databases or downloaded from the VertNet database (<http://portal.vertnet.org>). Geographic coordinates were rectified against the known distribution of *R. cherrii* and *R. mexicanus* (Hooper 1952; Hall 1981), to reduce georeferencing errors.

We characterize the species ecological niche using six bioclimatic variables from Worldclim 2.0 (Fick and Hijmans 2017; <http://www.worldclim.org>): BIO1 = Annual Mean Temperature, BIO5 = Max Temperature of Warmest Month, BIO6 = Min Temperature of Coldest Month, BIO12 = Annual Precipitation, BIO13 = Precipitation of Wettest Month, and BIO14 = Precipitation of Driest Month, with a spatial resolution of  $\sim 1 \text{ km}^2$ . The selection of variables was based on previous studies that highlight their importance for small mammals ecological analyses (Santos et al. 2017; Guevara et al. 2018; Stanchak and Santana 2018). Because the distribution of small mammals may also depend on elevation gradient and vegetation quality (Patterson et al. 1989; McCain 2005; Umetsu and Pardini 2007), we also used elevation as a topographic variable derived from a digital elevation model (data available at <http://www.worldclim.org>), and the Normalized Vegetation Difference Index (NDVI; Pet-torelli et al. 2005). The NDVI layer was obtained from the





**Figure 2.** Landmarks (black dots) and semi-landmarks (blue dots) digitized in ventral and dorsal views of the skull. Anatomical positions of landmarks described in Appendix 2. The specimen voucher (BYU 15439) is a male of *Reithrodontomys mexicanus*.

Climate Engine Platform available at <http://ClimateEngine.org> (Huntington et al. 2017), which represents the average conditions of this index in the last five years.

**Ecological niche differences.** Ecological niche characteristics of *R. cherrii* and *R. mexicanus* were summarized using the predictor variables described above (bioclimatic, elevation, and NDVI). To determinate the environmental ranges of each species, we calculated descriptive statistics (Mean, Standard Error, Maximum and Minimum values, Confidence Intervals constructed from the percentile method, Standard Deviation, and Coefficient of Variation) using information extracted from the occurrence records.

With the predictor variables, we generated an environmental background using ArcGIS 10.8 (ESRI 2020), which included the information extracted from 10,000 random points within a buffer of 100 km<sup>2</sup>, around each occurrence record. This buffer selection allows to consider regions with suitable environmental conditions for the species and to minimize the inclusion of areas where they would not be found due to the presence of physical barriers or biotic interactions (Kubiak et al. 2017).

The environmental background and the occurrence data were used to visualize the species ecological niche in a three-dimensional space constructed from the first three principal components (which explained over 95 % of the variance in the data). For this, the minimum-volume ellipsoid (Van Aelst and Rousseeuw 2009) and convex polyhedron (Soberón and Nakamura 2009) were displayed as a representation of the fundamental and

realized niche (Qiao et al. 2016) for each species, respectively. Analyses were performed using NicheA (Qiao et al. 2016), a software for exploring and analyzing the environmental and geographic spaces of virtual and real species. Niche similarity between species was quantified in NicheA, based on Jaccard index (Jaccard 1912), and using the minimum-volume ellipsoid calculation method. This index based on the superposition of ellipsoids in the environmental space, captures the fundamental niche rather than the realized.

Additionally, we performed PCA on the environmental data of the occurrence records, to determine whether the ecological niches were significantly different ( $P \leq 0.05$ ) between species, using the first two principal components (which explained 99.7 % of the total variance). Finally, we performed a Mann-Whitney U test for each variable in the program Statistica 8.0 (STATSOFT 2007), to know which of them contributed to the environmental niche differentiation between *R. mexicanus* and *R. cherrii*.

## Results

**Sexual dimorphism and allometry.** Females and males of *R. mexicanus* and *R. cherrii* did not show sexual dimorphism in shape or size of the skull for both ventral and dorsal views (Table 1). Consequently, we did not distinguish between sexes to perform the morphometric analyses. Likewise, none of the species demonstrated significant allometry between shape or size of any skull view, therefore, we used the Procrustes distances matrix in our analyses of interspecific comparison.

**Table 1.** Results of sexual dimorphism and allometry analyses in *Reithrodontomys mexicanus* and *R. cherrii*, using shape and size (centroid size) of the skull from ventral and dorsal views.

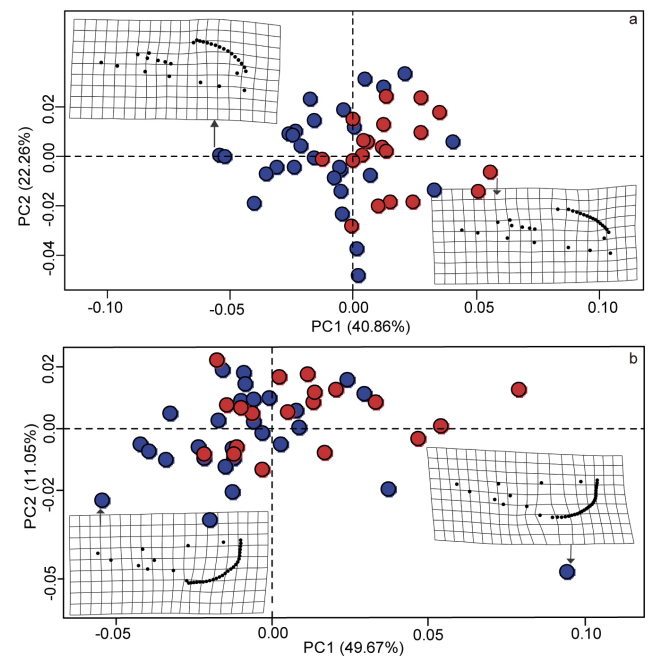
	SS	MS	R <sup>2</sup>	F	Z	P
<i>A. Reithrodontomys mexicanus</i>						
Ventral view						
Shape-Sex	0.002	0.002	0.051	1.416	0.870	0.208
Centroid size-Sex	2.856	2.856	0.028	0.764	0.400	0.404
Shape-Centroid size	0.002	0.002	0.048	1.319	0.809	0.207
Dorsal view						
Shape-Sex	0.002	0.002	0.054	1.554	0.985	0.162
Centroid size-Sex	8.509	8.509	0.064	1.779	0.805	0.207
Shape-Centroid size	0.003	0.003	0.079	2.272	1.556	0.078
<i>B. Reithrodontomys cherrii</i>						
Ventral view						
Shape-Sex	0.002	0.002	0.091	2.017	1.569	0.067
Centroid size-Sex	0.165	0.165	0.012	0.210	0.168	0.651
Shape-Centroid size	0.002	0.002	0.092	2.029	1.448	0.084
Dorsal view						
Shape-Sex	0.003	0.003	0.103	1.949	1.288	0.106
Centroid size-Sex	2.310	2.310	0.133	2.612	0.924	0.157
Shape-Centroid size	0.002	0.002	0.063	1.196	0.531	0.283

SS = sum of squares; MS = means squares; Z and P values based on 1000 permutations

**Skull shape and size differences.** For the ventral view of the skull, the first principal component (PC1) separated *R. cherrii* from *R. mexicanus* almost completely, which together with the second component (PC2) explained 63.12 % of shape variation (Figure 3a). For the dorsal view, the first two principal components overlapped in the skull shape for both species (Figure 3b). The PC1 segregated the two species better than the PC2, explaining 49.67 % and 11.05 % of the skull shape variation, respectively.

Procrustes ANOVA analyses revealed differences between *R. mexicanus* and *R. cherrii* for the ventral ( $F_{1,46} = 6.42$ ;  $P \leq 0.01$ ) and dorsal ( $F_{1,46} = 3.19$ ;  $P \leq 0.01$ ) views of the skull shape. Differences between mean shape for the ventral view were found mainly in the posterior region of the cranium (Figure 4). *R. mexicanus* displayed an expansion of the landmarks of the foramen magnum, and a relatively broader braincase than *R. cherrii*. In the dorsal view, the most notable differences were located on the anterior and middle region of the cranium (Figure 4). *R. cherrii* exhibited a relatively longer nasal bones, a broader configuration between the 3-4-5 landmarks that described the zygomatic plate and the interorbital region, and a relatively broader parietal bone. Skull size differences between species (Figure 5) were highly significant for the ventral view ( $F_{1,46} = 17.01$ ;  $P \leq 0.01$ ), and significant for the dorsal view ( $F_{1,46} = 4.50$ ;  $P \leq 0.05$ ).

**Ecological niche differences.** Most variables that characterized the ecological niche of each species were relatively distinct in mean and ranges (Table 2). The environmental space of *R. mexicanus* had ranges with higher values of temperature for the warmest and coldest months, although the



**Figure 3.** Principal component analysis of ventral (a) and dorsal (b) views of the skull. Red dots = *Reithrodontomys mexicanus* and blue dots = *R. cherrii*. Deformation grids represent the minimum and maximum variation in skull shape along the first component.

mean annual temperature was similar for both species. With respect to precipitation, the environmental space of both species showed similar means for the driest month, but not for the wettest month. Annual precipitation values were notably different, the environmental niche of *R. cherrii* exhibited a higher mean value than that reported for the geographic region where *R. mexicanus* is distributed. The

**Table 2.** Descriptive statistics used to summarize the ecological niche characteristics of *Reithrodontomys mexicanus* and *R. cherrii* based on the environmental information from occurrence records.

	Environmental Variables	Mean $\pm$ SE	Min - Max	CI	SD	CV
<i>R. mexicanus</i>	Annual Mean Temperature	17.87 $\pm$ 0.53	10.44 - 25.43	12.36 - 21.57	3.61	20.26
	Max Temperature of Warmest Month	25.94 $\pm$ 0.62	18.80 - 36.00	19.50 - 31.30	4.21	16.25
	Min Temperature of Coldest Month	9.58 $\pm$ 0.50	1 - 18.90	5.50 - 13.20	3.42	35.68
	Annual Precipitation	1906.39 $\pm$ 128.08	482 - 4875	1145 - 2943	868.68	45.57
	Precipitation of Wettest Month	346.09 $\pm$ 22.38	103 - 886	223 - 546	151.82	43.87
	Precipitation of Driest Month	34.80 $\pm$ 4.45	2 - 144	6 - 68	30.16	86.85
	Elevation	1696.91 $\pm$ 82.87	438 - 3083	997 - 2434	562.09	33.12
<i>R. cherrii</i>	NDVI	0.77 $\pm$ 0.02	0.34 - 0.90	0.58 - 0.87	0.12	15.90
	Annual Mean Temperature	17.88 $\pm$ 0.56	8.41 - 22.03	12.51 - 20.42	3.28	18.36
	Max Temperature of Warmest Month	24.35 $\pm$ 0.62	14.1 - 29	18.10 - 27.20	3.61	14.85
	Min Temperature of Coldest Month	11.92 $\pm$ 0.56	2.3 - 16.3	6.70 - 14.60	3.28	27.55
	Annual Precipitation	2721.65 $\pm$ 90.67	1961 - 4052	2197 - 3562	528.75	19.43
	Precipitation of Wettest Month	426.48 $\pm$ 11.73	317 - 592	340 - 520	68.42	16.04
	Precipitation of Driest Month	46.29 $\pm$ 5.50	10 - 131	10 - 100	32.07	69.29
Elevation	1629.76 $\pm$ 96.25	900 - 3297	1170 - 2573	561.28	34.43	
NDVI	0.67 $\pm$ 0.03	0.23 - 0.87	0.38 - 0.83	0.17	25.87	

SE = Standard Error, Min = Minimum, Max = Maximum, CI = Confidence Intervals (Percentile method), SE = Standard Deviation, CV = Coefficient of Variation

elevation gradient occupied by *R. cherrii* ranged from 900 to almost 3,300 masl, whereas in *R. mexicanus* it ranged from over 400 to around 3,000 masl. Distribution areas of *R. mexicanus* presented NDVI ranges slightly higher than those of *R. cherrii*, although in both species the NDVI means were above 0.6.

The ecological niche visualization using the first three components showed that *R. mexicanus* occurs in a broader environmental space than *R. cherrii* (Figure 6a). The minimum-volume ellipsoids displayed a partial overlap of the environmental conditions of each species, with a low niche similarity of Jaccard index (0.08). The statistical comparison of the principal component scores showed significant differences ( $U = 313$ ;  $P = 0.02$ ) between the environmental niche of each species for the first component, while no differences were found ( $U = 458$ ;  $P = 0.25$ ) for the second component (Figure 6b, 6c). In the pairwise comparisons, all variables were statistically different, except for BIO1, BIO14, and elevation (Figure 7).

## Discussion

Geometric morphometrics has shown great applicability in mammal taxonomic studies, mainly for anatomical structures such as the cranium, dentition, or mandibles (e. g., [Cordeiro-Estrela et al. 2008](#); [Barčiová 2009](#); [Kryštufek et al. 2021](#)). As part of an integrative taxonomy, this morphological tool has allowed researchers to corroborate hypotheses derived from phylogenetic studies and to establish species boundaries ([Camul and Polly 2005](#); [Pavan and Marroig 2016](#)). This taxonomic approach also includes other lines of evidence, such as ecological data ([Dayrat 2005](#)). In particular, the environmental niche characteristics can be useful to delimit cryptic species or phylogenetically related groups, especially when their relationships are known, something that has been confirmed in several rodent species ([Martínez-Gordillo et al. 2010](#)). This could be the case of the harvest mice *R. mexicanus* and *R. cherrii*, for which genetic differences are well established, but lack modern morphometrics and ecological data. In this study, we tested the premise that differences in the skull and the environmental characteristics would be consistent with the genetic divergence documented between these species ([Arellano et al. 2003, 2005, 2006](#)).

Our findings of sexual non-dimorphism in both species coincide with that previously described for *Reithrodontomys* ([Hooper 1952](#)). In *R. mexicanus*, the absence of sexual dimorphism was reported by [Arellano et al. \(2012\)](#) analyzing the morphometric variation in a population from Sierra Juárez, Oaxaca. Whereas for *R. cherrii*, here we report for the first time sexual non-dimorphism for cranial characteristics.

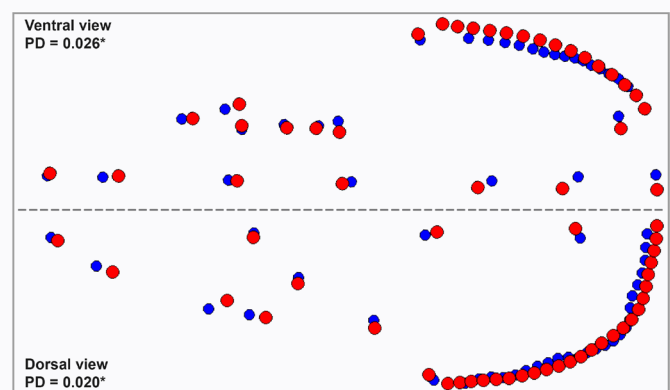
The PCA revealed some overlap in the skull shape for both ventral and dorsal views. However, the cranial structures on the ventral view were more useful to differentiate *R. cherrii* from *R. mexicanus*. The greater discriminative power of the ventral side versus the dorsal of the skull has been noted for other cricetid rodents ([Martínez and](#)

[Di Cola 2011](#)). Particularly for *Reithrodontomys*, [Mayares \(2012\)](#) evaluated the morphometric variation in the skull ventral view among *R. sumichrasti* populations distributed on both sides of the Isthmus of Tehuantepec in Mexico and correctly differentiated populations grouped according to genetic clades suggested as different species by [Hardy et al. \(2013\)](#).

Despite the overlap in skull shape, we found statistically significant differences between *R. cherrii* and *R. mexicanus* for ventral and dorsal views. [Gardner and Carleton \(2009\)](#) used craniodental measurements to compare different *Reithrodontomys* taxa distributed in Costa Rica and Panama, including *R. cherrii*, *R. m. garichensis*, and *R. m. potrero-grandei*. The skull of *R. cherrii* was differentiated by “its overall robust size and evenly arched dorsal profile, especially over the braincase and occiput; it is notably broad across the braincase and zygomata but has a proportionally shorter rostrum” ([Gardner and Carleton 2009:167](#)). These characteristics partially differ from our results, since *R. cherrii* has a narrower foramen magnum region and braincase than *R. mexicanus*, while the nasal bone was relatively longer. [Hooper \(1952\)](#) compared specimens of *R. cherrii* from central Costa Rica to *R. m. lucifrons* central Honduras. [Hooper \(1952\)](#) described *R. m. lucifrons* as possessing a rostrum and incisive foramen that were slightly smaller than that of *R. cherrii*, consistent with the differences we documented for the ventral view of the skull.

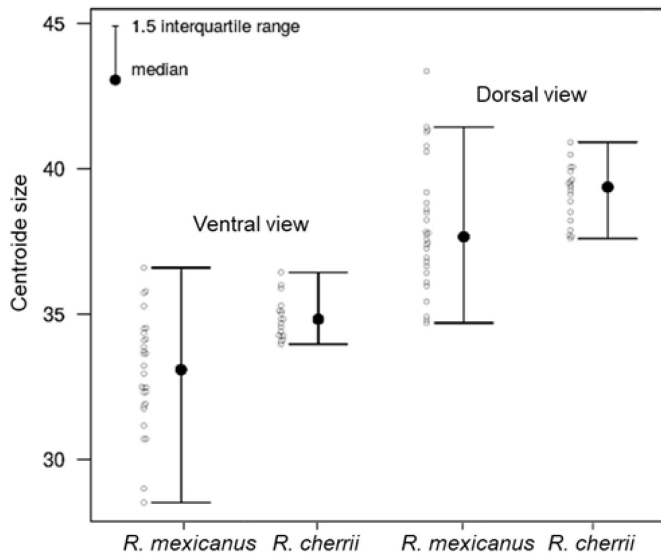
The differences in size (CS) for both views of the skull showed that *R. cherrii* tends to have a larger cranium than *R. mexicanus*. These results are consistent with the linear measurements given by [Hooper \(1952\)](#) and traditional morphometric analyses carried out by [Gardner and Carleton \(2009\)](#). [Hooper \(1952\)](#) highlighted *R. cherrii* as one of the largest subspecies within *R. mexicanus*, comparing it in body size and skull length with *Peromyscus maniculatus*.

Although our morphometric analyses statistically differentiate *R. cherrii* from *R. mexicanus*, both species showed overlap in shape and size of the skull. The degree of morphological overlap among species of *Reithrodontomys* has



**Figure 4.** Differences in mean shapes of ventral and dorsal views of the skull, using the Procrustes distances. Red dots = *Reithrodontomys mexicanus* and blue dots = *R. cherrii*, PD = Procrustes distances, asterisks = significant differences based on pairwise permutation test ( $P < 0.05$ ).





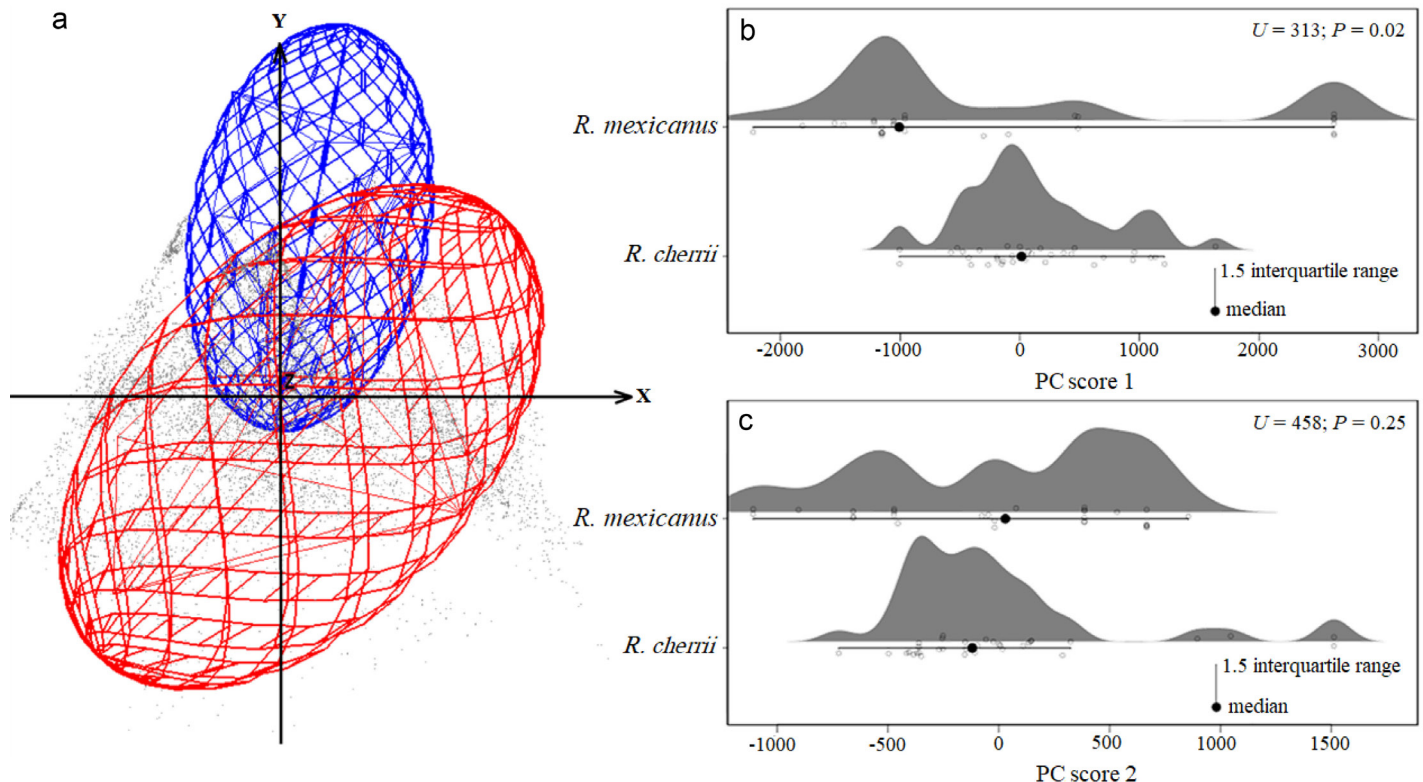
**Figure 5.** Skull size differences (based on centroid size variable) between *Reithrodontomys mexicanus* and *R. cherrii* for ventral and dorsal views. Statistical significance considered with  $P \leq 0.05$ .

been evident since the earliest monographs of the genus (Howell 1914; Hooper 1952). This may be why the taxonomic identification of *Reithrodontomys* species is difficult, leading to an underestimation of the actual number of species within this genus. For *R. mexicanus*, Hooper (1952) reported cranial and body measurements very similar or with very wide ranges among all its subspecies, including

the former *R. m. cherrii*. The strong similarity of morphological (cranial and body measurements) and pelage coloration among these subspecies explains the inclusion of *R. cherrii* within *R. mexicanus* (Howell 1914), even though it was originally described as a species (Allen 1891).

*Reithrodontomys mexicanus* has one of the widest distributions within the subgenus *Aporodon* (Arellano 2015), whereas *R. cherrii* is restricted to the highlands of central Costa Rica and the Cordillera de Talamanca (Gardner and Carleton 2009; Villalobos-Chaves et al. 2016). Throughout its distribution, *R. mexicanus* is associated with a variety of vegetation-types such as humid oak forests, cloud forests, deciduous arid forests, and deciduous lowland forests (Hooper 1952), which coincides with its broader environmental space here reported compared to that of *R. cherrii*. *R. mexicanus* also occupies areas with high mean values of temperature and low values of precipitation compared to those for the distribution area of *R. cherrii*. The overlap of the minimum-volume ellipsoids in a small region, as well as the low Jaccard similarity index value, may be due to shared environmental characteristics between cloud forests and montane pluvial forests, the main plant formations where *R. mexicanus* and *R. cherrii* occurs, respectively.

The bioclimatic variables used in this study have been previously used in mammal ecological studies (Santos et al. 2017; Guevara et al. 2018; Stanchak and Santana 2018). BIO5, BIO6, BIO14, and BIO15 are variables that may be limiting the distribution of cloud forest species (Guevara et al.



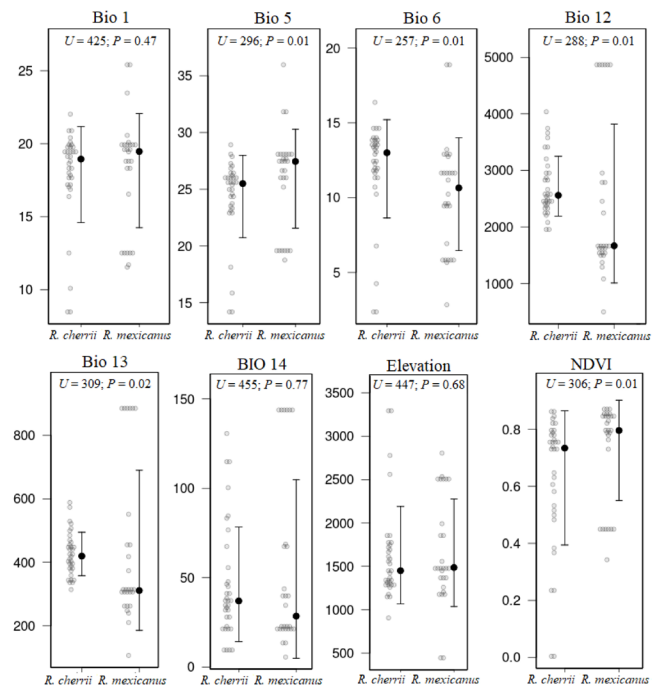
**Figure 6.** Environmental niche of *Reithrodontomys mexicanus* (red ellipsoid) and *R. cherrii* (blue ellipsoid) displayed in a three-dimensional space and based on the occurrence records and a background data (a). On the right (b and c) show the statistical comparisons using the scores of the first two principal components derived from the environmental variables. Statistical significance considered with  $P \leq 0.05$ .

2018). Of these four variables, only BIO14 (Precipitation of the driest month) was not significantly different, while the annual precipitation values were the most divergent among regions occupied by these species. These values were higher than 2,000 mm per year for *R. cherrii*, coinciding with the physical-geographical characteristics of the Central Valley (Gómez 1986), which is the climatic region where it is distributed in Costa Rica.

Additionally, we used elevation and NDVI variables to characterize the environmental space of each species. Although the minimum and maximum elevation values were higher for *R. cherrii*, the mean was similar between species. The altitudinal gradient of *Aporodon* species is variable, but most of them inhabit above 1,000 masl (Hooper 1952; Hall 1981). This could explain the lack of statistical differences when comparing this variable between the two species. Elevation has been widely used in studies focused on small rodents (McCain 2005), and it is useful to characterize the habitat in this group. However, this variable should be treated with caution when carrying out ecological analyses, such as niche modeling, due to the high association to temperature and precipitation, especially for small mammals distributed at high elevations (Rubidge et al. 2011; Santos et al. 2017; Guevara et al. 2018).

The NDVI is related to different vegetation parameters (Pettorelli 2013), with values ranging between 0.2 and 0.8, where higher numbers are indicators of photosynthetic activity linked to vegetation types such as temperate forest, rain forest, among others (Meneses-Tovar 2011). Beyond the statistical differences found for this index between *R. mexicanus* and *R. cherrii*, it is important to highlight that the natural areas where these two species are distributed could be considered high quality ecosystems following Pettorelli et al. (2007), considering that the mean values were greater than 0.6. However, quantitative and qualitative indicators are needed to assess integrative ecosystem health (Lu et al. 2015). The NDVI application in animal ecology appears to be helpful, especially since it can be linked to animal distribution and abundance (Pettorelli et al. 2005). Its use in carnivorous and omnivorous mammal species has been well explored but little is known for small mammals (Pettorelli et al. 2011). Our results confirm the utility of this index in habitat characterization and encourages their inclusion in ecological studies for small rodents.

Howell (1914) included *R. cherrii* within *R. mexicanus* as one of its largest and brightest subspecies (Hooper 1952). In mammals, environment can include changes in structures such as skull and jaw, leading to ecophenotypic variations (Camul and Polly 2005). This phenotypic change due to habitat could justify *R. cherrii* misclassification at the subspecific level. However, the high genetic divergence reported between this former subspecies and *R. mexicanus*, translates into different evolutionary histories that vindicates it at the species-level, now as a member of the *R. tenuirostris* species group (Arellano et al. 2003, 2005). We found these two species to be different based on the skull



**Figure 7.** Pairwise comparisons of variables used to characterize the environmental space between *Reithrodontomys mexicanus* and *R. cherrii*. Variable names are in the methods section. Statistical significance considered with  $P \leq 0.05$ .

morphometry and environmental. Thus, complementing the previous molecular evidence as a whole integrative taxonomy approach that supports *R. cherrii* as a different species from *R. mexicanus*.

## Acknowledge

We thank the following institutions and curators for allowing specimen examinations: Colección de Mamíferos de El Colegio de la Frontera Sur, San Cristóbal (C. Lorenzo); Mammal Collection American Museum of Natural History (R. S. Voss); and Museum of Zoology, University of Michigan (P. Tucker and C. Thompson). DM-B was supported by Consejo Nacional de Ciencia y Tecnología (CONACYT) Scholarship Program 2018-000012 01NACF-11852.

## Literature cited

- ADAMS, D. C., AND E. OTÁROLA-CASTILLO. 2013. Geomorph: An R package for the collection and analysis of geometric morphometric shape data. *Methods in Ecology and Evolution* 4:393-399.
- ALLEN, J. A. 1891. Notes on a collection of mammals from Costa Rica. *Bulletin of the American Museum of Natural History* 3:203-218.
- ALLEN, J. A. 1895. On the species of the genus *Reithrodontomys*. *Bulletin of the American Museum of Natural History* 7:7-143.
- ALSTRÖM, P., P. C. RASMUSSEN, U. OLSSON, AND P. SUNDBERG. 2008. Species delimitation based on multiple criteria: the Spotted Bush Warbler *Bradypterus thoracicus* complex (Aves: Megaluridae). *Zoological Journal of the Linnean Society* 154:291-307.
- ANDERSON, M. J. 2001. A new method for non-parametric multivariate analysis of variance. *Austral Ecology* 26:32-46.



- ARELLANO, E. 2015. Genus *Reithrodontomys* Giglioli, 1874. Pp. 61-63, in *Mammals of South America*, vol. 2: Rodents (Patton, J. L., U. F. Pardiñas, and G. D'Elia, eds.). University of Chicago Press. Illinois, U.S.A.
- ARELLANO, E., D. S. ROGERS, AND F. A. CERVANTES. 2003. Genic differentiation and phylogenetic relationships among tropical harvest mice (*Reithrodontomys*: subgenus *Aporodon*). *Journal of Mammalogy* 84:129-143.
- ARELLANO, E., F. GONZÁLEZ-COZÁTL, AND D. S. ROGERS. 2005. Molecular systematics of Middle American harvest mice *Reithrodontomys* (Muridae), estimated from mitochondrial cytochrome b gene sequences. *Molecular Phylogenetics and Evolution* 37:529-540.
- ARELLANO, E., D. S. ROGERS, AND F. X. GONZÁLEZ-CÓZATL. 2006. Sistemática Molecular del género *Reithrodontomys*. Pp. 27-35, in *Genética y Mamíferos Mexicanos: Presente y Futuro* (Vázquez-Domínguez, E., and D. J. Hafner, eds.). New Mexico Museum of Natural History and Science Bulletin 32:1-73.
- ARELLANO, E., J. A. GUERRERO, AND D. S. ROGERS. 2012. Variación morfométrica y alometría del crecimiento de *Reithrodontomys mexicanus* (Rodentia: Muridae) de Oaxaca, México. Pp. 35-45, in *Estudio Sobre la Biología de Roedores Silvestres Mexicanos* (Cervantes, F. A., and C. Ballesteros-Barrera, eds.). Creativa Impresores S. A., México City, México.
- BARČIOVÁ, L. 2009. Advances in insectivore and rodent systematics due to geometric morphometrics. *Mammal Review* 39:80-91.
- BOGDANOWICZ, W., J. JUSTE, R. D. OWEN, AND A. SZTENCCEL. 2005. Geometric morphometrics and cladistics: testing evolutionary relationships in mega- and microbats. *Acta Chiropterologica* 7:39-49.
- BOOKSTEIN, F. L. 1991. *Morphometric tools for landmark data. Geometry and biology.* Cambridge University Press. New York, U.S.A.
- BOOKSTEIN, F. L. 1997. Landmark methods for forms without landmarks: Morphometrics of group differences in outline shape. *Medical Image Analysis* 1:225-43.
- BRADLEY, R. D. 2017. Genus *Reithrodontomys*. Pp. 367-383, in *Handbook of the Mammals of the World: Rodents II* (Wilson, D. E., T. E. Lacher, and R. A. Mittermeier, eds.). Lynx Edicions. Barcelona, Spain.
- CAMUL, R., AND P. D. POLLY. 2005. Phylogenetic and environmental components of morphological variation: skull, mandible, and molar shape in Marmots (*Marmota*, Rodentia). *Evolution* 59:2460-2472.
- COLLYER, M. L., AND D. C. ADAMS. 2018. RRPP: An R package for fitting linear models to high-dimensional data using residual randomization. *Methods in Ecology and Evolution* 9:1772-1779.
- CORDEIRO-ESTRELA, P., M. BAYLAC, D. CHRISTIANE, AND J. POLOP. 2008. Combining geometric morphometrics and pattern recognition to identify interspecific patterns of skull variation: case study in sympatric Argentinian species of the genus *Calomys* (Rodentia: Cricetidae: Sigmodontinae). *Biological Journal of the Linnean Society* 94:365-378.
- DAYRAT, B. 2005. Towards integrative taxonomy. *Biological Journal of Linnean Society* 85:407-415.
- DE QUEIROZ, K. 1998. The general lineage concept of species, species criteria, and the process of speciation. *Endless forms: species and speciation.*
- DE QUEIROZ, K. 2007. Species concepts and species delimitation. *Systematic Biology* 56:879-886.
- ESRI. 2020. ArcGIS Desktop Release 10.8. Environmental System Research Institute, Inc. Redlands. California, U.S.A.
- FICK, S. E., AND R. J. HIJMANS. 2017. WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. *International Journal of Climatology* 37:4302-4315.
- GARDNER, A. L., AND M. D. CARLETON. 2009. A new species of *Reithrodontomys*, subgenus *Aporodon* (Cricetidae: Neotomiinae), from the highlands of Costa Rica, with comments on Costa Rican and Panamanian *Reithrodontomys*. *Bulletin of the American Museum of Natural History* 331:157-182.
- GÓMEZ, L. D. 1986. *Vegetación de Costa Rica, apuntes para una biogeografía costarricense: Vegetación y Clima de Costa Rica Volumen 1.* Editorial Universidad Estatal a Distancia. San José, Costa Rica.
- GOODALL, C. 1991. Procrustes methods in the statistical analysis of shape. *Journal of the Royal Statistical Society Series B (Statistical Methodology)* 53:285-339.
- GRAHAM, C. H., ET AL. 2004. Integrating phylogenetics and environmental niche models to explore speciation mechanism in dendrobatid frogs. *Evolution* 58:1781-1793.
- GUEVARA, L., B. E. GERSTNER, J. M. KASS, AND R. P. ANDERSON. 2018. Toward ecologically realistic predictions of species distributions: A cross-time example from tropical montane cloud forests. *Global Change Biology* 24:1511-1522.
- HALL, E. R. 1981. *The Mammals of North America* 2nd ed. John Wiley and Sons, Inc. New York, U.S.A.
- HARDY, D. K., F. X. GONZÁLEZ-CÓZATL, E. ARELLANO, AND D. S. ROGERS. 2013. Molecular phylogenetics and phylogeography structure of Sumichrast's harvest mouse (*Reithrodontomys sumichrasti*: Cricetidae) based on mitochondrial and nuclear DNA sequences. *Molecular Phylogenetics and Evolution* 68:282-292.
- HOOPER, E. T. 1952. A systematic review of harvest mice (genus *Reithrodontomys*) of Latin America. *Miscellaneous Publications Museum of Zoology, University of Michigan* 77:1-255.
- HOOPER, E. T. 1959. The glans penis in five genera of cricetid rodents. *Occasional Papers of the Museum of Zoology, University of Michigan* 613:1-11.
- HOWELL, A. H. 1914. Revision of the American harvest mice (genus *Reithrodontomys*). *North American Fauna* 36:1-97.
- HUNTINGTON, J. L., ET AL. 2017. Climate Engine: cloud computing and visualization of climate and remote sensing data for advanced natural resource monitoring and process understanding. *Bulletin of the American Meteorological Society* 98:2397-2410.
- JACCARD, P. 1912. The distribution of the flora in the alpine zone. 1. *New Phytologist* 11:37-50.
- KRYŠTUFEK, B., G. SHENBROT, T. KLENOVŠEK, AND F. JANŽEKVIČ. 2021. Geometric morphometrics of mandibular shape in the dwarf fat-tailed jerboa: relevancy for trinomial taxonomy. *Zoological Journal of the Linnean Society* 192:1363-1372.
- KUBIAK, B. B., ET AL. 2017. Can niche modeling and geometric morphometrics document competitive exclusion in a pair of subterranean rodents (genus *Ctenomys*) with tiny parapatric distributions? *Scientific Reports* 7:1-13.
- LEACHÉ, A. D., ET AL. 2009. Quantifying ecological, morphological, and genetic variation to delimit species in the coast horned

- lizard species complex (*Phrynosoma*). Proceedings of the National Academy of Sciences 106:12418-12423.
- LU, Y., ET AL. 2015. Ecosystem health towards sustainability. Ecosystem Health and Sustainability 1:1-15.
- MARTÍNEZ, J. J., AND V. DI COLA. 2011. Geographic distribution and phenetic skull variation in two close species of *Graomys* (Rodentia, Cricetidae, Sigmodontinae). Zoologischer Anzeiger—A Journal of Comparative Zoology 250:175-194.
- MARTÍNEZ-GORDILLO, D., O. ROJAS-SOTO, AND A. ESPINOSA-DE LOS MONTE-ROS. 2010. Ecological niche modelling as an exploratory tool for identifying species limits: an example based on Mexican murid rodents. Journal of Evolutionary Biology 23:259-270.
- MAYARES, D. I. 2012. Variación intraespecífica de *Reithrodontomys sumichrasti* (Rodentia: Cricetidae) con base en un análisis de morfometría geométrica. Bachelor thesis. Universidad Autónoma del Estado de Morelos. Cuernavaca, México.
- MAYDEN, R. L. 1997. A hierarchy of species concepts: the denouement in the saga of the species problem. Pp 381-423, in Species: The Units of Biodiversity (Claridge, M. F. H. A. Dawah, and M. R. Wilson, eds.). Chapman and Hall CRC Press, London, United Kingdom.
- MAYR, E. 2000. The biological species concept. Pp 17-29 in Species concepts and phylogenetic theory: a debate (Wheeler, Q. D., and R. Meier, eds.). Columbia University Press, New York, U.S.A.
- MCCAIN, C. M. 2005. Elevational gradients in diversity of small mammals. Ecology 86:366-372.
- MENESES-TOVAR, C.L. 2011. NDVI as indicator of degradation. Unasylva 62:39-46.
- MERRIAM, C. H. 1901. Descriptions of 23 new harvest mice (genus *Reithrodontomys*). Proceedings of the Washington Academy of Sciences 3:547-558.
- MILLER, J. R., AND M. D. ENGSTROM. 2008. The relationships of major lineages within Peromyscine rodents: a molecular phylogenetic hypothesis and systematic reappraisal. Journal of Mammalogy 89:1279-1295.
- PATTERSON, B. D., P. L. MESERVE, AND B. K. LANG. 1989. Distribution and abundance of small mammals along an elevational transect in temperate rainforests of Chile. Journal of Mammalogy 70:67-78.
- PAVAN, A. C., AND G. MARROIG. 2016. Integrating multiple evidences in taxonomy: species diversity and phylogeny of mustached bats (Mormoopidae: *Pteronotus*). Molecular Phylogenetics and Evolution 103:184-198.
- PETTORELLI, N. 2013. The normalized difference vegetation index. Oxford University Press. Oxford, United Kingdom.
- PETTORELLI N., ET AL. 2005. Using the satellite-derived Normalized Difference Vegetation Index (NDVI) to assess ecological effects of environmental change. Trends in Ecology and Evolution 20:503-510.
- PETTORELLI N., ET AL. 2007. Early onset of vegetation growth versus rapid green-up: impacts on juvenile mountain ungulates. Ecology 88:381-390.
- PETTORELLI, N., ET AL. 2011. The Normalized Difference Vegetation Index (NDVI): unforeseen successes in animal ecology. Climate Research 46:15-27.
- QIAO, H., ET AL. 2016. NicheA: Creating virtual species and ecological niches in multivariate environmental scenarios. Ecography 39:805-813.
- R DEVELOPMENT CORE TEAM. 2018. R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria. [www.R-project.org/](http://www.R-project.org/)
- RAMÍREZ-ARRIETA, V. M. 2020. Extended Boxplot Graphics. <https://vmra.shinyapps.io/univariados/>. Last accessed February 25, 2021.
- RISSLER, L. J., AND J. J. APODACA. 2007. Adding more ecology into species delimitation: ecological niche models and phylogeography help define cryptic species in the Black Salamander (*Aneides flavipunctatus*). Systematic Biology 56:924-942.
- RIVERA, P. C., ET AL. 2018. Molecular phylogenetics and environmental niche modeling reveal a cryptic species in the *Oligoryzomys flavescens* complex (Rodentia, Cricetidae). Journal of Mammalogy 99:363-376.
- ROHLF, F. J. 2015. The tps series of software. Hystrix – Italian Journal of Mammalogy 26:9-12.
- ROHLF, F. J., AND D. SLICE. 1990. Extensions of the Procrustes method for the optimal superimposition of landmarks. Systematic Zoology 39:40-21.
- RUBIDGE, E. M., ET AL. 2011. The role of climate, habitat, and species co-occurrence as drivers of change in small mammal distributions over the past century. Global Change Biology 17:696-708.
- SANGSTER, G. 2018. Integrative taxonomy of birds: the nature and delimitation of species. Pp. 9-37, in Bird species. Fascinating Life Sciences (Tietze, D. T., ed.). Springer, Cham. New York, U.S.A.
- SANTOS, M. J., A. B. SMITH, J. H. THORNE, AND C. MORITZ. 2017. The relative influence of change in habitat and climate on elevation range limits in small mammals in Yosemite National Park, California, U.S.A. Climate Change Responses 4:1-12.
- SAUSSURE DE, H. 1860. Note sur quelques mammifères du Mexique. Revue et Magasin de Zoologie Serie 2 12:97-110.
- SCHLAGER, S. 2016. Morpho: calculations and visualisations related to geometric morphometrics. R package version 2.3.1.1. Freiburg, Germany, Stefan Schlager, <https://CRAN.R-project.org/package=Morpho>.
- SEIFERT, B. 2014. A pragmatic species concept applicable to all eukaryotic organisms independent from their mode of reproduction or evolutionary history. Soil Organisms 86:85-93.
- SITES JR., J. W., AND J. C. MARSHALL. 2003. Delimiting species: a Renaissance issue in systematic biology. Trends in Ecology and Evolution 18:462-470.
- SITES JR., J. W., AND J. C. MARSHALL. 2004. Operational criteria for delimiting species. Annual Review of Ecology, Evolution, and Systematics 35:199-227.
- SOBERÓN J., AND M. NAKAMURA. 2009. Niches and distributional areas: concepts, methods, and assumptions. Proceedings of the National Academy of Sciences U.S.A 106:19644-19650.
- STANCHAK, K. E., AND S. E. SANTANA. 2018. Do ecogeographical rules explain morphological variation in a diverse, Holarctic genus of small mammals? Journal of Biogeography 46:110-122.
- STATSOFT, I. N. C. 2007. STATISTICA (data analysis software system). Version 7:1984-2004.
- STRUCK, T. H., ET AL. 2018. Finding evolutionary processes hidden in cryptic species. Trends in Ecology and Evolution 33:153-163.
- UMETSU, F., AND R. PARDINI. 2007. Small mammals in a mosaic of forest remnants and anthropogenic habitats—evaluating matrix quality in an Atlantic Forest landscape. Landscape Ecology 22:517-530.

- VAN AELST, S., AND P. ROUSSEEUW. 2009. Minimum volume ellipsoid. *Wiley Interdisciplinary Reviews: Computational Statistics* 1:71-82.
- VILLALOBOS-CHAVES, D., ET AL. 2016. Clave para la identificación de los roedores de Costa Rica. Escuela de Biología, Universidad de Costa Rica, Ciudad Universitaria Rodrigo Facio, Costa Rica.
- ZELDITCH, M. L., D. L. SWIDERSKI, H. D. SHEETS, AND W. L. FINK. 2004. *Geometric morphometrics for biologists: A primer*. Elsevier Academic Press, New York, U.S.A.
- ZHAO, Q., H. ZHANG, AND J. WEI. 2019. Climatic niche comparison across a cryptic species complex. *PeerJ* 7:p.e7042.

*Associated editor: Monica Diaz*

*Submitted: June 13, 2021; Reviewed: July 19, 2021.*

*Accepted: September 13, 2021; Published on line: November 29, 2021*



## Appendix 1

List of *Reithrodontomys mexicanus* and *R. cherrii* voucher specimens and their associated collecting localities used in the geometric morphometric analyses. Abbreviations before catalogue number represent the mammal collection housing the specimens: AMNH = American Museum of Natural History; BYU = Brigham Young University; CMC = Colección de Mamíferos del Centro de Investigación en Biodiversidad y Conservación; ECOSUR = Colección Mastozoológica de El Colegio de la Frontera Sur, Unidad San Cristóbal; UMMZ = Museum of Zoology, University of Michigan.

Voucher	Species	Locality
UMMZ-109894	<i>R. mexicanus</i>	Los Esesmiles, Chalatenango, El Salvador
UMMZ-109897	<i>R. mexicanus</i>	Los Esesmiles, Chalatenango, El Salvador
UMMZ-109900	<i>R. mexicanus</i>	Los Esesmiles, Chalatenango, El Salvador
UMMZ-109903	<i>R. mexicanus</i>	Los Esesmiles, Chalatenango, El Salvador
UMMZ-109905	<i>R. mexicanus</i>	Los Esesmiles, Chalatenango, El Salvador
UMMZ-116882-116884	<i>R. mexicanus</i>	Santa María de Ostuma, 9 km N of Matagalpa, Nicaragua, 1,400 m
UMMZ-118145-118146	<i>R. mexicanus</i>	Municipio La Libertad, Hacienda El Injerto, río Aguacate, Huehuetenango, Guatemala, 1600 m.
UMMZ-118147-118150	<i>R. mexicanus</i>	Barillas, Hacienda Santa Gregoria, Huehuetenango, Guatemala
UMMZ-118152-118153	<i>R. mexicanus</i>	Barillas, Hacienda Santa Gregoria, Huehuetenango, Guatemala
UMMZ-118154-118155	<i>R. mexicanus</i>	Finca Concepción, Tukurú, Alta Verapaz, Guatemala
AMNH-142460	<i>R. mexicanus</i>	Coto Brus, Cañas Gordas, Puntarenas, Costa Rica
AMNH-142462-142463	<i>R. mexicanus</i>	Coto Brus, Cañas Gordas, Puntarenas, Costa Rica
AMNH-142465	<i>R. mexicanus</i>	Coto Brus, Cañas Gordas, Puntarenas, Costa Rica
AMNH-142468-142472	<i>R. mexicanus</i>	Coto Brus, Cañas Gordas, Puntarenas, Costa Rica
BYU-15426	<i>R. mexicanus</i>	Municipio Santiago Comaltepec, 11 km SW (by road.) La Esperanza, Oaxaca, México
BYU-15436	<i>R. mexicanus</i>	Municipio Teotitlán de Flores Magón, 1.5 km S Puerto de la Soledad, Oaxaca, MX
BYU-15439	<i>R. mexicanus</i>	Municipio Ixhuacán, 18 km NW Teocelo, Veracruz, México
BYU-20781	<i>R. mexicanus</i>	Rancho La Providencia, Chiapas, México, 1775 m
CMC-872	<i>R. mexicanus</i>	Municipio Huatusco, 1.9 km N Las Cañadas (Eco reserva), Veracruz, México
CMC-874	<i>R. mexicanus</i>	Municipio Huatusco, 1.9 km N Las Cañadas (Eco reserva), Veracruz, México
CMC-877	<i>R. mexicanus</i>	Municipio Huatusco, 1.9 km N Las Cañadas (Eco reserva), Veracruz, México
ECOSUR-2842	<i>R. mexicanus</i>	Santo Tomás Oxchuc, Mercado Indígena, Chiapas, México
ECOSUR-3000	<i>R. mexicanus</i>	Ejido Sombra Chica, 1 km NW Tumbalá, Chiapas, México
ECOSUR-931	<i>R. mexicanus</i>	Las Grutas. PN Lagos de Montebello, 3.45 Km N El Vivero, Chiapas, México
AMNH-7905	<i>R. cherrii</i>	Cerro La Carpintera, Cartago, Costa Rica
AMNH-123503	<i>R. cherrii</i>	Cerro La Carpintera, Cartago, Costa Rica
AMNH-7902-7904	<i>R. cherrii</i>	Cerro La Carpintera, Cartago, Costa Rica
AMNH-7908	<i>R. cherrii</i>	Cerro La Carpintera, Cartago, Costa Rica
AMNH-131739	<i>R. cherrii</i>	Escazú, San José, Costa Rica
AMNH-135258	<i>R. cherrii</i>	Vázquez de Coronado, Nubes, San José, Costa Rica
AMNH-135924	<i>R. cherrii</i>	Alajuela, Sabanilla, Costa Rica
AMNH-138088	<i>R. cherrii</i>	Escazú, Los Higueros, San José, Costa Rica
AMNH-139284	<i>R. cherrii</i>	Montes de Oca, Sabanilla, San José, Costa Rica
AMNH-139289	<i>R. cherrii</i>	Montes de Oca, Sabanilla, San José, Costa Rica
AMNH-141878	<i>R. cherrii</i>	Alajuelita, Santa Teresa, Peralta, Costa Rica
AMNH-141880-141881	<i>R. cherrii</i>	Alajuelita, Santa Teresa, Peralta, Costa Rica
AMNH-141883	<i>R. cherrii</i>	Alajuelita, Santa Teresa, Peralta, Costa Rica
AMNH-19187-19188	<i>R. cherrii</i>	Montes de Oca, San Pedro, San José, Costa Rica
AMNH-19192	<i>R. cherrii</i>	Montes de Oca, San Pedro, San José, Costa Rica

## Appendix 2

Anatomical and numerical position (see Figure 2) of landmarks and semi-landmarks used in the geometric morphometric analyses for ventral and dorsal views of the skull.

Ventral		Dorsal	
1	Rostralmost point of the upper incisor tooth next to the midline	1	Rostralmost point of the nasal bone
2	Anteriormost point of the incisive foramen	2	Anteriormost point of suture between nasal bone and nasal process of the incisive
3	Posteriormost point of the incisive foramen	3	Rostral end of zygomatic plate in a dorsal projection
4	Rostral end of zygomatic plate in a ventral projection	4	Anteriormost point of the orbit in a dorsal projection
5	Anteriormost point of the orbit in a ventral projection	5	Narrowest point of the interorbital region
6	Caudalmost point of the orbit in a ventral projection	6	Rostralmost point of the parietal bone
7	Posterior end of zygomatic bar in a ventral projection	7	Caudalmost point of the orbit in a dorsal projection
8-20	Semi-landmarks	8	Posterior end of zygomatic bar in a dorsal projection
21	Lateral margin of the basioccipital	9-31	Semi-landmarks
22	Lateral margin of the foramen magnum	32	Caudal end of the curvature of the occipital bone
23	Posteriormost point of the occipital foramen in the midline	33	Intersection of the sagittal and parietal-interparietal sutures
24	Anteriormost point of the occipital foramen in the midline	34	Intersection of the coronal and sagittal sutures
25	Midpoint of suture between basisphenoid and basioccipital	35	Intersection of the naso-frontal suture in the midline
26	Posteriormost extent of palate at the midline		
27	Posteriormost point of the third molar		
28	Contact point between second and third molars		
29	Contact point between first and second molars		
30	Anteriormost point of the first molar		

### Appendix 3

Geographical coordinates (Datum WGS-84) of localities used in the ecological analysis for *Reithrodontomys mexicanus* and *R. cherrii*.

Species	Longitude	Latitude	Species	Longitude	Latitude
<i>R. mexicanus</i>	-89.133	14.383	<i>R. mexicanus</i>	-92.016	15.566
<i>R. mexicanus</i>	-85.925	13.005	<i>R. mexicanus</i>	-90.066	15.300
<i>R. mexicanus</i>	-91.315	15.803	<i>R. mexicanus</i>	-96.447	17.555
<i>R. mexicanus</i>	-97.220	18.356	<i>R. mexicanus</i>	-92.340	15.216
<i>R. mexicanus</i>	-97.064	19.525	<i>R. mexicanus</i>	-91.720	16.130
<i>R. mexicanus</i>	-96.984	19.258	<i>R. cherrii</i>	-83.984	9.881
<i>R. mexicanus</i>	-92.346	16.794	<i>R. cherrii</i>	-84.216	10.076
<i>R. mexicanus</i>	-92.315	17.270	<i>R. cherrii</i>	-84.144	9.894
<i>R. mexicanus</i>	-87.106	14.208	<i>R. cherrii</i>	-84.136	9.913
<i>R. mexicanus</i>	-96.305	17.599	<i>R. cherrii</i>	-84.029	9.947
<i>R. mexicanus</i>	-96.016	17.176	<i>R. cherrii</i>	-84.021	9.944
<i>R. mexicanus</i>	-92.340	15.216	<i>R. cherrii</i>	-83.962	9.987
<i>R. mexicanus</i>	-93.120	17.190	<i>R. cherrii</i>	-84.083	9.933
<i>R. mexicanus</i>	-91.700	16.100	<i>R. cherrii</i>	-83.849	9.979
<i>R. mexicanus</i>	-92.640	15.650	<i>R. cherrii</i>	-83.798	9.768
<i>R. mexicanus</i>	-93.070	15.800	<i>R. cherrii</i>	-83.981	9.896
<i>R. mexicanus</i>	-92.692	16.684	<i>R. cherrii</i>	-84.153	9.913
<i>R. mexicanus</i>	-92.400	16.963	<i>R. cherrii</i>	-84.406	10.221
<i>R. mexicanus</i>	-90.031	15.089	<i>R. cherrii</i>	-84.059	9.705
<i>R. mexicanus</i>	-92.851	16.990	<i>R. cherrii</i>	-83.850	9.983
<i>R. mexicanus</i>	-92.975	17.208	<i>R. cherrii</i>	-83.902	9.833
<i>R. mexicanus</i>	-91.932	15.485	<i>R. cherrii</i>	-83.916	9.839
<i>R. mexicanus</i>	-86.339	13.080	<i>R. cherrii</i>	-84.244	10.102
<i>R. mexicanus</i>	-89.366	14.400	<i>R. cherrii</i>	-83.982	9.950
<i>R. mexicanus</i>	-90.455	14.558	<i>R. cherrii</i>	-84.107	9.868
<i>R. mexicanus</i>	-89.623	15.136	<i>R. cherrii</i>	-84.183	9.950
<i>R. mexicanus</i>	-96.605	17.579	<i>R. cherrii</i>	-83.548	9.450
<i>R. mexicanus</i>	-96.842	18.168	<i>R. cherrii</i>	-83.877	9.823
<i>R. mexicanus</i>	-97.040	18.618	<i>R. cherrii</i>	-83.916	9.650
<i>R. mexicanus</i>	-97.028	19.037	<i>R. cherrii</i>	-83.881	9.833
<i>R. mexicanus</i>	-96.798	17.811	<i>R. cherrii</i>	-83.903	9.879
<i>R. mexicanus</i>	-90.062	15.385	<i>R. cherrii</i>	-83.983	9.890
<i>R. mexicanus</i>	-97.024	18.869	<i>R. cherrii</i>	-83.803	9.753
<i>R. mexicanus</i>	-91.394	15.571	<i>R. cherrii</i>	-84.139	9.883
<i>R. mexicanus</i>	-89.481	15.166	<i>R. cherrii</i>	-84.333	10.277
<i>R. mexicanus</i>	-91.560	15.984	<i>R. cherrii</i>	-83.707	9.566
<i>R. mexicanus</i>	-90.251	15.236	<i>R. cherrii</i>	-83.968	9.888
<i>R. mexicanus</i>	-96.436	17.386	<i>R. cherrii</i>	-84.233	10.183
<i>R. mexicanus</i>	-92.591	16.051	<i>R. cherrii</i>	-84.465	9.920