

About the specific status of *Baiomys musculus* and *B. brunneus*

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The southern pygmy mouse, *Baiomys musculus*, is distributed in arid and semiarid lowlands, from southern Nayarit and central Veracruz in México to northwestern Nicaragua, excluding the Yucatán Peninsula and the Caribbean tropical lowlands. Previous reports suggest that *B. musculus* includes two clades that may be eligible for specific status, although this remains uncertain. We used mitochondrial DNA (cytochrome b) and morphometric data to test whether two lineages exist within the species. Molecular data support the existence of two monophyletic groups with genetic distances of 6.69 % between them: clade I, a western clade found in Colima, Jalisco, and Michoacán; and clade II, an eastern clade found in Guerrero, Morelos, Oaxaca, and Veracruz. Morphologically, clade I individuals are larger than clade II. Moreover, these clades seem to be allopatric, and their geographic boundaries are located in the complex topography of western México. Examination of previous reviews in addition to the data from this study suggest that it may be appropriate to recognize each clade as a species: clade I as *B. musculus* (Merriam, 1892) and clade II as *B. brunneus* (Allen and Chapman, 1897). Future studies with nuclear or genomic data, including Central American populations, would verify this taxonomic hypothesis.

El ratón pigmeo del sur, *Baiomys musculus*, se distribuye en las tierras bajas áridas y semiáridas, desde el sur de Nayarit y el centro de Veracruz en México, hasta el noroeste de Nicaragua, excluyendo la península de Yucatán y las tierras bajas tropicales del Caribe. Previamente fue reportado que existen dos clados genéticos que podrían representar especies diferentes, aunque esto sigue siendo incierto. Utilizamos DNA mitocondrial (citocromo b) y datos morfométricos para analizar la existencia de dos linajes al interior de la especie. Los datos moleculares confirman la existencia de dos grupos monofiléticos con distancias genéticas entre ellos de 6.69 %: el clado I, o el clado del oeste que se distribuye en Colima, Jalisco y Michoacán; y el clado II, o clado del este que habita en Guerrero, Morelos, Oaxaca y Veracruz. Los análisis morfométricos mostraron que los individuos del clado I son más grandes que los del clado II. Además, estos clados parecen ser alopátricos, y sus límites geográficos se localizan en la compleja topografía del oeste de México. Después de integrar previas investigaciones con nuestros datos, sugerimos apropiado renombrar al clado I como *B. musculus* (Merriam, 1892), y al clado II como *B. brunneus* (Allen and Chapman, 1897). Es necesario realizar estudios con datos nucleares o genómicos, incluyendo a las poblaciones de Centro América, para poder validar esta hipótesis taxonómica.

Keywords: Mitochondrial DNA; morphometric data; southern pygmy mouse; taxonomic change; western México.

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Introduction

The mice in the genus *Baiomys* belong to the cricetid tribe *Baiomyini* ([Pardiñas et al. 2017](#)) and includes some of the smallest North American rodents in the subfamily *Neotominae*, commonly known as pygmy mice ([Packard 1960](#)). *Baiomys* comprises two extant species: the northern pygmy mouse (*B. taylori*) and the southern pygmy mouse (*B. musculus*; [Packard 1960](#); [Pardiñas et al. 2017](#)). The southern pygmy mouse primarily inhabits arid and semiarid lowlands ([Packard and Montgomery 1978](#)), from southern Nayarit and central Veracruz in México to northwestern Nicaragua, excluding the Yucatán Peninsula and the Caribbean tropical lowlands ([Packard and Montgomery 1978](#); [Pardiñas et al. 2017](#)). This distribution overlaps with *B. taylori* in the west-central Mexican states of Colima, Jalisco, Michoacán, and Nayarit ([Pardiñas et al. 2017](#)). In this sympatric area, *B. musculus* and *B. taylori* are more strongly morphologically differentiated from each other than are other allopatric populations of each species ([Packard 1960](#)).

Baiomys musculus originally was described as *Sitomys musculus* (Merriam, 1892), and later the subgenus *Baiomys* was defined based on morphological differences ([True 1894](#)). *Baiomys* was recognized as an independent genus by [Mearns \(1907\)](#), who also was the first to use the binomial *Baiomys musculus*. Two years later, *Baiomys* was considered a subgenus of *Peromyscus* ([Osgood 1909](#)), but [Miller \(1912\)](#) subsequently re-recognized it as an independent genus. Currently, eight subspecies are recognized within *B. musculus*: *B. m. musculus* (Merriam, 1892; type specimen from Colima, México); *B. m. brunneus* (Allen and Chapman, 1897; type specimen from Veracruz, México); *B. m. nigrescens* (Osgood, 1904; type specimen from Chiapas, México); *B. m. grisescens* Goldman, 1932 (type specimen from Tegucigalpa, Honduras); *B. m. infernalis* Hooper, 1952 (type specimen from Oaxaca, México); *B. m. pallidus* Russell, 1952 (type specimen from Morelos, México); *B. m. handleyi* Packard, 1958 (type specimen from El Quiche, Guatemala); and *B. m. pullus* Packard, 1958 (type specimen from Esteli, Nicaragua; Figure 1).

Baiomys musculus mice have been studied from different perspectives, including assessment of morphometry ([Osgood 1909](#); [Hooper 1952](#); [Packard 1960](#)), karyotypes ([Lee and Elder 1977](#)), allozymes ([Calhoun et al. 1989](#)), demographic features and habitat preferences ([García-Estrada et al. 2002](#); [Schnell et al. 2008](#)), geometric morphometrics ([Abuzeineh 2006](#)), singing behavior ([Miller and Engstrom 2007](#)), intra-specific niche modeling ([Martínez-Gordillo et al. 2010](#)), landscape genetics ([Vargas et al. 2012](#)), and ecotoxicology ([Galván-Ramírez 2020](#)). With respect to taxonomic relationships, analysis of the mitochondrial gene cytochrome b (*CytB*), detected two clades within *B. musculus*, one located in the Mexican states of Jalisco and Michoacán, and the other in Chiapas, Guerrero, Oaxaca, and Puebla ([Amman and Bradley 2004](#)). The genetic divergence between these two clades (genetic p-distance = 6.46 %) suggests that both units may be eligible for species status ([Amman and Bradley 2004](#)), however, the eight subspecies are still recognized ([Pardiñas et al. 2017](#)).

Our objective was to revisit the taxonomic status of these two monophyletic groups within *B. musculus* using additional genetic and morphological data. New *CytB* sequences were obtained from GenBank and others were generated herein, including individuals from the previously unanalyzed states of Colima, Morelos, and Veracruz, and the previously unsampled subspecies *B. m. brunneus*. The mitochondrial *CytB* gene was chosen because of its availability and its proven utility to clarify phylogenetic relationships in other Neotominae rodents ([Edwards and Bradley 2002](#); [Arellano et al. 2005](#); [Bradley et al. 2007](#); [Rogers et al. 2007](#); [Vallejo and González-Cózatl 2012](#)). Although more than 1,700 specimens of *B. musculus* have been analyzed in previous morphological studies ([Osgood 1909](#); [Hooper 1952](#); [Packard 1960](#)), and it was validated that differences in size and coloration among the eight subspecies exist, none of these morphological studies specifically attempted to detect morphometric evidence to confirm or reject the hypothesis of two genetic clades ([Amman and Bradley 2004](#)).

Materials and Methods

DNA sequence data. Mitochondrial sequences of the complete *CytB* gene (1,143 base pairs) were obtained from five specimens (four *B. musculus* and one *B. taylori*) housed in the mammal collection of the Museo de Zoología, Facultad de Ciencias, Universidad Nacional Autónoma de México, Ciudad de México, México (MZFC). A Qiagen DNEasy Blood & Tissue kit (Qiagen, Germantown, Maryland) was used to extract whole genomic DNA following the manufacturer's recommended protocols. Polymerase chain reaction (PCR) was used to amplify this gene using the primers MVZ05 ([Smith and Patton 1993](#)) and H15915 ([Irwin et al. 1991](#)). Each PCR had a final reaction volume of 13 µL and contained 6.25 µL of GoTaq Green Master Mix (Promega, Madison, WI, U.S.A.), 4.75 µL of H₂O, 0.5 µL of each primer (10 µM), and 1 µL of DNA stock. The PCR thermal profile included 2 minutes of initial

denaturation at 95 °C, followed by 38 cycles of 30 seconds of denaturation at 95 °C, 30 seconds of annealing at 50 °C, and 68 seconds for extension at 72 °C. We included a 5-minute final extension step at 72 °C. PCR products (3 µL) were visualized using electrophoresis in 1 % agarose gels stained with SYBR Safe DNA Gel Stain (Life Technologies, Carlsbad, CA, U.S.A.). Each PCR product was purified with 1 µL of a 20% dilution of ExoSAP-IT (GE Healthcare Bio-Sciences Corp. Piscataway, NJ, U.S.A.), then incubated for 30 minutes at 37 °C followed by 15 minutes at 80 °C. Samples were cycle-sequenced using 6.1 µL of H₂O, 1.5 µL of 5x buffer, 1 µL of 10 µM primer, 0.4 µL of ABI PRISM Big Dye v. 3.1 (Applied Biosystems, Foster City, CA, U.S.A.), and 1 µL of the purified template. The cycle-sequencing profile included 1 minute of initial denaturation at 96 °C, followed by 25 cycles of 10 seconds for denaturation at 96 °C, 5 seconds for annealing at 50 °C, and 4 minutes for extension at 60 °C. Cycle sequencing products were purified using an EtOH-EDTA precipitation protocol and were read with an ABI 3130xl genetic analyzer (Applied Biosystems, Foster City, CA, U.S.A.). DNA sequences were edited, aligned, and visually inspected using MEGA X ([Kumar et al. 2018](#)) and FINCHTV 1.4 ([Patterson et al. 2004](#)). Nineteen additional *CytB* sequences of *B. musculus* were recovered from GenBank ([Amman and Bradley 2004](#); [Miller and Engstrom 2008](#); [Light et al. 2016](#)), so in total we analyzed 23 individuals of *B. musculus* (representing five subspecies: *B. m. brunneus*, *B. m. infernalis*, *B. m. musculus*, *B. m. nigriscens*, and *B. m. pallidus*; Figure 1) and one *B. taylori* individual was used as the outgroup (Appendix I). With this new sampling, we almost doubled the genetic samples analyzed by [Amman and Bradley \(2004\)](#), including samples from new localities, states, and subspecies previously not analyzed (Figure 1).

Maximum likelihood (ML) and Bayesian inference (BI) were used to estimate phylogenetic relationships of *B. musculus*. Prior to phylogenetic analyses, the best model and partition scheme (maximally divided by codon position) among all available models in MrBayes 3.2 ([Ronquist et al. 2012](#)) was selected based on the Bayesian Information Criterion (BIC) in PartitionFinder 2 ([Lanfear et al. 2016](#)). The IQ-TREE 1.6.12 ([Nguyen et al. 2015](#)) was used to estimate the ML gene tree, with branch support estimated by 1,000 replicates of nonparametric bootstrap. In MrBayes 3.2, three hot chains and one cold chain were used in two independent runs of 10 million generations, sampling data every 1,000 iterations. Convergence of MCMC results was determined by examining trace plots and sample sizes in Tracer 1.7 ([Rambaut et al. 2018](#)). The final topology was obtained using a majority rule consensus tree and considering a burn-in of 25 % (with effective sample sizes > 200). To evaluate levels of genetic differentiation, p-distances were estimated in Mega X using the pairwise deletion option and the Kimura 2-parameter model ([Kimura 1980](#)). These setting were chosen to facilitate comparison with previous works ([Bradley and Baker 2001](#); [Baker and Bradley 2006](#)).

Morphological analyses. Following the removal of subadult and damaged specimens, morphometric variation was analyzed in 47 specimens of *B. musculus* from three subspecies (*B. m. brunneus*, *B. m. musculus*, *B. m. pallidus*; Figure 1; Appendix II). Twelve cranial measurements as defined by [Ávila-Valle et al. \(2012\)](#) and [Hurtado and Pacheco \(2017\)](#) were obtained using a digital caliper (0.01 mm resolution) as follows: condyle-incisive length (CIL), braincase depth (BCD), braincase breadth (BCB), zygomatic breadth (ZB), interorbital constriction (IOC), rostral breadth (BR), maxillary toothrow length (MTL), breadth across M3-M3 (BMM), breadth of M1 (BM1), length of auditory bulla (LAB), dentary greatest length (DGL), and dentary height (DH). Age classes were assigned to the specimens following tooth eruption and wear patterns, and we only analyzed adult specimens. To determine if the molecular results were congruent with the morphological data, we specifically tested for morphological differences between the clades detected in the molecular analyses. Because we were interested in recognizing measurements useful to detect groups within *B. musculus*, and sexual size dimorphism has not been supported in this species ([Packard 1960](#); [Abuzeineh 2006](#)), females and males were analyzed together.

All univariate analyses and summary statistics were performed in R 3.6.2 ([R Core Team 2014](#)). QQ-plots and the Shapiro-Wilk test were used to analyze the normality of the data in each group (clades I and II) and the Levene test from the [CAR 3.0-6](#) package ([Fox and Weisberg 2019](#)) to test for homogeneity of variances between groups. All variables fulfilled the normality assumption, and all variables except for IOC and MTL fulfilled the assumption of homogeneity of variance. A Student's t-test was used to determine whether the two clades differed in each of the morphological measurements, using the Welch approximation to degrees of freedom to account for the heteroscedasticity for IOC and MTL (using var.equal = FALSE in the t.test function of R). A significance threshold (α) of 0.05 was implemented. Boxplots were plotted in [ggplot2 \(Wickham 2011\)](#) to better visualize the results.

Results

DNA sequence data. The final alignment included 207 variable characters, 92 singleton sites, and 115 parsimony informative characters. The best evolutionary model schemes were K80+I, F81+I, and GTR+G applied to the first, second,

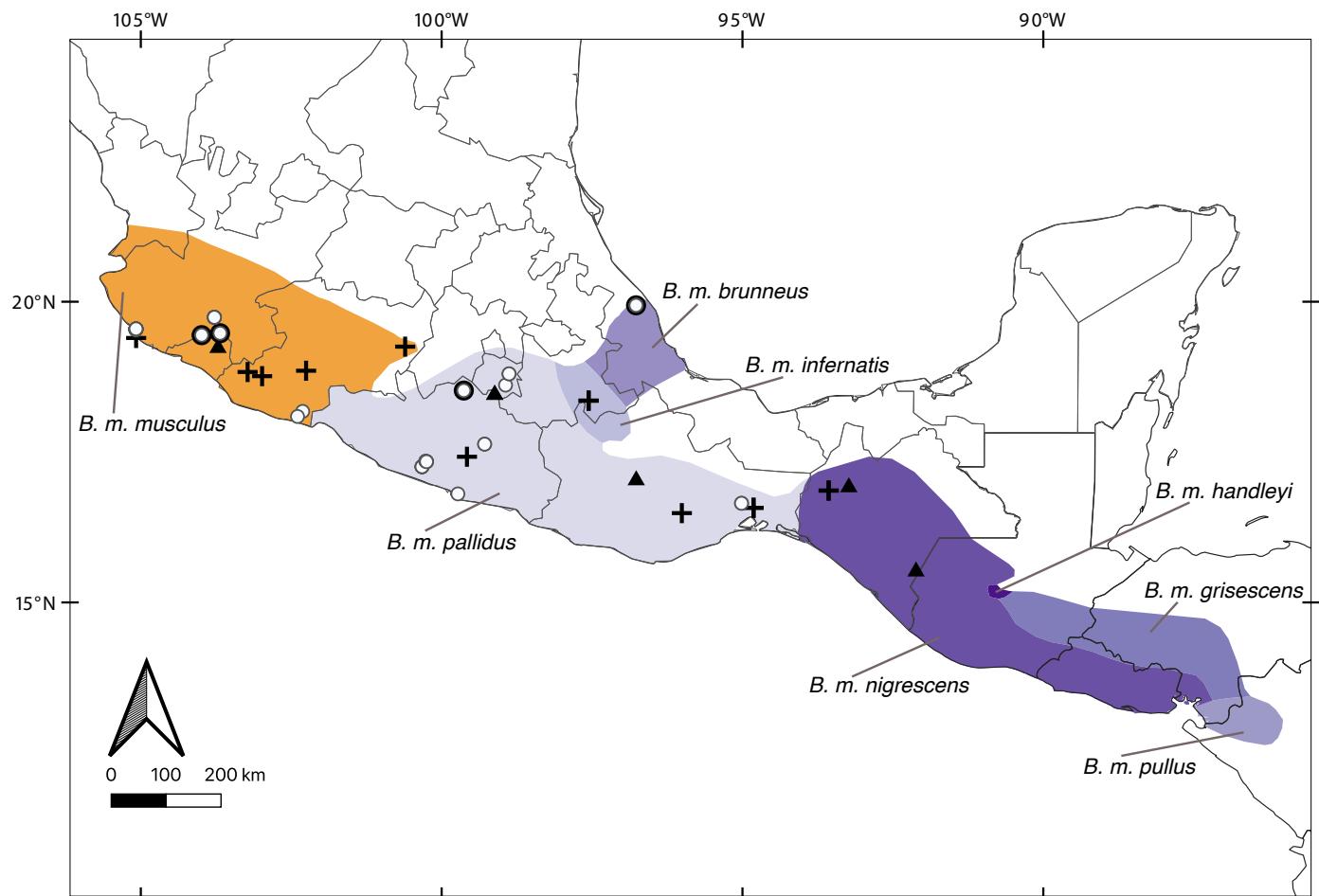


Figure 1. Geographic ranges of the eight recognized subspecies of *Baiomys musculus*, and specimens analyzed in this study. Individuals with cytochrome b sequences are represented by black crosses (sequences from Amman and Bradley 2004), black triangles (sequences from Miller and Engstrom 2008; Light et al. 2016), and black dots (this study). White dots represent individuals with morphological data (this study).

and third codon positions, respectively. Topologies from ML and BI trees were almost identical, but nodal support values were higher in BI (Figure 2). As previously reported, two well-supported clades were recovered within *B. musculus* (Amman and Bradley 2004). These clades were geographically structured, with clade I including samples from western México (Colima, Jalisco and Michoacán), and clade II including the rest of the samples. Clade II contained two sub-clades, one from central México (Guerrero, Morelos, and Puebla; clade II.a), and the other from eastern México (Chiapas, Oaxaca, and Veracruz; clade II.b). The K2P genetic distances between *B. taylori* and the two clades within *B. musculus* were > 11 %, the distance between clades I and II was 6.69 %, and between clades II.a and II.b was 3.98 %.

Morphological analyses. Based on molecular results, individuals were assigned to two *a priori* groups: clade I (Colima, Jalisco, and Michoacán; $n = 26$, 1 of them sequenced) and clade II (Guerrero, Morelos, Oaxaca, and Veracruz; $n = 21$, 2 of them sequenced; Appendix 2). Although some measurements overlapped between clades, individuals from clade I had significantly larger measurements than clade II in nine morphometric variables (Figure 3 and Table 1; all of the morphometric variables measured, except for BR, BMM, and LAB).

Discussion

The two mitochondrial lineages within *B. musculus* previously detected by Amman and Bradley (2004; Figure 2) were recovered, because their clade C is equivalent to our clade I. In addition, the K2P genetic distance between clades I and II was similar to those observed in other recognized sister species in Neotominae, such as in the genus *Megadontomys* (Vallejo and González-Cózatl 2012), *Neotoma* (Hernández-Canchola et al. 2021), *Peromyscus* (Bradley et al. 2007), among others (Bradley and Baker 2001; Baker and Bradley 2006). These two clades seem to be allopatric, but more thorough sampling is needed to determine if there is a distinct boundary between them. The complex topography between the Sierra Madre del Sur and the Transmexican Volcanic Belt, in addition to the mouth of the Balsas River, could be acting as geographic barriers to gene flow between clades in the southern pygmy mouse (Amman and Bradley 2004; Figure 4), and this limit aligns well with boundaries between other cryptic sister mammal taxa such as the mouse opossums (Arcangeli et al. 2018) and the Osgood's deer mice (Ruiz-Vega et al. 2018). Additionally, multiple diversification events in western México generated many Mexican endemic mammal species, including

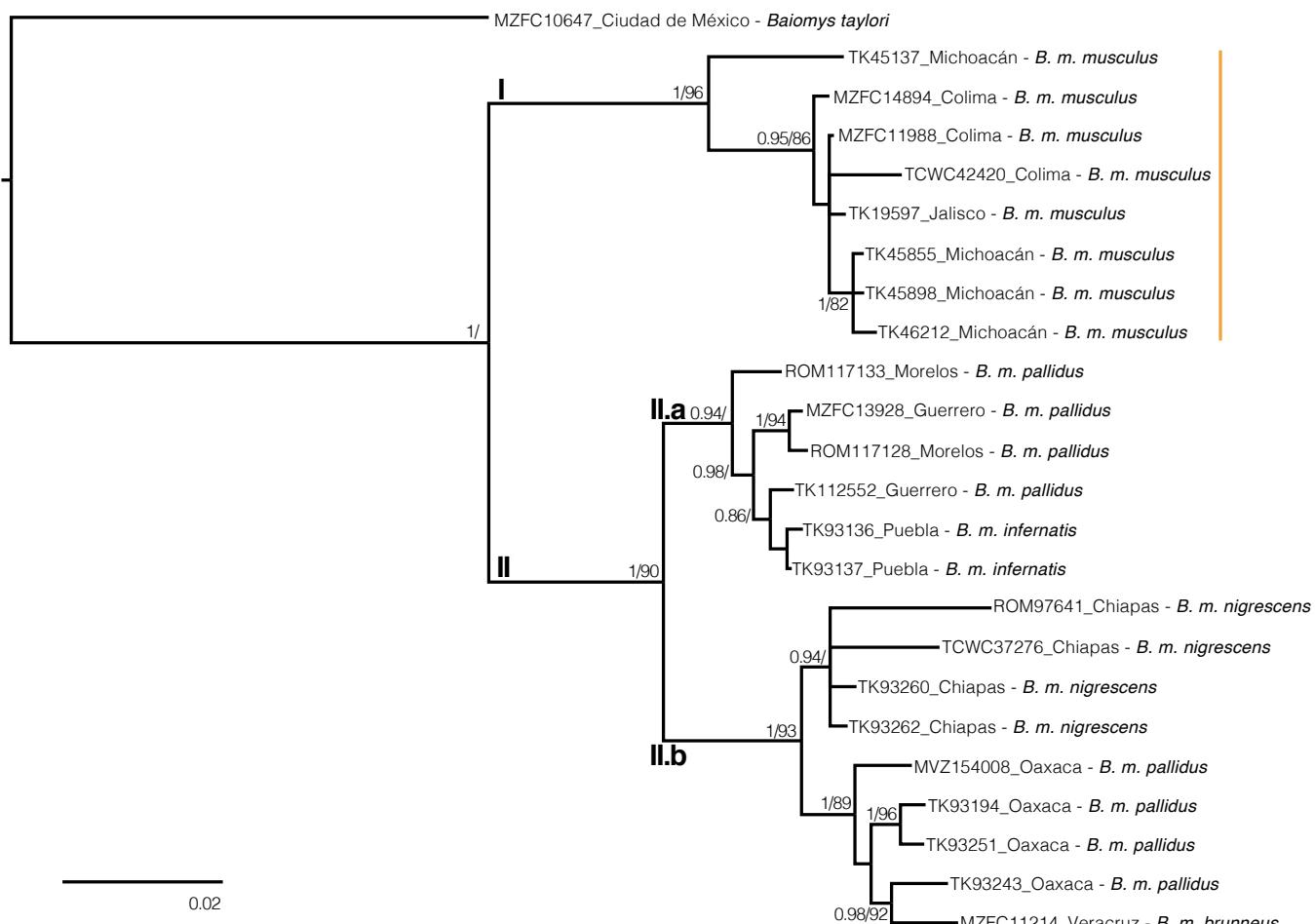


Figure 2. Majority rule consensus tree for *Baiomys musculus*, obtained from the Bayesian analysis of the mitochondrial cytochrome b sequences. Support values are shown as posterior probabilities followed by bootstrap values from a maximum likelihood analysis. Support values < 0.8/80 are not shown. Tip labels show the catalog number, the Mexican state of each sample and the taxa of each tip. Clade I is in orange and clade II in purple.

other neotomine rodents (*Osgoodomys banderanus*, *Xenomys nelsoni*, *Hodomys allenii*, and *Peromyscus perfulvus*), in addition to the pygmy spotted skunk (*Spilogale pygmaea*), two species of mouse opossums (*Tlacuatzin*), the Mexican shrew (*Megasorex gigas*), and the banana bat (*Musonycteris harrisoni*), among others (Ceballos 2014; Arcangeli et al. 2018). This evidence suggests that the evolutionary history of clades I and II within *B. musculus* could have been influenced by the complex topography of western México, as in many other mammal taxa.

Baiomys musculus from clade I are distributed in Colima, Jalisco, and Michoacán, and specimens from these Mexican states were the largest individuals examined. Although the sample size was relatively small, our results are consistent with previous morphological evaluations that analyzed larger numbers of specimens from Guatemala and México ($n = 299$, Osgood 1909), México ($n = 351$, Hooper 1952), and

from El Salvador, Guatemala, Honduras, México, and Nicaragua ($n = 1,748$; Packard 1960). In these previous morphological reviews, individuals from western México (Nayarit, Jalisco, Colima, and Michoacán) were larger in external and cranial dimensions than other Mexican and Central American specimens. This morphological differentiation of the populations from western México has been recognized for many years, as evidenced by their recognition as the subspecies *B. m. musculus* (Osgood 1909; Hooper 1952; Packard 1960; Figure 1).

Packard (1960) also noted increasing size in *B. musculus* from south to north. This general trend follows Bergmann's rule. However, *B. m. musculus* is the most distinct subspecies of the southern pygmy mouse, and it was proposed that its difference could be related to character displacement (Packard 1960) that could magnify the Bergmann's rule, because in western México *B. musculus* and *B. taylori*

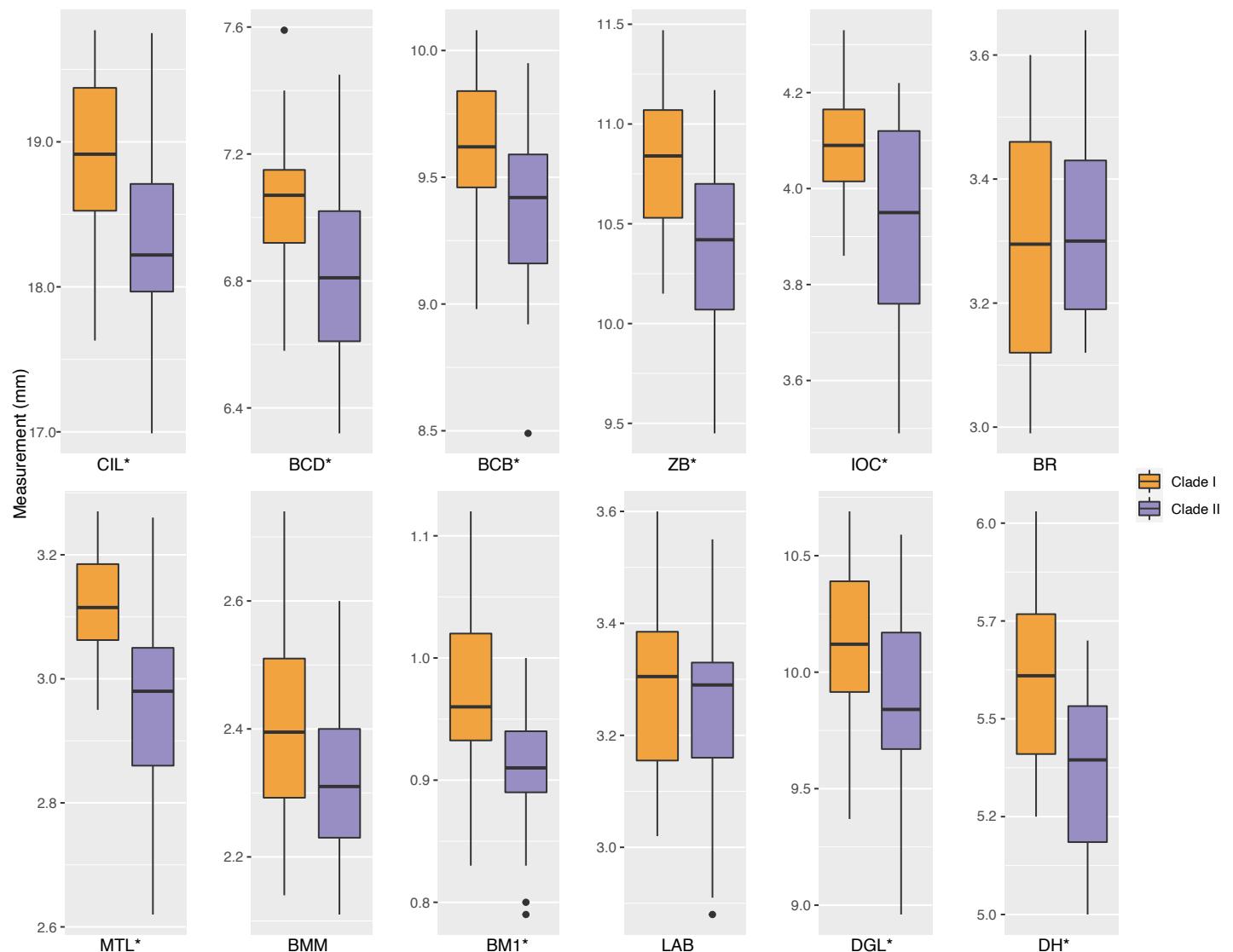


Figure 3. Boxplots summarizing the cranial and mandibular measurements of *Baiomys musculus*. Each plot represents a morphometric variable: condyle-incisive length (CIL), braincase depth (BCD), braincase breadth (BCB), zygomatic breadth (ZB), interorbital constriction (IOC), rostral breadth (BR), maxillary toothrow length (MTL), breadth across M3-M3 (BMM), breadth of M1 (BM1), length of auditory bulla (LAB), dentary greatest length (DGL), and dentary height (DH). P -values that were significant different (< 0.05) between clades are shown with an asterisk. Horizontal lines represent medians, boxes span the interval between the 25th and 75th percentiles, and the range of the vertical lines show the minimum and maximum values for each variable. Black dots show measurements that are farther from the mean than 1.5 times the interquartile range.

Table 1. Summary statistics and test statistics of morphological variables measured in *B. musculus* (condyle-incisive length, CIL; braincase depth, BCD; braincase breadth, BCB; zygomatic breadth, ZB; interorbital constriction, IOC; rostral breadth, BR; maxillary toothrow length, MTL; breadth across M3-M3, BMM; breadth of M1, BM1; length of auditory bulla, LAB; dentary greatest length, DGL; and dentary height, DH). Mean and standard deviation (sd) are shown for each clade, followed by the Student's t statistic (t), degrees of freedom (df; estimated using the Welch approximation for IOC and MTL to account for heteroscedasticity), and the P-value (P). P-values that were significant (< 0.05) are shown in bold type.

	Clade I		Clade II				
	Mean	sd	Mean	sd	t	df	P
CIL	18.90	0.576	18.30	0.687	3.20	44.0	0.003
BCD	7.05	0.229	6.83	0.293	2.84	45.0	0.007
BCB	9.65	0.265	9.36	0.344	3.20	44.0	0.003
ZB	10.80	0.361	10.40	0.480	3.32	44.0	0.002
IOC	4.09	0.116	3.95	0.219	2.76	28.9	0.010
BR	3.30	0.185	3.32	0.163	-0.35	45.0	0.729
MTL	3.12	0.088	2.97	0.155	3.92	30.2	<0.001
BMM	2.40	0.152	2.32	0.128	1.86	45.0	0.070
BM1	0.98	0.072	0.91	0.056	3.84	45.0	<0.001
LAB	3.28	0.169	3.23	0.175	0.92	45.0	0.365
DGL	10.10	0.358	9.85	0.445	2.25	42.0	0.030
DH	5.60	0.226	5.37	0.217	3.33	40.0	0.002

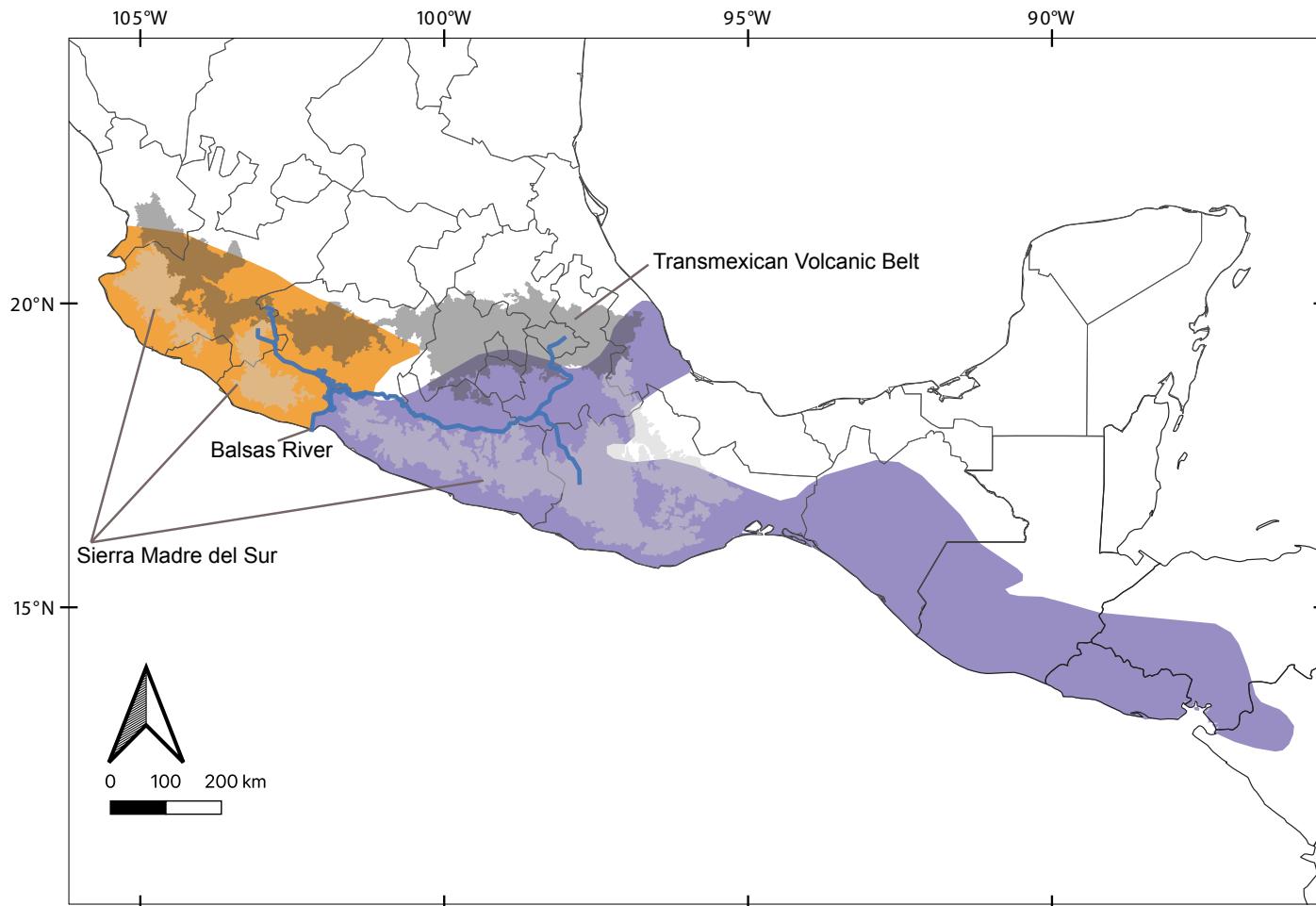


Figure 4. Revisited geographic ranges in the southern pigmy mice. Map colors show the preliminary suggested geographic ranges within *Baiomys musculus* of clade I (orange) and *B. brunneus* clade II (purple). The main biogeographic barriers discussed in this work also are shown.

are sympatric and they are more morphologically distinct than where they are allopatric (Hooper 1952; Packard 1960). Analyses of cranial geometric morphometrics rejected the character displacement hypothesis between *B. musculus* and *B. taylori* (Abuzeineh 2006), but this proposal has not been tested in postcranial structures, or with other methodologies, and deserves attention to understand the processes that originated sympatric populations of *B. musculus* clade I / *B. taylori*, and the allopatric populations of *B. musculus* clade II / *B. taylori*. Martínez-Gordillo et al. (2010) tested whether clades I and II had different ecological niches, and although clade I inhabits warmer and drier environments than clade II, the ecological models showed nesting of the niches of the two clades (ecological niche modeling of clade I recovered most of the distribution of clade II, and vice-versa). Interestingly, *B. taylori*, which inhabits from the United States of America to central México, also has two detected clades with a lower genetic divergence than *B. musculus* (2.82 %; Amman and Bradley 2004), but the ecological niches were different between the two (Martínez-Gordillo et al. 2010). These contrasting genetic and environmental results between the northern and southern pygmy mice could suggest that different evolutionary processes are acting in each taxon within *Baiomys*.

In addition to the high levels of mitochondrial divergence ([Amman and Bradley 2004](#)) and morphological differences (external size, coat color, and certain cranial measurements; see [Packard 1960](#) for more details) between *B. m. musculus* and the remaining subspecies, a substantial subdivision between *B. musculus* samples from Jalisco and Colima ($n = 9$) versus Chiapas, Oaxaca, and Veracruz ($n = 11$) was reported using allozymes ([Calhoun et al. 1989](#)). In the present work, more than using our limited sampling to draw conclusions about the taxonomic status of *B. m. musculus* (Figures 2, 3, Table 1), we aim to integrate the multiple and impressive reviews that have analyzed the variation within *B. musculus* ([Osgood 1909](#); [Hooper 1952](#); [Packard 1960](#); [Calhoun et al. 1989](#); [Amman and Bradley 2004](#)).

Considering all of these data, collected independently over more than a century, it appears that clade I, the Mexican endemic pygmy mice that inhabits in Colima, Jalisco, Michoacán, and Nayarit, could be named as *B. musculus* (Merriam 1892), and that all other populations of the southern pygmy mouse (including the brunneus, griseocens, handleyi, infernatis, nigrescens, pallidus, and pullus populations) seem to merit specific status and, following taxonomic priority, could be referred to as *B. brunneus* (Allen and Chapman, 1897). However, studies of additional data sets, such as nuclear / genomic data or Central American populations, will be required to definitively confirm the taxonomic status of these specimens. The substructure detected within clade II (Figure 2) also deserves additional attention: clade II.a appears to be restricted to the Balsas Basin and clade II.b to southeastern México. This phylogeographic pattern also has been reported in other mammal species, such as the Mesoamerican yellow-shouldered bat ([Hernández-Canchola and León-Paniagua 2017](#)) and the nine-banded armadillo ([Arteaga et al. 2012](#)) and this genetic differentiation does not agree with the geographic boundaries between subspecies of *B. musculus* (Figures 1, 2), so future studies will be needed to verify their validity. Basic aspects of the neotomine rodents, such as the number of species that inhabit North and Central America, are still not clear ([Miller and Engstrom 2008](#); [Platt et al. 2015](#); [Sullivan et al. 2017](#)). This knowledge gap will likely continue to exist in taxa that are rare and/or have restricted distributions ([Gardner and Carleton 2009](#); [Fernández 2014](#)), unless the use of Next Generation Sequencing methods allow DNA data to be obtained from ancient specimens hosted in mammal collections ([Castañeda-Rico et al. 2020](#)). However, in more common and abundant species, as *B. musculus*, it will be easier to obtain and analyze data to solve these taxonomic uncertainties, which will allow us to understand the processes that generate and maintain biodiversity ([Upham et al. 2019](#)).

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Appendix 1

Specimens with the mitochondrial cytochrome b analyzed in this work.

Taxa	Mexican state	Lat	Long	Catalog #	Tissue #	Genbank
<i>B. taylori</i>	Ciudad de México	19.325	-98.986	MZFC 10647	5043	MZ056876
<i>B. musculus</i>	Colima	19.478	-103.683	MZFC 11988	4439	MZ056877
<i>B. musculus</i>	Colima	19.445	-103.989	MZFC 14894	6295	MZ056878
<i>B. musculus</i>	Colima	19.233	-103.717	TCWC 42420		MZ056878
<i>B. musculus</i>	Jalisco	19.398	-104.962	TTU 37737	TK19597	AF548478
<i>B. musculus</i>	Michoacán	19.254	-100.491		TK45137	AF548484
<i>B. musculus</i>	Michoacán	18.763	-102.868		TK45855	AF548483
<i>B. musculus</i>	Michoacán	18.833	-103.109		TK45898	AF548485
<i>B. musculus</i>	Michoacán	18.854	-102.137		TK46212	AF548482
<i>B. b. brunneus</i>	Veracruz	19.941	-96.769	MZFC 11214	3281	MZ056880
<i>B. b. infernatis</i>	Puebla	18.356	-97.442	TTU 82658	TK93136	AF548488
<i>B. b. infernatis</i>	Puebla	18.356	-97.442	TTU 82659	TK93137	AF548489
<i>B. b. nigrescens</i>	Chiapas	15.517	-92.117	ROM 97641		EF989933
<i>B. b. nigrescens</i>	Chiapas	16.917	-93.233	TCWC 37276		KU298967
<i>B. b. nigrescens</i>	Chiapas	16.861	-93.453		TK93260	AF548486
<i>B. b. nigrescens</i>	Chiapas	16.861	-93.453		TK93262	AF548487
<i>B. b. pallidus</i>	Guerrero	18.525	-99.632	MZFC 13928	3614	MZ056879
<i>B. b. pallidus</i>	Guerrero	17.424	-99.464	TTU 90341	TK112552	AF548490
<i>B. b. pallidus</i>	Morelos	18.454	-99.119	ROM 117128		EF989934
<i>B. b. pallidus</i>	Morelos	18.454	-99.119	ROM 117133		EF989935
<i>B. b. pallidus</i>	Oaxaca	17.033	-96.767	MVZ 154008		KU298966
<i>B. b. pallidus</i>	Oaxaca	16.486	-95.893		TK93194	AF548481
<i>B. b. pallidus</i>	Oaxaca	16.575	-94.701		TK93251	AF548479
<i>B. b. pallidus</i>	Oaxaca	16.575	-94.701		TK93253	AF548480

Appendix 2

Specimens with morphological data analyzed in this work. Specimens that have cytochrome b sequences are showed with an asterisk.

Taxa	state	Lat	Long	Sex	Catalog #	Field #
<i>B. musculus</i>	Colima	19.446	-103.989	Female	MZFC 14896	MLR160
<i>B. musculus*</i>	Colima	19.478	-103.683	Male	MZFC 11988	MYACH430
<i>B. musculus</i>	Jalisco	19.531	-105.083	Female	MZFC 9818	MCHA001
<i>B. musculus</i>	Jalisco	19.742	-103.778	Male	MZFC 10458	FCR074
<i>B. musculus</i>	Jalisco	19.742	-103.778	Female	MZFC 10459	FCR075
<i>B. musculus</i>	Jalisco	19.742	-103.778	Female	MZFC 10460	FCR076
<i>B. musculus</i>	Jalisco	19.548	-105.082	Male	MZFC 10685	CHAM036
<i>B. musculus</i>	Jalisco	19.548	-105.082	Male	MZFC 10686	CHAM060
<i>B. musculus</i>	Jalisco	19.546	-105.082	Female	MZFC 12368	MCHAM119
<i>B. musculus</i>	Jalisco	19.546	-105.082	Female	MZFC 12369	MCHAM123
<i>B. musculus</i>	Jalisco	19.546	-105.082	Female	MZFC 12370	MCHAM126
<i>B. musculus</i>	Jalisco	19.546	-105.082	Female	MZFC 12371	MCHAM127
<i>B. musculus</i>	Jalisco	19.546	-105.082	Male	MZFC 12373	MCHAM132
<i>B. musculus</i>	Jalisco	19.546	-105.082	Male	MZFC 12374	MCHAM133
<i>B. musculus</i>	Jalisco	19.550	-105.080	Male	MZFC 12775	MCHAM111
<i>B. musculus</i>	Jalisco	19.550	-105.080	Male	MZFC 12777	MCHAM110
<i>B. musculus</i>	Jalisco	19.550	-105.080	Female	MZFC 12778	MCHAM108
<i>B. musculus</i>	Jalisco	19.550	-105.080	Female	MZFC 12779	MCHAM107
<i>B. musculus</i>	Jalisco	19.547	-105.081	Male	MZFC 12781	MCHAM098
<i>B. musculus</i>	Jalisco	19.550	-105.080	Male	MZFC 12783	MCHAM103
<i>B. musculus</i>	Jalisco	19.742	-103.778	Female	MZFC 12795	FCR085
<i>B. musculus</i>	Jalisco	19.742	-103.778	Male	MZFC 12798	FCR092
<i>B. musculus</i>	Jalisco	19.742	-103.778	Female	MZFC 12799	FCR097
<i>B. musculus</i>	Jalisco	19.742	-103.778	Male	MZFC 13616	FCR073
<i>B. musculus</i>	Michoacán	18.178	-102.310	Female	MZFC 10196	MBB108
<i>B. musculus</i>	Michoacán	18.093	-102.396	Female	MZFC 10197	MBB113
<i>B. b. brunneus</i>	Veracruz	19.941	-96.769	Female	MZFC 11094	MRM031
<i>B. b. brunneus*</i>	Veracruz	19.941	-96.769	Male	MZFC 11214	MRM038
<i>B. b. brunneus</i>	Veracruz	19.941	-96.769	Female	MZFC 11219	MRM032
<i>B. b. pallidus</i>	Guerrero	16.806	-99.731	Male	MZFC 727	2205-132MTB
<i>B. b. pallidus</i>	Guerrero	17.342	-100.252	Female	MZFC 2356	953JJG
<i>B. b. pallidus</i>	Guerrero	17.258	-100.327	Male	MZFC 2357	1150JJG
<i>B. b. pallidus</i>	Guerrero	17.342	-100.252	Female	MZFC 2358	949JJG
<i>B. b. pallidus</i>	Guerrero	17.342	-100.252	Male	MZFC 2359	957JJG
<i>B. b. pallidus</i>	Guerrero	17.258	-100.327	Male	MZFC 2360	1105JJG
<i>B. b. pallidus</i>	Guerrero	17.258	-100.327	Female	MZFC 2361	1104JJG
<i>B. b. pallidus</i>	Guerrero	17.258	-100.327	Male	MZFC 2362	1103JJG
<i>B. b. pallidus</i>	Guerrero	17.352	-100.266	Male	MZFC 2363	916JJG
<i>B. b. pallidus</i>	Guerrero	17.342	-100.252	Male	MZFC 2364	958JJG
<i>B. b. pallidus*</i>	Guerrero	18.525	-99.632	Female	MZFC 13928	GHC035
<i>B. b. pallidus</i>	Guerrero	17.633	-99.286	Male	MZFC 14875	MLR113
<i>B. b. pallidus</i>	Guerrero	17.633	-99.286	Male	MZFC 14879	MLR109
<i>B. b. pallidus</i>	Morelos	18.614	-98.938	Female	MZFC 9591	RAG375
<i>B. b. pallidus</i>	Morelos	18.802	-98.880	Female	MZFC 13918	GHC076
<i>B. b. pallidus</i>	Morelos	18.802	-98.880	Female	MZFC 13919	GHC086
<i>B. b. pallidus</i>	Oaxaca	16.650	-95.017	Male	MZFC 10055	NIZA043
<i>B. b. pallidus</i>	Oaxaca	16.650	-95.017	Male	MZFC 10056	NIZA044