UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO
POSGRADO EN CIENCIAS BIOLÓGICAS

FACULTAD DE CIENCIAS

BIOLOGÍA EVOLUTIVA

FILOGEOGRAFÍA COMPARADA DE ROEDORES MONTANOS DE LA ZONA DE TRANSICIÓN MESOAMERICANA

TESIS

QUE PARA OPTAR POR EL GRADO DE:

DOCTORA EN CIENCIAS

PRESENTA:

ZAMIRA ANAHÍ ÁVILA VALLE

TUTOR PRINCIPAL DE TESIS: **DOCTOR ADOLFO GERARDO NAVARRO SIGÜENZA**
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México D. F., 7 de mayo de 2014

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POSGRADO EN CIENCIAS BIOLÓGICAS
FACULTAD DE CIENCIAS
DIVISIÓN DE ESTUDIOS DE POSGRADO

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ASUNTO: Oficio de Jurado

Dr. Isidro Ávila Martínez
Director General de Administración Escolar, UNAM
P r e s e n t e

Me permito informar a usted que en la reunión ordinaria del Comité Académico del Posgrado en Ciencias Biológicas, celebrada el día **10 de febrero de 2014**, se aprobó el siguiente jurado para el examen de grado de **DOCTORA EN CIENCIAS** del (la) alumno (a) **ÁVILA VALLE ZAMIRA ANAHÍ** con número de cuenta **93150695** con la tesis titulada: **"FILOGEOGRAFÍA COMPARADA DE ROEDORES MONTANOS DE LA ZONA DE TRANSICIÓN MESOAMERICANA",** realizada bajo la dirección del (la**) DR. ADOLFO GERARDO NAVARRO SIGÜENZA:**

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

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…No existe falta de tiempo, existe falta de interés. Porque cuando la gente realmente quiere, la madrugada se vuelve día; el martes se vuelve sábado y un momento se vuelve oportunidad… - Dalai Lama

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RESUMEN

El presente trabajo consta de tres capítulos, en los dos primeros se realizó un análisis sistemático con la finalidad de obtener filogenias resueltas que se usarían, junto con secuencias de roedores ya publicados, en el tercer capítulo.

En el primer capítulo se estudia a la especie *Peromyscus furvus* desde dos aspectos, primero se analizó la variación geográfica de la especie considerando caracteres morfométricos tradicionales, mientras que en la segunda se obtuvo una filogenia con datos moleculares que muestra las relaciones entre las poblaciones de *P. furvus*, destacando la separación de las poblaciones atribuidas anteriormente a *P. latirostris*, la cual se propone sea una especie independiente.

En el siguiente capítulo se analizó la relación entre las poblaciones designadas a la especie *Peromyscus mexicanus*, cuya filogenia refleja la complejidad entre sus poblaciones. Los resultados señalan la carencia de una estructuración geográfica, siendo más bien los aspectos ecológicos los que rigen la separación de las poblaciones.

Finalmente, el tercer capítulo muestra la filogeografía comparada de cinco especies de roedores montanos con base en la divergencia y diversidad genéticas. Se generaron mapas genéticos (divergencia y diversidad) por especie y múltiple sobre las zonas montanas de México y se sugiere que, por un lado, las zonas montanas de Oaxaca son un sitio de unión entre poblaciones del centro de México con el sur y hacia Centroamérica y, por otro, éstas zonas promueve una diversificación alta, debido a la influencia de aspectos ecológico-geográficos.

ABSTRACT

This work contains three chapters, the first and second chapter comprise systematic analysis in order to obtain resolved phylogenies. These phylogenies, along with other published rodent sequences were analized to generate the third chapter.

In the first chapter we studied *Peromyscus furvus* species from two perspectives, first one, was examined the geographic variation of the species based on morphometric characters, while in the second we obtained a phylogeny with molecular data where the monophyly of *P. furvus* shown as well as the separation of populations attributed to *P. latirostris*, which is proposed to be a separate species.

In the next chapter, was analyzed the relationship of populations designated as *Peromyscus mexicanus* species whose phylogeny reflects the complexity among populations. The results indicates the lack of geographic structuring, being more ecological aspects governing the separation of populations.

Finally, the last chapter shows the comparative phylogeography of five species of montane rodents based on genetic divergence and diversity. Genetic maps (divergence and diversity) per species and multiple were generated on montane areas of Mexico and suggests that, on the one hand, montane areas of Oaxaca are a binding site among populations from central Mexico to the south and Central America and, secondly, these zones promotes a highly diversified through the influence of ecological and geographical aspects .

Introducción general

INTRODUCCIÓN

La biogeografía se encarga del estudio de la distribución de los seres vivos y sus causas, las cuales pueden ser explicadas a nivel histórico o ecológico. La descripción de los patrones de distribución de especies y taxa supraespecíficos pretende dar una explicación del origen de su distribución (Morrone 2004a). Actualmente, existe la tendencia a integrar los enfoques histórico y ecológico de la biogeografía para entender mejor los procesos biogeográficos (Halffter 2003, Morrone 2004a). Por ello, se propone categorizar los procesos como medida para explicar tendencias histórico-evolutivas, utilizando subgrupos dentro de subgrupos (Lieberman 2003, Morrone 2004a). Así, se busca que, a partir de datos particulares (*e. g.*, secuencias genéticas de una especie), se reconstruyan historias evolutivas (i. e. árboles filogenéticos resueltos), con la intención de encontrar patrones evolutivos que pudieran afectar a biotas completas.

La sistemática molecular ha tenido mucho auge en disciplinas que buscan esclarecer las relaciones de ancestro-descendencia de los taxones desde el punto de vista evolutivo, las cuales son tomadas como base para contestar preguntas generales sobre aspectos taxonómicos y biogeográficos, así como para entender la distribución y el establecimiento de las especies en las áreas. El reconocimiento de trazos genealógicos, a través de las fronteras genéticas en niveles taxonómicos como especies, géneros y familias, se realiza principalmente con el DNA mitocondrial (DNAmt), seguido del DNA nuclear (DNAn) y RNA's mensajero, de transferencia y ribosomal (Bermingham & Moritz 1998). Los primeros estudios que proponen la integración de las filogenias moleculares específicas con la historia o características de las áreas de distribución de las especies originaron a la filogeografía (Avise et al. 1987, Avise 2000, Crisci et al. 2003), la cual surge a finales de los años 1980, y cuya importancia radica en la búsqueda de los principios y procesos que determinan la distribución geográfica de los linajes genealógicos y divergencia genética dentro y entre especies cercanas (Avise et al. 1987, Avise 2000,

Lapointe & Rissler 2005, Michaux & Filippucci 2005). Éste tipo de filogeografía, conocida como filogeografía intraespecífica, permite visualizar solamente la historia y estructura evolutivas entre las poblaciones de una especies (Crisci et al. 2003). Si consideramos que la robustez de una hipótesis biogeografía depende en gran medida de la repetición de modelos en diferentes grupos (Bernatchez & Wilson 1998, Zink 2002, Lapointe & Rissler 2005, Michaux & Filippucci 2005), entonces, las predicciones biogeográficas basadas en una sola especie pueden ser falsas o endebles (Bermingham & Moritz 1998, Arbogast & Kenagy 2001, Hickerson et al. 2006). Por lo anterior, para obtener un análisis comparado en Filogeografía, se evaluaba la congruencia entre los árboles filogeográficos de las especies comprendidas en un área y con ello se explica el surgimiento de las mismas tomando en cuenta los procesos de ensamblaje vistos en especies actuales (Avise et al. 1987, Bermingham & Moritz 1998, Bernatchez & Wilson 1998, Sullivan et al. 2000, Avise 2000, Arbogast & Kenagy 2001, Zink et al. 2001, Zink 2002, Hoffmann & Baker 2003, Hickerson et al. 2006). Acompañando a las representaciones filogeográficas se encuentran datos obtenidos con herramientas de la genética de poblaciones, que permiten enfatizar el análisis de procesos tales como evolución de áreas, distribución de especies en una región, especiación, radiación adaptativa y extinción (Evans et al. 1997, Bermingham & Moritz 1998, Moritz & Faith 1998).

El desarrollo del presente trabajo está desglosado en tres partes, en las dos primeras partes se obtuvieron filogenias resueltas de dos especies de peromiscinos, la primera endémica a los bosques mesófilos de montaña del oriente de México, mientras que la segunda presenta una distribución más amplia tanto geográfica como en ecosistemas. El tercer capítulo se realizó con los resultados obtenidos en los dos primeros capítulos y con secuencias publicadas de roedores con la finalidad de dar mejor aproximación a los análisis filogeográficos.

El primer capítulo comprende el análisis de las relaciones filogenéticas entre las poblaciones designadas bajo la especie *Peromyscus furvus*, desde dos perspectivas, en la primera se examinó la variación geográfica de 17 localidades grupo, con base en caracteres morfométricos tradicionales. Se realizó un análisis de agrupamiento, en donde las poblaciones presentaron un arreglo en tamaño y con el cual se construyeron 5 cinco grupos morfométricos. Éstos grupos se retomaron para la segunda parte, la cual se realizó con datos moleculares y que sirvieron para obtener una filogenia que muestra la monofilia de *P. furvus*, haciendo énfasis en la separación de las poblaciones del norte de la distribución de la especie correspondiente a uno de los grupos morfométricos, el cual anteriormente se describió como *P. latirostris*. Por lo tanto, consideramos que la especie conocida como *P. furvus* en realidad contiene a otra especie (*P. latirostris*) por lo que proponemos reestablecer a *P. latirostris* como una especie independiente de *P. furvus*.

En el segundo capítulo se analizaron las relaciones filogenéticas de cuatro subespecies de *Peromyscus mexicanus*, ubicadas en la zona montanas del este de México y cuya distribución abarca tanto zonas tropicales como zonas templadas, por lo que la especie se encuentra en diferentes ambientes y altitudes. Con el análisis de varianza molecular (AMOVA) se observa la existencia de una estructuración entre las poblaciones de la especie; sin embargo, los datos de divergencia de las poblaciones carecen de una estructuración geográfica. La filogenia refleja conflictos tanto entre las poblaciones atribuidas a *P. mexicanus*, como con otras especies de peromiscinos analizadas. La mayoría de las poblaciones tienen un arreglo relacionado con aspectos ecológicos más que un arreglo geográfico, además de que algunas poblaciones oaxaqueñas se asociaron con especies consideradas como grupo externo. Por lo anterior, se propone en el trabajo que los caracteres que permiten distinguir a la especie están subordinados a los aspectos ecológicos generando especies crípticos por un lado y grupos parafiéticos por otro.

En el último capítulo se analizó la divergencia y diversidad genéticas de cinco especies de roedores cuya distribución se ubicara en las zonas montanas de la porción nororiental de la zona de transición mesoamericana. Para cada especie se obtuvieron mapas genéticos (divergencia y diversidad) que, junto con las ecorregiones y provincias fisiográficas, permitieran reconocer las zonas de rompimiento del flujo genético para así inferir patrones de distribución generales en roedores. Resaltamos en el trabajo que la zona de estudio, sobretodo la porción oaxaqueña, contiene la mayor divergencia y diversidad genéticas, reflejado en los mapas múltiples, por lo que es factible suponer que ésta zona promueve la diversificación *in situ*, producto de aspectos ecológico-geográficos.

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Capítulo 1. Geographic variation and molecular evidence of the Blackish Deer Mouse complex (*Peromyscus furvus*, Rodentia: Muridae)

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Original Investigation

Geographic variation and molecular evidence of the Blackish Deer Mouse complex (*Peromyscus furvus*, Rodentia: Muridae)

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ABSTRACT

Several authors have discussed whether *Peromyscus furvus* is a monotypic species rather than a polytypic entity, that it includes more than one species. Here, we analyze these questions by means of traditional morphometrics and by genetic analyses using ND3-ND4 mtDNA genes as markers. In spite of a generalized overlap of the measurable characters among populations, our analyses show that the northernmost populations, which was assignable to *P. latirostris*, consistently show larger dimensions overall. The amount of genetic differentiation revealed by our molecular data, support conclusive evidence to suggest this taxon is a valid species. Our results also disclose that morphometric and molecular segregation between *P. furvus* and *P. angustirostris* is still incomplete. Finally, the two populations from the state of Oaxaca showed more morphometric affinity with those attributable to *P. furvus* and revealed a discrete degree of genetic differentiation. Nevertheless, their systematic position is not clear yet.

Introduction

Peromyscus furvus J.A. Allen and Chapman, 1897 is currently regarded as a monotypic species endemic to Mexico (Musser 1964; Hall 1971; Ramírez-Pulido and Müdespacher 1987; Cervantes et al. 1994; Arita and Ceballos 1997; Martínez-Coronel et al. 1997; Harris and Rogers 1999; Harris et al. 2000; Musser and Carleton 2005). They are inhabitants of the moist and temperate highland forests (elevations from 226 to 2960 m), in a thin strip along the Sierra Madre Oriental that contacts the Mexican Transvolcanic Belt in the middle and ends in the Northern Sierra of Oaxaca (Allen and Chapman 1897; Dalquest 1950; Hall and Álvarez 1961; Huckaby 1980; Hall 1981), mainly associated with temperate semi-humid forests of coniferous trees (*Pinus* sp., *Abies religiosa*, and *Juniperus* sp.) and especially in cloud forests between 1300 and 2950 m, even in conditions where the vegetation includes secondary vegetation of shade-coffee plantations (*Coffea* sp.), so long as the herbaceous stratus remains thick (Musser 1964; Hall 1971); the main climate types in these plant communities belong to the C(fm), temperate-humid with rain all the year round, and to the C(w), temperate-subhumid with rain during the fall, respectively (García 1973).

All populations known as the Blackish Deer Mouse share a dark coloration of the fur (Allen and Chapman 1897; Huckaby 1980; Hall 1981), big ears, and a long slightly scaled tail, together with a large average size (229–300, 114–162, 26–33, and 20–23, Hall 1981). In general, the skull is bulky, strong, and heavy with anteriorly expanded zygomata and a narrow interorbital constriction. It is distinctive for its bell shaped nasals, in some cases as wide as the interorbital constriction, especially in adult and old specimens. However, this feature shows ontogenetic variation and is not quite distinguishable in all populations. This description includes populations originally recognized as three different species (Hall and Kelson 1959; Hall 1981): (a) *P. furvus* Allen and Chapman, 1897 from Xalapa, Veracruz and its surroundings, together with other populations from the mountains of this state and from the highlands of northern Puebla, (b) *P. latirostris* Dalquest, 1950, from Xilitla, San Luis Potosí, restricted to a limited area at the northernmost range for the taxon; and (c) *P. angustirostris* Hall and Álvarez, 1961, from Zacualpan, Veracruz, and its surroundings. At present *P. furvus* is

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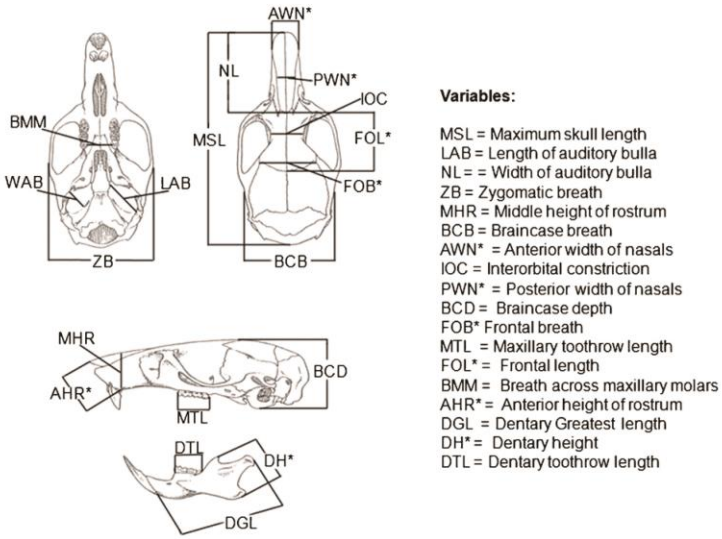

Fig. 1. Measurements obtained from the skull and used in morphometric analyses.

considered a monotypic species, in spite of previous morphological, biochemical, and molecular evidence (Musser 1964; Hall 1971; Harris and Rogers 1999; Harris et al. 2000) that have disclosed a certain amount of variation among the populations, especially those from the northern (i.e., former *P. latirostris*) and southern limits of its range (populations in Oaxaca). Yet, these studies have not arrived at a conclusive taxonomic rearrangement, due to a combination of facts; for instance, they: (a) covered only selected populations, instead of conducting a rigorous survey throughout the geographic distribution of the species; (b) used only one kind of taxonomic character (i.e., morphologic, allozymes, or genetic); (c) examined specimens from different age groups; and, (d) might

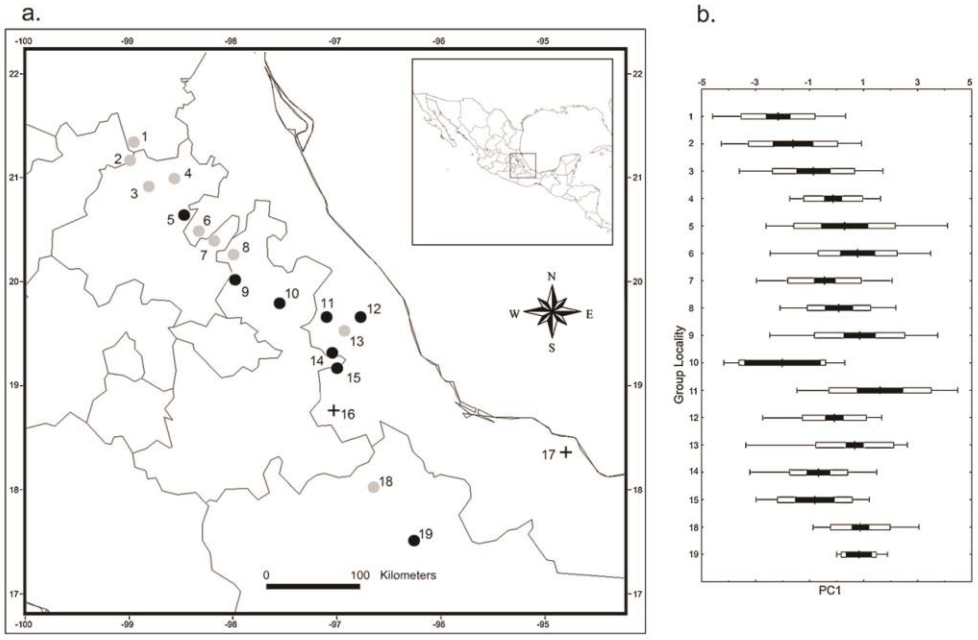

Fig. 2. Location of group localities (1–19), (a) along a NW–SE transect, and their Dice–Leraas diagrams (b) depicted from their first principal component (PC1). Light dots indicate group localities used in morphometric and molecular analyses, while dark dots were only used in the former. See text.

have used genetic markers whose resolution prevented them from detecting diagnostic genetic divergence.

Here, we analyze the morphometric variation among populations representing the entire geographic distribution of the Blackish Deer Mouse. We also analyzed molecular variation in mtDNA genes as markers; these included the type localities, as well as some previously unexplored localities. We also analyze the taxonomic status of *Peromyscus furvus*, specifically addressing the question of whether it is a monotypic species (Musser 1964; Hall 1971, 1981; Harris and Rogers 1999; Harris et al. 2000), a polytypic species, or more than one species.

Materials and methods

Specimens examined

We analyzed 554 adult individuals (*sensu* Hoffmeister 1951; Hooper 1957) for morphometric evaluation, and 62 specimens in the molecular assays (Fig. 1, Tables 1 and 2), including the types and topotypes of taxa identified as *P. furvus* Allen and Chapman, 1897; *Peromyscus latirostris* Dalquest, 1950, and *P. angustirostris* Hall and Álvarez, 1961; hereafter, referred to as *furvus*, (or *Pf*); *latirostris*, (or *Pl*); *angustirostris* (or *Pa*), respectively. Most of them were examined in 14 mammalian collections from Mexico and the United States (Appendix A), and include some individuals that we collected in the field. All specimens were assigned to *furvus* or to its synonyms, according to literature (Table 1), by curators in scientific collections or by us. Those specimens represented the full geographic range of the taxon (Tables 1 and 2, Fig. 2a).

Sampling (SLs) and group localities (GLs)

We recorded the sampling localities ($n = 136$) directly from the skin tag, standardized the distance in *km* and the altitude in *m* to plot them on maps (1:250,000; INEGI 1982), *sensu* Ramírez-Pulido et al. (1989), and associated layers of similar ecological conditions along the current geographic range of *P. furvus*, using vegetation, climate, and elevation data from CONABIO (1999) in ArcView (ver. 3.2). Habitat resemblance, together with closeness of ≤5 km between the SLs, allowed us to hypothesize genetic flow among populations. Then, we pooled the SLs within 19 group localities (GLs) along a NW–SE transect (Table 1, Fig. 2a, Appendix A). Since GL 16 (Tequila) and GL 17 (Tebanca) in Veracruz had just one adult individual and we excluded them both from the analyses, they are represented by a plus symbol (+) in Fig. 2a, only as geographic reference.

Morphometric analyses

We took 19 measurements from the skull and jaw (Hooper 1952; Williams and Ramírez-Pulido 1984; Castro-Campillo et al. 1999, Fig. 1), using a Helios caliper (0.001 mm). Since previous *ad hoc* calculations showed no morphometric sexual dimorphism ($\alpha \geq 0.05$, unpublished data), we pooled together all adults in further analyses. To explore and summarize overall geographic morphometric variation among GLs, we constructed Dice–Leraas diagrams (Fig. 1b) along the NW–SE transect, using the measurements of central tendency of each population computed from the coefficients of the first principal component, PC1: a vertical line represents the mean, an horizontal line shows the extreme values, an open box is one standard deviation from the mean, and a black box is two standard errors from the mean (Allard 1986; Castro-Campillo 1987; Hafner 1992; Carleton and Stanley 2005). The principal component analysis (PCA), together with the projection of a minimum spanning tree (MST) onto the population centroids (unpublished data, Sneath and Sokal 1973; Neff and Marcus 1980; Sokal and Rohlf 1981) was also used to explore the multivariate variation among GLs and to determine the most correlated variables to the first three PCs. Then a Cluster Analysis (CA, Fig. 3) allowed us to join the most phenetically similar GLs ($r \geq 0.7$) in a phenogram by means of the UPGMA algorithm (Sneath and Sokal 1973; Rohlf 1997). Outcomes from these exploratory techniques prompted us to test *a priori* groups of GLs (Sneath and Sokal 1973), especially those derived from the MST and the CA, through Canonical Discriminant Analyses (CDA, Fig. 4). The PCA with MST were obtained in PAST program (Hammer et al. 2001); CA and CDA were run with Statistica package (StatSoft 1998) and all the resulting diagrams were edited in PhotoShop (ver. 8, 2006).

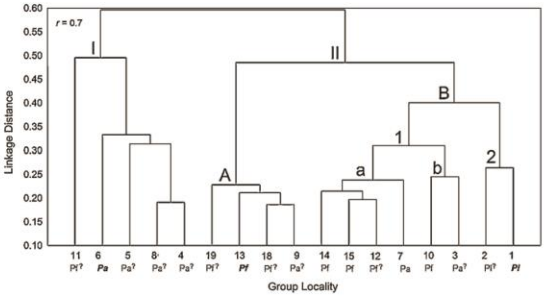

Fig. 3. Cophenetic coeficient and phenetic similarity among the 17 group localities. See text.

DNA extraction and amplification

We used skin and phalanges from voucher specimens, as well as fresh and frozen tissues for the analyses of genetic variation (Appendix A). DNAmt extraction was accomplished through a Dneasy® Qiagen Blood and Cell Culture DNA Mini Kit. Then DNAmt was amplified by polymerase chain reaction (PCR), using a GeneAmp 9700 (Applied Biosystems) and a Techgene Block TG 20 × 0.5 ml. (ver. 10.19, Techne). The 50 μl PCR mixture for each sample contained 1.5 μl template DNAmt, 5 μl of 2 mM 10× dNTP, 1 μl each primer (10 pmol/μl), 5 μl reaction buffer (10×), 5 μl $MgCl_2$ (20×, 30 mM), and 1 μl taq-polymerase (5 U/μl, Amplificasa). Oligonucleotides were obtained from Engel *et al.* (1998): Gly 5ĭ-TAACTAGTACAAGTGACTTC-3ĭ, optimal temperature (OT) = 48 °C, and León-Paniagua et al. (2007): F2 5ĭ-CCAAAAATGTCACCTGTAACC-3ĭ, OT = 49 °C; R1 3ĭ-TGATATAAGGTGTGAACGG-5ĭ, OT = 48 °C; R3 3ĭ-TGTTTTGATTACCTCATCG-5ĭ, OT = 49 °C. General protocol was 94 °C (1 min); 40 cycles of 94 °C (30 s), TO (30 s), and 72 °C (30 s), extension 72 °C (7 min), and 4 °C (α). PCR product was purified with a Millipore® kit and subjected to sequence reaction using Big Dye 3.1 (Applied Biosystems), according to manufacturer protocol. The products were cleaned in Sephadex columns (Princeton Separation Inc., Adelphia, NJ). Sequencing of base pairs was carried out at the Laboratory of Molecular Biology, Instituto de Biología, Universidad Nacional Autónoma de Mexico.

Phylogenetic analyses

Sequences of mitochondrial genes ND3-ND4 were edited and cleaned in BioEdit 7.0.9.0 Sequence Alignment Editor © (Hall 1999), and the matrix of sequences was aligned with Muscle (Edgar 2004). Trees of phylogenetic inference were generated by maximum parsimony (MP) with TNT program (Goloboff et al. 2003, 2008) through traditional search without weighing the characters

Table 1
Populations currently regarded as *Peromyscus furvus* used in morphometric ($n = 554$) and molecular ($n = 62$) analyses with a summary of results and conclusions.[a]

GL	Locality, State	Morphometrics		Molecular				Refs.	CA	IB	MPs	MPm	Taxon
		SL	*N*	Id	SL	*N*	P						
1	*Xilitla, San Luis Potosí*	1–4, 6–15	41	Xil	13	13	a	*b*, e, f, h	IIB2	I	II	I	*Pl*
2	Santa Inés, Querétaro	17–21	19	Sin	16, 18, 19	5	b	*e*, f	IIB2	I	II	I	
3	Otongo, Hidalgo	22, 28, 29, 35–38, 40	23	Oto	39	3	c		IIB1b	II–III	III–V	V	*Pfa*
4	Tlanchinol, Hidalgo	23–27, 30–34	50	Tlan	35	3	d	*h*	I	II–III	III–V	V	
				Huaz	36	3	e						
5	Molango, Hidalgo	41–46	18					*g*	I				
6	*Zacualpan, Veracruz*	47, 48, 50	21	Zac	49	7	f	*c*, d–f, h	I	II–III	III–V	V	
7	Tenango de Doria, Hidalgo	51–58	50	TDor	58	4	g	*c*, d–g	IIB1a	II–III	III–V	V	
8	Xicotepec de Juárez, Puebla	59, 61	22	ESal	60	7	h	*c*	I	III	III	III	
										II–III	III–V	V	
9	Huahuchinango, Puebla	62–69	32					*d*, e–h	IIA				*Pa?/Pf?*
10	Zacapoaxtla, Puebla	70, 71	7					*g*	IIB1b				*/Pf?/P?*
11	Las Minas, Veracruz	72, 73, 75	20						I				
12	Naolinco, Veracruz	74, 76–83	51						IIB1a				*Pff*
13	*Xalapa, Veracruz*	84–87, 89–98	80	MYer	88	6	i	*a*, d–h	IIA	IV	IV	IV	
				Xico	98	5	j						
14	Ixhuacán de los Reyes, Veracruz	99–101	24					*g*	IIB1a				*Pf?/Pf?*
15	Coscomatepec, Veracruz	102–106	14					*g*	IIB1a				
16	Tequila, Veracruz	107	1										*/P?*
17	Tebanca, Veracruz	108	1										
18	Puerto de la Soledad, Oaxaca	109–128	47	Psol	115	5	k	*f*, h	IIA	V	I	II	*Pf?/P?*
19	La Esperanza, Oaxaca	129–133	33						IIA				

[a] References: a, Allen and Chapman 1897; b, Dalquest 1950; c, Hall and Alvarez 1961; d, Musser 1964; e, Hall, 1971; f, Hall 1981; g, Martínez-Coronel et al., 1997; h, Harris and Rogers 1999 together with Harris et al. 2000.

and with tree bisection and reconnection branch swapping (TBR). Robustness of tree topologies was assessed through 1000 bootstrap replicates (BSR), using 100 beginnings for each replicate. In addition, a maximum posterior probability tree (PPT) was constructed using MrBayes (Ronquist and Huelsenbeck 2003). Construction of the Bayesian tree (BI) was made through three independent runs, each one with four Metropolis-coupled, Markov Chains, using a default temperature ($T = 0.02$) and sampling trees every 100 generations (Fig. 6): the first run used the default settings and was stopped after 2,500,000 generations; the second run was stopped after 5,000,000 generations and was based on a model of six parameters substitution, a gamma distribution divided into four categories, and changing rates at variable sites (GTR + I + Γ); the third run was stopped after 6,000,000 generations, using the substitution model that best fit the data by the Bayesian Information Criterion (BIC), according to JModel Test ver. 0.1.1 (Guindon and Gascuel 2003; Felsenstein 2005; Posada 2008). In addition to the runs, the initial 25% of all trees was burn-in, and the posterior probabilities were obtained with the remaining trees. Most of the 14 specimens used as outgroups for rooting were gathered from GenBank and included up to two individuals of each of the following species: *Osgoodomys banderanus* (1), *Podomys floridanus* (2), *Habromys lophurus* (1), *Neotomodon alstoni* (2), *Megadontomys nelsoni* (1), *Peromyscus mexicanus* (2), *P. eremicus* (1), *P. melanocarpus* (2), and *P. boylii* (2).

Population genetics analyses

The haplotype matrix was obtained in the DNAsp program (ver. 5.0, Rozas et al. 2003; Librado and Rozas 2009), the nucleotidic composition and proportion of sites, and the values of genetic distances between sequences and between groups (Fig. 5), were obtained with the software MEGA (ver. 4.0, Tamura et al. 2007), using the model of Tamura and Nei (1993). The genetic diversity indexes were computed under the same model in the software ARLEQUIN (ver. 3.11, Excoffier et al. 2005) and by means of 10,000 permutations. The resulting trees from the analyses of MP and BI, together with genetic divergence among populations ($\pi \leq 0.03$), were employed to define entities to be used in further analyses. Differences within entities (Φ_{ST}), among entities within a clade (Φ_{SC}), and among clades (Φ_{CT}) were examined through an Analysis of Molecular Variance (AMOVA, Excoffier et al. 1992), followed by the computation of a pair wise Φ_{ST} to determine which pair of entities were different from each other. In addition, to assess population stability, we

Table 2
Observed genetic variability within populations (P) currently assigned to *Peromyscus furvus*.[a]

P(GL)	Locality	Id	*N*	*S*	*H*	$Hd \pm SD$	$K \pm SD$	$\pi \pm SD$
a(1)	Xilitla	Xil	13	28	10	0.95 ± 0.05	7.90 ± 3.93	0.008 ± 0.004
b(2)	Santa Inés	Sin	5	28	5	1.00 ± 0.13	11.60 ± 6.35	0.011 ± 0.007
c(3)	Otongo	Oto	4	35	4	1.00 ± 0.18	17.80 ± 10.10	0.017 ± 0.012
d(4)	Tlanchinol	Tlan	3	5	3	1.00 ± 0.01	3.30 ± 2.32	0.003 ± 2.323
e(4)	Huazalingo	Huaz	3	47	3	1.00 ± 0.27	31.30 ± 19.08	0.030 ± 0.023
f(6)	Zacualpan	Zac	7	70	7	1.00 ± 0.08	25.40 ± 12.72	0.024 ± 0.014
g(7)	Tenango de Doria	TDor	4	19	4	1.00 ± 0.18	10.00 ± 5.81	0.010 ± 0.007
h (8)	El Salto	ESal	7	37	7	1.00 ± 0.08	18.80 ± 9.49	0.017 ± 0.010
i(13)	Mesa de la Yerba	MYer	6	18	6	1.00 ± 0.10	7.70 ± 4.17	0.007 ± 0.005
j(13)	Xico	Xico	5	31	4	0.90 ± 0.16	13.00 ± 7.08	0.012 ± 7.078
k(18)	Puerto de la Soledad	Psol	5	62	5	1.00 ± 7.08	27.00 ± 14.33	0.026 ± 0.016

[a] Number of specimens (*N*), of segregating sites (*S*), and of haplotypes (*H*), together with Haplotype diversity (*Hd*), average number of differences (*K*), and nucleotide diversity (*Pi*), are shown

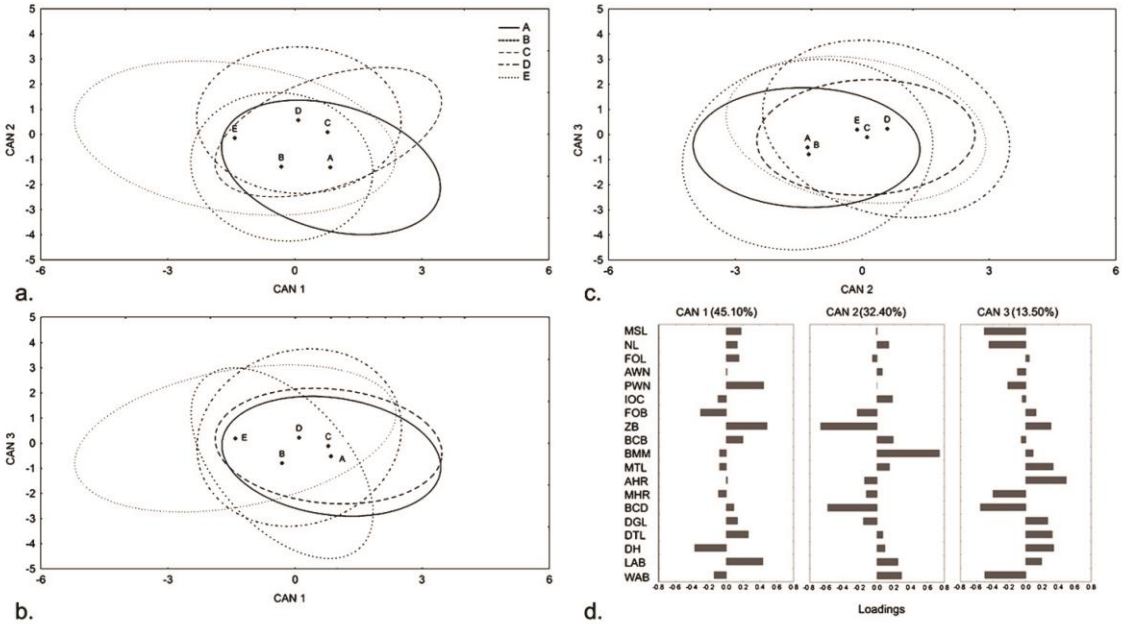

Fig. 4. Projections of 95% equiprobability ellipses around the population centroids of five *a priori* groups (A–E) in the multivariate space generated by the first three canonical functions (CAN1–3, a–c), and the relative contribution of 19 morphometric variables (d).

carried out a Mismatch analysis (Fig. 6) for each entity (Rogers and Harpending 1992) and calculated the significance of the estimated parameters, as well as the *P* value (Excoffier et al. 2005), by computing the sum of square deviations (*SSD*) statistic and the raggedness statistic (H_{rg}; Harpending 1994). Tajima's *D* (Tajima 1989) and *Fu*'s *FS* (Fu 1997) statistics, as well as their *P* values, were calculated to examine the neutrality of DNAmt sequences (Fig. 6).

Results

Morphometric analyses

Size had an overall important effect on all morphometric results and upon phenetic similarity among the GLs, regardless of taxonomic designations (*latirostris, Pl*; *angustirostris*, *Pa*; or *furvus*, *Pf*; Table 1) or geographic closeness of locations (Fig. 2a, Table 1). Dice–Leraas diagrams (Fig. 2b) showed almost no morphometric gaps among the GLs, geographically assignable to *Pl* (GLs 1–2), *Pa* (GLs 3–9), or *Pf* (GLs 10–15, 18–19) along the NW–SE transect, exception the gap between GLs 10 and GL 11 (both *Pf*). Furthermore, GL 10 from GL 9 (*Pf*), GL 3 (*Pa*) from GLs 1–2 (both *Pl*), and GL 11 from GL 12 (both *Pf*), showed a tendency to segregate from their closest neighbor.

This wide overlapping was also evident in the biplots of the PCA, where the first three principal components explained 72.5%. The variables accounting for most of the overall size variation of the skull, emphasized width of braincase (ZB, BCB, and BMM), followed by its length (MSL, NL, and DGL) in the first two PC. Conversely, the outstanding variables in PC3 stressed the importance of the length and width of the rostrum (NL, FOB, FOL, and AWN), in combination with height of the braincase (BCD). Location of GLs along the three PCs depicted the main width (PC3) and length axes of skull (PC2), with prevailing magnitude of ZB and MSL (PC1). In the PC1–PC2 plot, the separation along the latter component of GLs 13, 12, 9, 19, and 18 (Fig. 2) are mainly due to BCB. Between PC1–PC3 plot, the type localities of *furvus* (GL 13) and *angustirostris* (GL 6) fold together into the same direction of the space, while type locality of *latirostris* (GL 1) remains clearly to the left, furthermore, the relative position of the GLs is due to the differences in the rostrum and the depth of the braincase explained by PC3, so that some geographically closer GLs (i.e., GLs 1–2, 18–19) spread out along this component. The influence of BCB and both NL and AWN with other rostrum related variables showed in the PC2-PC3 plot, produce a non-geographic arrangements among GL's.

MST revealed three main branches shoot off from GL 7 (*Pa*), each one including one type locality (GLs, 1 = *latirostris*, 6 = *angustrostris*, and 13 = *furvus*, respectively), and the relative position of the GLs in the branches are very clear in the PC1–PC2 biplot. The branch connections are associated mainly with skull size. Overall largest skulls include GLs 1–2 (*Pl*), 10 (*Pf*), 3 (*Pa*), 14 and 15 (both *Pf*). Then GL 7 (*Pa*) and GL 12 (*Pf*) arranged closer to the former because of their larger MSL, FOL, AHR, DGL, and ZB; however, they showed lower magnitude in other variables. Conversely, the smallest skulls were found at GL 11 (*Pf*), while those from GLs 4, 5 (both *Pf*), GL 6 (*Pa*), and GL 8 (*Pf*), were similarly shorter in FOL, ZB, and AHR. An interesting opposite pattern was found for BCB, since the usually larger-sized skulls in GLs 1–2 (*Pl*), 3, 7 (both *Pa*), 10, and 12–15 (all *Pf*), turned out to be the smallest, while the contrary occurred in the smaller-sized mice from GLs 4, 6 (both *Pa*), 5, 8, and 11 (all *Pf*).

The cluster analysis (Fig. 3) had a high cophenetic correlation coefficient ($r = 0.7$) and GLs were ordinated from smallest skulls at the left of the phenogram to largest ones towards the right, respectively. Accordingly, GLs with the smaller skulls clumped in cluster I, like as MST, including the topotypes of *angustirostris* (GL 6) and nearby populations from Hidalgo (GLs 4, 5) and Puebla (GL 8) together with the farther GL 11 (Veracruz), although this one is geographically closer to the *furvus* type locality (Fig. 2). The remaining GLs were then encompassed in cluster II (Fig. 3), which included the

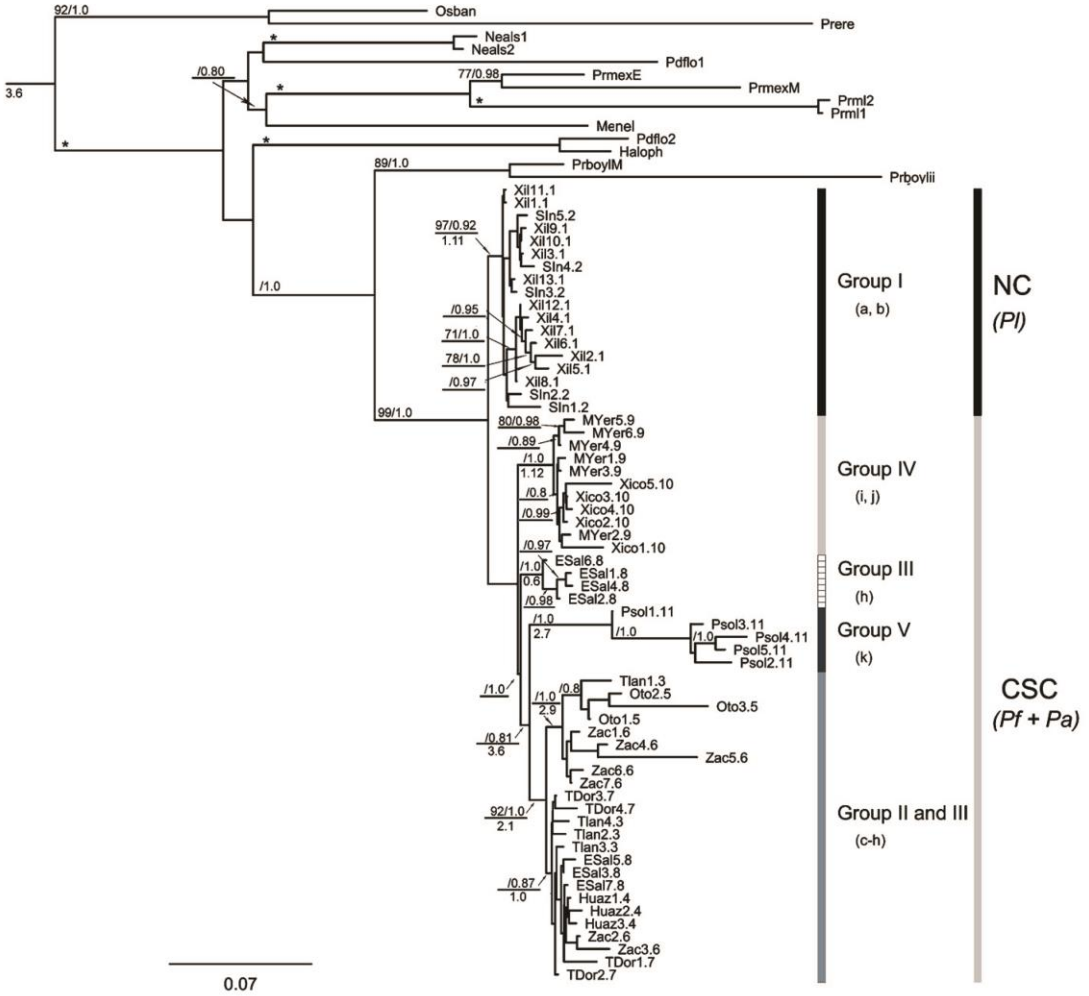

Fig. 5. Phylogeny with the highest likelihood score (−Ln = 16437.923) from a Bayesian analysis. * = best support in MP and BI trees (100 and 1.0, respectively). Numbers above branches indicate the MP bootstrap support (left) and the posterior probabilities (right), respectively. The numbers below branches represent sequences divergence (%).

other two branches of the MST, arranged in several groups, according to the increasing size pattern. Hence, middle-sized specimens, most of them assignable to *furvus* (*Pf*), grouped in cluster A, which enclosed the type locality (GL 13, *Pf*), together with the two samples from Oaxaca (GLs 18–19, both *Pf*?), and one population in northern Puebla (GL 9 *Pa*?). The following cluster B split into sets 1 and 2, and the former of these also divided into subsets "a" and "b". Subset "a" reunited the nearby GLs 14, 15, 12, all assignable to *furvus*, attached to GL7 (*Pa*), which was also the node for GLs 14, 15, and 12 in the MST. Subsequently subset "b" included GL 10 (*Pf*) and GL 3 (*Pa*?) with larger skulls. Finally, in set 2, both the largest-sized and northernmost populations, the two of them assignable to *latirostris* (GLs 1–2, *Pl*), separated from the rest; these two GLs were located at a terminal offshoot in the MST.

The five *a priori* sets of GLs obtained from the cluster analysis (Fig. 3) and the MST in the PCA were respectively renamed within the CDA (Fig. 4) as group A (GLs 1–2), group B (GLs 3, 10), group C (GLs 7, 12, 15, 14), group D (GLs 9, 18, 13, 19), and group E (GLs 4, 8, 5, 6, 11). Wilks' lambda for this analysis was highly significant ($P < 0.0000$) and the first three canonical functions (CAN1–3, Fig. 4a–c) explained 91% of the variation in the reduced space (Fig. 4d). In general, the diagnostic power of variables changed according to canonical functions, but ZB coincided in CAN1 and CAN2, while the same occurred for BCD and WAB in CAN 2 and CAN3, respectively. The variables with most discriminant power (Fig. 4d) and positive sign were ZB, PWN, and LAB in CAN1, BMM in CAN2, and AHR in CAN3, while those with negative sign were DH and FOB in CAN1, ZB and BCD in CAN2, and BCD, MSL, WAB, and NL in CAN3. Even though there is extensive overlap among the ellipses depicting the dispersion of A–E groups in the hyperspace, population centroids showed different arrangements, according to combinations of canonical functions (Fig. 4a–c). In both the biplots of CAN1 *vs.* either CAN2 or CAN3, groups C and A were closer to each other than to any other group, then group D was a little less

closer to group B, and, finally, group E tended to separate from the former four in the opposite side along CAN1. In both biplots, group E was closer to either groups C or D than to the allied groups A and B, along CAN2 and CAN3. Morphometric resemblance between groups A and B was emphazised in the biplot of CAN2-CAN3, where the centroids of groups E, C, and D appeared more separated from them; here, groups E and C were closer than any to group D.

Phylogenetic analyses

In the MP analysis, the strict consensus tree, generated from 1045 most parsimonious trees (not shown; 2925 steps, CI = 42, RI = 75), showed polytomies (+) among the specimens from either close or far away localities; i.e. $((((k)+ab)h+ij)c-h)$; these polytomies were partially resolved with the majority consensus rule (MCR > 70%); i.e., $((((ab)k)h)ij)c-h)$. On the other hand, the best supported tree obtained with BI was found using TIM + I + G [r(A↔C) = 0.429, r(A↔G) = 5.004, r(A↔T) = 0.429, r(C↔G) = 1.000, r(C↔T) = 5.004, r(G↔T) = 1.000; pi(A) = 0.380, pi(C) = 0.2657, pi(G) = 0.0594, pi(T) = 0.2949; α = 0.901; pinvar = 0.348] model run. Both phylogenetic inference trees (MP, BI) had respective values of BSR and PPT higher than 70% and showed strong support in most nodes (Fig. 5). All trees were consistent in placing *P. boylii* as sister to *P. "furvus"*, and *Osgoodomys banderanus* as outgroup. All samples identified as *P. furvus* constitute a monophyletic clade with two clear groups, derived from the rearrangement of the localities in the two methods. The first group is formed by the specimens from the two northernmost localities (*a–b* in Tables 1 and 2), consistently mixed in group I and henceforth referred to as the Northern Clade (NC); indeed, the NC is the first to separate in either topology (MCR 70% and BI) being also the most related to outgroups. The second assemblage (Figs. 2 and 5, Tables 1 and 2) comprises all the specimens from central (*c–j*) and southern localities (*k*) and will be referred hereafter as the Central-Southern Clade (CSC). Most of the specimens intermingle within the CSC, independently of their geographic origin, especially those from localities *c–g*, and some specimens from locality *h* consistently pooled together, while the rest joined with those from localities *c–g*. Also, specimens from localities *i* and *j* combined to each other only, while individuals from locality *k* always remained associated with themselves.

In spite of their shared patterns, topologies obtained with each method of phylogenetic inference differ slightly according to the place taken by the specimens from localities *k* and *i–j*, as well as those from *h* that attach together within the CSG. For instance, in the strict consensus MP tree, specimens from locality *k* are the first to shoot off from the NC (group I) and are subsequently followed by those from localities *a–b*, and then in part by those from *h*, *i–j*, and *c–h*. As for the BI, position of the groups within the CSC start with specimens from localities *i–j*, followed by those from *h* plus those from *c–h*, that are also bonded to a clear off shoot formed by the individuals from locality *k*. That is, differences between the respective CSC resulting from the two methods are due to the segregation of specimens coming from either the southernmost locality (*k* in MP) or from the type locality of *furvus* and its surroundings (populations *i–j* in BI).

Diversity index

The matrix of individual sequences was aligned at 1047 base pairs (bp), corresponding to 306 bp for ND3, 68 bp for tRNA-Arg, 297 bp for ND4L, and 372 bp for the first part of ND4. Values for nucleotide composition obtained in the ingroup were (T = 31.7, C = 23.7, A = 35.3, G = 9.3). A total of 59 sites was conservative, 41 sites were variable, 21 phylogenetically informative, and 20 singleton; nucleotide frequencies were: transitions = 3.5, transvertions = 1.4, and ratio = 2.3.

Average number of pairwise differences and the estimated values of evolutionary divergence allowed us to compute sequence distances between and among ingroups (Fig. 5). The smallest mean distances within groups were observed in the individuals from localities *a* (0.3%) and *e* (0.8%). On the other hand, the least distance among populations occurred between localities *a–b* (1.4%), and between localities *e–h* (1.5%). Locality *k* showed higher genetic distances from the other ingroup localities (8.1–11.6%).

A total of 58 unique haplotypes (H) were derived from the 62 analyzed sequences (Table 2), indicating the existence of some genetic divergence among the localities within the ingroup; however, the π value found among some haplotypes is small ($\leq$0.03). As derived from these and the other genetic analyses (Table 2), the existence of two mayor clades (NC and CSC, Fig. 5) were supported. However, populations contained in the latter presented moderate genetic divergence and were separated in four entities (groups II, III, IV and V, see below).

Population structure

According to previous results (Fig. 5), as well as geographic proximity, the sampled populations (Fig. 2, Tables 1 and 2) were rearranged into five groups (I–V, Fig. 6), within the two mentioned major clades, as follows: Clade NC included only group I = Xilitla (*a*) + Santa Inés (*b*); Clade CSC included groups II–V as follows: group II = Otongo (*c*) + Tlanchinol (*d*) + Huazalingo (*e*); group III = Zacualpan (*f*) + Tenango de Doria (*g*) + El Salto (*h*); group IV = Mesa de la Yerba (*i*) + Xico (*j*); group V = Puerto de la Soledad (*k*). The genetic variation of the AMOVA among groups (Φ_{GT}, σ = 15.09, df = 3), among populations within groups (Φ_{SG}, σ = 0.87, df = 1), and within populations (Φ_{ST}, σ = 8.92, df = 57) explained 60.65%, 3.5%, and 35.84% of variation, respectively. This test proved the presence of a significant structure within populations (Φ_{ST} = 0.642, p = 0.000) and among populations within the groups (Φ_{SG} = 0.089, p = 0.0411), but variation among the groups (Φ_{GT} = 0.607, p = 0.841) was not significant. Furthermore, the pairwise Φ_{ST}-matrix (not presented) provided no evidence for significant differences between groups II and III (Φ_{ST} = 0.049; $p \geq 0.05$), while differences among the remaining pair of groups show significant values ($p < 0.001$).

Mismatch distributions (Fig. 6) showed an unimodal shape of the expected or simulated values for groups I and IV, while the curve was multimodal for groups II, III, and V. In general, all groups turned out to bear low and non-significant values ($p \geq 0.05$) for the H_{ri} and *SSD* statistics, with the exception of group I, for which *SSD* was significant (p = 0.03). Similarly, Tajima's D test indicated negative values for all groups, except for group I (p = 0.04), none were significant. In the case of Fu's *FS* test, groups I, II, III, and IV have negative values, which are significant, only for the former (p = 0.03).

Discussion

Morphometric analyses

The high degree of morphological overlap among *Peromyscus furvus, P. angustirostris,* and *P. latirostris* prompted Musser (1964) and Hall (1971) to merge them, thus rendering *P. furvus* a monotypic species. Nevertheless, Hall (1971) pointed out the northern populations showed distinctive morphological characters, such as a narrower interorbital constriction (IOC) and nasals quite expanded at the tip (AWN; i.e., bell-shaped). Regardless of the extensive size overlap, our morphometric results also show a certain degree of differentiation among these populations formerly described as a different taxon (e.g., *P. latirostris*), but also for populations from Oaxaca. However, in our analyses the most distinctive characters are those responsible for the three main axes of magnitude: (1) the

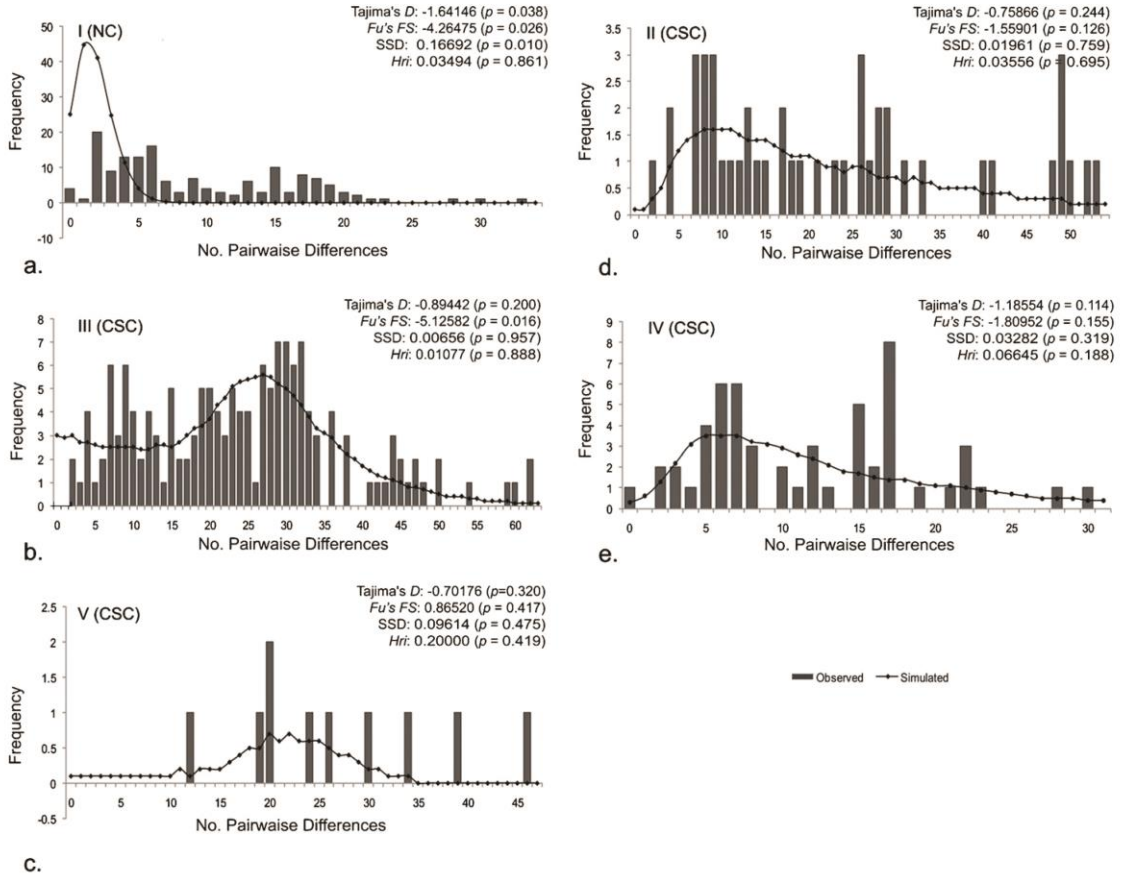

Fig. 6. Mismatch distribution for the ND3–ND4 haplotypes for the populations currently assigned to *Peromyscus furvus* and analyzed here. Bars represent the observed frequency; while the solid line connecting diamonds, depicts the expected frequency under the expansion model. Also shown are the neutrality tests (Tajima's *D* and Fu's *FS*) and statistical values of mismatch distribution (SDD and H_{rg}).

maximum skull length (MSL) and the length of nasals (NL); (2) the breadth between the zygomata (ZB) and that between the maxillary molars (BMM), together with the breadth of the frontal bone (FOB); and (3) finally, the depth of the braincase (BCD).

Moreover, in spite of the overall mensural similarity among all the examined populations, our analyses also show that the pattern of geographic variation outlines subtle degrees of geographic differentiation that anticipate more than a single morphometric unit within the purported monotypic *P. furvus*. For instance, it is interesting that the size of the skull aggregates populations that could reasonably match up with *P. angustirostris*, that *P. furvus* associates with the populations from Oaxaca, and that *P. latirostris* clearly segregates from other populations assignable to *P. angustirostris* or *P. furvus* (Fig. 3). Our traditional morphometric results stress size concealment among entities designated as *P. furvus*. In fact, these cryptic entities are better revealed by the molecular analyses such as in other *Peromyscus* species (Riddle et al. 2000; Hafner et al. 2001; Bradley et al. 2004; Shipp-Pennock et al. 2005), and it is possible that further studies using geometric morphometrics would be helpful to distinguish morphological subtleties among them (Cardini et al. 2009; Furman et al. 2010; Velazco et al. 2010).

Phylogenetic analyses

Our results show both topology discrepancies and entity agreements with previous phylogenetic studies of *P. furvus*. In their allozymic study, Harris and Rogers (1999) tried to establish the limits of the species, comparing seven populations which correspond here to GLs (populations) 1(*a*), 4(*c*), 6(*f*), 7(El Potrero, Hidalgo), 9 (Huauchinango, Puebla), 13 (Xalapa, Veracruz), and 18(*k*), respectively. Besides their distinctive topology, both their phenogram and MP cladogram coincide with our phenogram and cladograms in the clean segregation of the topotypic specimens of *latirostris* (Xilitla), *angustirostris* (Zacualpan), and *furvus* (Xalapa), as well as the unnamed specimens from Puerto de la Soledad, Oaxaca. Their dendrograms also agree with ours in the mixture of the populations between the NC and group IV in the CSC (II–III), including populations assigned to *angustirostris* and *furvus*. However, since they assumed that too few fixed differences existed among them and no diagnostic fixed alleles were found, they concluded that *P. furvus* should remain as monotypic.

In a second contribution, Harris *et al.* (2000) developed a cladistic analysis based on *Cyt-b*, adding specimens from Metepec,

Hidalgo (GL6) to the seven previous samples. Their MP and ML trees still define what we could call henceforth *furvus* (IV), *angustirostris-furvus* (II–III, GLs 4, 6, 7, 8), *latirostris* (I), and the unnamed populations from Oaxaca (V), with the difference that specimens from Huauchinango, Puebla (GL9), which we did not analyze, also segregate, making up five entities within *P. furvus*, which they refer to as ESUs. Unfortunately again, Harris et al. (2000) focus on the genetic and phylogenetic divergence of the populations from Oaxaca and not deeply enough on the relationships among the other populations.

Here, our molecular results show the genetic differentiation of the northern populations from San Luis Potosí and Querétaro (NC), as opposed to our traditional morphometric results. These populations not only aggregate in a single clade (NC) strongly supported and independent from the remaining populations (CSC) but also have the closest genetic distance between them. In fact, the NC is well supported by its values of nucleotidic and haplotypic diversity, indicating genetic similarity within (0.3% in GL1 and 2.5% in GL2) and between (1.4%) these northernmost populations. These divergence values are similar to those found in other *Peromyscus* complexes (e.g., *P. boylii*, Tiemann-Boege et al., 2000; *P. eremicus*, Riddle et al., 2000; *P. leucopus*, Shipp-Pennock et al., 2005; *P. maniculatus*, Zhen et al., 2003, Lucid and Cook, 2004, 2007; *P. schmidlyi*, Bradley et al., 2004; *P. slevini*, Hafner et al., 2001; *P. truei* group Durish et al., 2004) that used cytb as a genetic marker. In comparison with those studies, in which *Cyt-b* were used (), we observed a sequence divergence within species lower than 3.5 and between species higher than 4%. If we consider a divergence sequence $\geq$3.0 to separate species in ND3–ND4 genes (Nachman et al. 1994; Hogan et al. 1997; Corneli and Ward 2000; Chirhart et al. 2001), genetic divergence found between the NC and the CSC is high (>3.5%) and help to distinguish cryptic species complexes (Peppers et al. 2002; Zheng et al. 2003; Lucid and Cook 2004; Bradley et al. 2004; Shipp-Pennock et al. 2005). Additionally, the mismatch distribution indicates the existence of a recent expansion, and support separation of NC as a different species (*Peromyscus latirostris* Dalquest, 1950).

Biogeography

Past climatic and geologic factors might have driven the evolution of this taxon in the Mesoamerican highlands. For instance, the geological history of the Sierra Madre Oriental is quite ancient and complex, with its origin starting far before the Jurassic-Cretaceous (Demant and Robin 1975; Ferrusquía-Villafranca 1998). The emerging elevations, together with the subsequent diversification of climates, eventually favored the establishment of quite unique floristic assemblages, especially those of the temperate cloud forests (TCF, Alcántara-Ayala and Luna-Vega 1997; Contreras-Medina et al. 1999; Luna-Vega et al. 1989, 1994, 1999, 2001a,b; Mayorga-Saucedo et al. 1998; Ruiz-Jiménez et al. 2000; Cartujano et al. 2002; Acosta 2004), which in turn became inhabited by distinctive faunistic assemblages (Canseco-Márquez et al. 2004; García-Moreno et al. 2004; León-Paniagua et al. 2004; Sánchez-González et al. 2007). Although the former distribution of the TCF was quite extensive during the Triassic, it became progressively shrunken and fragmented in the Neogene, becoming the present amalgamation of ancient and modern species disjunctly distributed along the highlands of both coastal slopes in Mexico (Espinosa et al. 2004; Luna-Vega et al. 1999, 2001a; Rzedowsky 1996).

It seems that diversification in wild mice, particularly of those inhabiting the TCF at the highlands of Sierra Madre Oriental, eastern end of the Mexican Transvolcanic Belt, and northern Sierras of Oaxaca, had started by the Mio-Pliocene when environmental conditions were drier and warmer, and culminated during the climatic shifts of the Pleistocene (Schuster 1999; Still et al. 1999). By that time, low temperatures caused shrinking and breakup of the distribution range of the TCF, so that the remaining patches are presently considered as relict communities that host a striking amount of species, especially endemics (Luna-Vega et al. 2001a,b; Rzedowsky 1996). Such environmental changes together with the results of some studies in other species of mice (Engel et al. 1998; Harris et al. 2000; Hoffman and Koeppl 1985; Hogan et al. 1997; Jaarola et al. 2004; Schmidly et al. 1993) suggest that the diversification of former *Peromyscus furvus* could have started in the Plio-Pleistocene. In fact, evidence gathered from both floristic and faunistic studies suggest that the climatic changes of the Pleistocene triggered the beginning of a diversification period (Sullivan et al. 2000,1997) for different taxa of mice and other sedentary vertebrates. For instance, possibly as a result of the recent climatological processes that provoked the reduction of the TCF, both the populations of humming birds *Lampornis* (García-Moreno et al. 2006) and the deermice *Habromys* (Leon-Paniagua et al. 2007; Rogers et al. 2007) now show different amounts of diversification together with high endemism along their extensive distribution throughout the Mesoamerican montane zones. Moreover, as depicted by the five genetically distinct populations of the common bush-tanager, *Chlorospingus ophthalmicus* (García-Moreno et al. 2004), its diversification was also accompanied by geographic segregation in the corresponding patches of the fragmented mesophilic vegetation, similarly to what seems to have occurred among the studied populations of what was formerly regarded as *P. furvus*.

Taxonomic implications

In spite of the overlapping in morphometric characters, our molecular data suggest an important segregation among the populations in two major clades, the Northern (NC) and the Central-South Clade (CSC). Therefore, it is reasonable to consider that *P. furvus* is a complex of two species (*P. furvus* and *P. latirostris*). These entities are conserved within the CSC in all analyses, despite the inconsistencies between them.

P. latirostris Dalquest (1950) is thus restricted to the temperate woodlands of the Sierra Madre Oriental, from 220 to 1400 m in Xilitla, San Luis Potosí, and Santa Inés, Querétaro. Its specific status is also supported by the *Cyt-b* study by Harris et al. (2000), as well as by our morphometric and genetic data.

Therefore, *P. furvus* J.A. Allen and Chapman, 1897, is represented by the populations from Xalapa and vicinity (*v. gr.*, Mesa de la Yerba, Naolinco and Xico) from southern to northern Puebla; they inhabit the temperate moist forests at the highlands (220–1200 m). Therefore, *P. angustirostris* Hall & Álvarez, 1961, occurring in the temperate moist forests from Hidalgo to northern Puebla, is herein considered a synonym of *P. furvus*. However, our data were unable to clarify the systematic status of the populations from Puerto de la Soledad, Oaxaca, in spite of a long phylogenetic distance that suggests its existence as an independent lineage as noted by Harris and Rogers (1999) and Harris et al. (2000).

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Appendix A. Specimens examined

Individuals are organized according to A. Morphometric analyses (Table 1) and B. Molecular analyses (Table 2) in which they were used; in either case, data are mentioned in a NW–SE direction (Fig. 2).

Acronyms and full names for the scientific collections are as follows: UAMI, Colección de Mamíferos de la Universidad Autónoma Metropolitana; MZFC, Museo de Zoología "Alfonso L. Herrera" de la Facultad de Ciencias, Universidad Nacional Autónoma de México; CNM, Coolección Nacional de Mamíferos, Instituto de Biología, Universidad Nacional Autónoma de México; ENCB, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional; UV, Centro de Investigaciones Biológicas de la Universidad de Veracruz; CEAMISH, Centro de Educación Ambiental e Investigación de la Sierra de Huautla, Universidad Autónoma de Morelos; USNM, United States National Museum, Smithsonian Institution and Biological Resources; AMNH, American Museum of Natural History; CM, Carnegie Museum of Natural History; LSUMZ, University of Chicago; Museum of Natural Science, Louisiana State University; MVZ, Museum of Vertebrate Zoology, University of California at Berkeley; TCWC, Texas Cooperative Wildlife Collection, Texas A&M University; TTU, The Museum, Texas Tech University; KU, Museum of Natural History, University of Kansas; UMMZ, Museum of Zoology, University of Michigan; BYU, Birmingham Young University.

A.1. Morphometric analyses (n = 554)

Group localities (GL, $n = 19$) and Mexican States in bold face, are followed by the total number of specimens examined within parenthesis. Then Specific Localities (SLs) are progressively alluded (1–128, Table 1) with the number of specimens and the acronyms of mammal collections within parenthesis. Underlined italics designate type localities and some SLs have an asterisk (*), indicating they were also sampled for in molecular analyses or that the same specimens were used in both morphometric and molecular tests; these SLs are also mentioned in the next section.

GL1 (41): San Luis Potosí (41): **1**. Grande Miramar, región Xilitla, (6LSUMZ, 1TCWC); **2**. Llano coneja, región cerro coneja (2LSUMZ); **3**. Llano Bajo, región conejo (2LSUMZ); **4**. 8 km NW Xilitla (5ENCB); **6**. Las Pozas, 1 km N Xilitla (1ENCB); **7**. Apetzco, 0.5 km N, 2 km W Xilitla (5UAMI); ***8***. *Apetzco, región Xilitla (2KU, 3LSUMZ, 1TCWC)*; **9**. 6 km W Xilitla (1CNM); **10**. Xilitla (1LSUMZ); **11**. Cerro Miramar, región Xilitla (1LSUMZ); **12**. Cerro San Antonio (1LSUMZ); **13**. 5.6 km SW Xilitla (1UMMZ), **14**. 9.65 km W Ahuacatlán (3LSUMZ); **15**. 11 km S, 8 km W Xilitla (1MZFC, 4UAMI). **GL2 (19): Querétaro (16).- 16**. El Pemoche (5MZFC)*; **17**. 2.8 km NW Santa Inés (3MZFC); **18**. 2.5 km NW Santa Inés (6MZFC)*; **19**. 2 km W Santa Inés (2MZFC)*. **Hidalgo (3): 20**. 13.5 km SE Pisaflores (1UAMI); **21**. 3 km S Santa Ana de Allende (2UAMI). **GL3 (23): Hidalgo.- 22**. 4 km N Tepehuacán de Guerrero (1UAMI); **28**. 1.5 km N Chilijapa (5UAMI); **29**. 1 km N Chilijapa (1UAMI); **35**. 1.5 km S, 3.8 km W Tlanchinol (4UAMI); **36**. 2 km S, 3 km W Tlanchinol (6UAMI); **37**. 1 km S, 6 km W Otongo (3UAMI); **38**. 1 km S, 3.5 km W Otongo (2UAMI); **40**. 4.5 km N Ixtlahuaco (1TCWC). **GL4 (50): Hidalgo.- 23**. 5 km N, 3 km E Tlanchinol (1UAMI); **24**. 4 km N, 2 km E Tlanchinol (5UAMI); **25**. 4 km N, 1.5 km E Tlanchinol (1UAMI); **26**. 2.5 Km N, 1.5 km W Tlanchinol (5UAMI); **27**. 12 km WSW Tehuetlán (2TTU); **30**. 4 km NE Tlanchinol (5ENCB); **31** 3 km N 1 km E Tlanchinol (3UAMI); **32**. 13 km WSW Tehuetlán (9TTU); **33**. 1 km N, 3 km E Tlanchinol (9TTU); **34**. 1 km N, 2 km E Tlanchinol (10TTU). **GL5 (18): Hidalgo.- 41**. 2 km N Xochicoatlán (1ENCB); **42**. 6 km S, 2.7 km E Molango (1ENCB); **43**. 3.5 km SE Tianguistengo (1UAMI); **44**. 4 km N Zacualtipán (4TTU); **45**. 5 km E Zacualtipán (4TTU); **46**. 1.2 km S, 8.4 E Zacualtipán (1CNM, 6ENCB). **GL6 (21): Veracruz (21): 47**. 3.2 km SE Huayacocotla (3UMMZ); ***48***. *Zacualpan (2MZFC)*; **50**. 3 km W Zacualpan (16KU). **GL7 (50): Hidalgo.- 51**. 3 km N San Bartolo Tutotepec (2ENCB); **52**. 2 km N, 2 km W San Bartolo Tutotepec (3ENCB); **53**. San Bartolo, cueva El Cirio (5MZFC); **54**. 25.4 km NE Metepec (2TCWC); **55**. 22.8 km NE Metepec (3TCWC); **56**. 21 km NE Metepec (3TCWC, 22UMMZ); **57**. El Texmé (2MZFC); **58**. Tenango de Doria (8MZFC)*. **GL8 (22): Puebla (22): 59**. 8 km N Huauchinango; **61**. 5.6 km SW Xicotepec de Juárez (8AMNH, 5CM, 7TCWC). **GL9 (32): Puebla (32): 62**. 0.2 km N Honey (1UAMI); **63**. 3 km N, 3 km E Huauchinango (1ENCB); **64**. Huauchinango (1UMMZ); **65**. 2 km SW Huauchinango (2ENCB); **66**. 3.2 km SW Huauchinango (9UMMZ); **67**. 5.5 km SW Huachinango (4TCWC); **68**. 5.6 km SW Huachinango (4MZFC); **69**. 9.2 km SW Huauchinango (10UMMZ). **GL10 (7): Puebla (7): 70**. 5.5 km N Zacapoaxtla (6UAMI); **71**. 5 km N Zacapoaxtla (1UAMI). **GL11 (20): Veracruz (20): 72**. 1.6 km NE Las Minas (12USMN); **73**. 1 km NE Las Minas (4USMN); **75**. 1.6 km E Las Minas (3USMN, 1UV). **GL12 (51): Veracruz (51): 74**. 4 km N Naolinco (2UAMI); **76**. 1 km W Tlacolulan (6UAMI); **77**. 1 km S Tlacolulan (6UAMI); **78**. 5 km W Naolinco (3CNM); **79**. Naolinco (1UV); **80**. 4 km N Jilotepec (2UAMI); **81**. Jilotepec (17UV); **82**. 8 km SW Naolinco (11UAMI); **83**. 4 km N, 7.5 km W Actopan (3ENCB).

GL13 (80): Veracruz (80): 84. 5 km N Xalapa (5KU, 4TCWC); **85**. Banderillas, 6 km NW Xalapa (1CNM); **86**. 1.5 km SE Banderillas (6UAMI); **87**. Plan de Sedeño (1UV); **89**. 2 km W de El Paisano (3UV); **90**. 2 km N, 7.5 W Xalapa (4ENCB); ***91***. *Xalapa (15AMNH)*; **92**. 1 km W Tengonapa (11UV); **93**. Tlachinola (1UV); **94**. 5 km S Xalapa (1UMMZ); **95**. Las Limas, Coatepec (1UV); **96**. 2 km W Xico (11KU); **97**. 1.6 km W Xico (15UMMZ); **98**. Xico (1USMN). **GL14 (24): Veracruz (24): 99**. 2 km NW Ixhuacán de los Reyes (8UAMI); **100**. 1 km W Ixhuacán de los Reyes (10UAMI); **101**. Ixhuacán de los Reyes (6UV). **GL15 (14): Puebla (5): 102**. 1.5 km SE Quimixtlán (5UAMI); **Veracruz (9): 103**. 9.6 km Calcahualca, Pte. San Bernardo (1CNM); **104**. Teopantitla, 1.5 km E Atotonilco (5ENCB); **105**. 5.5 km N, 6 km E Coscomatepec (1UAMI); **106**. 3 km N, 3.5 km E Coscomatepec (2 M UAMI). **GL16 (1): Veracruz (1): 107**. 5 km N Tequila (1CNM). **GL17 (1): Veracruz (1): 108**. 1 km E, 2 km S Tabanca [Tebanca] (1TTU). **GL 18 (47): Oaxaca (47)**: **109**. 6.5 km Puente Fierro-Santa María Chilchota (1CNM); **110**. 5 km NW Puerto de la Soledad (1CNM); **111**. 3 km NW Puerto de la Soledad (5CNM); **112**. 1 km NE Puerto de la Soledad (1CNM); **113**. Puerto de la Soledad (4CNM); **114**. Puerto de la Soledad (3CNM); **115**. Puerto de la Soledad (4CNM, 2MZFC)*; **116**. Puerto de la Soledad (1CNM); **117**. 0.5 km S Puerto de la Soledad (2CNM); **118**. 1 km SE Puerto de la Soledad (3CNM); **119**. 5 km N, 1 km W Huautla (1UAMI); **120**. 3 km N, 1 km W Huautla (1UAMI); **121**. 1.5 km S Puerto de la Soledad (7CNM); **122**. 1.5 km Puerto de la Soledad-San Bernardino (2CNM); **123**. Puerto de la Soledad-San Bernardino (3CNM); **124**. 2 km Puerto de la Soledad-San Bernardino (1CNM); **125**. 2.5 km SW Plan de Guadalupe (2CNM); **126**. 4.5 km SE Plan de Guadalupe (1CNM); **127**. Aguaduende (1CNM); **128**. 0.5 km W, 0.5 km N Vista Hermosa (1TTU).

GL 19 (33): Oaxaca (33): **129**. 3 km N, 16 km E San Pedro Yolox (1TTU); **130**. 2 km S La Esperanza (3TTU); **131**. 5 km S, 3 km W La Esperanza (10UAMI); **132** 2.5 km N, 1 km E La Esperanza (9UAMI, 9UV); **133**. 1 km N, 1 km E Llano de las Flores (1TTU).

A.2. Molecular analyses (76)

Tissue samples involved: (i) dried first phalange of the second digit of forepaws and/or ventral skin (UAMI, MZFC); (ii) frozen (MZFC) viscera: heart and/or kidney; (iii) alcoholic liver (UAMI, BYU, CEAMISH). The 62 individuals assigned to *Peromyscus furvus* and used as ingroups for the molecular analyses are listed as mentioned before, but with the insertion of bold identifying letters (a–k, Table 2) and the abbreviations used in the cladogram (Fig. 6). In addition to the number of specimens examined and the acronyms of the scientific collections, the GenBank accession number also appears within the parenthesis. Finally, since outgroups were mostly gathered from the GeneBank, we only list them, followed by the abbreviations used in cladograms and their accession number within parenthesis.

Ingroups (62).-GL1(13), San Luis Potosí, a. Xil (13): 5. Ejido Aguayo, 6.2 km N Xilitla (13BYU, JN885476 – JN885488). **GL2(5), Querétaro, b. Sin (5): 16**. El Pemoche (1MZFC, JN885490)*, **18**. 2.5 km NW Santa Inés (2MZFC, JN885489, JN885491)*, **19**. 2 km W Santa Inés (2MZFC, JN885482, JN885493)*. **GL3(3), Hidalgo, c. (3): 39**. 1.5 km S, 3.8 km W Otongo (3UAMI, JN885501 – JN885503). **GL4(7), Hidalgo, d. Tlan (4): 35**. 4 km E Tlanchinol (4UAMI, JN885494 – JN885497); **e. Huaz (3) 36**. Carr. Tehuatlan-Huazalingo 10 km NW (3MZFC, JN885498 – JN885500). **GL6(7), Veracruz, f. Zac (7): 49**. 3.2 E km Zacualpan (7MZFC, JN885504 – JN885510). **GL7(4), Hidalgo, g. TDor (4): 58.** Tenango de Doria (4MZFC, JN885511 – JN885514)*. **GL8(7), Puebla, h. ESal (7): 60**. El Salto (7MZFC, JN885515 – JN885521). **GL 13, Veracruz, i. MYer (6): 88**. Mesa de la Yerba (6CEAMISH, JN885522 – JN885527). **GL13, Veracruz, j. Xico (5): 98.** Xico (5CEAMISH, JN885528 – JN885532). **GL18, Oaxaca, k. Psol (5): 115.** Puerto de la Soledad (5MZFC, JN885533 – JN885537)*.

Outgroups (14). *Osgoodomys banderanus* (Osban, U83860). *Podomys floridanus* (Podflo1, U83865; Podflo2, KMH1042). *Habromys lophurus* (Haloph, U83863). *Neotomodon alstoni* (Neals1, JN885474; Neals2, JN885475). *Megadonthomys nelsoni* (Menel, DQ793119). *Peromyscus mexicanus* (PrmexE, U83862; JN885471). *Peromyscus eremicus* (Prere, U8386). *Peromyscus melanocarpus* (Prml1, JN885472; Prml2, JN885473). *Peromyscus boylii* (Prboylii, U83864; PrboylM, JN885470).

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Capítulo 2. Systematics of *Peromyscus mexicanus* (Rodentia: Cricetidae) using ND3-ND4 mitochondrial genes

Systematics of *Peromyscus mexicanus* (Rodentia: Cricetidae) using ND3-ND4 mitochondrial genes.

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Abstract

The systematic status of the Mexican deer mouse *Peromyscus mexicanus*, which occurs in different environmental conditions through the highlands and lowlands of Mexico and Central America, is controversial. Morphological analyses and recent reviews of the species suggest the existence of seven subspecies, although the geographic boundaries of the distribution for each one are unclear. In order to determine whether the species constitutes a monophyletic clade, we analyzed the phylogenetic relationships and genetic divergence among four of the subspecies, *Peromyscus m. mexicanus*, *P. m. totontepecus*, *P. m. saxatilis*, and *P. m. azulensis*, using mtDNA genes. Both Bayesian and Maximum Parsimony revealed that some individuals considered as *P. mexicanus* are closer to other species and thus constitute a paraphyletic taxon; whereas *P. melanocarpus* joined to the clade with the majority of *P. mexicanus* specimens, highlighting the complexity of the species' limits in the taxon.

Key words: paraphyletic, *Peromyscus mexicanus*, cryptic species, species limits

Introduction

The *mexicanus* group in the genus *Peromyscus* has been considered a complex assemblaje; since to some of its species have unique discrete morphological characters, while other have overlapping features (Hooper 1968; Huckaby 1980; Rogers & Engstrom 1992; Ordóñez-Garza et al. 2010). Huckaby (1980) highlighted heterogeneity of mexicanus: on one hand, he indicated the existence of qualitative discrete characters to differentiate *P. megalops*, *P. melanocarpus*, *P. ochraventer*, and *P. yucatanicus* from other members of the group, while, on the other, the differences among *P. mexicanus*, *P. guatemalensis*, *P. grandis*,

P. zarhynchus, and *P. gymnotis* were subjective, because they were only based in size and color. Rogers & Engstrom (1992), performed a phenetic analysis of the group using allozymic data, and contrasted their results to preliminary cladistic examination, showing that the *P. mexicanus* group conformed a paraphyletic taxon, since to one of its populations was closer to *P. gymnotis*. In addition, *P. yucatanicus*, which is corrently considered by Lawlor (1965), Hopper (1968), and Huckaby (1980) as a separate species, joined to *P. mexicanus*, being the genetic divergence between both species low. Ordóñez-Garza et a*l*. (2010), evaluated the relationships among *P. guatemalensis*, *P. grandis*, and *P. zarhynchus* by both morphometric and molecular analyses, and used some members of the *P. mexicanus* group (*P. mexicanus*, *P. nudipes*, and *P. gymnotis*) as comparing.These authors also demonstrated the close relationship between *P. mexicanus* and *P. gymnotis* (i. e. Rogers & Engstrom 1992) but they found a clear separation between *P. mexicanus* and *P. nudipes*, as opposed to Huckaby (1980), while they supported the opinion of Osgood (1909) and Hopper (1968). Nevertheless the clear genetic relationship between *P. mexicanus* and *P. gymnotis*, these taxa are not still reciprocally monophyletic.

Peromyscus mexicanus Saussure, 1860 is a polytypic species which includes seven subspecies (*P. m. angelensis*, *P. m. azulensis*, *P. m. mexicanus*, *P. m. putlaensis*, *P. m. saxatilis*, *P. m. teapensis*, and *P. m. totontepecus*; Ramírez-Pulido & Müdespacher 1987; Cervantes et al. 1994; Arita & Ceballos 1997; Ramírez-Pulido et al. 2005; Ceballos & Oliva 2005; Trujano-Álvarez & Álvarez-Castañeda 2010), distributed in different vegetation types over a vast region. Extending as far as the mountains of Panama, this region includes the lowlands and highlands of the Sierra Madre Oriental (SMOr), Sierra Madre del Sur (SMS), the Trans-Mexican Volcanic Belt (TVB), the northern Highlands of Oaxaca (NHO), and the Sierra Madre de Chiapas (SMC) in Mexico, as well as an extensive area to the south throughout Guatemala, El Salvador, Honduras, Nicaragua, and Costa Rica. (Reid 1997; Rojas-Rojas & Barbosa-Rodríguez 2007; Trujano-Álvarez & Álvarez-Castañeda 2010). The occurrence of the species, often confused with similar syntopic or closely species mainly *P. ochraventer*, *P. evidens*, *P. guatemalensis*, *P. oaxacensis*, *P. aztecus*, *P. megalops*, *P. aztecus*, and *P. furvus* (Huckaby 1980), has been reported in a wide range of environmental conditions, from dry to humid, but always within forests and often in nearby plantations (Huckaby 1980, Hall 1981; Carleton 1989; Trujano-Álvarez & Álvarez-Castañeda 2010). The taxonomic status of the populations of the species and its closely related taxa

has been evaluated morphologically. Huckaby (1980) analized the species complex within the *mexicanus* group, and reviewed the synonimies among *Peromyscus mexicanus*, recognizing only six subspecies. Recently, Trujano-Álvarez & Álvarez-Castañeda 2010 in its general review of the species, considering among other data, the morphology, ecology, and cariotypes, incorporated *P. megalops azulensis* as a valid subspecies of *P. mexicanus* (Trujano-Álvarez & Álvarez-Castañeda 2010). Due to the scarcity of taxonomic and systematic reviews on the species, together with its wide morphological and environmental variation, the number of subspecies is unclear (6 to 10), and it is also possible that the taxon contains more than one species (Huckaby 1980, Trujano-Álvarez & Álvarez-Castañeda 2010). Therefore, here we evaluated the phylogeny of some populations of *P. mexicanus*, which corresponds to *Peromyscus m. mexicanus*, *P. m. totontepecus*, *P. m. saxatilis*, and *P. m. azulensis*, through molecular systematic and genetic analyses, using sequences of the mtDNA ND3-ND4 genes.

2. Materials and methods

2.1. Specimen collection and sequencing

We analyzed sequences of 46 specimens from 23 populations (Fig. 1, Table 1) along the mountains of Hidalgo, Puebla, Oaxaca, and Chiapas, Mexico, as well as Chalatenango, El Salvador. This samples represents four of the seven recognized subspecies (*Peromyscus mexicanus mexicanus*, *P. m. totontepecus*, *P. m. saxatilis*, and *P. m. azulensis*). The samples were obtained from five Mexican Mammals Collections (Appendix 1). We used two DNA extraction techniques: a) NaCl and Chloroform Isoamilic alcohol with recent skin and tissue, and b) Dneasy® Qiagen Blood and Cell Culture DNA Mini Kit with old skin. We amplified three mtDNA genes and one tRNA (ND3, tArg-RNA, ND4L, and the first part of ND4) with a polymerase chain reaction (PCR), using either GeneAmp 9700 (Applied Biosystems) or Techgene Block TG 20x0.5 ml. (ver. 10.19, Techne) thermal cycler. The PCR products were carried out in 12.5µl volume, including 7.325µl of ultrapure water, 1µl of DNAmt template, 0.825µl of 2mM (10x) dNTP mix, 0.625µl of 20pmol/µl each primer, 1.25µl of 10 x reaction buffer, 0.75µl of 20x 25mM $MgCl_2$, and 0.1µl of 5U/µl *Taq* DNA polymerase (Amplificasa or Vivantis). Oligonucleotides employed (as well as their optimal temperature), are reported in Ávila-Valle *et al.* (2012). DNA sequencing was processed by the commercial services provided by either Macrogen Inc. (South Korea), or the High-Throughput Genomics Unit at the University of Washington (http://www.htseq.org). The

program MEGA (ver. 5, Tamura et al. 2011) was used to edit and clean the sequences, and the alignment was obtained by the program MUSCLE (Edgar 2004).

2.2 Phylogenetic analyses.

The phylogenetic analyses were generated by Bayesian inference (BI) through the MrBayes program (Ronquist & Huelsenbeck 2003). We obtained 5 million generations x 4 chains (one cold and three heated), sampling every 100 generations, with a burning of 25% (12,500 trees). The posterior probabilities (*PP*) were generated with the remaining 37,500 trees. The best substitution model that fit the data by the Bayesian Information Criterion (BIC) was generated according to the *J*ModelTest program (ver. 0.1.1, Guindon & Gascuel 2003; Felsenstein 2005; Posada 2008). Outgroups used for rooting trees were gathered from GenBank (see Appendix 1).

2.3 Diversity indexes and Population genetics analyses.

The nucleotidic composition, proportion of sites, and the genetic distance values among sequences and groups were conducted using the program MEGA (ver. 5.0, Tamura et al. 2011), under the Tamura-Nei model (Tamura & Nei 1993). This molecular model was also used in the ARLEQUIN program (ver. 3.11, Excoffier et al. 2005) to generate the genetic diversity indexes after 10,000 permutations. The haplotype matrix and the general demographic values were obtained using the DNAsp program (ver. 5.0, Rozas et al. 2003; Librado & Rozas 2009). Differences within haplotypes (Φ_{ST}), among haplotypes within each population (Φ_{SC}), and among populations (Φ_{CT}), were examined through an Analysis of Molecular Variance (AMOVA, Excoffier et al. 1992), followed by the computation of a pairwise-Φ_{ST} to determine which pair of entities were different from each other. In addition, to assess population stability, we carried out a Mismatch analysis for each entity (Rogers & Harpending 1992) and calculated the significance of the estimated parameters, as well as the *P* value (Excoffier et al. 2005), by computing the raggedness (H_{rg}; Harpending 1994) and Ramos-Onsins and Rozas test (R_2; Ramos-Onsins and Rozas 2002) statistics. Tajima's *D* (Tajima 1989) and Fu's *FS* (Fu 1997) statistics, as well as their *P* values, were calculated to examine the neutrality of DNAmt sequences.

3. Results

3.1 Phylogenetics Analyses

In the BI analysis, the best supported tree was obtained using TPM2uf + I + G model run, from the following values: r(A↔C) = 0.7436, r(A↔G) = 8.6134, r(A↔T) = 0.7436, r(C↔G) = 1.0000, r(C↔T) = 8.6134, r(G↔T) = 1.0000; pi(A) = 0.3753, pi(C) = 0.2646, pi(G) = 0.0647, pi(T) = 0.2954; α = 0. 8390; pinvar = 0.3080. The highest likelihood topology was found in the first run (Fig. 2) and shows some individuals assigned to *P. mexicanus* intermingled with outgroups. The clade (nodes, N) with the highest number of samples considered to be *P. mexicanus*, had little support (*PP*=52), indicating poor consistency within the species; moreover, the *PP* of N 2 and 3 also had low support (>50). These are three groups inside N 2 (Fig. 2). Group A was composed by specimens from Chiapas (L's 14, 15, 19-22) and Group B included specimens from Oaxaca (L's 7-9, 13), both are contained in N 1. Group C is comprised by samples from Mexico [Hidalgo (L 11), Oaxaca (L 5), and Chiapas (L 18 and 23)] and Central America [Chalatenango (L 17) and Engel *et al*. (1998) specimen (L 16)]. The populations grouped at N 3 have an environmental arrangement, linked to type of vegetation mainly, rather to a geographical aspect. One individual from L 12 is shown in a separate branch, together with the above groups (N 4), while two individuals from the same locality are associated with *P. furvus*, as well as the sample from L 10. Some individuals from Oaxaca (L's 1-4 and 6) were joined nearby to *P. beatae*, *P. melanophrys*, *Habromys lophurus*, and *H. schmidlyi* (Fig. 2).

3.2 Diversity indexes and Population genetics analyses.

The sequence matrix used contained 1086 base pairs (bp). The nucleotide diversity values were T=31.2, C=24.1, A=35.0, and G=9.6. There were 580 monomorphic sites (53.4 %) and 505 polymorphic sites (46.5 %), the latter with 375 phylogenetically informative sites (34.5 %) and 130 singleton sites (12.0 %). Further, the nucleotidic frequencies include 880 identical, 77 transitional, and 22 transversional (transition/transversion = 3.48), with a transition matrix of TT=270.00, TC=29.00, TA=4.00, TG=1.00, CT=30.00, CC=202.00, CA=5.00, CG=1.00, AT=4.00, AC=4.00, AA=324.00, AG=9.00, GT=1.00, GC=2.00, GA=9.00, GG=83.00. The above values show the existence of a high genetic differentiation among *Peromyscus mexicanus* specimens, indicating that the species as we currently recognize it may not represent a monophyletic clade.

We obtained 42 unique haplotypes from 46 specimens –of which nine contained only one sequence per locality– representing a high haplotypic diversity (H_d=0.9961); nonetheless, the nucleotide diversity was low

(π=0.089), indicating that despite an elevated number of haplotypes, the nucleotide differences among the sequences are few (Table 1). The Φ_{ST} was the only significant component explained by the AMOVA (δ^2 =3.90102) and showed the lowest value of the variation contribution (17.7%); however, it contained the highest fixation index value (0.82303), indicating the existence of genetic differentiation within haplotypes (Table 2). The pairwise-Φ_{ST} matrix (Table 3) revealed that Ejido Ojo de Agua in Chiapas (L 20) was significantly different from some populations of Oaxaca (L's 2, 7, 12, and13), Hidalgo (L 11), and Chiapas (L's 15, 22, and 23). Meanwhile, Río Camarones, Hidalgo (L 11) showed significant differences with Ejido Nicolás Bravo, as well as Ejido Loma Bonita, Chiapas (L 23) with San Martín Caballero, Oaxaca (L 12). The remaining populations showed non-significant differences. Mismatch distribution (Fig. 3) shows multi-modal aspect (H_{rd}=0.0014; R_2=0.0975); in addition, Tajima's *D* (-1.02254) and Fu's F_S (-13.988) were of no significance, indicating a non-expanded demographic process.

4. Discussion

4.1. Evidence of crypticity and/or paraphilly within the species

Although the studies mentioned in the introduction suggest both morphological and genetic complexity among the members of *Peromyscus mexicanus* group (Lawlor 1965; Hooper 1968; Huckaby 1980; Rogers & Engstrom 1992; Ordóñez-Garza et al. 2010), lack of recent taxonomic studies within the species *P. mexicanus* (not only in the group), has prevented a better understanding of its phylogeny, and therefore the delimitation of the species remains confusing (Huckaby 1980; Trujano-Álvarez & Álvarez-Castañeda 2010). In one hand, Bayesian Inference (Fig. 2), showed that *Peromyscus mexicanus* specimens do not join together in a natural clade, including the specimens of *P. melanocarpus*, but rather constitute a paraphyletic group. Additionally, the presence of short branch lengths among the terminals indicates the hazardously existence (and permanence) of several haplotypes in the populations; the latter implicates either a rapid divergence rate in the species with few genetic changes among speciation events (Pérez-Suárez et al. 1994; Edwards et al. 1997; Scheffer & Wiegmann 2000) or the establishment of a high effective population size (Bulgin et al. 2003; Donnelly et al. 2004; Steppan et al. 2004). Moreover, the low values of the *PP* indicate little support of the characters which contribute to the phylogenetic resolution, but nonetheless a large number of phylogenetically informative sites (Bulgin et al. 2003). Furthermore, the genetic divergence within species presents a high rate of change (8.4%),

whereas morphological characters used by Huckaby (1980) to separate the members of *Peromyscus mexicanus* group seem to have a lower evolutionary divergence rate (Ordóñez-Garza et al. 2010).

Some authors suggest that diversification of the members of *P. mexicanus* group (as well as all neotomines) occurred North to South, and was the result of the topology and climate diversity along Mesoamerica; however, the relationships between the taxon expansion and speciation processes proposed are still unclear (Carleton 1989; Huckaby 1980; Dawson 2005; Ordóñez-Garza et al. 2010). A geographic morphological variation analysis (unpublished data), where males and females were examined separately, using multivariate statistics, pointed out different locality arrangements between the two sex groups, suggesting that the environmental factors together with some speciation processes are important not only at species level but also at the genus level. Apparently, within *P. mexicanus* populations, influence of environment factors has been the main feature sharping along its distribution. The first clade contains individuals that inhabit either temperate forests or cloud forests (Group C), while the other (N 1) comprises populations from tropical environments (Fig. 2).

As in other neotomines (Riddle et al. 2000; Hafner et al. 2001; Peppers et al. 2002; Bradley et al. 2004; Shipp-Pennock et al. 2005; Ávila-Valle et al. 2012), the former arguments provide evidence of the crypticity of species contained in what we now call *P. mexicanus*, since neither overall morphology nor traditional morphometrics have contributed in the correct reconstruction of the phylogeny (Hooper 1968; Huckaby 1980; Ordóñez-Garza et al. 2010). The phylogeny obtained (Fig. 2) shows how some of the populations assigned to *P. mexicanus* are grouped with other species such as *P. furvus*, *P. melanophrys*, *P. beatae*, *Habromys schmidly* and *H. lophurus*, rather than with themselves. Furthermore, the joining of *P. melanocarpus* to populations from clade A, highlights of the paraphylletic occurrence within peromiscine and supports presence of high cryptic morphological characters within neotomines. On the other hand, the high genetic divergence rate can be a consequence of the paraphilly array species of the Mexican group associated with introgression (Good 1989; Edwards et al. 2001) or retention of ancestral polymorphisms (Hulva et al. 2004), especially between *P. mexicanus* and *P. furvus*. Also, the convergence in the morphologic characters, might be hiding recognition of other species (Rogers & Engstrom 1992; Funk & Omland 2003; Roe & Sperling 2007).

4.2 Further studies

In the hight of evidence found here, we emphasize the phylogenetic controversy within the species of *P. mexicanus*. The *mt*DNA is considered a powerful tool to gather evolutionary process; however, these markers are unusable in the presence of introgression of lineage sorting evidence (Moran & Kornfield 1993). Therefore, we believe that addition of nuclear markers to this analysis –either microsatellites or SNP's (Mims et al. 2010), which have the capacity to find each parental lineage–could facilitate the delimitation of the species. Additionally, geometric morphometrics analyses to evaluate which characteristics do support the species would be complementary and essential in order to facilitate the identification of *P. mexicanus* (Cardini et al. 2009; Furman et al. 2010b; Velazco et al. 2010).

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Journal name: Journal of Mammalian Evolution

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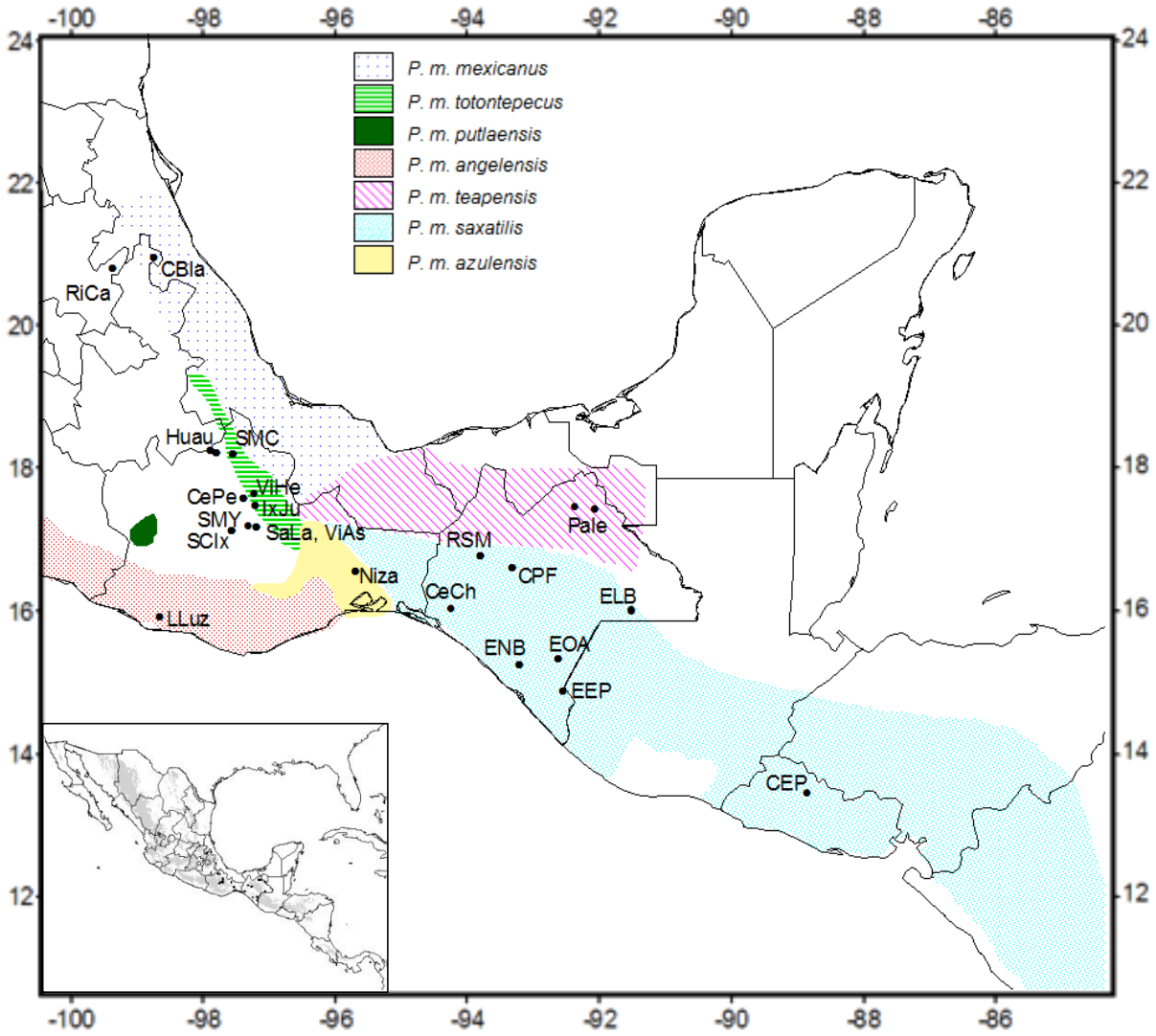

Figure 1. *Peromyscus mexicanus* and its recognized subspecies distribution (Modified from Trujano-Álvarez & Álvarez-Castañeda 2010) Black dots indicate localities analyzed in this work. See Table 1 for abbreviation.

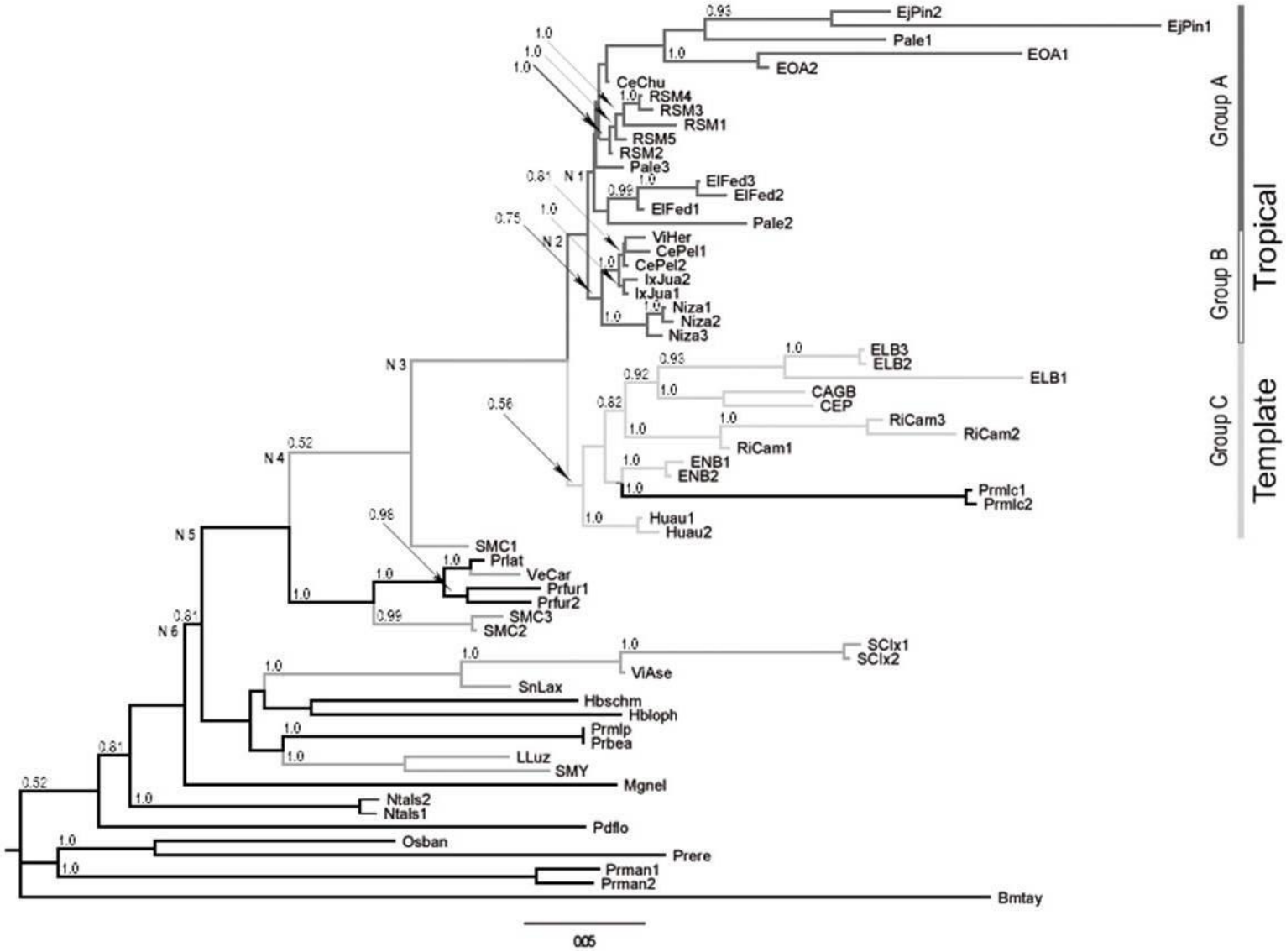

Figure 2. Phylogeny with the highest likelihood score (−Ln = 13545.477) from a Bayesian analysis. Gray branches (dark, medium and light) indicate specimens considered *Peromyscus mexicanus*. Numbers in the nodes (N 1-6) indicate posterior probabilities support (see Table 1 and Appendix A for abbrevations).

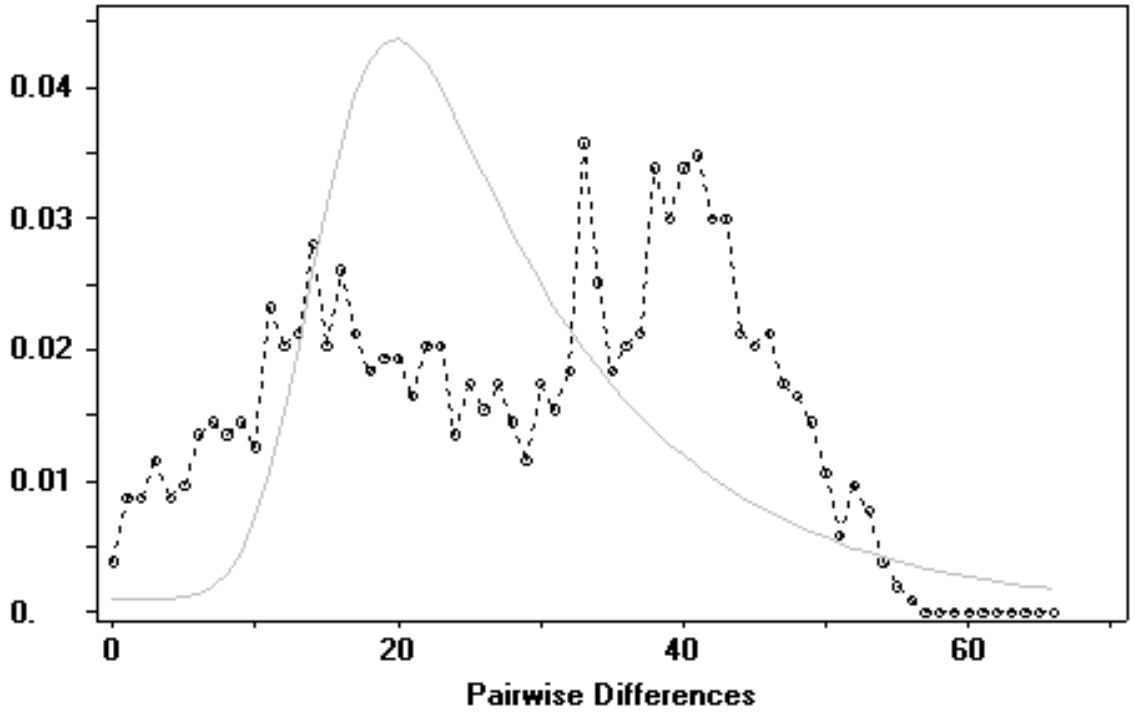

Figure 3. Mismatch distribution for the ND3-ND4 haplotypes for the populations of *Peromyscus mexicanus*. The gray solid line represents observed frequency, while the broken line connecting the diamonds depicts expected frequency under the expansion model. For neutrality tests (Tajima's *D* and Fu's F_S) and statistical values of mismatch distribution (*Hrg* and R_2) see text.

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Table 1. Observed genetic variability within localities (L) currently assigned to *Peromyscus mexicanus*. Number of specimens (*N*), of segregating sites (*S*), and of haplotypes (*H*), together with Haplotype diversity (*Hd*), average number of differences (*K*), and nucleotide diversity (π), are shown. Localities with one sample only show *N* and *H*.

L (number and name locality)	**Country(State)**	**Id**	***N***	***H***	***S***	***Hd***	***K***	***π***
1. La Luz	Mexico(Oaxaca)	LLuz	1	1	-	-	-	-
2. Santa Catarina Loxicha	Mexico(Oaxaca)	SCIx	2	2	1	1.00	1.00	0.003
3. Santiago Laxopa	Mexico(Oaxaca)	SnLax	1	1	-	-	-	-
4. Viejo Aserradero	Mexico(Oaxaca)	ViAse	1	1	-	-	-	-
5. Huautla de Jiménez	Mexico(Oaxaca)	Huau	2	2	5	1.00	5.00	0.014
6. Santa María Yavesía	Mexico(Oaxaca)	SMY	1	1	-	-	-	-
7. Cerro Pelón	Mexico(Oaxaca)	CePel	2	2	10	1.00	10.00	0.027
8. Vista Hermosa	Mexico(Oaxaca)	ViHer	1	1	-	-	-	-
9. Ixtlán Juárez	Mexico(Oaxaca)	IxJua	2	2	5	1.00	5.00	0.014
10. Venustiano Carranza	Mexico(Puebla)	VeCar	1	1	-	-	-	-
11. Río Camarones	Mexico(Hidalgo)	RiCam	3	3	10	1.00	6.67	0.018
12. San Martín Caballero	Mexico(Oaxaca)	SMC	3	3	38	1.00	25.33	0.069
13. Nizanda	Mexico(Oaxaca)	Niza	3	3	9	1.00	6.00	0.016
14. Cerro Chumpipe	Mexico(Chiapas)	CeChu	1	1	-	-	-	-
15. Palenque	Mexico(Chiapas)	Pale	3	3	37	1.00	25.00	0.068
16. Central America GB	Central America	CAGB	1	1	-	-	-	-
17. Cerro El Pital	El Salvador(Chalatenango)	CEP	1	1	-	-	-	-
18. Ejido Nicolás Bravo	Mexico(Chiapas)	ENB	2	1	0	0.00	0.00	0.000
19. Ejido Ojo de Agua	Mexico(Chiapas)	EOA	2	2	6	1.00	6.00	0.016
20. Ranchería San Martín	Mexico(Chiapas)	RSM	5	4	6	0.90	2.60	0.007
21. Ejido El Pinabete	Mexico(Chiapas)	EjPin	2	2	8	1.00	8.00	0.022
22. El Federalista	Mexico(Chiapas)	ElFed	3	3	16	1.00	10.67	0.029
23. Ejido Loma Bonita	Mexico(Chiapas)	ELB	3	2	4	0.67	2.67	0.007
Total			46	42	136	0.99	28.47	0.089

Table 2. AMOVA results calculated in Arlequin v. 3.3, by population and by haplotype.

Source of variation	d.f.	*SSD*	δ^2	% total	Fixation Indices
Φ_{CT}	20	849.811	11.49651	52.15	0.52154
Φ_{SC}	2	23.309	6.64583	30.15	0.63012
Φ_{ST}	23	89.724	3.90102**	17.70	0.82303
Total	45	962.844	22.04336		

Table 3. F_{ST} pair-wise population values, estimated by Tamura & Nei algorithm (above diagonal), and their *p*-values (below diagonal). The plus (+) indicates localities with significative differences ($p \leq 0.05$).

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
1:	LLuz		0.99	1	1	0.98	1	0.85	1	0.94	1	0.94	0.62	0.92	1	0.45	1	1	1	1	0.96	0.94	0.85	0.99
2:	SCIx	-		0.99	0.33	0.99	0.99	0.93	0.99	0.97	0.99	0.98	0.79	0.96	0.98	0.73	0.99	0.99	0.99	0.99	0.96	0.95	0.92	0.99
3:	SnLax	-	-		1	0.98	1	0.89	1	0.95	1	0.95	0.58	0.91	1	0.61	1	1	1	1	0.96	0.95	0.86	0.99
4:	ViAse	-	-	-		0.99	1	0.87	1	0.95	1	0.97	0.66	0.94	1	0.59	1	1	1	1	0.96	0.93	0.88	0.99
5:	Huau	-	-	-	-		0.98	0.79	0.97	0.89	0.99	0.88	0.52	0.86	0.95	0.3	0.96	0.97	0.97	0.97	0.9	0.96	0.72	0.99
6:	SMY	-	-	-	-	-		0.87	1	0.95	1	0.94	0.65	0.92	1	0.48	1	1	1	1	0.96	0.93	0.86	0.99
7:	CePel	-	-	-	-	-	-		0.51	-0.1	0.88	0.77	0.35	0.59	-0.2	-0.1	0.71	0.74	0.77	0.65	0.57	0.91	0.19	0.93
8:	ViHer	-	-	-	-	-	-	-		0.79	1	0.87	0.33	0.79	1	0.04	1	1	1	1	0.87	0.94	0.54	0.99
9:	IxJua	-	-	-	-	-	-	-	-		0.95	0.81	0.31	0.64	0.34	-0.2	0.89	0.89	0.89	0.83	0.63	0.94	0.14	0.97
10:	VeCar	-	-	-	-	-	-	-	-	-		0.96	0.4	0.92	1	0.63	1	1	1	1	0.95	0.96	0.87	0.99
11:	RiCam	-	-	-	-	-	-	-	-	-	-		0.66	0.85	0.82	0.49	0.88	0.89	0.85	0.9	0.88	0.94	0.73	0.96
12:	SMC	-	-	-	-	-	-	-	-	-	-	-		0.52	0.03	0.24	0.4	0.49	0.52	0.39	0.64	0.71	0.41	0.72
13:	Niza	-	-	-	-	-	-	-	-	-	-	-	-		0.64	0.36	0.83	0.86	0.85	0.81	0.8	0.93	0.41	0.93
14:	CeChu	-	-	-	-	-	-	-	-	-	-	-	-	-		-0.7	1	1	1	1	0.64	0.93	0.11	0.99
15:	Pale	-	-	-	-	-	-	-	-	-	-	-	-	-	-		0.09	0.27	0.27	0.07	0.35	0.64	0.02	0.76
16:	CAGB	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		1	1	1	0.92	0.94	0.64	0.99
17:	CEP	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		1	1	0.92	0.94	0.73	0.99
18:	ENB	-	-	-	-	-	-	-	-	-	-	**+**	-	-	-	-	-	-		1	0.88	0.96	0.67	0.99
19:	EOA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		0.84	0.95	0.6	0.99
20:	RSM	-	**+**	-	-	-	-	**+**	-	-	-	**+**	**+**	**+**	-	**+**	-	-	-	-		0.96	0.63	0.96
21:	EjPin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		0.87	0.98
22:	ElFed	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**+**	-		0.9
23:	ELB	-	-	-	-	-	-	-	-	-	-	-	**+**	-	-	-	-	-	-	-	**+**	-	-	

Appendix A. Specimens examined

Data samples of *Peromyscus mexicanus* (collection and accession number) and the outgroup (accession number) used in this work are shown in the following list. Acronyms and full names for the scientific collections are as follows: UAMI, Colección de Mamíferos de la Universidad Autónoma Metropolitana; MZFC, Museo de Zoología "Alfonso L. Herrera" de la Facultad de Ciencias, Universidad Nacional Autónoma de México; Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional, Oaxaca (CIIDIR-O), Instituto Politécnico Nacional; El Colegio de la Frontera Sur, San Cristóbal de las Casas (ECOSUR), and Instituto de Historia Natural y Ecología (IHNyE). See Table 1 for localities

Peromyscus mexicanus specimens list (n = 46):

LLuz (MZFC8657), SCIx1 (CIIDIRO2540), SCIx2 (CIIDIRO2541), SnLax (CIIDIRO3582), ViAse (CIIDIRO3579), SMY (MZFC8203), SMC1 (MZFC8689), SMC2 (MZFC8695), SMC3 (MZFC8697), RiCam1 (MZFC8995), RiCam2 (MZFC8997), RiCam3 (MZFC9000), Huau1(UAMI10252), Huau2 (UAMI10259), CePel1 (CIIDIRO3441), CePel2 (CIIDIRO3297), ViHer (CIIDIRO3294), IxJua1 (MAPP020), IxJua2 (MAPP021), VeCar (UAMI5008), Niza1 (MZFC9297), Niza2 (MZFC9304), Niza3 (MZFC9302), CeChu (IHNE2187), Pale1 (ECOSUR1674), Pale2 (ECOSUR1705), Pale3 (IHNE2340), CAGB (U8386), CEP (MZFCXXX), ENB1 (ECOSUR1404), ENB2 (ECOSUR1408), EOA1s (ECOSUR1619), EOA2 (ECOSUR1620), EjPin1 (IHNE2008), EjPin2 (IHNE2009), ElFed1 (ECOSUR1883), ElFed2 (ECOSUR1886), ElFed3 (ECOSUR1888), RSM1 (ECOSUR1496), RSM2 (ECOSUR1499), RSM3 (ECOSUR1501), RSM4 (ECOSUR1503), RSM5 (ECOSUR1504), ELB1 (ECOSUR1512JBC109), ELB2 (ECOSUR1333), ELB3 (ECOSUR1328).

Outgroup specimens list (n = 17):

Baiomys taylori (Bmtay: U83829), *Habromys lophurus* (Hblop: U83863), *H. schmidlyi* (Hbsch: DQ793115), *Megadontomys nelsoni* (Mgnel: DQ793119), *Neotomodon alstoni* (Ntals1: JN885474, Ntals2: JN885475), *Osgoodomys banderanus* (Osban: U83860), *Peromyscus beatae* (Prbea: U83864), *P. eremicus* (Prere: U83861), *P. furvus* (Prfur1: JN885522, Prfur2: JN885504, Prlat: JN885476), *P. maniculatus* (Prman1: U40249, Prman2: U40251), *P. melanocarpus* (Prmlc: JN885472, JN885473), *P. melanophrys* (Prmlp: xxxxx), and *Podomys floridanus* (Pdflo: PFU83865).

Capítulo 3. Filogeografía comparada de roedores mexicanos basada en la divergencia y diversidad genéticas

Filogeografía comparada de roedores mexicanos, basada en la divergencia y diversidad genéticas

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RESUMEN

Se ha propuesto que la Filogeografía Comparada vincula procesos micro y macroecológicos e infiere historias biogeográficas comunes a partir tanto de grupos taxonómicos codistribuidos, como de la sistemática molecular y la genética de poblaciones. La genética del paisaje, derivada de la genética de poblaciones, liga elementos ambientales y/o geográficos con la divergencia y diversidad genéticas para generar modelos espaciales de los patrones genéticos, cuyos resultados pueden ayudar a la Filogeografía Comparada en la búsqueda de procesos histórico-evolutivos. Se analizaron la divergencia y diversidad genética de cinco especies de roedores, todos ellos distribuidos en las zonas montanas del oriente de México para para reconocer sitios genéticamente diversos y rompimientos en el flujo genético de los mismo para reconocer la influencia de éstás zonas sobre la estructura poblacional de las especies. Se generaron redes de haplotipos por especie, valores de sitios polimórficos, matrices de haplotipos y diversidad haplotípica. Además, se utilizaron datos geográficos para generar mapas de divergencia y diversidad genética individual y múltiple. Los AMOVAs indican estructuras que pueden asociarse con factores ecológicos-geográficos, excepto en *P. mexicanus*. Los mapas resaltan que la unión entre la FVTM, SMOr y ZMOx contienen los valores más altos de divergencia y diversidad sugiriendo que ésta zona puede promover la generadora de nuevas especies.

INTRODUCCIÓN

La filogeografía comparada vincula procesos micro y macroecológicos e infiere historias biogeográficas comunes a partir grupos taxonómicos codistribuidos, a partir de la sistemática molecular y la genética de poblaciones (Avise *et al*. 1987, Evans *et al*. 1997, Bermingham & Moritz 1998, Moritz & Faith 1998, Avise 2000, Brunsfeld *et al*. 2001, Zink 2002, Arbeláez-Cortés 2012, Cutter 2013). El análisis en trabajos filogeográficos de los valores de divergencia y diversidad genéticos, dados por la genética de poblaciones, permiten tener un mejor entendimiento acerca del comportamiento de las poblaciones al momento de vincularlas con su filogenia (Bernatchez & Wilson 1998, Moritz & Faith 1998, Arbogast & Kenagy 2001, Zink *et al*. 2001, Hoffmann & Baker 2003, Bowen & Karl 2007, Whiteman *et al*. 2007, Cortés-Rodríguez *et al*. 2008, Hull *et al*. 2008, Lejeusne *et al*. 2011, Zhao *et al*. 2011).

Derivada de la genética de poblaciones encontramos a la genética del paisaje, la cual liga elementos ambientales y/o geográficos con la divergencia y diversidad genéticos para generar modelos espaciales de los patrones genéticos (Petren *et al*. 2005, Lee-Yaw *et al*. 2009, Richards-Zawacki 2009, Zellmer & Knowles 2009, Poelchau & Hamrick 2012), cuyos resultados pueden ayudar a la filogeografía comparada en la búsqueda de procesos histórico-evolutivos (Manel *et al*. 2003, Guillot *et al*. 2005a, b, Kidd & Ritchie 2006, Miller *et al*. 2006, Kozak *et al*. 2008, Vandergast *et al*. 2008, Vandergast *et al*. 2011), sobre todo para reconocer barreras de flujo génico, áreas de alta diversidad genética (hotspots) y estructuración poblacional, asociadas a aspectos ecológico-geográficos como temperatura, climas, tipos de vegetación, ecorregiones, hidrología, fisiografía, entre otras (Vandergast *et al.* 2007, Vandergast *et al*. 2008, Lee-Yaw *et al*. 2009, Scoble & Lowe 2010 Vandergast *et al*. 2013*,* Arbeláez-Cortés *et al*. 2014).

La genética del paisaje permite entender la dispersión y la diferenciación de las poblaciones en términos del paisaje (Weiss & Ferrand 2007). La correlación entre el paisaje y la conectividad entre poblaciones con la divergencia y diversidad genéticas generan resultados que pueden ser reflejo de la heterogeneidad ambiental por lo tanto, la importancia del paisaje sobre la estructura genética dependerá de si la especie es especialista o generalista. (Arnaud 2003, Geffen *et al*. 2004, Weiss & Ferrand 2007). Vandergast y colaboradores en diversos trabajos han generado y utilizado una herramienta para ArcGis (Genetic Landscapes GIS Toolbox 9.3, Vandergast *et al*. 2011) que permite mapear paisajes genéticos a partir de diversas fuentes de datos moleculares (i. e. secuencias de DNAmt, DNAn, DNAcl y microsatélites) en diferentes especies codistribuidas para así examinar patrones de concordancia que permitan localizar las regiones geográficas relevantes en la diversidad de especies (Vandergast *et al*. 2011, Vandergast *et al*. 2013)

En este trabajo, analizaremos la filogeografía comparada a través de los mapas de divergencia y diversidad genéticas de cinco especies de roedores (*Glaucomys volans*, *Peromyscus furvus*, *P. mexicanus*, *Reithrodontomys mexicanus* y *R. sumichrasti*) para inferir el o los posibles factores que intervienen en la reconstrucción de los patrones de distribución genética de roedores en las zonas montanas del oriente de México, desde el sur de Sierra Madre Oriental hasta el centro de Oaxaca.

MÉTODOS

ZONA DE ESTUDIO

El área de estudio ocupa una extensión del 32,335 km^2 (Fig. 1) y comprende a la Sierra Madre Oriental (SMOr), el sector oriental de la Faja Volcánica Transmexicana (FVTM) y las zonas montanas de Oaxaca (ZMOax). Las ecorregiones (Fig. 3) de la World Wild Foundation-CONABIO-CCA (1997) sirvieron para delimitada la zona de estudio.

OBTENCIÓN DE DATOS

Se seleccionaron especies cuya distribución está parcial o totalmente dentro de las zonas montanas consideradas para éste trabajo (Fig 1), mientras que las secuencias genéticas para cada especie fueron obtenidas de trabajos sistemáticos que contuvieran los datos geográficos (localidad y su georreferencia) completos o que pudieran obtenerse de bases de datos como Global Biodiversity Information Facility (GBIF, www.gbif.org), Mammal Networked information System (MANIS, manisnet.org), Unidad de Informática para la Biodiversidad (UNIBIO, unibio.unam.mx) y National Science Research Laboratory at the Museum of Texas Tech University (TTU, http://www.nsrl.ttu.edu). Para *Glaucomys volans* (Kerhoulas & Arbogast 2010), *Peromyscus furvus* (Harris *et al*. 2000, Arnaud 2003), *Reithrodontomys mexicanus* (Arellano *et al*. 2005, Miller & Engstrom 2008, Hardy *et al*. 2013) y *R. sumichrasti* (Sullivan *et al*. 2000, Miller & Engstrom 2008, Hardy *et al*. 2013) las secuencias se obtuvieron de la base de datos del GeneBank (http://www.ncbi.nlm.nih.gov/). En el caso de *P. mexicanus* (ver Cap. 2) las secuencias se generaron en el laboratorio del Museo de Zoología, UNAM.

Las secuencias fueron alineadas con la implementación de Muscle (Edgar 2004) en el programa MEGA 6. (Tamura *et al*. 2013). Por medio del programa DNAsp 5.10.1 (Librado, Rozas 2009) se agruparon los individuos por localidad y se obtuvieron los valores de sitios polimórficos (invariables y variables), número de haplotipos, diversidad haplotípica y la matriz de haplotipos para cada especie (Cuadro 1). Ésta última se analizó en el programa Arlequin 3.5 (Excoffier, Lischer 2010) para generar los datos genéticos que se utilizaron para los mapas genéticos (siguiente sección). Además, para cada especie se realizó un Análisis de Varianza Molecular (AMOVA) para establecer si existe estructuración y se obtuvieron los valores de los estadísticos *Fst* (Cuadro 2). Por otro lado, se generaron redes de haplotipos (Fig. 2) con el programa Network 4.6.1.1 (Bandelt *et al*. 1999).

MAPAS DE DISTRIBUCIÓN GENÉTICOS

Los datos genéticos obtenidos con Arlequin 3.5 (Excoffier, Lischer 2010) junto con datos geográficos (localidades y georreferencias) se utilizaron para generar los mapas de divergencia y diversidad genética por especie (MDgS y MDsS respectivamente, Fig. 4) y múltiple (MDgM y MDsM respectivamente, Figs. 5) en el programa ArcGIS 9.3 (ESRI 2009) por medio de la caja de herramientas para genética del paisaje (Genetic Landscapes GIS Toolbox 9.3, Vandergast *et al*. 2011). Para la divergencia se calculó la matriz de divergencia genética interpoblacional, mientras que para la diversidad genética se obtuvieron los valores de diversidad nucleotídica (π) intrapoblacional. En ambos análisis se utilizó el algoritmo de Tamura-Nei (Tamura, Nei 1993), cuya escala genética va de 0-1 donde 0 es baja y 1 es alta divergencia o diversidad (Vandergast *et al*. 2011, Vandergast *et al*. 2013), por lo que se categorizaron en cinco intervalos tanto de divergencia como de diversidad, agrupados por rompimientos naturales con el método de Jenk (Fig 5a). Los mapas resultantes se compararon con las ecorregiones bosques montanos de Veracruz (bmV) y de Oaxaca en su porción norte (bmON) y sur (bmOS), asi como los bosques de pino-encino de la SMOr (bpeSMOr), de la FVTM (bpeFVNM) y de la Sierra Madre de Oaxaca localizada en las ZMOax (bpeSMO) y las provincias fisiográficas Karst Huasteco (KH),Chiconquiaco (PrC), lagos y volcánes del Anáhuac (LVA) y SierrasOrientales (PrSO), éste último obtenido del portal de geoinformación de la Comisión Nacional para el Conocimiento y Uso de la Biodiversidad (CONABIO, http://www.conabio.gob.mx/informacion/gis/).

RESULTADOS

GENÉTICA DE POBLACIONES Y REDES DE HAPLOTIPOS

Los valores de diversidad genética por especie (Cuadro 1) muestran que las especies presentan una diversidad haplotípica alta, con bajos niveles de diversidad nucleotídica, señalando que a pesar de tener muchos haplotipos, las diferencias entre ellos son mínimas. Por otro lado, si bien las secuencias analizadas contienen diferentes

genes y longitudes en términos de los pares de bases, el porcentaje de sitios informativos es mayor a 2%, por lo que existen suficientes sitios para hacer buenas inferencias filogenéticas. Las AMOVA's (Cuadro 2) por especie señalan que en todas ellas existen diferencias dentro de las poblaciones (F_{ST}) y en algunos casos como en *P. mexicanus* y *R. sumichrasti* también hay significancia entre grupos (F_{CT}). Por su parte, los grupos formados por las redes de haplotipos de cada especie (Fig 2), se asocian en gran medida con las ecorregiones (Fig. 3) situadas sobre los en los macizos montañosos de México y hasta Centroamética,. Los haplotipos asociados a las ZMOax que se encuentran al sur del área de estudio sobre los bmON y bmOS, bpeSMO y bpeSMS, están asociados hacia el norte con haplotipos que se encuentran en los bmV, bpeSMOr y en los bpeFVTM como lo señalan *P. fuvus*, *P. mexicanus* y *R. sumichrasti*, y hacia el sur con ecorregiones ubicadas desde Chiapas hasta Centroamérica como se muestra en *P. mexicanus*, *R. sumichrasti*, *R. mexicanus* y *G. volans.* En el caso de la red de *P. mexicanus*, los haplotipos de las ZMOax se mezclan entre los demás haplotipos, por lo que ver alguna concordancia geográfica resulta complejo; sin embargo, se puede apreciar que las ZMOax asocian a las poblaciones de las zonas montanas de este de México con los macizos montañosos de Chiapas y hacia Centroamérica.

ANÁLISIS DE LA DIVERGENCIA Y DIVERSIDAD GENÉTICAS

DIVERGENCIA GENÉTICA

La divergencia genética permite relacionar poblaciones fragmentadas para reconocer los rompimiento en el flujo génico, estableciéndose una relación inversa entre éste y la divergencia, es decir, entre más alta sea la divergencia, el flujo génico entre las poblaciones disminuye, mientras que a mayor flujo génico la divergencia es menor (Vandergast *et al*. 2008, Vandergast *et al.* 2013, Kraft *et al*. 2010). De las cinco especies, *Reithrodontomys sumichrasti* y *Peromyscus mexicanus* contribuyen mayormente en el

acomodo de las zonas de divergencia en el MDgM, siendo *Glaucomys volans* la que menos apoya.

El mapa de divergencia múltiple (MDgM) ocupa el 89.30% (28,873.87 km^2) sobre el área de estudio. Las zonas de divergencia muy baja se localiza al oeste del KH, ocupando el este de los BPESMOR, norte de los bpeFTVM y oeste de los bmV con un área de 1007.65 km^2, que representa el 3.49% del mapa obtenido, basado en los MDgS's de *Reithrodontomys sumichrasti*, *Peromyscus furvus* y *P. mexicanus*. Las de divergencia baja se apoya en los MDgS's *R. sumichrasti* y *P. mexicanus*, ocupando el 19.45% (5615.88 km^2) de la distribución y se encuentran desde el norte hasta el este de los bpeFVTM, así como en los bmV, norte y centro de bmON, principalmente en el KH y PrC. La divergencia media presenta una extensión de 9163.32 km^2 (31.74%) y se divide en tres secciones, la primera y más amplia se localiza en los bpeFVTM y bmON que se distribuyen al sur del KH, oeste de la PrC y este de los LVA y es avalada por el MDgS de *P. mexicanus* principalmente, seguida del MDgS de *R sumichrasti*. Las siguientes dos áreas las apoya principalmente el MDgS de *R. mexicanus*, seguida por el MDgS de *R. sumichrasti* y se encuentra en los bmOS y bpeSMO en la PrSO, restringidas a altitudes entre 1000 y 2000 msnm. Las zonas de divergencia alta y muy alta, con una extensión de 6891.85 (23.87%) y 6195.18 (21.46%) respectivamente se localizan mayormente en los bpeSMO y bmOS sobre la PrSO, además del sureste de los bpeFVTM y sur de bmON sobre los LVA y PrC, apoyadas principalmente por los MDgG's de *P. mexicanus* y *P. furvus* seguida de *R. sumichrasti*.

DIVERSIDAD GENÉTICA

La diversidad genética, permite establecer la calidad del pool genético de los fragmentos y, al diferencia de la divergencia genética, entre mayor diversidad mejor la calidad del pool genético, mientras que a menor diversidad los fragmentos son más pobres (Davis *et al*. 2008, Vandergast *et al*. 2008, Vandergast *et al*. 2013). En éste caso,

las cinco especies están contribuyendo en la conformación del MDsM, siendo *R. sumuchrasti* la que más aporta sobre todo para las zonas de diversidad alta, seguida de *G. volans*. La que menos aporta por tener la menor distribución es *R. mexicanus* para éste análisis

La diversidad muy baja y baja representan el 16.21% (5061.08 km^2) y 15.4% (4808.49 km^2) del MDsM respectivamente y las encontramos principalmente sobre los bmON en la PrC, seguida de algunos manchones en el este de los bpeFVTM, norte y centro de los bmV y centro de los bmOS que se encuentran en el KH y los LVA. La diversidad media, que es apoyada principalmente por *R. sumichrasti* seguida de *G. volans*, es la más abundante con una extensión de 14,801.25 km^2 (47.39%) y se localiza al centro-norte de los bmV, este de los bpeFVTM, los bmOS y los bpeSMO en las provincias KH, LVA y PrSO. La diversidad alta y muy alta se ubica principalmente al centro del MDsM sobre provincias PrC, PrSO y LVA al sur de los bmON y norte del bpeSMO apoyado especialmente por *R. sumichrasti* y *G. volans*, aunque también se encuentran varios de éstos manchones sobre los bmV y centro de los bpeSMO, basados en los MDsS's de *P. furvus* para el primero y de *P. mexicanus* y *G. volans* para el segundo.

DISCUSIÓN

GEOGRAFÍA VS. ECOLOGÍA

En México la biodiversidad y procesos de especiación están muy relacionados con la variedad de ecosistemas, producto a su vez de una compleja historia geológica (Halffter 2003, Espinoza *et al*. 2006, Cortés-Rodríguez *et al*. 2008), que en el caso de la consolidación de los macizos montañosos de México entre el Mioceno y el Plioceno, asociados a las fluctuaciones climáticas del Plioceno-Pleistoceno, el cambio en la distribución y los tipos de vegetación y a la influencia humana en la actualidad (Fa &

Morales 1998, Cox 2000, Montellano-Ballesteros & Jiménez-Hidalgo 2006, Cortés-Rodríguez *et al*. 2008), hacen que grupos como los roedores, que para éstas zonas representan un taxón importante por su alta riqueza de especies, endémicas en muchos casos (Fa & Morales 1998, Montellano-Ballesteros & Jiménez-Hidalgo 2006), sea interesante para diversos autores (Hogan *et al*. 1997, Engel *et al*. 1998, D'Elía 2000, Harris *et al*. 2000, Sullivan *et al*. 2000, Arellano *et al*. 2006, León-Paniagua *et al*. 2007, Rogers *et al*. 2007). Los cricétidos, albergan la mayoría de la especies de roedores, siendo la subfamilia Neotominae de origen Neártico una de las más predominantes (Fa & Morales 1998, Espinoza *et al*. 2006, Montellano-Ballesteros & Jiménez-Hidalgo 2006). Muchos autores intentar encontrar patrones filogeográficos relacionados con los aspectos geológico-geográficos, por lo que explicar la formación de especies crípticas y/o de patrones parafiléticos es complicado, sobre todo si las especies se establecen posteriormente a cualquier influencia de éstos aspectos sobre un área, por lo que considerar aspectos ecológicos y genéticos es crucial para el entendimiento de la distribución de éstos mamíferos.

Al observar las redes de haplotipos (Fig. 2) y relacionarlas con los estadísticos de F_{ST}; (cuadro 2), reconocemos en *R. sumichrasti*, *R. mexicanus*, *P. furvus* y *G. volans* una estructuración vinculada con las provincias fisiográficas (Fig. 1b) y las ecorregiones (Fig. 3), resaltando a las ZMOax como conectoras de diferentes áreas. León-Paniagua y Morrone (2009) indican que éstas zonas no representan una unidad natural, sirviendo más bien como un puente entre especies de las zonas montanas de la SMOr y FVTM con las de Chiapas hasta Panamá como lo muestran las redes de haplotipos de *G. volans*, *R. sumichrasti* y *R. mexicanus*, que al compararlas con sus filogénicas (ver Arellano *et al*. 2006 y Hardy *et al*. 2013), se observa una alta correlación entre los haplogrupos sugeridos en éste trabajo y los nodos encontrados para cada especies, reforzando lo

reportado por León-Paniagua y Morrone (2009) sobre que las ZMOax tienen afinidad tanto con la SMOr y FVTM, por un lado, como con Chiapas-Centroamérica por otro. Solamente en *P. mexicanus* la red de haplotipos (Fig. 2e) está pobremente asociación con la geográfica, aunque presente una F_{ST} significativa. Ávila-Valle *et al*. (cap. 2) denotan que las poblaciones asignadas a *P. mexicanus* presentan una estructuración que está más asociada con aspectos ecológicos, lo que nos permite pensar en la importancia de éstos como modeladores de la distribución de la especie. En éste sentido Michaux *et al*. (2005) evalúan poblaciones de dos especies de roedores y tratan de determinar si las incongruencias entre los patrones filogeográficos de especies simpátridas y cercanamente relacionadas, son consecuencia de tener diferentes hábitos ecológicos. Los autores denotan que los patrones encontrados para cada especie, al ser diferentes, hacen evidente que algunas barreras como el Mar Mediterráneo y Negro, o las montañas de los Pirineos, Alpes y Carpachos, están ligadas a historias filogeográficas de algunos organismos durante el Plio-Pleistoceno. Finalmente, resaltan que las diferencias entre las especies pueden ser asociadas a eventos alopátridos muy antiguos, los cuales posteriormente fueron ensombrecidos por los cambios climáticos involucrados en el modelo de expansión-contracción durante el Cuaternario, lo que provocó que las poblaciones analizadas tuvieran cambios de densidad poblacional y, por lo tanto, se crea que son especies simpátridas en la actualidad.

Filogeografía comparada y Genética del Paisaje

El debate sobre la diferencia entre la filogeografía y la genética del paisaje es reciente y algunos autores las consideran dos disciplinas que coinciden en búsqueda y entendimiento de la distribución de la variación genética sobre los ambientes naturales, siendo su principal diferencia la escala de tiempo en que resuelven (Manel *et al*. 2003, Wang 2010, Garrido-Garduño & Vázquez-Domínguez 2013). De manera tradicional, la

genética del paisaje y la filogeografía han implementado diferentes tipos de marcadores. Los primeros generalmente usan microsatélites, mientras que la filogeografía considera el DNAmt o DNAcp en la inferencia de patrones históricos (Wang 2010). Asimismo, la Filogeografía Comparada, pretende evidenciar historias evolutivas comunes, puesto que solo permite el uso de un solo tipo de marcador para las especies analizadas (Avise *et al*. 1987, Evans *et al*. 1997, Bermingham & Moritz 1998, Bernatchez & Wilson 1998, Moritz & Faith 1998, Sullivan *et al*. 2000, Avise 2000, Arbogast & Kenagy 2001, Zink *et al*. 2001, Zink 2002, Hoffmann & Baker 2003, Hickerson *et al*. 2006). Es decir, la combinación de diferentes marcadores de diferentes especies ha sido una limitación para la Filogeografía (intraespecífica y comparada) y la genética del paisaje. Recientemente, Vandergast *et al.* (2011) desarrollaron una herramienta (Genetic Landscapes GIS Toolbox) que permite el uso de varios marcadores para la evaluación y búsqueda de patrones de distribución genéticos que reflejen patrones evolutivos, sobre todo al comparar los resultados de divergencia y diversidad genética múltiple con mapas de aspecto ecológico-geográfico (Wang 2010).

Con apoyo de los MDgS (Fig. 4a), observamos que las zonas de alta divergencia en el MDgM (Fig. 5a) se encuentran en la unión del sector oriente de la FVTM con la SMOr y ZMOax, siendo ésta última la que presenta mayor extensión de ésta divergencia, abarcando los bpeSMO y bmOS sobre la PrSO. Asimismo, el MDsM (Fig. 5b), a pesar de que los MDsS (Fig 4 b) muestran que las tendencias individuales son ligeramente diferentes entre las especies, indica que las zonas de alta diversidad se encuentra, al igual que en la divergencia, en la coyuntura entre las tres zonas montanas. Por lo anterior, podemos sugerir que el efecto de la asociación de éstas tres zonas montanas sobre la divergencia y diversidad genética, ha conducido a grupos como las especies del género *Habromys* (León-Paniagua *et al*. 2007, Rogers *et al*. 2007), algunas especies del

subgénero *Aporodon* (Arellano *et al*. 2005) e incluso a las especies *P. mexicanus* (Ávila-Valle *et al*. Cap. 2), *R. mexicanus* (Arellano *et al*. 2005) y *R. sumichrasti* (Hardy *et al*. 2013) hacia la generación de nuevas especies o a impulsar el proceso de especiación, sobre todo si consideramos que las condiciones ecológicas en la unión de los macizos montañosos de la SMOr, FVTM y ZMOax han tenido la capacidad de generar diferentes ambientes (ecorregiones) que promueven la diversidad *in situ* (Harris & Rogers 2000, Dawson 2005, León-Paniagua *et al*. 2007).

CONCLUSIONES

Aunque el registro fósil indica que los clados mayores de roedores divergieron en el paleoceno entre 55-60 ma (Honeycutt *et al*. 2007), la variedad de especies de roedores actuales data del Plio-Pleistoceno (Engel *et al*. 1998, Dawson 2005, Martínez *et al*. 2009, Kerhoulas & Arbogast 2010), por lo que más que la influencia de aspectos geológico-geográficos sobre diversificación de roedores, son los componente ecológicos, como las fluctuaciones climáticas del Pleistoceno, las que han contribuido en el surgimiento de nuevas especies. Los análisis filogenéticos y filogeográficos de *P. furvus* (Harris & Rogers 2000, Arnaud 2003), *P. mexicanus* (cap. 2), *R. mexicanus* (Arellano *et al*. 2005), *R. sumichrasti* (Hardy *et al*. 2013) y *G. volans* (Kerhoulas & Arbogast 2010) junto con las redes de haplotipos resaltan a las ZMOax como puente entre las poblaciones de las zonas montanas del este de México y las de Chiapas-Centroamérica al presentar haplotipos compartidos con ambas secciones y, por ello, éstas zonas ensombrecen las relaciones entre las demás poblaciones debido a que se mezclan sin ningún patrón aparente. León-Paniagua y Morrone (2009) han recapitulado que las ZMOax no son una unidad natural puesto que elementos de la SMOr se asocian más con poblaciones de la Sierra Mazateca, mientras que Sierra Madre del Sur se relaciona con el nudo Zempoaltépetl-Sierra de Juárez. Asimismo, los mapas genéticos muestran cómo la

divergencia y diversidad en los bmOS y bpeSMO, localizados en las ZMOax es alta, sugiriendo cómo estos aspectos genéticos son modelados por condiciones ecológicas, aportando suficiente información para realizar un primer acercamiento sobre los posibles factores y patrones que están involucrados en el establecimiento y segregación de las especies.

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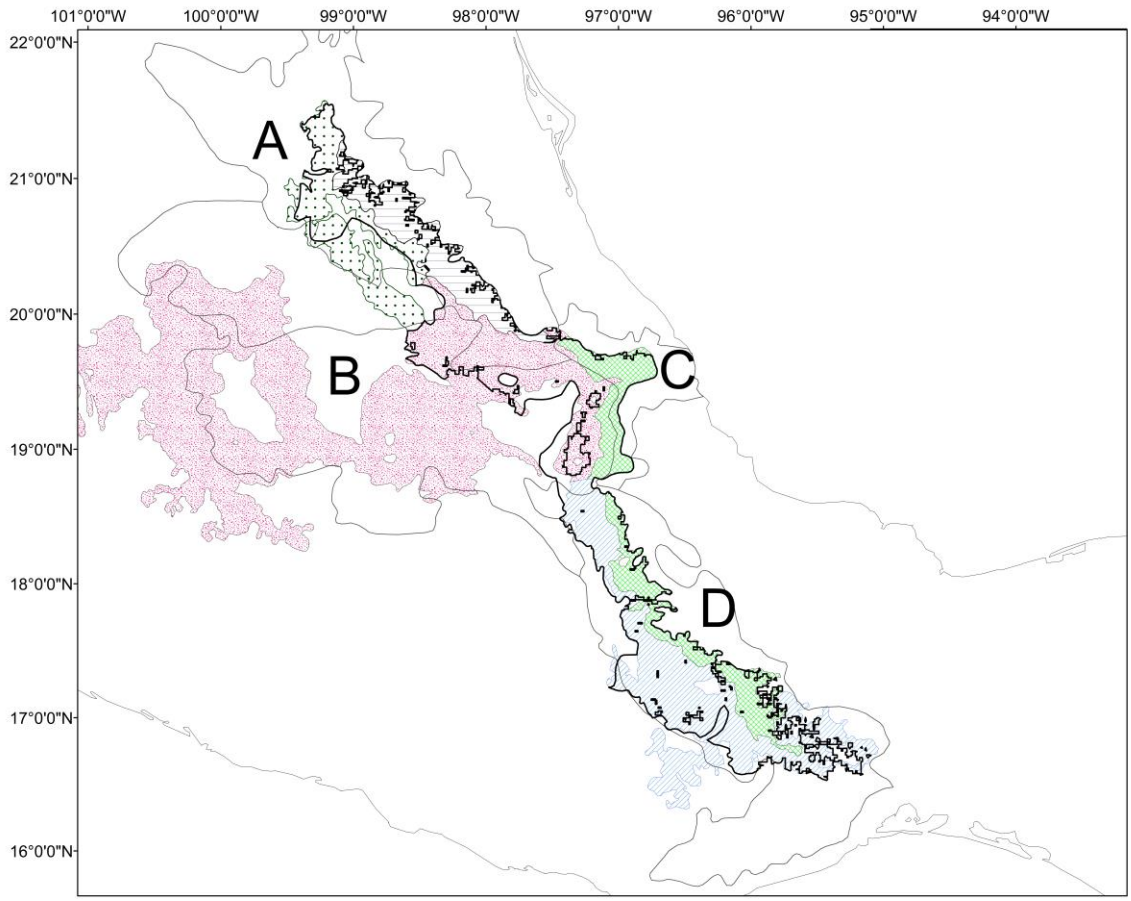

Figura 1. Área de estudio (contorno negro) recortada a partir de las ecorregiones de la WWF (magenta=BpeFVTM, azul marino=BMV, verde obscuro). Las letras indican provincias fisiográficas (contorno gris) donde ocurre el área de distribución (A = Karst huasteco; B = Lagos y Volcanes del Anáhuac; C = Chiconquiaco; D = Sierras Orientales).

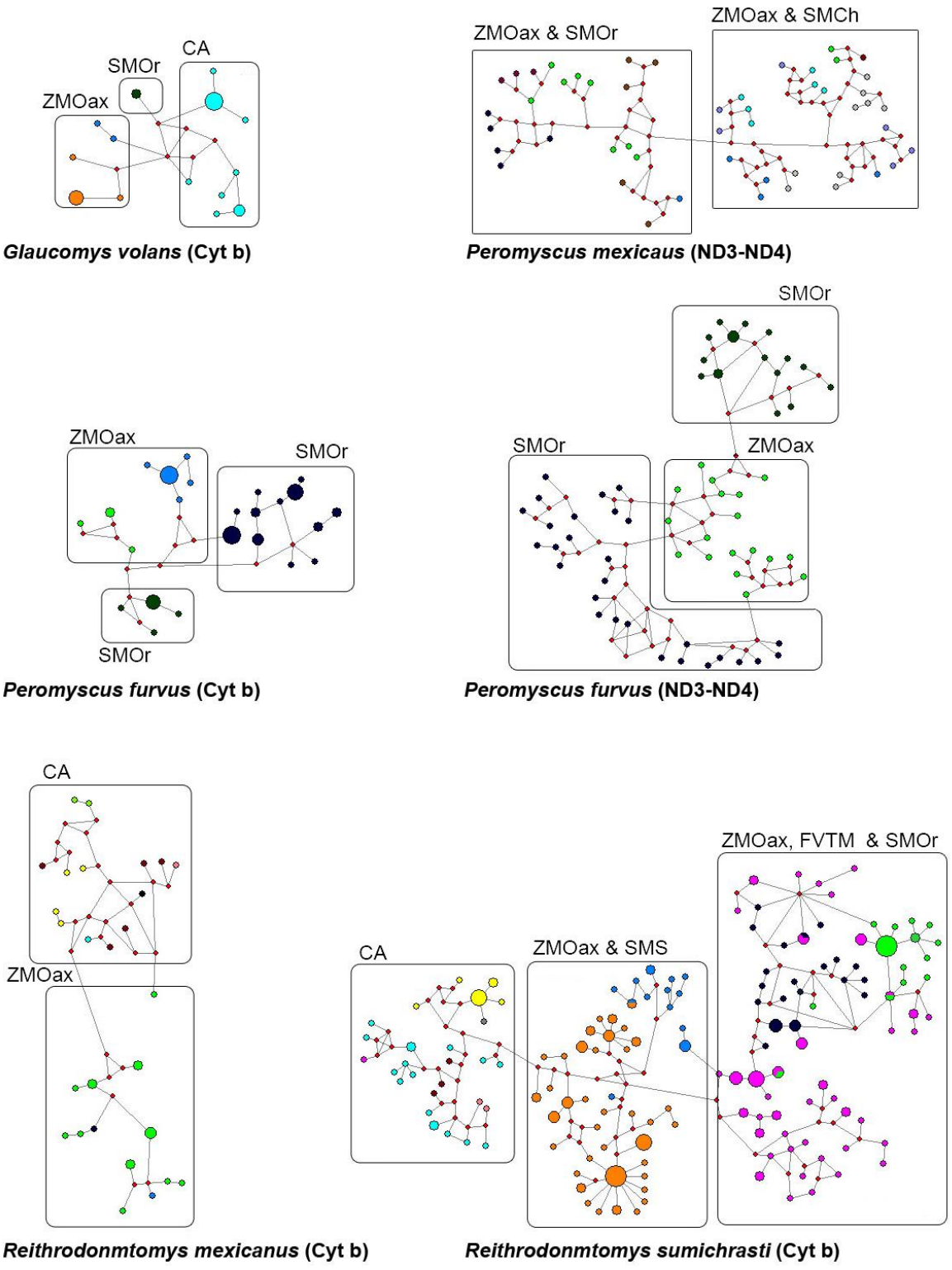

Figura 2. Redes de haplotipos de las cinco especies analizadas (Ver texto para referencias). Los colores denotan las ecorregiones de la Figura 3.

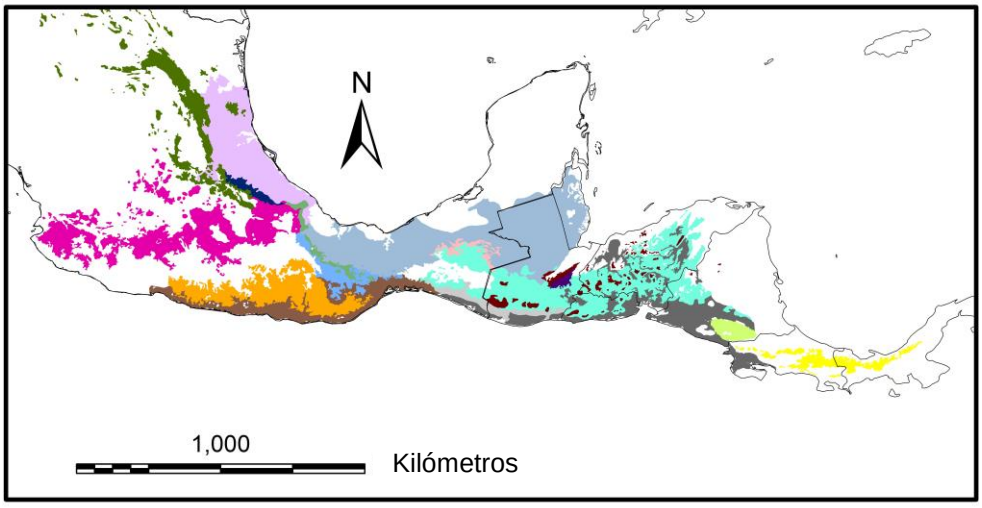

Figura 3. Ecorregiones vinculadas con la red de haplotipos (Fig. 2). Bosques montanos de Veracruz (BMV, azul marino), Bosques montanos de Oaxaca (BMO, verde), Bosques montanos de Chiapas (BMC, rosa), Bosques montanos de Centroamérica (BMCA, rojo marrón), Bosques montanos de Talamanca (BMT, amarillo), Bosques de pino-encino de la Faja VolcánicaTransmexicana (BpeFVTM, magenta), Bosques de pino-encino Sierra Madre de Oaxaca (BpeMO, azul), Bosques de pino-encino Sierra Madre del Sur (BpeSMS, naranja), Bosques de pino-encino de Centroamérica (BpeCA, cian), Bosques de pino-encino Sierra Madre Oriental (BPESMOR, verde obscuro), Bosques húmedos de Veracruz (BHV, lila), Bosques húmedos Petén-Veracruz (BHPV, azul grisáceo), Bosques húmedos Sierra Madre de Chiapas (BHSMC, gris claro), Bosques secos Pacífico Sur (BSPS, café), Bosques secos Centroamérica (BSCA, gris), Matorral espinoso Valle Motagua (MEVM, Morado), Lago Cocibolca (LC, verde claro)

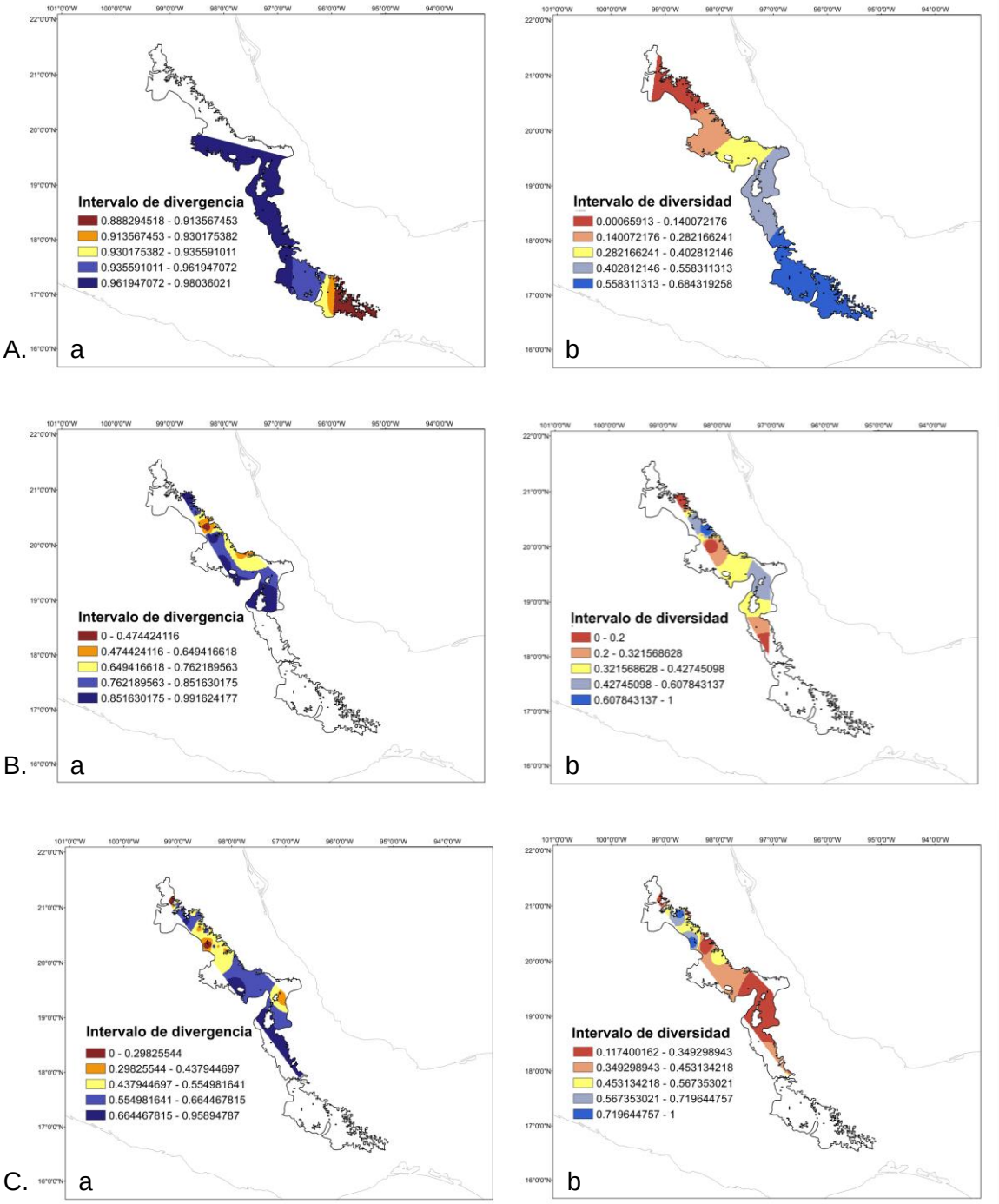

Figura 4. Proyecciones genéticas individuales para **A.** *Glaucomys volans*, **B.** *Peromyscus furvus* Cytb, **C.** *P. furvus* ND3-4, **D.** *P. mexicanus,* **E.** *R. mexicanus*, **F.** *R. sumichrasti*. **a**. Divergencia genética muy alta (morado), alta (azul), media (amarillo), baja (naranja) y muy baja (marrón). **b**. Diversidad genética muy alta (azul), alta (azul claro), media (amarilla) y baja (naranja) y muy baja (roja). Ver texto.

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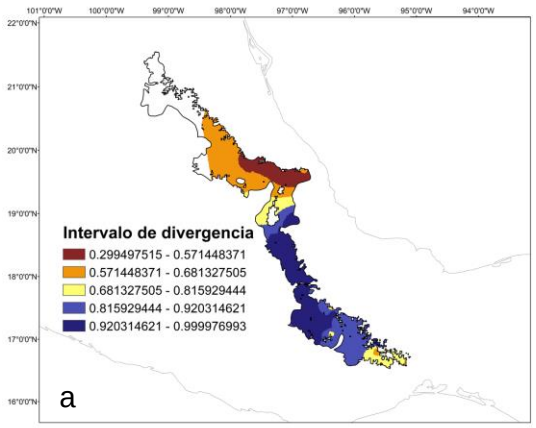

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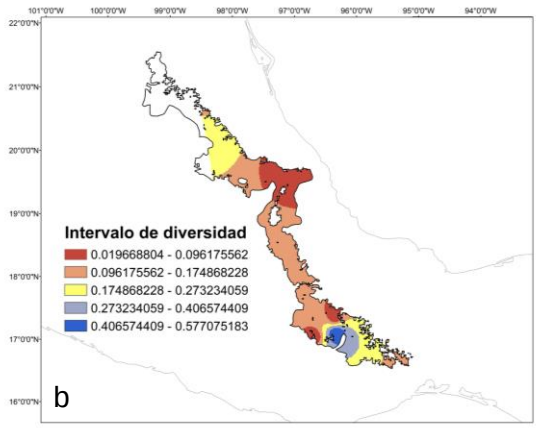

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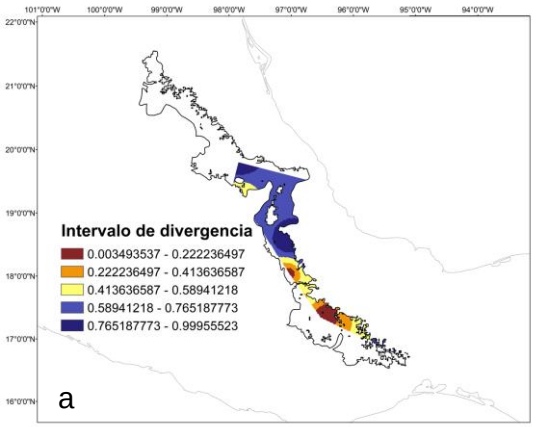

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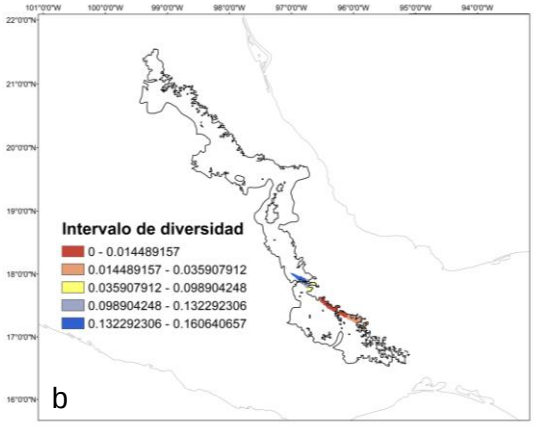

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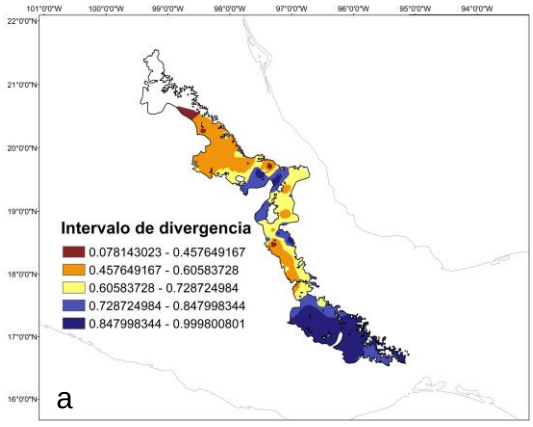

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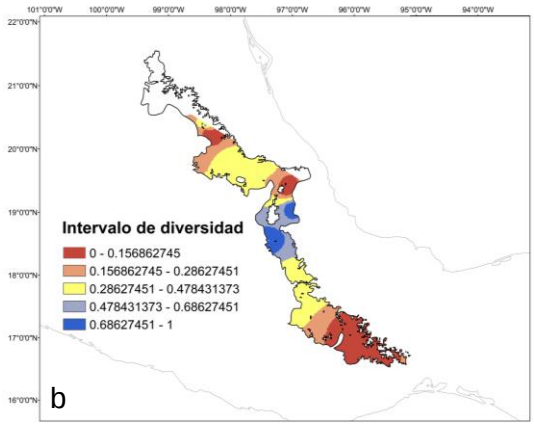

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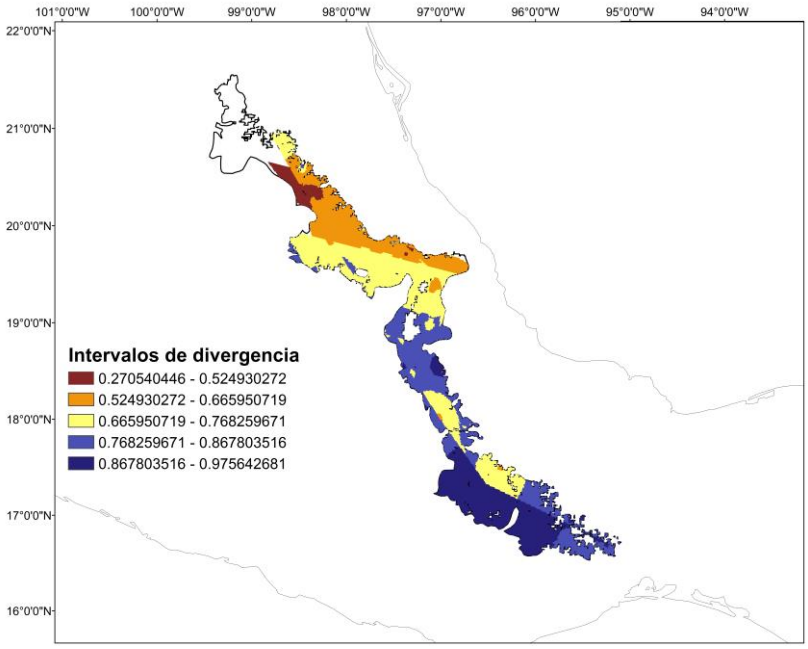

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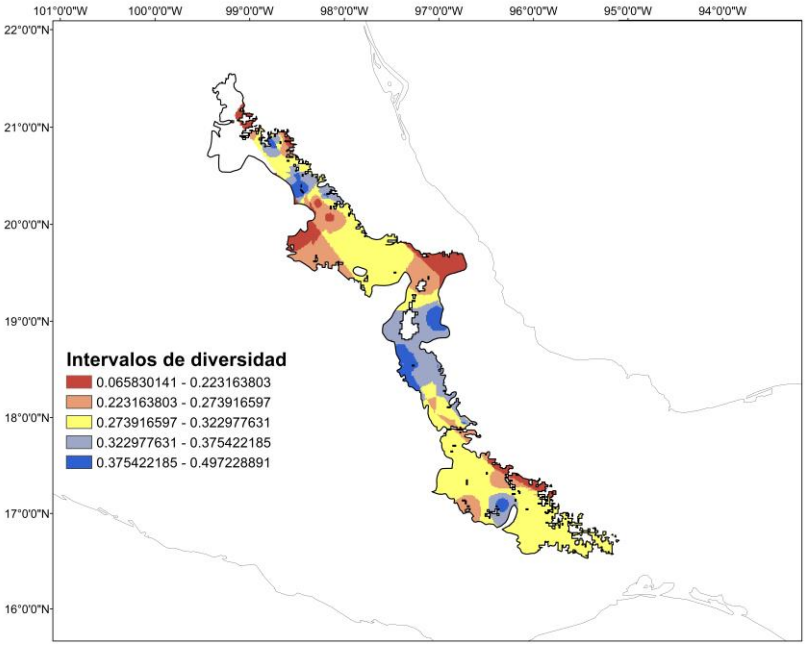

b.

Figura 5. Proyección genética múltiple generada a partir del promedio de los mapas de genéticos por especie (Fig. 4). **a**. Divergencia genética muy alta (morado), alta (azul), media (amarillo), baja (naranja) y muy baja (marrón). **b**. Diversidad genética muy alta (azul), alta (azul claro), media (amarilla) y baja (naranja) y muy baja (roja). Ver texto.

Cuadro 1. Valores de diversidad genetica para los taxa (Tx): *Glaucomys volans* (Gv), *Peromyscus furvus* (Pf), *P. mexicanus* (Pmx), *Reithrodontomys mexicanus* (Rmx) y *R. sumichrasti* (Rs) considerando los genes (Gen) citocromo b (cyt b) y las subunidades subunidades 3, 4L y 4 de la NADH deshidrogenasa (ND3-4). Localidades (L), número de individuos (N), número de haplotipos (*H*), diversidad haplotípica (*Hd*), diversidad nucleotídica (π), pares de bases (PB), sitios invariables (SI), sitios variables (SV), sition únicos (SU), sitios filogenéticamente informativos (SFI)

Tx	**Gen**	**L**	**N**	***H***	***Hd***	***π***	**PB**	**SI**	**SV**	**SU**	**SFI**
Gv	Cyt b	9	28	12	0.823	0.014	571	294	21	7	14 (2.45)
Hb	Cyt b	18	35	27	0.980	0.076	1143	510	171	26	145 (12.69)
Hb	ND3-4	17	29	23	0.983	0.090	1330	320	108	13	95 (7.14)
Pf	Cyt b	8	53	23	0.935	0.0367	719	533	71	10	61 (8.48)
Pf	ND3-4	17	64	60	0.998	0.0380	1049	791	250	97	153 (14.59)
Pm	ND3-4	32	50	45	0.995	0.083	1086	205	156	48	108 (9.94)
Rm	Cyt b	15	32	26	0.986	0.094	1143	731	312	59	253 (22.13)
Rs	Cyt b	231	64	100	0.982	0.039	1152	416	163	51	112 (9.72)

Cuadro 2. Análisis de Varianza Molecular (AMOVA) de cada taxón (Tx) e índice de fijación (FI) entre grupos (F_{CT}), entre poblaciones dentro de grupos (F_{CS}) y dentro de las poblaciones (F_{ST}). Los valores en negritas y cursivas tienen una significación $p \leq 0.05$.

Sp	***F_{CT}* (FI)**	***F_{SC}* (FI)**	***F_{ST}* (FI)**
GvCytb	4.83 (0.95)	-0.19 (-0.77)	***0.44 (0.91)***
HbCytb	44.64 (0.91)	2.26 (0.51)	***2.13 (0.96)***
HbND3-4	***65.86 (1.00)***	-5.98 (0.00)	***5.98 (0.91)***
PfCytb	13.24 (0.81)	2.14 (0.67)	***1.03 (0.94)***
PfND3-4	3.92 (0.18)	10.95 (0.60)	***7.28 (0.67)***
PmND3-4	***49.07 (0.79)***	-7.12 (-0.54)	***20.33 (0.67)***
RmCytb	46.45 (0.71)	-11.30 (-0.61)	***29.87 (0.54)***
RsCytb	***28.22 (0.97)***	-2.98 (-3.00)	3.97 (0.86)

Discusión y conclusiones generales

DISCUSIÓN GENERAL

El establecimiento de la biota en la zona de transición mesoamericana está fuertemente influenciada por la historia geológica, procesos tectónicos y los cambios cíclicos en el clima, vegetación, y nivel del mar (Halffter 2003, Hasbún *et al.*2005, Schuster & Cano 2005). Tanto el clima cálido y húmedo del Plioceno como las fluctuaciones en el nivel del mar y climáticas del Pleistoceno generaron múltiples colonizaciones y extinciones, lo que ha promovido el incremento de la biodiversidad, originada por la especiación *in situ* de biotas neárticas y neotropicales, formando un componente mesoamericano (Savage 1966, Marshall 1988, Engel *et al.* 1998, D'Elia 2000, Pauly *et al.* 2004, Crawford & Smith 2005, Kemp 2005, Kirby & MacFadden 2005, Singer & Avery 2005, Reeves & Bermingham 2006, Escalante *et al.* 2007, Kirby *et al.*2008, Lession 2008, Rull 2008, Castoe *et al.* 2009). Uno de los grupos taxonómicos más diversos y que en Latinoamérica es muy importantes por su alta endemicidad y riqueza de especies son los roedores (Fa & Morales 1998, Cox 2000, Kemp 2005, Montellano-Ballesteros & Jiménez-Hidalgo 2005), los cuales en muchos casos responden a las condiciones ecológicas, que pueden producir especiación criptica así como procesos de parafilia en diversos grupos, haciendo difícil la interpretación de patrones filogeográficos (Michaux *et al.* 2005, Bryant et al. 2011) y el interés en encontrar patrones filogenéticos y filogeográficos afectados por los aspectos ecológico-geográficos para explicar tanto patrones parafiléticos como la cripticidad de las especie es complicado. El grupos de los neotominos de origen neártico es uno de los más predominantes en Mesoamérica (Fa & Morales 1998, Espinoza-Medinilla *et al.* 2005, Montellano-Ballesteros & Jiménez-Hidalgo 2005) como lo son *Peromyscus furvus* y *P. mexicanus*. La obtención de una hipótesis filogenética, ya sea por parsimonia, máxima verosimilitud o por inferencia bayesiana, nos permite reconocer la divergencia existente entre los diferentes nodos de las especies analizadas.

En el caso de *Peromyuscus furvus*, que es una especie endémica a las zonas montanas de la Sierra Madre Oriental, se observa que, si bien existe una relación geográfica en el establecimiento de los clados, el considerar la altitud y tipos de vegetación local pueden ayudar en el entendimiento de su filogenia. Por otro lado, el caso de *P. mexicanus*, que es una especie generalista y con un alto grado de superposición de caracteres entre sus especies cercanas, es más complejo. En los análisis de Inferencia Bayesiana forma clados aparentemente parafiléticos, e incluso algunos de sus individuos se asocian más a otras especies. Sin embargo, al considerar aspectos ecológicos siendo el tipo de vegetación el factor más importante para la especie, podemos observar el establecimiento de dos grupos uno tropical, que se divide en las localidades de Oaxaca y Chiapas, y otro templado, que lo vemos reacomodado de manera geográfica en ejemplares de Hidalgo, Oaxaca y Chiapas.

La combinación de la divergencia y diversidad junto con propuestas filogenéticas como las obtenidas con la inferencia bayesiana, son un complemento idóneo en la búsqueda de estructuración genética sobre todo cuando se tiene especiación críptica, como resultado de factores ambientales, y taxa de amplia distribución (Omland 2000, Shaffer *et al*. 2004, Kittlein & Gaggiotti 2008). Por lo anterior, herramientas como la genética del paisaje, que utiliza valores de divergencia y diversidad genética para generar sus proyecciones (Manel *et al*. 2003, Wang 2010, Garrido-Garduño & Vázquez-Domínguez 2013), ayuda a visualizar rupturas genéticas, sean producto de una estructuración geográfica como en *P. furvus* o ambiental como para *P. mexicanus*, ésta última derivada de especiación crítica, por lo que es una herramienta que sirve en el esclarecimiento de patrones filogeográficos (Mora *et al.* 2007, Vandergast *et al*. 2007, Vandergast *et al*. 2013)

CONCLUSIONES GENERALES

1. *Peromyscus furvus* presenta una variación geográfica que evidencia morfométricamente, la posible separación de las poblaciones del norte del resto de las localidades bajo su designación
2. La filogenia molecular de *P. furvus* muestra que las poblaciones del norte se separan del resto de la localidades y que además se ubican en la parte más ancestral de la filogenia
3. Genéticamente, las localidades del norte presentan valores altos de divergencia, aunado a la presencia de una estructura geográfica, con respecto a la demás distribución de la especie por lo que se propone el restablecimiento de *P. latirostris* como una especie independiente de *P. furvus*
4. La filogenia de las subesdpecies analizadas de *P. mexicanus* muestra que la especie es parafilética y que además la mayoría de sus poblaciones presenta un arreglo ecológico, asociado a el tipo de vegetación y elclima, segregrándose en dos ambientes:
    a. Templado, que alberga poblaciones de zonas montanas que se asocian a secuencias de la especie *P. melanocarpus*
    b. Tropical, asociado a zonas de tierras bajas y que presenta dos subgrupos, uno con localidades de Oaxaca y otro con poblaciones de Chiapas
5. Las poblaciones designadas bajo la especie de *P. mexicanus* posiblemente alberguen a más de una especie, debido a que presentan altos niveles de divergencia y diversidad genéticas
6. Algunas de las poblaciones de *P. mexicanus* de las zonas montanas de Oaxaca se asocian a secuencias del grupo externo, apoyando la idea de la parafilia y especiación críptica contenida en el taxón.

7. La asociación de las poblaciones localizadas en las zonas montanas de Oaxaca con poblaciones de la Sierra Madre Oriental, Faja Volcánica Transmexicana y hacia las Sierras de Chiapas, como lo muestran las redes de haplotipos, indica que las zonas oaxaqueñas contienen elementos de diferentes orígenes biogeográficos, por lo que se considera por algunos autores como una unidad no natural.
8. Las proyecciones genéticas de divergencia y diversidad muetran que, además, las zonas montanas de Oaxaca contienen altos niveles de diferenciación, lo que permite considerar a éstas zonas como generadoras de nuevas especies, muchas de ellas vinculadas a aspectos ecológicos, los cuales pueden propiciar el establecimiento de especiación críptica, sobretodo en especies generalistas como *P. mexicanus*.

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