UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO

POSGRADO EN CIENCIAS BIOLÓGICAS

FACULTAD DE CIENCIAS

DIVERSIFICACIÓN DEL GRUPO *MELANOPHRYS* (*Peromyscus melanophrys, P. perfulvus* y *P. mekisturus*, RODENTIA: CRICETIDAE: NEOTOMINAE)

TESIS

QUE PARA OPTAR POR EL GRADO DE:

DOCTORA EN CIENCIAS

PRESENTA:

SUSETTE SAMÍ CASTAÑEDA RICO

TUTOR PRINCIPAL DE TESIS: **Dr. Adolfo Gerardo Navarro Sigüenza**
Facultad de Ciencias, UNAM

COMITÉ TUTOR: **Dra. Ella Gloria Vázquez Domínguez**
Instituto de Ecología, UNAM

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POSGRADO EN CIENCIAS BIOLÓGICAS
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DIVISIÓN DE ESTUDIOS DE POSGRADO

OFICIO FCIE/DEP/439/14

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 **16 de junio de 2014**, se aprobó el siguiente jurado para el examen de grado de **DOCTORA EN CIENCIAS** del (la) alumno (a) **CASTAÑEDA RICO SUSETTE SAMI** con número de cuenta **96320228** con la tesis titulada: **"Diversificación del grupo Melanophrys *(Peromyscus melanophrys, P. perfulvus y P. mekisturus*, RODENTIA:CRICETIDAE:NEOTOMINAE)"**, realizada bajo la dirección del (la**) DR. ADOLFO GERARDO NAVARRO SIGÜENZA :**

Presidente:	DR. JUAN JOSÉ MORRONE LUPI
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Suplente	DR. JOAQUÍN ARROYO CABRALES

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

A t e n t a m e n t e
"POR MI RAZA HABLARA EL ESPÍRITU"
Cd. Universitaria, D.F. a 21 de agosto de 2014

Dra. María del Coro Arizmendi Arriaga
Coordinadora del Programa

FACULTAD DE CIENCIAS
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Layka y Maya

INDICE

RESUMEN

Las reconstrucciones filogenéticas permiten entender la evolución de la biota y su historia biogeográfica. Asimismo, los estudios filogeográficos proveen información sobre la distribución, tiempos de divergencia e historia demográfica de los linajes evolutivos reconocidos mediante estudios filogenéticos. Así, generar información filogenética y filogeográfica de una especie o grupo de especies permite documentar los procesos de especiación y diversificación involucrados. El complejo *Peromyscus melanophrys* (*P. melanophrys, P. perfulvus* y *P. mekisturus*) es el único grupo de peromicinos endémico de México, que además se encuentra restringido a zonas de tierras bajas. Es uno de los grupos menos estudiados dentro del género *Peromyscus*, incluso la especie *P. mekisturus* no había sido incluida en trabajos filogenéticos previos. En el presente estudio se utilizaron genes mitocondriales (ND3, tRNA-Arg, ND4L y un fragmento del ND4) y un gen nuclear (GHR) para evaluar la filogenia y filogeografía del complejo *Peromyscus melanophrys*. Para ello se analizaron 1,315 pb de los genes mitocondriales y 829 pb del gen nuclear en un total de 165 individuos provenientes de 65 localidades únicas. Se utilizaron diferentes herramientas filogenéticas y filogeográficas: análisis de Máxima Verosimilitud, Inferencia Bayesiana, diversidad y estructura genética, tiempos de divergencia, análisis de demografía histórica, redes de haplotipos, entre otros.

Los resultados filogenéticos mostraron que el complejo *Peromyscus melanophrys* es un grupo monofilético. Se reconocieron dos clados dentro de *P. perfulvus* y dos dentro de *P. melanophrys*, uno de los cuales incluyó a *P. mekisturus*. Los tiempos de divergencia estimados, indican que el complejo se originó durante el Plioceno (4.8 [4.4-5.3] millones de años), mientras que los principales eventos de diversificación ocurrieron a finales del Plioceno y durante el Pleistoceno.

Los resultados filogeográficos indicaron que el complejo *Peromyscus melanophrys* está formado por seis linajes (Depresión del Balsas Oeste, Costa del Pacífico, tierras bajas de la Sierra Madre de Chiapas-Planicie Costera de Tehuantepec, Depresión del Balsas Este, tierras bajas de la Sierra Madre Occidental y Altiplano Mexicano), algunos de los cuales mostraron crecimiento poblacional reciente. Los cambios climáticos durante el Pleistoceno y algunos factores ecológicos potencialmente moldearon la diversificación dentro del complejo.

ABSTRACT

Phylogenetic reconstructions illuminate the biogeographic history of the biota and eventually their evolution. Also, phylogeographic studies provide information about the distribution, time of divergence and demographic history of evolutionary lineages described by phylogenetic analyses. Thus, considering phylogenetic and phylogeographic information of species or group of species is critical in order to document their speciation and diversification processes. The *Peromyscus melanophrys* complex (*P. melanophrys, P. perfulvus* y *P. mekisturus*), is the only peromicine group endemic to Mexico that is restricted to lowlands. This group is one of the less-studied within *Peromyscus*, even the poorly known *P. mekisturus* has not been previously included in a phylogenetic analysis. In this work, we used mitochondrial genes (ND3, tRNA-Arg, ND4L and partial ND4) and a nuclear gene (GHR) to evaluate the phylogeny and phylogeography of the *Peromyscus melanophrys* complex. For this purpose, 1,315 bp from mitochondrial genes and 829 from nuclear gene of 165 individuals from 65 unique localities were analyzed. Different phylogenetic and phylogeographic tools were used: Maximum Likelihood analysis, Bayesian Inference, genetic diversity and structure, time of divergence, demographic history analyses, haplotype networks, inter alia.

The phylogenetic results showed that the *Peromyscus melanophrys* complex is a monophyletic group. We recovered two distinct clades within *P. perfulvus* and two within *P. melanophrys,* one of which contains the *P. mekisturus* sample. In accordance with the divergence times estimated, the group diverged during the Pliocene (4.8 [4.4-5.3] million years ago) and the main diversification events within the group occurred at the end of the Pliocene and through the Pleistocene.

The phylogeographic results showed that the *Peromyscus melanophrys* complex encompasses six lineages (Western Balsas Basin, Pacific Coast, Tehuantepec Coastal Plain-Sierra Madre de Chiapas lowlands, Eastern Balsas Basin, Sierra Madre Occidental lowlands and Mexican Plateau), some of which show a population expansion signal. Climatic oscillations during the Pleistocene and some ecological factors have potencially modeled diversification within the complex.

CAPÍTULO 1

Introducción General

INTRODUCCIÓN GENERAL

La diversificación es un incremento evolutivo en el número de especies de un grupo dado, usualmente acompañado por divergencia fenotípica (Futuyma, 2009). Generalmente surge como una radiación adaptativa, cuya principal causa es la divergencia ecológica, por lo que es rápida si hay nichos o espacio ecológico disponible (zonas adaptativas) (Schluter, 2000; Futuyma, 2009). En general, durante estos eventos de especiación, el flujo genético es interrumpido por barreras geográficas (históricas y actuales) así como por eventos climáticos y ecológicos (Patton y da Silva, 2005). Los principales factores que fomentan su desarrollo son evitar la competencia, la evolución de adaptaciones clave, especialización y coevolución (Mayr, 1942; Simpson, 1944; Benton, 1990; Signor, 1990; Futuyma, 1998).

El conocimiento de la diversificación y la distribución de los taxones y las biotas a través del tiempo se basa en el entendimiento de la distribución geográfica y de las relaciones entre linajes evolutivos (Riddle *et al*., 2000). Así una de las herramientas más útiles para entender la historia biogeográfica de los grupos y la evolución de las biotas son las reconstrucciones filogenéticas. Los árboles filogenéticos, especialmente los que incluyen diferentes especies dentro de un grupo; aunado a la información geográfica y ecológica que se tenga de dichas especies proveen información acerca de los eventos y causas de especiación que pudieron haber ocurrido dentro del grupo (Barraclough y Nee, 2001; Berlocher, 1998; Brooks y McLennan, 1991). Adicionalmente, los árboles obtenidos a partir de secuencias de DNA contienen información sobre tiempos de divergencia y pueden ser usados para estimar tasas de especiación (Barraclough y Nee, 2001; Hey, 1992; Nee, 2001).

Por otro lado, para incorporar la historia filogenética y la genética de poblaciones bajo una perspectiva biogeográfica se desarrolló la filogeografía, cuyo objetivo es

relacionar las filogenias moleculares con la historia o características de las áreas de distribución de las especies (Avise et al., 1987; Avise, 2000). Los análisis filogeográficos permiten delimitar grupos evolutivos de una forma más precisa o más fina, así como detallar la historia evolutiva de cada especie; a diferencia de los análisis filogenéticos que determinan eventos de especiación a mayor escala (Avise, 2000; Barraclough y Nee, 2001).

Los roedores constituyen el orden más grande de mamíferos, incluyen 32 géneros y aproximadamente 2,277 especies, lo cual representa el 42% de las especies de mamíferos existentes (Wilson y Reeder, 1993; Musser y Carleton, 2005). Poseen una distribución amplia, con excepción de algunas islas y las regiones polares. Este grupo está adaptado a una gran variedad de hábitats desde los terrestres, arbóreos, escansoriales (adaptados a escalar), fosoriales hasta semiacuáticos. A pesar de que presentan una gran adaptabilidad y una alta diversidad, los caracteres morfológicos que presentan, en general, llegan a ser marcadamente homogéneos dentro de algunos grupos.

La roedores múridos (ratas y ratones) comprenden uno de los grupos más grandes y diversos dentro de este orden, aunque sus relaciones filogenéticas son poco claras (Miller y Engstrom, 2008). Dentro de éstos, el estado taxonómico de los cricétidos, en especial el de los neotominos de Norte y Centro América ha recibido especial interés debido a la gran diversidad ecológica, conductual, genética y taxonómica que presentan, aunado a su amplia distribución (Musser y Carleton, 2005; Reeder et al., 2006). Dentro de la subfamilia Neotominae, el género *Peromyscus* es uno de los grupos de roedores más diversos y comunes en el continente Americano, se extiende desde Canadá hasta Panamá y se encuentra prácticamente en todos los hábitats (desde zonas alpinas hasta selvas bajas y desde zonas montañosas hasta el nivel del mar). La gran diversidad de

ambientes en la que se distribuye dicho grupo, aunado a la variación morfológica, conductual y fisiológica que presentan los miembros de este grupo, podría reflejar una historia evolutiva aparentemente rápida (Kirkland and Layne, 1989). Al ser un género Neártico, se ha propuesto que los ciclos climáticos del Pleistoceno desplazaron a las poblaciones de América del Norte hacia el sur durante los avances glaciares, y hacia el norte durante el calentamiento interglacial, sirviendo como una "bomba de especiación", lo cual se ve apoyado por los restos fósiles registrados y la distribución actual de *Peromyscus* (Dawson, 2005; Hibard, 1968).

Varias especies de este género han sido objeto de numerosos estudios de sistemática y biogeografía, así como modelos en ecología, evolución, conducta, fisiología, reproducción, bioquímica, etc. (Alderman et al., 1987; Carleton, 1989; Kaufman y Kaufman, 1989; MacMillen y Garland, 1989; Millar, 1989; Sullivan et al., 1997); además de ser reservorios de vectores de enfermedades fatales que afectan a los humanos como el hantavirus (Jay et al., 1997; Salazar-Bravo et al., 2004), entre otras.

El género *Peromyscus* es muy complejo desde el punto de vista taxonómico y con gran variación entre y dentro de las especies (Carleton, 1989; Hogan *et al*., 1993; Musser y Carleton, 2005), consiste de dos subgéneros (*Haplomylomys* y *Peromyscus*), donde el número de especies fluctúa entre 53 (Hafner *et al.*, 2001) y 57 (Musser y Carleton, 2005; Ramírez-Pulido *et al*., 2005). En su revisión del género, Osgood (1909) propone que las especies se clasifiquen en diferentes grupos, en función de las características morfológicas que tienen en común. Posteriormente, a través de otros estudios basados en características morfológicas, cariotipos, aloenzimas y secuencias del citocromo b, se reconocieron 13 grupos de especies: *californicus, eremicus, crinitus, hooperi, aztecus, furvus, megalops, mexicanus, melanophrys, boylii, truei, leucopus y maniculatus* (Carleton, 1989; Hogan *et al*., 1993; Musser y Carleton, 1993; Dawson, 2005; Bradley *et*

al., 2007). El número de grupos así no se ha modificado, sin embargo las especies han sido constantemente reclasificadas, dependiendo principalmente del tipo de análisis (*i.e.* morfológico, molecular). La gran diversidad del grupo ha interesado no sólo a los taxónomos, sino a paleontólogos, y biogeógrafos, quienes han trabajado sobre sus relaciones filogenéticas y evolución. Sin embargo, en muchos casos las propuestas son variables y cambiantes: por un lado la estabilidad y conformación de cada uno de los grupos de especies y por otro, el reconocimiento de las especies y subespecies descritas (Riddle *et al*., 2000; Bradley *et al*., 2007; Álvarez-Castañeda y González-Ruíz, 2008; Miller y Engstrom, 2008). Por lo anterior, la relación entre las especies dentro de cada grupo no ha logrado resolverse y en consecuencia su historia filogenética y biogeográfica.

Uno de los grupos de especies que ha sido menos estudiado y que es el único endémico de México, es el grupo "*Peromyscus melanophrys*", el cual incluye a: *Peromyscus melanophrys,* que presenta seis subespecies (*P. m. coahuilensis, P. m. consobrinus, P. m. melanophrys, P. m. micropus, P. m. xenurus* y *P. m. zamorae*), cuya distribución abarca el norte, centro y hasta el sur del país a través del Altiplano, desde Durango y Chihuahua hasta Oaxaca y Chiapas (Osgood, 1909); *P. pefulvus,* incluye dos subespecies (*P. p. chrysopus* y *P. p. perfulvus*) y se distribuye en la zona costera de Jalisco hasta Guerrero y a lo largo del río Balsas hasta el sur del Estado de México (Hooper, 1955); y *P. mekisturus* exclusivamente en el estado de Puebla (Hall, 1981). Es importante destacar que *P. mekisturus* nunca se había incluido en algún trabajo filogenético y que solamente se conocen dos ejemplares en el mundo: el ejemplar holotipo (Merriam, 1898) de Chalchicomula, Puebla (hoy Ciudad Serdán) y un ejemplar más de Tehuacán, Puebla (Hooper, 1947).

Los individuos de *P. melanophrys*, presentan una coloración muy variable, son de los de mayor tamaño dentro del género e incluso se distinguen por presentar una cola

extremadamente larga en relación a la proporción del cuerpo (Hall, 1981). Son estrictamente terrestres, habitan en zonas áridas y rocosas, asociadas a yuca, ocotillo, nopal, mezquite y algunas cactáceas. Se les encuentra en matorral xerófilo, bosque espinoso y pastizales, en altitudes desde los 50 hasta los 2700 metros sobre nivel del mar (msnm); son propios de las zonas de contacto entre climas áridos y templados (Baker, 1956; Jones y Webster, 1977; Hall, 1981). *P. perfulvus* incluye roedores de talla mediana, presentan una coloración color canela-grisácea. Se distinguen por tener una cola larga color sepia uniforme, cubierta de pelo con forma de pincel en la punta. Es una especie semiarborícola, lo que se corrobora al tener muy desarrollados los tubérculos plantares, facilitándoles así su desplazamiento en las ramas de los árboles. Habita lugares húmedos como selva mediana y selva baja, desde el nivel del mar hasta los 1300 msnm (Ceballos y Miranda, 2000; Osgood, 1945; Sánchez et al., 2009). Finalmente, *P. mekisturus* es considerada una especie arborícola, lo que se apoya con el tamaño excepcional de la cola (160% del tamaño del cuerpo), así como la longitud del quinto dedo de la pata trasera (Castro-Campillo et al., 2005). Habita en bosque de pino-encino y se le ha encontrado a 1700 msnm, sin embargo, Hooper (1968) estableció que se distribuye en ambientes áridos y rocosos como su especie hermana *P. melanophrys*.

Dadas las características de estas especies, como son: distribuciones geográficas tanto amplias como restringidas; la diferencia de hábitos que presentan, que van desde terrestres hasta arbóreos estrictos; así como la escasa información filogenética, filogeográfica y ecológica, es esencial resolver la diversificación del grupo y su relación con otros peromicinos. Además de resolver las relaciones filogenéticas y la filogeografía del grupo, este trabajo tiene como meta reconstruir su historia evolutiva y describir algunos de los patrones de diversificación de los roedores peromicinos y de las regiones

en que han evolucionado, dentro del contexto global de la dinámica evolutiva de la biota mesoamericana.

Los objetivos de este trabajo son: 1) evaluar la filogenia del grupo *Peromyscus melanophrys* (*P. melanophrys, P. perfulvus* y *P. mekisturus*) con el fin de reconocer linajes, procesos de diversificación y tiempo de divergencia entre las especies y subespecies que lo conforman, 2) describir los patrones filogeográficos del grupo, 3) evaluar su estructura genética, historia demográfica y la correlación entre distribución geográfica y patrones genéticos dentro de los linajes que conforman al grupo.

El presente trabajo está estructurado en cuatro capítulos, el capítulo 1 y 4 conforman la introducción y discusión generales, respectivamente, mientras que los capítulos 2 y 3 incluyen los estudios filogenético y filogeográfico, como se describe a continuación:

El capítulo 2 analiza las relaciones filogenéticas dentro del grupo *Peromyscus melanophrys* (*P. melanophrys, P. perfulvus* y *P. mekistutrus*). Se realizaron análisis de Máxima Verosimilitud e Inferencia Bayesiana, con los genes mitocondriales (ND3, tRNA-Arg, ND4L y una parte del ND4) y un gen nuclear (GHR). Se identificó que el grupo *Peromyscus melanophrys* es un grupo monofilético y se propusieron cambios taxonómicos dentro del mismo (Castañeda-Rico et al., 2014).

El capítulo 3 conforma la historia demográfica y filogeográfica del complejo *Peromyscus melanophrys*. Dado que con base en los resultados filogenéticos se sugieren posibles cambios taxonómicos (capítulo 2), en este capítulo se decidió analizar todas las poblaciones dentro del grupo como un solo conjunto de datos, sin considerar agrupaciones dadas por especies. Se realizaron análisis de Inferencia Bayesiana, redes filogenéticas (algoritmo Neighbor-Net) y de estructura (clusters), los cuales permitieron

identificar seis linajes genéticos. Se incluye la descripción de la estructura genética e historia demográfica para cada linaje y la datación del origen del grupo y su posterior diversificación.

REFERENCIAS

Alderman, J., Takagi, A., Lieber, C. 1987. Ethanol-metabolizing pathways in deermice. Journal of Biological Chemestry. 262:7497-7503.

Álvarez-Castañeda, S., González-Ruiz, N., 2008. Análisis preliminar de las relaciones filogenéticas entre los grupos de especies del género *Peromyscus*, in: Lorenzo, C., Espinoza, E., Ortega J. (Eds.), Avances en el estudio de los mamíferos de México II. CIB, Universidad Veracruzana, IPN, Ecosur, Unicach, UAEM, AMMAC, UAM, México, pp. 5-26.

Avise, J.C. 2000. Phylogeography: The history and formation of the species. Cambridge, MA, Harvard University Press.

Avise, J.C., Arnold, J., Ball, R.M., Bermingham, E., Lamb, T., Neigl, J.E., et al., (1987). Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. Annu. Rev. Ecol. 18, 489-522.

Baker, R.H. 1956. Mammals of Coahuila, Mexico. University of Kansas Publications, Museum of Natural History, 7: 339-347.

Barraclough, T. y Nee, S. 2001. Phylogenetics and speciation. Trends Ecol. Evol. 16 (7):391-399.

Benton, M. 1990. The causes of diversification of life. pp: 409-430. *En*: Taylor, P. y G. Larwood (eds.) Mejor evolutionary radiations. Clarendon Press, Oxford.

Berlocher, S.H. 1998. Can sympatric speciation be proven from phylogenetic and biogeographic evidence? Pp:99-113. *En*: Howard, D.J. y Bercholer, S.H. (eds.) Endless forms: Species and Speciation. Oxford University Press.

Bradley, R.D., Durish, N., Rogers, D., Millar, J., Engstrom M., Kilpatrick, W., 2007. Toward a molecular Phylogeny for *Peromyscus*: Evidence from mitochondrial cytochrome-b sequences. J. Mammal. 88, 1146-1159.

Brooks, D.R. y McLennan, D. A. 1991. Phylogeny, Ecology and Behaviour: a research program in comparative biology. Chicago University Press.

Carleton, M. D., 1989. Systematics and Evolution, en: Kirkland, G.L., Layne, J. (Eds.) Advances in the study of *Peromyscus* (Rodentia). Texas Tech University Press., Lubbock. pp. 7-141.

Castañeda-Rico, S., León-Paniagua, L., Vázquez-Domínguez, E., Navarro-Sigüenza, A.G. 2014. Evolutionary diversification and speciation in rodents of the Mexican lowlands: The *Peromyscus melanophrys* species group. Mol. Phyl. Evol. 70: 454-463.

Castro-Campillo, A., Martínez, H., y Ramírez-Pulido, J. 2005. *Peromyscus mekisturus*. Pp: 751. En: Ceballos, G. y Oliva, G. (Eds.) Los mamíferos silvestres de México. Fondo de Cultura Económica, CONABIO.

Ceballos, G. y Miranda, A. 2000. Guía de campo de los mamíferos de la Costa de Jalisco, México. Fundación Ecológica-Cuixmala, A. C. México.

Dawson, W., 2005. Peromyscine Biogeography, Mexican topography and Pleistocene climatology. En: Sánchez-Cordero, V., Medellín, R. (Eds.), Contribuciones Mastozoológicas en Homenaje a Bernardo Villa. UNAM-CONABIO. México, pp. 145-156.

Futuyma, D. 1998. Evolutionary Biology. 3 ed. Sinauer Associates. USA. Pp. 763.

Futuyma, D. 2009. Evolution. 2 ed. Sinauer Associates. USA. Pp. 633.

Hafner, D., Riddle, B., Álvarez-Castañeda, S., 2001. Evolutionary relationships of White footed mice (*Peromyscus*) on the islands in the Sea of Cortez, Mexico. J. Mammal. 82, 775-790.

Hall, E.R., 1981. Mammals of North America, second ed. John Wiley and Sons, New York.

Hey, J. 1992. Using phylogenetic trees to study speciation and extinction. Evolution. 46: 627:640.

Hibbard, C. 1968. Paleontology. pp: 6-26. En: King, J. (ed.) Biology of *Peromyscus* (Rodentia). Special Publication No.2 The American Society of Mammalogist.

Hogan, K., Heding, M., Koh, S., Davis, K., Greenbaum, I., 1993. Systematic and taxonomic implications of karyotypic, electrophoretic and mitochondrial DNA variation in *Peromyscus* from the Pacific Northwest. J. Mammal. 74, 819-831.

Hooper, E.T. 1955. Notes on mammals of western Mexico. Occas. Pap. Mus. Zool. Univ. Mich. 565, 1-26.

Hooper, E.T. Classification. Pp: 27-70. En: Biology of *Peromyscus* (Rodentia), J. A. King (ed.) American Special Publications. Num. 2. 593 pp.

Jay, M. *et al*. 1997. Seroepidemiologic studies of hantavirus infection among wild rodents in California. Emerging infectious diseases. 3:183-190.

Jones, G.S. y Webster, J.D. 1977. Notes on distribution, hábitat and abundance in some mammals of Zacatecas, Mexico. Anales del Instituto de Biología, Universidad Nacional Autónoma de México, Serie Zoología, 47: 75-84.

Kaufman, D., Kaufman, G. 1989. Population biology. Pp.233-270 *en* Advances in the study of *Permoyscus* (Rodentia) (G.L. Kirkland y J. Layne, eds.) Texas Tech University Press, Lubbock.

Kirkland, G., Layne, J. 1989. Advances in the study of Peromyscus (Rodentia). Texas Tech University Press. Pp. 364.

MacMillen, R., Galard, T. 1989. Adaptative physiology. Pp.143-168. *en* Advances in the study of *Permoyscus* (Rodentia) (G.L. Kirkland y J. Layne, eds.) Texas Tech University Press, Lubbock.

Mayr, E. 1942. Systematics and the origin of species. Columbia University Press, New York.

Millar, J.1989. Reproduction and Development. Pp.169-232. *en* Advances in the study of *Permoyscus* (Rodentia) (G.L. Kirkland y J. Layne, eds.) Texas Tech University Press, Lubbock.

Miller,J., Engstrom, M., 2008. The relationships of major lineages within peromyscine rodents: A molecular phylogenetic hypothesis and systematic reappraisal. J. Mammal. 89, 1279-1295.

Musser, G., Carleton, M.D., 1993. Family Muridae, in: Wilson, D.E., Reeder, M., (Eds), Mammal species of the world: a taxonomic and geographic reference. Smithsonian Institution Press, Washington D.C., pp. 501-755.

Musser, G., Carleton M.D., 2005. Superfamily Muridae. en: Wilson, D.E., Reeder, M., (Eds), Mammal species of the world: a taxonomic and geographic reference, third ed. Johns Hopkins University Press, Baltimore, Maryland, pp. 894-1531.

Nee, S. 2001. On inferring speciation rates from phylogenies. Evolution. 55(4): 661-668.

Osgood, W. H. 1945. Two new rodents from Mexico. Journal of Mammalogy, 26: 299-301.

Patton, J., M. da Silva y J. Malcolm. 2000. Mammals of Rio Juruá and the evolutionary and ecological diversification of Amazonia Bulletin of the American Museum of Natural History. No. 244. American Museum of Natural History, New York.

Patton, J. y M. da Silva. 2005. The history of Amazonian mammals: mechanisms and timing of diversification. pp: 107-126. En: Bermingham, E., C. Dick y C. Moritz (eds.) tropical Rainforest, Past, Present and Future. The University of Chicago Press.

Ramírez-Pulido J., Arroyo-Cabrales, J., Castro-Campillo, A., 2005. Estado actual y relación nomenclatural de los mamíferos terrestres de México. Acta Zool. Mex. (n. s.). 21, 21-82.

Riddle, B., Hafner, D., Alexander L., 2000. Phylogeography and Systematics of the *Peromyscus eremicus* species group and the historical biogeography of North American Warm Regional deserts. Mol. Phylogenet. Evol. 17, 145-160.

Salazar-Bravo, J., *et al*. 2004. Serosurvey of wild rodents for hantaviruses in Panama, 2000-2002. Journal of Wild Life Diseases. 40:103-109.

Sánchez, C., Schnell, G y Romero, L. 2009. *Peromyscus perfulvus* (Rodentia: Cricetidae). Mammalian Species. 833: 1-8.

Schluter, D. 2000. The ecology of adaptative radiation. Oxford University Press, Oxford.

Signor, P. III. 1990. The geological history of diversity. Annual Review in Ecology, Evolution and Systematic 21:509-539.

Simpson, G. 1944. Tempo and mode in evolution. Columbia University Pres. New York.

Sullivan, J., Market, A., Kilpatrick, C. 1997. Phylogeography and molecular systematics of *Peromyscus aztecus* group (Rodentia:Muridae) inferred using parsimony and likelihood. Systematic Biology. 46:426-440.

CAPÍTULO 2

Artículo: Evolutionary diversification and speciation in rodents of the Mexican lowlands: The *Peromyscus melanophrys* species group.

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Evolutionary diversification and speciation in rodents of the Mexican lowlands: The *Peromyscus melanophrys* species group

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ABSTRACT

Despite some studies of the species groups within the genus *Peromyscus* have been performed, both evolutionary relationships among species within groups and group composition have remained controversial. In this study, we address phylogenetic relationships among species in the *Peromyscus melanophrys* group (*P. melanophrys*, *P. perfulvus*, and *P. mekisturus*), using a molecular phylogenetic analysis. This analysis is the first to include the poorly known *P. mekisturus*. We conducted maximum likelihood and Bayesian inference analyses with the ND3, tRNA-Arginine, ND4L, and partial ND4 mitochondrial genes, and the GHR nuclear gene. We consistently recovered a *P. melanophrys* group that is monophyletic with respect to the set of outgroups. Also, we recovered two distinct clades within *P. perfulvus* and two within *P. melanophrys*, one of which contain *P. mekisturus* among other *P. melanophrys*, all with geographic consistency. According to our divergence time estimates, the *P. melanophrys* group diverged during the Pliocene and the main diversification events within the group occurred at the end of the Pliocene and through the Pleistocene.

1. Introduction

Knowledge of diversification and distribution of taxa over time is based on understanding the geographic and evolutionary relationships among lineages (Riddle et al., 2000). Thus, phylogenetic reconstructions are necessary to illuminate the biogeographic history of groups and eventually, the evolution of full biotas. The order Rodentia is particularly interesting in light of its high species number, widespread distribution, and ecological diversity. However, phylogenetic relationships of this order remain poorly understood (Carleton and Musser, 2005; Merritt, 2010).

The most common and speciose genus in the subfamily Neotominae is the genus *Peromyscus*. It is abundant from central Canada and Alaska south to Panama, occupying a wide variety of habitats, and has been used as a model organism for studies in many areas of research (Alderman et al., 1987; Carleton, 1989; Kaufman and Kaufman, 1989; MacMillen and Garland, 1989; Millar, 1989; Sullivan et al., 1997; Dawson, 2005). The genus consists of two subgenera (*Haplomylomys* and *Peromyscus*), and the number of extant species varies according to the source, from 53 to 57, with about 16 extinct species (Carleton, 1989; Hogan et al., 1993; Musser and Carleton, 1993, 2005; Hafner et al., 2001; Ramírez-Pulido et al., 2005). *Peromyscus* is a very complex genus from a phylogenetic perspective, with marked morphological, ecological, and molecular inter- and intraspecific variation (Carleton, 1980, 1989; Rogers et al., 2005; Miller and Engstrom, 2008). The relationships and status of several taxa formerly included within *Peromyscus* are also controversial (Carleton, 1980, 1989; Hogan et al., 1993; Musser and Carleton, 1993, 2005; Riddle et al., 2000; Rogers et al., 2005; Miller and Engstrom, 2008).

Osgood (1909) reviewed *Peromyscus* and, based mainly on similarities of morphological characters, placed related species into the following species groups: *maniculatus*, *leucopus*, *boylii*, *truei*, *melanophrys*, *lepturus*, *mexicanus*, and *megalops*. He did not clearly define the use of supraspecific grouping, but in a previous work (Osgood, 1900) mentioned the arrangement of species into groups "... in order to show the affinities of the species...". Whether he was referring to morphological, evolutionary, or ecological affinities is unclear (Carleton, 1989). At present, several lines of evidence (morphological, karyotypes, allozymes, and, most recently, cytochrome b [cyt b] sequences) collectively suggest the recognition of 13 monophyletic species groups within the *Peromyscus* genus: *californicus*, *eremicus*, *crinitus*, *hooperi*, *aztecus*, *furvus*, *megalops*, *mexicanus*, *melanophrys*, *boylii*, *truei*, *leucopus*, and *maniculatus* (Carleton, 1989; Musser and Carleton, 1993, 2005; Hogan et al., 1993; Dawson, 2005; Bradley et al., 2007). Many studies of *Peromyscus* have employed these species groups with slightly different

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meanings and underlying assumptions regarding evolutionary or speciation processes (Hooper and Musser, 1964; Hooper, 1968; Huckaby, 1980; Hall, 1981; Carleton, 1989), mainly because it has facilitated a broad study of the genus, so testing and verifying the monophyly of these groups is important. Moreover, the groups have been modified several times based on new evidence and many of the species have been repeatedly relocated (Carleton, 1989; Riddle et al., 2000; Álvarez-Castañeda and González-Ruiz, 2008) and, surprisingly, little is known about phylogenetic relationships within groups.

The *Peromyscus melanophrys* group, endemic to Mexico, is one of the less-studied within *Peromyscus*. However, different authors (Osgood, 1909; Carleton, 1989; Musser and Carleton, 1993, 2005; Bradley et al., 2007) have suggested its monophyly, comprising three species: (1) *P. melanophrys*, which has six subspecies (*P. m. coahuilensis*, *P. m. consobrinus*, *P. m. melanophrys*, *P. m. micropus*, *P. m. xenurus*, and *P. m. zamorae*) distributed from the northern parts of the Central Mexican Plateau (Durango and Chihuahua) to the states of Oaxaca and Chiapas in the south (Osgood, 1909); (2) *P. perfulvus* with two subspecies (*P. p. chrysopus* and *P. p. perfulvus*) and is distributed along the Pacific coast from Jalisco to Guerrero and inland in the Balsas River Basin south of the Estado de México (Hooper, 1955); and (3) *P. mekisturus*, which is known solely from two specimens. Merriam's (1898) holotype was from Chalchicomula and Hooper's (1947) record was from Tehuacán, both in Puebla (Fig. 1). Although the species-group association (i.e., sister group relationship) between *P. melanophrys* and *P. perfulvus* is supported by diverse evidence (Hooper, 1955, 1968; Hooper and Musser, 1964; Zimmerman, 1974; Lee and Elder, 1977; Musser and Carleton, 2005; Bradley et al., 2007; Álvarez-Castañeda and González-Ruiz, 2008), knowledge of the relationships of *P. mekisturus* remains scarce. In addition, most studies of the group have been based on morphological characters, with the exception of Bradley et al. (2007), who used cyt b sequences, but did not include *P. mekisturus* in their analysis.

Given the lack of modern studies, it is not surprising that evolutionary relationships among species of the *melanophrys* group remain controversial. Given some characteristics of the species, such as distribution, habitat differences, and morphology, understanding the diversification of the group and the relationship with other peromyscine rodents is of great interest. In accordance, the aim of this study was to evaluate: (1) monophyly of the group with respect to the set of outgroups used, considering that *P. mekisturus* has never been included in a molecular phylogenetic analysis; and (2) phylogenetic relationships among the three species in the *melanophrys* group, using molecular techniques, to discern evolutionary lineages.

2. Methods

2.1. Sample collection

We obtained samples from two sources: 14 tissue samples collected from natural populations in the states of Puebla, Coahuila, Zacatecas, Jalisco, and Nayarit (fieldwork from June to December 2009), and Jalisco (March 2010, Chamela Biological Station), and obtained 87 tissue, bone, and skin samples from museum specimens (12 national and foreign mammal collections; see Acknowledgements). We provide collection locations of specimens examined in Appendix A and Fig. 1. We used sampling methods designed specifically for arboreal (see Castañeda-Rico et al., 2011 for details), semi-arboreal, and terrestrial rodent species. We placed Sherman live traps (7.6 × 8.9 × 22.9 cm; H. B. Sherman Traps, Tallahassee, Florida) on trees or on the ground, depending on the species. We took a tissue sample from an ear of each individual trapped at the Chamela Biological Station and stored it in 100% ethanol. For all other localities, voucher specimens were collected and deposited in the Mammal Collection of the Museo de Zoología "Alfonso L. Herrera", Facultad de Ciencias, UNAM (MZFC). Techniques we used are in compliance with guidelines published by the

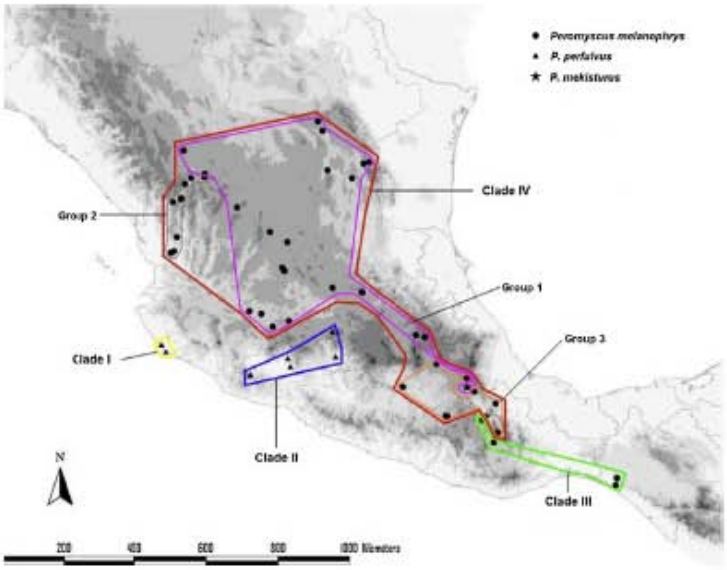

Fig. 1. Sampling localities for the *P. melanophrys* group (*Peromyscus melanophrys*, *P. perfulvus* and *P. mekisturus*) in Mexico. Clades and groups obtained from BI and ML analyses based on mitochondrial and nuclear genes are shown. Base map is the Digital Elevation Model from USGS (http://eros.usgs.gov/#/Find_Data/Products_and_Data_Available/gtopo30/hydro).

American Society of Mammalogists for use of wild mammals in research (Kelt and Hafner, 2010; Sikes et al., 2011).

2.2. DNA extraction, amplification and sequencing

We extracted DNA from tissue, fresh or dry skin and bone samples using the DNeasy Blood and Tissue Kit (Qiagen, Valencia, California), except for the sample of *P. mekisturus* for which we used the QIAamp DNA Investigator Kit (Qiagen, Valencia, California). We amplified and sequenced mitochondrial (mtDNA) genes ND3, ND4L, arginine tRNA, and partial ND4 from all tissue and fresh skin samples using the primers Gly, ND4LRev2 and NAP2 (Engel et al., 1998) and F2 (León-Paniagua et al., 2007). Specific primers were designed for dry skin or bone samples, with the programs Primer 3 and IDT Oligo-analyzer 3.1, because most of the museum samples consisted of fragmented and/or degraded DNA (Appendix B). We analyzed all mtDNA genes as a single locus because they evolve at the same rate and all are subunits of the same protein (Cao et al.1994; Russo et al., 1996; Engel et al., 1998; León-Paniagua et al., 2007). We amplified and sequenced the nuclear (nDNA) gene GHR (Growth Hormone Receptor) only from fresh tissue samples using the primers GHR1f and GHRend1f (Jansa et al., 2009; Fernández et al., 2012). We used *Osgoodomys banderanus*, *Habromys simulatus*, *Peromyscus furvus*, *P. mexicanus*, *P. eremicus*, and *P. leucopus* as outgroups; their sequences were obtained either from sequencing at our laboratory or from GenBank (Appendix A).

We amplified DNA in a 25 μL reaction volume containing the following: 25–50 ng of template DNA for mtDNA genes and 100 ng for the nuclear gene, 1 unit of Taq DNA polymerase (Vivantis), 1.5 mM of $MgCl_2$, 200 μM of each deoxynucleoside triphosphate, and 0.25 μM of each primer. For *P. mekisturus* we used Taq Gold (Quiagen, Valencia, California). Polymerase chain reaction (PCR) conditions for mitochondrial DNA (mtDNA) were as follows: Taq activation at 95 °C for 10 min, initial 3 min denaturation at 95 °C, followed by 40 cycles, each cycle consisting of 95 °C denaturing for 30 s, 50 °C annealing temperature for 30 s, and extension at 72 °C for 30 s, with a final 72 °C for 7 min. PCR parameters for mtDNA obtained from dry skin or bone were: denaturation at 95 °C for 10 min, followed by 15 cycles at 95 °C for 30 s, 50 °C for 1 min, and 72 °C for 1 min, followed by 25 cycles at 95 °C for 30 s, 55 °C for 1 min, and 72 °C for 1 min with a final extension at 72 °C for 5 min. PCR conditions for the GHR nuclear gene were denaturation at 94 °C for 5 min, followed by 35 cycles at 94 °C for 15 s, 60 °C for 1 min, and 72 °C for 1.5 min, with a final extension at 72 °C for 10 min (Fernández et al., 2012). We used agarose (1.5%) gels stained with ethidium bromide to visualize amplified products. After amplification, PCR products were purified and run on an ABI3730xl DNA analyzer (Applied Biosystems, Carlsbad, California) by a sequencing service provider, High Throughput Sequencing (HTSeq), Washington, USA (http://www.htseq.org). To avoid contamination of samples, standard authentication criteria for ancient DNA studies (bone or skin) were followed. In particular, we included negative controls in all amplifications to check for contamination. Multiple independent PCR amplifications for random samples (including *P. mekisturus*) were also conducted and samples were sequenced twice to ensure reproducibility and correct readings. Finally, for randomly selected sequences (specially for *P. mekisturus*), a search was performed independently for each segment that was amplified, using the Basic Local Alignment Search Tool (BLAST) at the NCBI website (http://blast.ncbi.nlm.nih.gov/Blast.cgi), to ensure correct identification of amplifications.

2.3. Phylogenetic analyses

We edited and cleaned sequences using BioEdit 7.0.9.0 (Hall, 1999) and performed multiple sequence alignment manually and using ClustalX2 (Thompson et al., 1997; Larkin et al., 2007). For all analyses, we only used unique haplotypes. We estimated monomorphic, polymorphic, and parsimony-informative sites with the program DNAsp 5.0 (Librado and Rozas, 2009).

We analyzed our sequence data using maximum likelihood (ML) and Bayesian inference (BI) methods. jModeltest 0.1.1 (Guindon and Gascuel, 2003; Posada, 2008) was used to select the best-fit model of evolution, based on the Akaike Information Criterion (AIC). The model selected was TIM1 + I + G, which was used for model-based analyses for mtDNA, along with the following parameters: base frequencies = A 0.3769, C 0.2713, G 0.0629, T 0.2889; nst = 6; rates = gamma with shape parameter (α) = 0.9780, and proportion of invariant sites = 0.4900. TPM3uf + G was the selected model for the nuclear gene, with the following parameters: base frequencies = A 0.2809, C 0.2869, G 0.2247, T 0.2076; nst = 6; rates = gamma with shape parameter (α) = 0.0170, and proportion of invariant site = 0. We implemented ML analyses using PhyML 3.0 (Guindon et al., 2010) using five random starting trees optimized through subtree pruning and regrafting and nearest-neighbor interchange, with four substitution categories. Clade support was assessed by bootstrapping with 500 replicates. We conducted BI analyses using MrBayes 3.1 (Ronquist and Huelsenbeck, 2003) and analyses consisted of four runs, each conducted with three heated and one cold Markov chains run for 15,000,000 generations, sampling every 100 generations and initiating with random, unconstrained, starting trees. Heating temperature was set at 0.02 to facilitate greater movement between the four Markov chains (Braun et al., 2010). Output parameters were visualized using Tracer 1.5 (Rambaut and Drummond, 2007). We determined resulting burn-in values (the point at which the model parameters and tree scores became stationary) as 25% of all sampled trees (37,500 trees), and we obtained posterior probabilities from the remaining trees. Because we found some differences between phylogenetic trees obtained with mtDNA and nDNA, we also conducted ML and BI analyses using a concatenation of the two sets of genes to improve phylogenetic accuracy. We performed a partition homogeneity test in PAUP*4.0a125 (Swofford, 2002) with 1000 replicates to test for conflicting signals from different data sets (mtDNA and nDNA data sets). Differences in topologies obtained were tested statistically for significance using the Shimodaira and Hasegawa test (1999) in PAUP4.0a125 (Swofford, 2002).

We calculated divergence between clades as the net number of nucleotide substitutions per site (*Da*) and the average number of nucleotide substitutions per site (*Dxy*), under a Jukes and Cantor model with DNAsp 5.0 (Librado and Rozas, 2009), using both the mtDNA and nDNA data sets. In addition, we calculated the genetic distance with the Kimura 2-parameter measure (Kimura, 1980) considering a gamma distribution value, as another estimate of differentiation between clades, and also because most phylogenetic studies of peromyscines have assessed this genetic distance (Bradley and Baker, 2001), thus we will be able to compare our results. Also we compared genetic distance within clades (at individual level) and between clades for Kimura 2-parameter distance. To incorporate some of the complexity of the sequences evolutionary model, we also estimated the Maximum Composite Likelihood distance. Both distances were calculated with MEGA v5.2 (Tamura et al., 2011). Based on the phylogenetic results obtained (see Section 3) and with the objective of specifically testing the position of *P. mekisturus* within Clade IV, we used Shimodaira–Hasegawa tests in PAUP*4.0 a125 (Swofford, 2002), and compared our mitochondrial tree topology with three artificially generated alternative topologies that we performed in MacClade 4.02 (Maddison and Maddison, 2002): (1) *P. mekisturus* is nested within the two *P. perfulvus* clades, being this clade a sister group of Clade I and Clade II: ((Clade III–CladeIV) (Clade II (Clade I – *P. mekisturus*))); (2) *P. mekisturus* is the sister group of both *P. melanophrys* (Clades III and IV):

((*P. mekisturus* (Clade III–Clade IV))(Clade I–Clade II)); and (3) *P. mekisturus* is nested within Clade III: (*P. melanophrys*) ((Clade IV (Clade III – *P. mekisturus*))(Clade I–Clade II)).

2.4. Molecular dating

We estimated divergence times for major phylogenetic clades (time to the most recent common ancestor, TMRCA) with the mtDNA data set by using a Yule tree prior and a reduced data set (to avoid imbalances due to low sequence variation or phylogenies with short branch lengths; Sanderson, 2003; Fontanella et al., 2008; Guiher and Burbrink, 2008) with a few representative samples of each clade, using a Bayesian phylogenetic analysis and relaxed-clock dating implemented in BEAST 1.7.4.(Drummond et al., 2012). We used these methods because they estimate divergence times simultaneously incorporating rate heterogeneity and uncertainty in the substitution parameters, tree topology, and calibration ages (Drummond et al., 2006; Brandley et al., 2010). Dating molecular divergences requires *a priori* assumptions of ages of one or more clades to calibrate the relationship between age and molecular rate (Drummond et al., 2006). Because no adequate fossil dates, biogeographically-based dates, or known substitution rates were available, we used three node ages: the split of *Onychomys* (10.3 million years ago [Mya]), the separation between *Peromyscus* and *Habromys* (4.69 Mya), and the split between *H. simulatus* from Oaxaca and *H. simulatus* from Hidalgo (0.73 Mya), which were calibrated by a fossil-based estimate (*Copemys russelli* 14.8 Mya) (León-Paniagua et al., 2007). We ran the analysis 50,000,000 generations, sampling model parameters every 1000 generations. We checked convergence statistics for effective sample sizes using Tracer 1.5 (Rambaut and Drummond, 2007). A consensus tree with node height distribution was generated using TreeAnnotator 1.7.4 (available in BEAST package), after elimination of 25% as burn-in, and visualized in FigTree 1.3.1 (Rambaut, 2009).

3. Results

3.1. Sequence variation

We analyzed complete mtDNA sequences (1315 base pairs [bp]) from 98 individuals (22 *P. perfulvus*, 75 *P. melanophrys* and one *P. mekisturus*), in which 888 (67.57%) invariable sites and 403 (30.66%) variable sites were found, 380 (94.29%) of which were parsimony-informative. In all, 96 samples showed unique haplotypes, thus the remaining two samples were removed from the analyses. All sequences were deposited at GenBank (Appendix A).

We obtained nDNA sequences from 39 individuals (26 *P. melanophrys* and 13 *P. perfulvus*) for a total of 829 bp. Eighteen identical

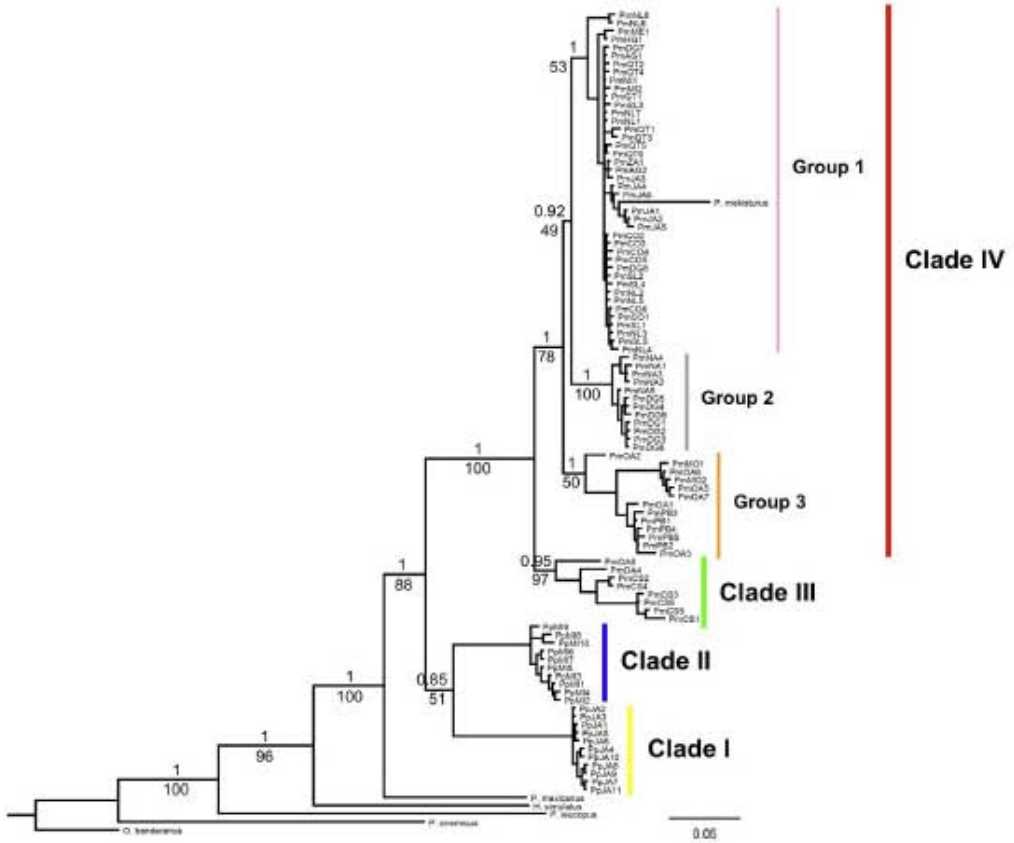

Fig. 2. Phylogenetic hypothesis for the *P. melanophrys* group based on BI and ML analyses of mitochondrial DNA sequence data. Numbers at nodes indicate support values (numbers above the branches indicate posterior probabilities, those below indicate bootstrap values).

sequences were removed from the data matrix to facilitate phylogenetic analyses, leaving a total of 12 unique haplotypes for *P. melanophrys* and nine for *P. perfulvus*). Across these haplotypes, 794 sites (90.34%) were invariable, 32 (3.86%) sites were variable, and 26 (81.25%) were parsimony-informative.

3.2. Phylogenetic relationships

ML and BI mtDNA analyses essentially converged on the same tree topology (Fig. 2), and we only describe the BI tree. Both ML (lnL = −9213.34) and BI uncovered a monophyletic *Peromyscus melanophrys* group (including individuals identified as *P. melanophrys*, *P. perfulvus*, and *P. mekisturus*), supported by high posterior probabilities (1). Samples of *P. perfulvus* were divided into two clades: Clade I including individuals from Jalisco and Clade II individuals from Michoacán. A Clade III was obtained including *P. melanophrys* individuals from Chiapas and Oaxaca and a last, Clade IV, encompassing the remaining *P. melanophrys* samples, together with the *P. mekisturus* haplotype. Within Clade IV, we identified three groups: the first including almost all *P. melanophrys* samples and the *P. mekisturus* haplotype, the second including individuals of *P melanophrys* from Durango and Nayarit, and the third including individuals from Oaxaca, Puebla, and Morelos (Fig. 2).

Results of the Shimodaira–Hasegawa test used to statistically evaluate alternative phylogenetic hypotheses about the position of *P. mekisturus*, showed in all comparisons that the "best" tree was our phylogenetic hypothesis ($p < 0.001$), in which *P. mekisturus* is placed within Clade IV. Values of the best tree are shown in Appendix C.

The nDNA analysis showed a phylogenetic tree (BI and ML [lnL = −1748.44]) with a similar topology as that of the mtDNA tree, although with lower resolution and relatively low bootstrap and posterior probabilities (Fig. 3). We could not include *P. mekisturus* in the nDNA analysis because the nature of the sample (a rather old –1947– and small sample) prevented correct amplification. Nonetheless, we identified a monophyletic *melanophrys* group (posterior probabilities and bootstrap values 1/87, respectively). Four major clades were resolved: Clade I includes individuals of *P. perfulvus* from Jalisco, Clade II includes individuals of *P. perfulvus* from Michoacán, Clade III individuals of *P. melanophrys* from Chiapas, and Clade IV consisting of the remaining *P. melanophrys* samples.

The same species membership was found within each clade (Figs. 2 and 3). However relationships among clades differed when using the mtDNA or nDNA datasets. The first showed Clade I and Clade II as sisters groups, and Clade III and Clade IV as sisters groups. The nDNA on the other hand, placed Clade II basally, but posterior probabilities and bootstrap values were higher in the first hypothesis (mDNA hypothesis), and relationships within Clade IV were also better resolved. The partition homogeneity test showed no significant differences between the mtDNA and nDNA data sets ($p = 0.408$), suggesting that the phylogenies obtained by each gene separately do not contradict the topology obtained in the concatenated analysis. ML (lnL = 8313.06) and BI analysis of the concatenated data set (Fig. 4) showed the same tree topology and phylogenetic relationships within the *Peromyscus melanophrys* group as those obtained with mtDNA (Clade I, Clade II, Clade III, and Clade IV). In addition, posterior probabilities and bootstrap values were higher (from 0.88 to 1 and 68 to 100, respectively for the main clades) than those obtained with either mtDNA or nDNA alone. Also, the Shimodaira–Hasegawa test ($p < 0.001$) suggests that the mitochondrial and nuclear tree topologies are different; however the best phylogenetic tree was that obtained with the mitochondrial data set (−lnL = 6280.66; difference = −lnL 924.68), which is the same as the one from the concatenated analysis (Appendix C).

The mtDNA divergence values obtained between clades ranged from 3.7% to 10.4% for *Da* and 6.9–12.2% for *Dxy*, while those obtained with the nuclear gene were 0.46–1.1% for *Da* and 0.75–1.5% for *Dxy* (Table 1). Clade IV is the most divergent with respect to the others. The genetic distance values showed comparatively consistent values between clades: Kimura 2-parameter were (mtDNA and nDNA, respectively): 10.1% and 1.1% between Clade I and Clade II; 4.2% and 1.7% between Clade III and Clade IV, while Maximum Composite Likelihood values were: 10.7% and 1% between Clade I and Clade II; 4.4% and 1.2% between Clade III and Clade IV (Table 2). Pairwise comparisons of genetic distance within clades (individual level) and between clades are shown in Fig. 5

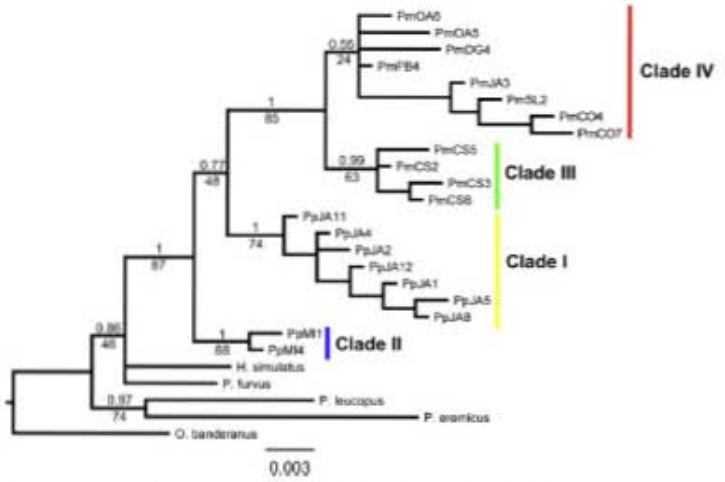

Fig. 3. Phylogenetic hypothesis for the *P. melanophrys* group based on BI and ML analyses of nuclear DNA sequence data. Numbers at nodes indicate support values (numbers above the branches indicate posterior probabilities, those below indicate bootstrap values).

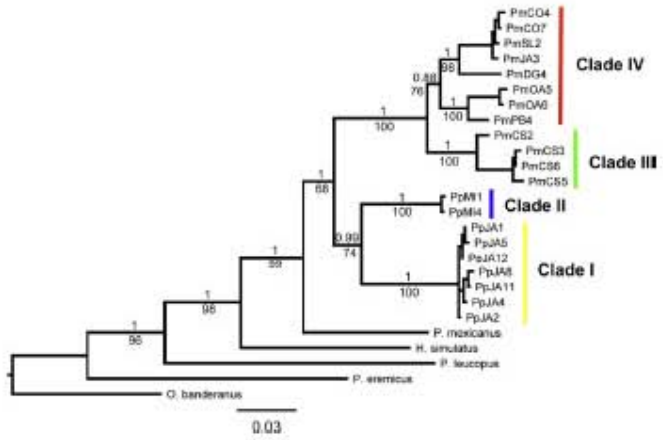

Fig. 4. Phylogenetic hypothesis for the *P. melanophrys* group based on BI and ML analyses from the concatenated sequence data set. Numbers at nodes indicate support values (numbers above the branches indicate posterior probabilities, those below indicate bootstrap values).

Table 1
Divergence values, shown as percentage, between clades within *P. melanophrys* group obtained from: (A) Mitochondrial genes and (B) Nuclear gene. Numbers above the diagonal are *Dxy* and below are *Da*.

	Clade I	Clade II	Clade III	Clade IV
A				
Clade I		9.70	12.20	12.10
Clade II	8.90		11.4	11.10
Clade III	10.40	9.30		6.90
Clade IV	10.20	9.10	3.70	
B				
Clade I		0.75	1.50	1.30
Clade II	0.56		1.20	1.50
Clade III	1	1.10		1
Clade IV	1.10	1.10	0.46	

Table 2
Genetic distances values, shown as percentage, between and within clades within *P. melanophrys* group. Numbers above the diagonal are from mitochondrial genes and numbers below are from nuclear gene. Numbers in the diagonal are distance values within each clade (mitochondrial/nuclear). (A) Kimura 2-parameter and (B) Maximum Composite Likelihood.

	Clade I	Clade II	Clade III	Clade IV
A				
Clade I	0.30/0.30	10.10	11.90	11.90
Clade II	1.10	1/0.10	11.10	10.60
Clade III	4	3.50	3.40/0.30	4.20
Clade IV	3.40	2.70	1.70	3.30/1.20
B				
Clade I	0.30/0.30	10.70	12.90	12.90
Clade II	1	1/0.01	11.60	11.11
Clade III	2.20	2.20	3.40/0.30	4.40
Clade IV	3	3.7	1.2	3.40/1.30

(Maximum Composite Likelihood values are not shown because they are not different from Kimura 2-parameter values).

3.3. Divergence times

We used 21 samples, representing the four main clades and the three groups detected in our phylogenetic hypothesis based on mtDNA sequence data, to estimate divergence times (Table 3 and Fig. 6). According to these estimates, the *Peromyscus melanophrys* group diverged from the other species 4.78 (95% HPD: 4.3–5.2) Mya. The split between Clade I and Clade II with respect to Clade III and Clade IV was dated 3.3 (95% HPD: 2.3–4.3) Mya, while the separation between Clade I and Clade II was 2.4 (95% HPD: 1.6–3.6) Mya. Finally, Clade III and Clade IV initially diverged 2 (95% HPD: 1.2–3) Mya. Subsequent diversification within Clade IV happened 1.7 (95% HPD: 1–2.7) Mya. Group 3 diverged from the rest and the divergence of Group 1 and Group 2 was estimated to have occurred 1.3 (95% HPD: 0.6–2.2) Mya.

4. Discussion

4.1. Peromyscus melanophrys as a monophyletic group

All phylogenetic analyses (ML and BI) using mtDNA, nDNA, and concatenated sequences (BI) data recovered a *Peromyscus melanophrys* group that is monophyletic, relative to the outgroups included. The results support the findings of Bradley et al. (2007), based on sequences of cyt b, although their analysis did not include *P. mekisturus*. Moreover, our results are in agreement with previous hypotheses based on morphological characters (Osgood, 1909; Hall and Kelson, 1952; Hooper and Musser, 1964; Hooper, 1968; Carleton, 1989). However, we are aware that given the evolutionary complexity of the genus *Peromyscus*, a definitive corroboration of the monophyly of the *melanophrys* group will need a more thorough taxonomic sampling that includes the most members of the genus and other closely related taxa as possible.

4.2. Variation and species limits within the Peromyscus melanophrys group

The *P. melanophrys* group encompasses three recognized species: *P. melanophrys* and *P. perfulvus*, considered sister taxa by Musser and Carleton (2005) and Bradley et al. (2007), although both studies were based on very few samples and relatively few subspecies. The third species, *P. mekisturus*, was included in the group based on morphological characters (Osgood, 1909; Carleton, 1989; Musser and Carleton, 1993, 2005). Our extensive sampling allowed us to determine more clearly the phylogenetic

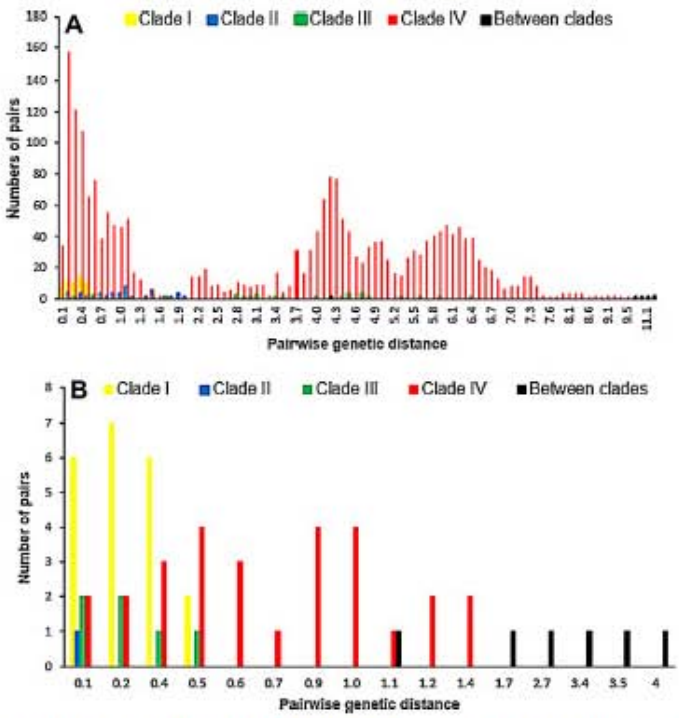

Fig. 5. Histogram of pairwise sequence divergences (Kimura 2-parameter percentage distance values), showing within clade (at individual level) and between clades comparisons of the *P. melanophrys* group. (A) Mitochondrial genes and (B) Nuclear gene.

Table 3
Estimated dates of divergence (time to de most recent common ancestor –TMRCA– and 95% confidence intervals in Mya) based on ages calibrated in León-Paniagua et al. (2007).

Clade	TMRCA	95% HPD
A	5.14	4.39–6.62
B	4.78	4.32–5.24
C	3.36	2.34–4.34
D	2.40	1.22–3.64
E	2.02	1.25–3.05
F	1.75	1.04–2.72
G	1.31	0.68–2.2
H	0.80	0.27–1.59
I	0.71	0.27–1.38
J	3.26	1.9–4.39
K	0.72	0.65–0.79

Clades depicted by letters correspond to those indicated in Fig. 6.

relationships within the group, given that we analyzed all six subspecies of *P. melanophrys*, both subspecies of *P. perfulvus* and *P. mekisturus*. Our results show two genetically divergent clades (Clade I and Clade II) that include all haplotypes of *P. perfulvus*, consistently resolved (reciprocal monophyly) and well supported by mtDNA and nDNA. The two clades are broadly disjunct, which precludes any test in sympatry. Differentiation between clades based on divergence and genetic distance measures (Da = 8.9%, Dxy = 9.7%, Kimura 2-parameters = 10.1%, Maximum Composite Likelihood = 10.7%) are similar to values observed for species of the same group in the *P. boylii*, *aztecus* and *truei* groups (between 7% and 8.1%; Tiemann-Boege et al., 2000). Durish et al. (2004) reported values of 7.7–10.6% between species of the *truei* group. For the current recognized subspecies within *P. perfulvus*, Hooper (1955) analyzed the morphology of the subspecies and documented that *P. p. chrysopus* is smaller and with upper surface of forefeet buffy, rather than white as in *P. p. perfulvus*, as well as differences in molars 1 and 2 (in *P. p. chrysopus* with ectostylids and complete mesolophs; in *P. p. perfulvus*, absent or mesolophs short, not reaching the labial border of the tooth). The absence of intermediate forms strongly suggests that the two clades obtained in our results are on separate evolutionary trajectories, a division that roughly coincides with the present subspecies limits. Since our data consistently resolve a well supported Clade I that is distinct from Clade II, we recommend elevating it to a species, *P. chrysopus* Hooper (1955), that is distributed in the Pacific Coast and is restricted to low altitudes (<200 m). Clade II (*P. perfulvus* Osgood, 1945), is distributed along the Balsas Depression and in an altitudinal range from 500 to 2000 m. However, a definitive statement of its species status would require more research on the potential for gene flow between the clades.

The case of *P. melanophrys* and *P. mekisturus* is more complex. Our results show two major clades (Clades III and IV), showing reciprocal monophyly and with 1 and 85 posterior probability and boostrap values. Divergence and genetic distance values between clades are 3.7% (Da), 6.9% (Dxy), 4.2% (Kimura 2-parameter), 4.4% (Maximum Composite Likelihood). However, these clades do not correspond well with the currently-recognized species and subspecies. Clade IV is one of the most striking findings in the present study, because the mysterious *P. mekisturus* falls in this clade, where all the samples are considered as *P. melanophrys*. Although the *P. mekisturus* haplotype shows a long branch, this could be the result of partial missing bases in the sequence (47 bp missing,

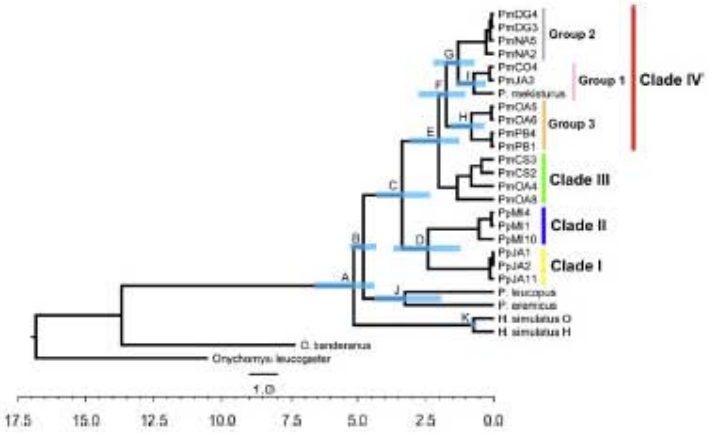

Fig. 6. Chronogram reconstructed from the mitochondrial DNA dataset inferred from BEAST. The horizontal bars show 95% confidence intervals. Letters at nodes depict values calculated as in Table 3.

for a total of 1268 bp sequenced). This hypothesis is supported by the Shimodaira–Hasegawa test, which showed that the best tree is the one including *P. mekisturus* within Clade IV. Carleton (1989) had suggested that *P. mekisturus* was the most divergent species within the group based on dental and cranial characters: more orthodont incisors, lack of a supraorbital shelf, shorter and wider mesopterygoid fossa, and greater development of an incisor capsule on the dentary. Nonetheless, the number of shared features (long tail, truncate rostrum, spacious sphenopalatine vacuities, sloping occiput, and molar configuration) and our molecular results all support its placement in the *P. melanophrys* group. Further, we have to consider that the historical distribution of *P. melanophrys* and *P. mekisturus* is sympatric at the two known localities of *P. mekisturus*, inhabiting rocky landscapes and arid environments (Hooper, 1968). In terms of morphology, Osgood (1909) found morphological similarities between *P. mekisturus* and *P. melanophrys* as the general color, the character of the pelage, and the very long coarsely-haired tail. He mentioned that even the skull bears a resemblance, but is smaller in *P. mekisturus*. Moreover, *P. mekisturus* has a very long tail and slightly beaded skull (Merriam, 1898), characteristics that are all also found in *P. melanophrys*, and which distinguish this species from nearly all the other peromyscine species from Mexico. Even some external measurements (Total length. Tail vertebrae and Ear) of *P. mekisturus* (222–249 mm, 135–155 mm, 17–19 mm) fall within those of *P. melanophrys* (235–280 mm, 122–163 mm, 18–21 mm). These morphological similarities along with our results suggest that *P. mekisturus* might not be as divergent as other authors had proposed in comparison with *P. melanophrys* (Carleton, 1989).

Clade IV has a wide distribution along the Mexican Plateau, the Sierra Madre Occidental, and the Trans-Mexican Volcanic Belt south to Puebla and Oaxaca, with limits on the interior lowlands adjacent to the Sierra Madre del Sur (Fig. 1). Clade IV is differentiated into three groups (Fig. 2) that do not bear any resemblance to the subspecific divisions proposed by Baker (1952), given that the differences found among them are usually rather subtle attributes of size and coloration, but have a geographic congruence. The first one is restricted to the Mexican Plateau south to the Tehuantepec Valley, in lowland and middle-elevations (<2500 m) and the second is restricted to the lowlands that extend throughout the Sierra Madre Occidental and with boundaries on the San Pedro Mezquital river basin in Durango and Nayarit. The third one is distributed along the Balsas River basin, surrounding the Sierra de Oaxaca (Morelos, Puebla and Oaxaca). Clade III includes exclusively *P. melanophrys* haplotypes, corresponding to populations distributed on the lowlands of the south Pacific coast, with a trans-Isthmic distribution in Oaxaca and Chiapas.

In synthesis, although our analyses suggest that *P. melanophrys sensu lato* (Clades III and IV) is composed of several evolutionary units, and that *P. mekisturus* is not a valid species, more individuals of *P. mekisturus* need to be found, so further molecular and morphological analyses would help to confirm its status a different species and clarify the whole panorama of relationships among these clades.

4.3. Biogeography

The present day-distribution of peromyscines has been impacted greatly by the dramatic climatic fluctuations since the apparition of the taxon in the mid-Pliocene to early Pleistocene (Hibbard, 1968; Dawson, 2005). Dawson (2005) argued that the unique Mexican topography has been an "incubator" of peromyscine species from their origin through the present, undergoing several periods of speciation and expansion via vicariant and dispersal events related to glacial cycles. This complex history of Pleistocene habitat fragmentation provides a context for biogeographic interpretation of the evolution of peromyscines (Sullivan et al., 1997) and our results are no exception. According to our divergence time estimates, the *P. melanophrys* group diverged during the Pliocene and the main diversification events within the group occurred at the end of the Pliocene and through the Pleistocene.

Based on the lineages recovered herein and the present distributional patterns, we consider the *P. melanophrys* group restricted to lowland and middle-elevation areas below 2500 m, thus highlands may represent significant barriers for the group. The emergence of the Trans-Mexican Volcanic Belt (between 11 and 1.8 Mya; Ferrari et al., 2000), and the presence of the Sierra Madre del Sur to the south, isolated the Balsas Depression (Ferrusquía-Villafranca, 1998), which could explain the initial separation of Clade I and Clade II from the Clade III and Clade IV in the mid-Pliocene. Similar patterns have been reported for other lowland animal species as the amphibian *Rana berlandieri* (Zaldivar-Riverón et al., 2004). Dawson (2005) explains that *P. perfulvus* has a trans-montane sympatric

connection with *P. melanophrys* via the Grande Santiago River Valley (lowlands).

We dated the divergence between Clades I and II at the end of the Pliocene and the beginning of the Pleistocene. However, a highland barrier is harder to identify between them, and while Clade I is restricted to the coast (< 200 m) Clade II is present at the foothills of the Trans-Mexican Volcanic Belt, although the mountains of western Michoacán may have provided some isolation. The alternative hypothesis is ecological speciation (e.g., *P. eremicus* group; Riddle et al., 2000), which has not yet been tested and at least appears to be a rare phenomenon in vertebrates (Peterson, 2011).

The lowlands of the Isthmus of Tehuantepec may act as a corridor for individuals of Clade III from Chiapas to southwestern Oaxaca, opposite to patterns in other species of vertebrates (Barrera-Guzmán et al., 2012; Arbeláez-Cortés et al., 2010; Cortés-Rodríguez et al., 2008). The Clade IV is distributed from the Tehuantepec coastal plain through the central valleys of Oaxaca, where the Sierra of Oaxaca may isolate it from Clade III; divergence of these two clades occurred during the Pleistocene. Devitt (2006) and Glazier (1980), among others, argued that Pliocene–Pleistocene vicariant events and more recently Quaternary climatic shifts have been shaping biodiversity patterns in the Nearctic-Neotropical transition zone, where the *P. melanophrys* group is distributed. Further phylogeographic analyses (Castañeda-Rico et al., in prep.) may clarify the biogeographic history of the group in greater detail.

Acknowledgments

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2013.10.004.

References

Alderman, J., Takagi, A., Lieber, C., 1987. Ethanol-metabolizing pathways in deermice. J. Biol. Chem. 262, 7497–7503.
Álvarez-Castañeda, S., González-Ruiz, N., 2008. Análisis preliminar de las relaciones filogenéticas entre los grupos de especies del género *Peromyscus*. In: Lorenzo, C., Espinoza, E., Ortega, J. (Eds.), Avances en el estudio de los mamíferos de México II. CIB. Universidad Veracruzana, IPN, Ecosur, Unicach, UAEM, AMMAC, UAM, México, pp. 5–26.
Arbeláez-Cortés, E., Nyári, A., Navarro-Sigüenza, A.G., 2010. The differential effect of lowlands on the phylogeographic pattern of a Mesoamerican montane sapecies (*Lepidocolaptes affinis*, Aves: Furnariidae). Mol. Phylogenet. Evol. 57, 658–668.
Baker, R.H., 1952. Geographic range of *Peromyscus melanophrys*, with description of new subspecies. Univ. Kans. Publ. Mus. Nat. Hist. 5, 251–258.
Barrera-Guzmán, A.O., Milá, B., Sánchez-González, L.A., Navarro-Sigüenza, A.G., 2012. Speciation in an avian complex endemic to the mountains of Middle America (*Ergaticus*, Aves, Parulidae). Mol. Phylogenet. Evol. 62, 907–920.
Bradley, R.D., Baker, R.J., 2001. A test of the genetic species concept: cytochrome-b sequences and mammals. J. Mammal. 82, 960–973.
Bradley, R.D., Durish, N., Rogers, D., Millar, J., Engstrom, M., Kilpatrick, W., 2007. Toward a molecular Phylogeny for *Peromyscus*: evidence from mitochondrial cytochrome-b sequences. J. Mammal. 88, 1146–1159.
Brandley, M., Guilher, T.J., Pyron, R.A., Winne, C.T., Burbrink, F.T., 2010. Does dispersal across an aquatic geographic barrier obscure phylogeographic structure in the diamond-backed watersnake (*Nerodia rhombifer*)? Mol. Phyl. Evol. 57, 552–560.
Braun, J., Mares, A.M., Coyner, B.S., Van Den Bush, R.A., 2010. New species of *Akodon* (Rodentia: Cricetidae: Sigmodontinae) from central Argentina. J. Mammal. 91, 387–400.
Cao, Y., Adachi, J., Janke, A., Paabo, S., Hasegawa, M., 1994. Phylogenetic relationships among eutherian orders estimated from inferred sequences of mitochondrial proteins: instability of a tree based on a single gene. J. Mol. Evol. 39, 519–527.
Carleton, M.D., 1980. Phylogenetic relationships in neotominae-peromyscine rodents (Muroidea) and a reappraisal of the dichotomy with New World Cricetinae. Misc. Publ. Mus. Zool. Univ. Mich. 146, 1–43.
Carleton, M.D., 1989. Systematics and Evolution. In: Kirkland, G.L., Layne, J. (Eds.), Advances in the study of *Peromyscus* (Rodentia). Texas Tech University Press, Lubbock, pp. 7–141.
Carleton, M.D., Musser, G.G., 2005. Order Rodentia. In: Wilson, D.E., Reeder, D.M. (Eds.), Mammal Species of the World, third ed. The Johns Hopkins University Press, USA, pp. 745–752.
Castañeda-Rico, S., León-Paniagua, L., Ruedas, L.A., Vázquez-Domínguez, E., 2011. High genetic diversity and extreme differentiation in the two remaining populations of *Habromys simulatus*. J. Mammal. 92, 963–973.
Cortés-Rodríguez, N., Hernández-Baños, B.E., Navarro-Sigüenza, A.G., Peterson, A.T., García-Moreno, J., 2008. Phylogeography and population genetics of the Amenthyst-throated Hummingbird (*Lampornis amethystinus*). Mol. Phylogenet. Evol. 48, 1–11.
Dawson, W., 2005. Peromyscine Biogeography, Mexican topography and Pleistocene climatology. In: Sánchez-Cordero, V., Medellín, R. (Eds.), Contribuciones Mastozoológicas en Homenaje a Bernardo Villa. UNAM-CONABIO, México, pp. 145–156.
Devitt, T.J., 2006. Phylogeography of Western Lyresnake (*Trimorphodon biscutatus*): testing arid land biogeographical hypotheses across the Nearctic-Neotropical transition. Mol. Ecol. 15, 4387–4407.
Drummond, A.J., Ho, S.Y.W., Phillips, M.J., Rambaut, A., 2006. Relaxed phylogenetics and dating with confidence. PloS Biol. 4, e88.
Drummond, A.J., Suchard, M.A., Xie, D., Rambaut, A., 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. Mol. Biol. Evol. 29, 1969–1973.
Durish, N.D., Halcomb, K.E., Kilpatrick, C.W., Bradley, R.D., 2004. Molecular systematics of the *Peromyscus truei* species group. J. Mammal. 85, 1160–1169.
Engel, S.R., Hogan, K.M., Taylor, J.F., Davis, S.K., 1998. Molecular systematics and paleobiogeography of the South American *Sigmodontine Rodents*. Mol. Biol. Evol. 15, 35–49.
Fernández, J.A., Cervantes, F.A., Hafner, M.S., 2012. Molecular systematics and biogeography of the Mexican endemic kangaroo rat, *Dipodomys phillipsii* (Rodentia: Heteromyidae). J. Mammal. 93, 560–571.
Ferrari, L., Cinticelli, S., Vaggelli, G., Peyrone, C.M., Manetti, P., 2000. Late Miocene volcanism and intera-arc tectonics during the early development of the Trans-Mexican Volcanic Belt. Tectonophysics 318, 161–185.

Ferrusquía-Villafranca, V.I., 1998. Geología de México: Una sinopsis. In: Ramamoorthy, T.P., Bye, R., Lot, A., Fa, J. (Eds.), Diversidad Biológica de México: Orígenes y Distribución. UNAM, México, pp. 3–108.
Fontanella, F., Feldman, C., Siddall, M., Burbrink, F., 2008. Phylogeography of *Diadophis punctatus*: extensive lineage diversity and repeated patterns of historical demography in a trans-continental snake. Mol. Phylogenet. Evol. 46, 1049–1070.
Glazier, D.S., 1980. Ecological shifts and the evolution of geographically restricted species of North American *Peromyscus* (mice). J. Biogeogr. 7, 63–83.
Guiher, T., Burbrink, F., 2008. Demographic and phylogeographic histories of two venomous North American snakes of the genus *Agkistrodon*. Mol. Phylogenet. Evol. 48, 543–553.
Guindon, S., Gascuel, O., 2003. A simple, fast and accurate method to estimate large phylogenies by maximum likelihood. Syst. Biol. 52, 696–704.
Guindon, S., Dufayard, J.F., Lefort, V., Anisimova, M., Hordijk, W., Gascuel, O., 2010. New algorithms and methods to estimate Maximum-Likelihood Phylogenies: Assessing the Performance of PhyML 3.0. Syst. Biol. 59, 307–321.
Hafner, D., Riddle, B., Álvarez-Castañeda, S., 2001. Evolutionary relationships of White footed mice (*Peromyscus*) on the islands in the Sea of Cortez, Mexico. J. Mammal. 82, 775–790.
Hall, E.R., 1981. Mammals of North America, second ed. John Wiley and Sons, New York.
Hall, T.A., 1999. BioEdit: a userfriendly biological sequence alignment editor and 886 analysis program for Windows 95/98/NT. Nucl. Acids Symp. 41, 95–98.
Hall, E.R., Kelson, K.R., 1952. Comments on the taxonomy and geographic distribution of some North American rodents. Univ. Kans. Publ. Mus. Nat. Hist. 5, 343–371.
Hibbard, C.W., 1968. Paleontology. In: King, J.A. (Ed.), Biology of *Peromyscus* (Rodentia). American Society of Mammalogist Special Publication 2, USA, pp. 6–26.
Hogan, K., Hering, M., Koh, S., Davis, K., Greenbaum, I., 1993. Systematic and taxonomic implications of karyotypic, electrophoretic and mitochondrial DNA variation in *Peromyscus* from the Pacific Northwest. J. Mammal. 74, 819–831.
Hooper, E.T., 1947. Notes on Mexican mammals. J. Mammal. 28, 40–57.
Hooper, E.T., 1955. Notes on mammals of western Mexico. Occas. Pap. Mus. Zool. Univ. Mich. 565, 1–26.
Hooper, E.T., 1968. Classification. In: King, J.A. (Ed.), Biology of *Peromyscus* (Rodentia). American Society of Mammalogist Special Publication 2, USA, pp. 27–74.
Hooper, E.T., Musser, G.G., 1964. Notes on classification of the rodents genus *Peromyscus*. Occas. Pap. Mus. Zool. Univ. Mich. 635, 1–13.
Huckaby, D.G., 1980. Species limits in the *Peromyscus mexicanus* group (Mammalia: Rodentia: Muroidea). Contrib. Sci. Nat. Hist. Mus. Los Angeles County 326, 1–24.
Jansa, S.A., Giarla, T.C., Lim, B.K., 2009. The phylogenetic position of the rodent genus *Typhlomys* and the geographic origin of Muroidea. J. Mammal. 90, 1083–1094.
Kaufman, D., Kaufman, G., 1989. Population biology. In: Kirkland, G.L., Layne, J. (Eds.), Advances in the study of *Peromyscus* (Rodentia). Texas Tech University Press, Lubbock, pp. 233–270.
Kelt, D.A., Hafner, M.S., 2010. The American Society of Mammalogists' Ad Hoc Committee for Guidelines on Handling Rodents in the Field. Updated guidelines for protection of mammalogists and wildlife researchers from hantavirus pulmonary syndrome (HPS). J. Mammal. 91, 1524–1527.
Kimura, M., 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol. 16, 111–120.
Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, A., Lopez, R., Thompson, J.D., Gibson, T.J., Higgins, D.G., 2007. Clustal W and Clustal X version 2.0. Bioinformatics 23, 2947–2948.
Lee, M.R., Elder, F.B., 1977. Karyotype of eight species of Mexican rodents (Muridae). J. Mammal. 58, 479–487.
León-Paniagua, L., Navarro-Sigüenza, A., Hernández-Baños, B., Morales, J., 2007. Diversification of the arboreal mice of the genus *Habromys* (Rodentia:Cricetidae:Neotominae) in Mesoamerican Highlands. Mol. Phylogenet. Evol. 42, 653–664.
Librado, P., Rozas, J., 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25, 1451–1452.
Maddison, D.R., Maddison, W.P., 2002. MacClade 4: Analysis of Phylogeny and Character Evolution. Version 4.02. Sunderland, MA: Sinauer Associates.
MacMillen, R., Garland Jr., T., 1989. Adaptative physiology. In: Kirkland, G.L., Layne, J. (Eds.), Advances in the study of *Peromyscus* (Rodentia). Texas Tech University Press, Lubbock, pp. 143–168.
Merriam, C.H., 1898. Description of twenty new species and a subgenus of *Peromyscus* from Mexico and Guatemala. P. Biol. Soc. Wash. 12, 115–125.
Merritt, J.F., 2010. The Biology of Small Mammals. The John Hopkins University Press, Baltimore, Maryland, USA.
Millar, J., 1989. Reproduction and Development. In: Kirkland, G.L., Layne, J. (Eds.), Advances in the study of *Peromyscus* (Rodentia). Texas Tech University Press, Lubbock, pp. 169–232.
Miller, J., Engstrom, M., 2008. The relationships of major lineages within peromyscine rodents: a molecular phylogenetic hypothesis and systematic reappraisal. J. Mammal. 89, 1279–1295.
Musser, G., Carleton, M.D., 1993. Family Muridae. In: Wilson, D.E., Reeder, M. (Eds.), Mammal Species of the World: A Taxonomic and Geographic Reference. Smithsonian Institution Press, Washington D.C., pp. 501–755.
Musser, G., Carleton, M.D., 2005. Superfamily Muridae. In: Wilson, D.E., Reeder, M. (Eds.), Mammal Species of the World: A Taxonomic and Geographic Reference, third ed. Johns Hopkins University Press, Baltimore, Maryland, pp. 894–1531.
Osgood, W.H., 1900. Revision of pocket mice of the genus *Perognathus*. North American Fauna 18, 1–72.
Osgood, W., 1909. Revision of the mice of the American genus *Peromyscus*. North American Fauna 28, 1–285.
Osgood, W.H., 1945. Two new rodents from Mexico. J. Mammal. 26, 299–301.
Peterson, T.A., 2011. Ecological niche conservatism; a time-structured review of evidence. J. Biogeogr. 38, 817–827.
Posada, D., 2008. jModelTest: phylogenetic model averaging. Mol. Biol. Evol. 25, 1253–1256.
Rambaut, A., Drummond, A.J., 2007. Tracer v1.5. <http://tree.bio.ed.ac.uk/software/tracer/>.
Rambaut, A., 2009. FigTree 1.3.1. <http://tree.bio.ed.ac.uk/software/figtree/>.
Ramírez-Pulido J., Arroyo-Cabrales, J., Castro-Campillo, A., 2005. Estado actual y relación nomenclatural de los mamíferos terrestres de México. Acta Zool. Mex. 21 (n.s.), 21–82.
Riddle, B., Hafner, D., Alexander, L., 2000. Phylogeography and Systematics of the *Peromyscus eremicus* species group and the historical biogeography of North American Warm Regional deserts. Mol. Phylogenet. Evol. 17, 145–160.
Rogers, D.S., Engstrom, M.D., Arellano, E., 2005. Phylogenetic relationships among Peromyscine rodents; Allozyme evidence. In: Sánchez-Cordero, V., Medellín, R. (Eds.), Contribuciones Mastozoológicas en Homenaje a Bernardo Villa. UNAM-CONABIO, México, pp. 437–449.
Ronquist, F., Huelsenbeck, J.P., 2003. Mr. Bayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572–1574.
Russo, C.A., Takezaki, N., Nei, M., 1996. Efficiencies of different genes and different tree-building methods in recovering a known vertebrate phylogeny. Mol. Biol. Evol. 13, 525–536.
Sanderson, M.J., 2003. R8s; inferring absolute rates of evolution and divergence times in the absence of a molecular clock. Bioinformatics 19, 301–302.
Shimodaira, H., Hasegawa, M., 1999. Multiple Comparison of Log-Likelihood with Applications to Phylogenetic Inference. Mol. Biol. Evol. 16, 1114–1116.
Sikes, R.S., Gannon, W.L., the Animal Care and Use Committee of the American Society of Mammalogists, 2011. Guidelines of the American Society of Mammalogists for the use of wild mammals in research. J. Mammal. 92, 235–253.
Sullivan, J., Market, A., Kilpatrick, C.W., 1997. Phylogeography and molecular systematics of *Peromyscus aztecus* group (Rodentia:Muridae) inferred using parsimony and likelihood. Syst. Biol. 46, 426–440.
Swofford, D.L., 2002. PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4.0a125. Sinauer Associates, Sunderland, Massachusetts.
Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S., 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. Evol. 28, 2731–2739.
Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., Higgins, D.G., 1997. The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucl. Acids. Res. 25, 4876–4882.
Tiemann-Boege, I., Kilpatric, C.W., Schmidly, D.J., Bradley, R.D., 2000. Molecular phylogenetics of the *Peromyscus boylii* species group (Rodentia: Muridae) based on mictochodrial cytpcrome *b* sequences. Mol. Phylogenet. Evol. 16, 366–378.
Zaldívar-Riverón, A., León-Regagnon, V., Nieto-Montes de Oca, A., 2004. Phylogeny of Mexican cranial leopard frogs of the *Rana berlandieri* group based on mtDNA sequences. Mol. Phylogenet. Evol. 30, 38–48.
Zimmerman, E.G., 1974. Chromosomes of the Mexican Plateu mouse, *Peromyscus melanophrys*, and a new sex-determining mechanism in mammals. Can. J. Genet. Cytol. 16, 797–804.

Appendix A

List of individuals used in this study indicating species, identity (ID), museum numbers registration, state from Mexico, location, mitochondrial and nuclear genes sequenced and GenBank accession number data.

Species	ID	Museum number	State	Location	ND3, ND4L, tRNA-Arginine and ND4 sequence	GHR sequence	GenBank accession number
Peromyscus melanophrys	PmOA1	828-CIOAX	Oaxaca	Viejo Zoquiapan, 6.5 km SW San Juan Atepec, Ixtlán	Complete		KF885880
	PmOA2	453-CIOAX	Oaxaca	San José del Chilar, 1 km N, 1 km E La Presa Matamba, Cuicatlán	Complete		KF885881
	PmOA3	454-CIOAX	Oaxaca	San Bartolomé, Ayoutla 2 km W, Teotitlán del Camino	Complete		KF885882
	PmOA4	449-CIOAX	Oaxaca	Ciudad de Oaxaca, Centro.	Complete		KF885879
	PmOA5	4464-CIOAX	Oaxaca	La Tetechera 1.75 km SW San Marcos Arteaga	Complete	Complete	KF885876 KF885924
	PmOA6	4467-CIOAX	Oaxaca	La Tetechera 1.75 km SW San Marcos Arteaga	Complete	Complete	KF885877 KF885925
	PmOA7	4469-CIOAX	Oaxaca	Los Encinos, 6.3 km SW San Marcos Arteaga	Complete	Complete	KF885878 KF885926
	PmOA8	CAS237-MZFC	Oaxaca	3 Km de Quiotepec, Estación de tren El Venado	Complete		KF885875
	PmCS1	1675-IHNE	Chiapas	Reserva de la Biósfera La Sepultura, Tonalá	Complete		KF885834
	PmCS2	TK20602-TTU	Chiapas	8.2 Mi SE, 2.5 Mi Tonalá, Río Ocuilapa	Complete	Complete	KF885829 KF885913
	PmCS3	TK20603-TTU	Chiapas	8.2 Mi SE, 2.5 Mi Tonalá, Río Ocuilapa	Complete	Complete	KF885830 KF885914
	PmCS4	TK20604-TTU	Chiapas	8.2 Mi SE, 2.5 Mi Tonalá, Río Ocuilapa	Complete	Complete	KF885831 KF885915
	PmCS5	TK20605-TTU	Chiapas	8.2 Mi SE, 2.5 Mi Tonalá, Río Ocuilapa	Complete	Complete	KF885832 KF885916
	PmCS6	TK20715-TTU	Chiapas	8.2 Mi SE, 2.5 Mi Tonalá, Río Ocuilapa	Complete	Complete	KF885833 KF885917
	PmPB1	4491-UAMI	Puebla	3 km N Asumbilla, Chapulco	Complete		KF885862
	PmPB2	4492-UAMI	Puebla	3 km N Asumbilla, Chapulco	Complete		KF885863
	PmPB3	4493-UAMI	Puebla	3 km N Asumbilla, Chapulco	Complete		KF885861

PmPB4	JAFF2113-LSU	Puebla	2 km E Zinacatepec	Complete	Complete	KF885859 KF885904
PmPB5	TK93149-TTU	Puebla	5 km SE San Antonia	Complete	Complete	KF885860 KF885905
PmME1	2852-UAMI	Estado de México	5 km SE Nopaltepec	Complete		KF885857
PmHG1	2851-UAMI	Hidalgo	4 km W Apan	Complete		KF885858
PmMI1	1072-UAMI	Michoacán	5 km SE Zamora	Complete		KF885841
PmMI2	1073-UAMI	Michoacán	5 km SE Zamora	Complete		KF885842
PmJA1	1157-UAMI	Jalisco	30 km N La Barca	Complete		KF885870
PmJA2	1158-UAMI	Jalisco	30 km N La Barca	Complete		KF885871
PmJA3	TK93076-TTU	Jalisco	2 km NW Mesoncito	Complete	Complete	KF885872 KF885903
PmJA4	34645-ENCB	Jalisco	3.5 km W Lagos de Moreno	Complete		KF885869
PmJA5	33350-ENCB	Jalisco	7 km W Ocotlán	Complete		KF885873
PmJA6	33351-ENCB	Jalisco	7 km W Ocotlán	Complete		KF885874
PmNL1	7114-INAH	Nuevo León	8 km N, 4.1 km E San Josecito	Complete		KF885851
PmNL2	7115-INAH	Nuevo León	8 km N, 4.1 km E San Josecito	Complete		KF885852
PmNL3	7117-INAH	Nuevo León	8 km N, 4.1 km E San Josecito	Complete		KF885853
PmNL4	7120-INAH	Nuevo León	8 km N, 4.1 km E San Josecito	Complete		KF885854
PmNL5	669-INAH	Nuevo León	8 km N, 4.1 km E San Josecito	Complete		KF885855
PmNL6	6444-INAH	Nuevo León	3.4 km N, 9.2 km W San Josecito	Complete		KF885856
PmNL7	TK137282-TTU	Nuevo León	8.7 km W Doctor Arroyo	Complete	Complete	KF885849 KF885906
PmNL8	TK137283-TTU	Nuevo León	8.7 km W Doctor Arroyo	Complete	Complete	KF885850 KF885907
PmAG1	JAFF9095-LSU	Aguascalientes	11 km N Rincón de Romas	Complete	Complete	KF885827 KF885918
PmAG2	36362-ENCB	Aguascalientes	3.8 km N, 1 km E Las Fraguas	Complete		KF885828
PmNA1	35087-CNM	Nayarit	Las Adjuntas II, 18.8 km E, 37.6 km N Tepic	Complete		KF885864
PmNA2	35088-CNM	Nayarit	Las Adjuntas II, 18.8 km E, 37.6 km N Tepic	Complete		KF885866
PmNA3	35078-CNM	Nayarit	Las Adjuntas II, 18.8 km E, 37.6 km N Tepic	Complete		KF885865
PmNA4	523921-USNM	Nayarit	Arroyo de Jiquite Río Grande de Santiago	Complete		KF885867
PmNA5	511691-USNM	Nayarit	Mesa del Nayar	Complete		KF885868
PmDG1	TD1820-CIDGO	Durango	1 km N, 4.2 km E Platanitos	Complete	Complete	KF885819 KF885921
PmDG2	TD1821-CIDGO	Durango	0.3 km N, 2.57 km E Platanitos	Complete	Complete	KF885820 KF885923
PmDG3	TD1822-CIDGO	Durango	0.5 km S, 2.75 km E Platanitos	Complete	Complete	KF885821 KF885920
PmDG4	TD1823-CIDGO	Durango	Chachacuaxtle	Complete	Complete	KF885822 KF885919
PmDG5	TD2328-CIDGO	Durango	3.5 W Agua Zarca	Complete	Complete	KF885818 KF885922
PmDG6	TD2330-CIDGO	Durango	Santiago Teneraca	Complete		KF885823
PmDG7	FCR271-MZFC	Durango	Castillo Nájera 2.7 km E	Complete		KF885817
PmDG8	TK48715-TTU	Durango	5.8 km N, 2.1 km E Vicente Guerrero	Complete		KF885824

	PmDG9	TK48848-TTU	Durango	2.2. km S, 2.5 km E Vicente Guerrero	Complete		KF885825
	PmCO1	FCR220-MZFC	Coahuila	8 km E, 1 km N de Buñuelos	Complete		KF885816
	PmCO2	FCR222-MZFC	Coahuila	Cruce de la Zacatera hacia San Francisco, 10.2 km SE, Gómez Farías	Complete		KF885811
	PmCO3	FCR223-MZFC	Coahuila	Cruce de la Zacatera hacia San Francisco, 10.2 km SE, Gómez Farías	complete		KF885812
	PmCO4	FCR225-MZFC	Coahuila	Cruce de la Zacatera hacia San Francisco, 10.2 km SE, Gómez Farías	Complete	Complete	KF885813 KF885901
	PmCO5	FCR226-MZFC	Coahuila	Cruce de la Zacatera hacia San Francisco, 10.2 km SE, Gómez Farías	Complete		KF885814
	PmCO6	FCR231-MZFC	Coahuila	Cruce de la Zacatera hacia San Francisco, 10.2 km SE, Gómez Farías	Complete		KF885815
	PmCO7	FCR228-MZFC	Coahuila	Cruce de la Zacatera hacia San Francisco, 10.2 km SE, Gómez Farías		Complete	KF885902
	PmZA1	FCR329-MZFC	Zacatecas	3.5 km NO de Santa Rosa	Complete		KF885826
	PmQT1	MQ1254-MZFC	Querétaro	11 km NW Bernal	Complete		KF885835
	PmQT2	MQ1259-MZFC	Querétaro	11 km NW Bernal	Complete		KF885836
	PmQT3	MQ1203-MZFC	Querétaro	12 km NW Bernal	Complete		KF885837
	PmQT4	MQ1205-MZFC	Querétaro	12 km NW Bernal	Complete		KF885838
	PmQT5	MQ1215-MZFC	Querétaro	12 km NW Bernal	Complete		KF885839
	PmQT6	MQ1216-MZFC	Querétaro	12 km NW Bernal	Complete		KF885840
	PmGT1	TK148868-TTU	Guanajuato	2 km NW San Miguel de Allende	Complete	Complete	KF885843 KF885912
	PmSL1	TK133374-TTU	San Luis Potosí	22.8 km N Real de Catorce	Complete	Complete	KF885844 KF885908
	PmSL2	TK133377-TTU	San Luis Potosí	22.8 km N Real de Catorce	Complete	Complete	KF885845 KF885909
	PmSL3	TK133378-TTU	San Luis Potosí	22.8 km N Real de Catorce	Complete	Complete	KF885846 KF885910
	PmSL4	TK133463-TTU	San Luis Potosí	22.8 km N Real de Catorce	Complete	Complete	KF885847 KF885911
	PmSL5	TK137267-TTU	San Luis Potosí	22.8 km N Real de Catorce	Complete		KF885848
	PmMO1	369AX-CIBC	Morelos	Sierra de Huautla, Axuchitlán	Complete		KF885883
	PmMO2	338AX-CIBC	Morelos	Sierra de Huautla, Axuchitlán	Complete		KF885884
Peromyscus perfulvus	PpJA1	MCP096-MZFC	Jalisco	Estación de Biología Chamela	Complete	Complete	KF885789 KF885888

	PpJA2	MCP108-MZFC	Jalisco	Estación de Biología Chamela	Complete	Complete	KF885790 KF885889
	PpJA3	MCP119-MZFC	Jalisco	Estación de Biología Chamela	Complete		KF885791
	PpJA4	MCP120-MZFC	Jalisco	Estación de Biología Chamela	Complete	Complete	KF885792 KF885891
	PpJA5	MCP122-MZFC	Jalisco	Estación de Biología Chamela	Complete	Complete	KF885793 KF885892
	PpJA6	TK11803-TTU	Jalisco	22 km S de Chamela	Complete		KF885794
	PpJA7	TK11807-TTU	Jalisco	22 km S de Chamela	Complete	Complete	KF885797 KF885895
	PpJA8	TK19581-TTU	Jalisco	22 km S de Chamela	Complete	Complete	KF885795 KF885893
	PpJA9	TK19583-TTU	Jalisco	22 km S de Chamela	Complete	Complete	KF885796 KF885894
	PpJA10	TK19657-TTU	Jalisco	22 km S de Chamela	complete	Complete	KF885798 KF885896
	PpJA11	TK19661-TTU	Jalisco	22 km S de Chamela	Complete	Complete	KF885799 KF885897
	PpJA12	MCP118-MZFC	Jalisco	Estación de Biología Chamela		Complete	KF885890
	PpMI1	MBB168-MZFC	Michoacán	La Huacana, Ichamio	Complete	Complete	KF885803 KF885898
	PpMI2	MBB169-MZFC	Michoacán	La Huacana, Ichamio	Complete	Complete	KF885802 KF885900
	PpMI3	MBB171-MZFC	Michoacán	La Huacana, Ichamio	Complete		KF885801
	PpMI4	MBB174-MZFC	Michoacán	La Huacana, Ichamio	Complete	Complete	KF885800 KF885899
	PpMI5	MAM9027-MZFC	Michoacán	Nuevo Hurecho	Complete		KF885804
	PpMI6	MAM9072-MZFC	Michoacán	Tierras Coloradas	Complete		KF885805
	PpMI7	MAM9066-MZFC	Michoacán	Tierras Coloradas	Complete		KF885806
	PpMI8	15515-UAMI	Michoacán	3 km NW Aguililla	Complete		KF885808
	PpMI9	27063-ENCB	Michoacán	Las Pilas, 14 km W Carácuaro	Complete		KF885807
	PpMI10	9703-ENCB	Michoacán	2 km N, 3.5 km W Agulilla	Complete		KF885809
Peromyscus mekisturus	*P. mekisturus*	88967-UMMZ	Puebla	Tehuacán	Missing 47 bp from ND4		KF885810
P. leucopus	*P. leucopus*						U40252.1 AY294927.1
P. eremicus	*P. eremicus*						U83861.1 EF989776.1
P. mexicanus	*P. mexicanus*	NIZA020-MZFC	Oaxaca	Agua Tibia, Nizanda, Asunción de Ixtaltepec	Complete		KF885887
P. mexicanus	*P. mexicanus*						EF989793.1
P. furvus	*P. furvus*	RAG197-MZFC	Querétaro	El Pemoche, Landa de Matamoros		Complete	KF885929

H. simulatus	*H. simulatus H*	024HBR-MZFC	Hidalgo	El Potrero, Tenango de Doria	Complete	Complete	KF885886 KF885928
H. simulatus	*H. simulatus O*						DQ793114.1
O. banderanus	*O. banderanus*	MCP099-MZFC	Jalisco	Estación de Biología Chamela	Complete	Complete	KF885885 KF885927
O. leucogaster	*Onychomys leucogaster*						U83858.1

Acronyms are as follow: MZFC, Museo de Zoología “Alfonso L. Herrera”, Facultad de Ciencias; Universidad Nacional Autónoma de México; UAMI; Universidad Nacional Autónoma Metropolitana Unidad Iztapalapa; INAH, Instituto Nacional de Antropología e Historia; ENCB Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional; CNM Instituto de Biología, Universidad Nacional Autónoma de México; CIOAX, Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional Unidad Oaxaca; CIDGO, Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional Unidad Durango; IHNE, Instituto de Historia Natural y Ecología, Ciapas; CIBC, Centro de Investigación en Biodiversidad y Conservación, Universidad Autónoma del Estado de Morelos; UMMZ, University of Michigan Museum of Zoology; TTU, The Museum of Texas Tech University; USNM, The National Museum of Natural History, Smithsonian Institute; LSU, Museum of Natural Science at Louisiana State University.

Appendix B

Primers designed for the amplification of the mitochondrial (ND3, tRNA-Arginine, ND4L and partial ND4) genes of species of the *P. melanophrys* group using Primer 3 and IDT Oligo-analyzer 3.1 programs.

Primer	SEQUENCE 5´→3´
1Pmlp_R	CRGYTCATTCTAGTCCTTTTTG
2Pmlp_F	CAGCTTTCATCCTAGTYTCAGTCC
2Pmlp_R	GCTARTCCAATGGCTGCTTC
3Pmlp_F	TAYCCCATTCCCATTGTYAT
3Pmlp_R	TGTGATTTTGGCTGGCTARA
4Pmlp_F	TAATACTTCTAGCCAGCCAAAATCA
5Pmlp_R	TTTATGGAGAATGGAAGGCG
6Pmlp_F	AAAGCAAATCCATACGAATGCGG
7Pmlp_R	AGTGATAGTATTATACCCTCTAAGC
8Pmlp_F	TTTTCTCACTTCTAGGGACCC
8Pmlp_R	TTAGCATTGTAGCAAGTTGAGG
9Pmlp_F	GAAGCAGCCATTGGATTAGC
9Pmlp_R	TTGAGAATAGTAGTGATAAGTTTGGGG
10Pmlp_F	TTTACGATTAGCCTGCTCTCTAC
10Pmlp_R	ATGATTAATTCGTTTGCAGAGAATG
11Pmlp_F	CCATTAATACTTCTAGCCAGCC
11Pmlp_R	AATTTTGGATGTAGATAAGGGCG
12Pmlp_F	ATTAAACGCAGGACTTTACTTCC

Appendix C

Results of Shimodaira-Hasewaga test used to evaluate three alternative phylogenetic hypotheses about the position of *P. mekisturus*. Tree 1 indicates the mitochondrial tree topology and tree 2 the artificial tree topology. Also, the difference in topologies obtained from mitochondrial versus nuclear DNA is included.

	Tree	-ln L	Diff –ln L	*p*
Hypothesis 1				
	1	8322.99	best	
	2	12852.22	4529.22	0.000 *
Hypothesis 2				
	1	8414.18	best	
	2	12148.15	3733.96	0.000 *
Hypothesis 3				
	1	8414.18	best	
	2	12147.49	3733.30	0.000 *
Mitochondrial/Nuclear				
	Mitochondrial	6280.65	best	
	Nuclear	7205.33	924.68	0.000 *

* ($p < 0.001$)

CAPÍTULO 3

Demographic history supports the speciation history in the *Peromyscus melanophrys* species complex

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DEMOGRAPHIC HISTORY SUPPORTS THE SPECIATION HISTORY IN THE *Peromyscus melanophrys* SPECIES COMPLEX

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ABSTRACT

In Mexico, the confluence of two major biotic regions (Neartic and Neotropical) has produced an astonishing level of diversity in several habitats. The *Peromyscus melanophrys* complex is the only extant group of *Peromyscus* species endemic to Mexico and restricted to lowlands, which have received a little interest from a phylogeographic and demographic point of view. This group offers an excellent opportunity to study spatial and temporal patterns of population structure and possible ecological and evolutionary processes that modeled patterns of historical divergence in these areas. We used mitochondrial genes ND3, tRNA-Arg, ND4L and partial ND4 to evaluate the phylogeographic patterns and demographic history of the six lineages that we found within the *Peromyscus melanophrys* complex. The Mexican Plateau lineage shows strong evidence of a recent population expansion. Meanwhile lineages from the Pacific Coast, Western Balsas Basin and Thehuantepec Coastal Plain-Sierra Madre de Chiapas lowlands show population stability or it is not possible to detect signal of demographic change. The origin of the group was dated during the mid-Pliocene (4.8 Mya), as the principal events of cladogenesis within the complex; however, the main diversification within each clade was dated during the Pleistocene.

Key words

Peromyscus, phylogeography, lineages, lowlands, diversification, Mexico.

1. Introduction

Reconstructing the demographic history of populations can allow us to gain useful insights into various evolutionary and population-genetic processes (Ho and Shapiro, 2011). For instance, by testing correlations between demographic and paleoclimatic events (Drummond et al, 2005; Milá et al., 2007) and by examining the factors driving past populations dynamics (Finlay et al., 2007; Stiller et al, 2010). The inference of past populations dynamics from genetic data has proved invaluable for testing hypotheses in a variety of biological disciplines (Drummond et al., 2005).

One example is phylogeography, a discipline that focuses on evaluating the relationship between geography and gene genealogies, especially at the intraspecific level, incorporating a phylogenetic and population genetic perspective into biogeography (Avise et al., 1987; Antonelli et al., 2010). It searches for patterns and processes governing the geographic distribution of genealogical lineages, time of divergence and demographic history of evolutionary lineages, incorporating historical processes (Avise, 1998, 2000; Avise et al., 1987). Mitochondrial DNA (mtDNA) remains the dominant marker, especially as few nuclear markers have sufficiently rapid rates of evolution for intraspecific phyologeographic studies studies (Beebee and Rowe, 2008; Antonelli et al., 2010). Notably, a phylogeographic approach in poorly known groups and undersampled geographic regions is likely to reveal new species-level taxa and provide information about the evolutionary processes generating biodiversity in these regions (Barrera-Guzmán et al., 2012).

In Mexico, phylogeographic and/or demographic studies with rodents inhabiting xeric valleys and tropical deciduous and semideciduous forests are scarce. Such habitats are interesting due to several particularities associated to them and in general are distributed at low elevations. Higher elevation areas lacking some of these particularities,

act as barriers (*e. g.* Sierra Madre Oriental, Sierra Madre Occidental, Trans-Mexican Volcanic Belt, Sierra de Oaxaca), resulting in several isolated habitat islands for lowlands biota.

The *Peromyscus melanophrys* complex, endemic to Mexico, is distributed on the lowlands of xeric valleys and tropical deciduous and semideciduous forests. This group encompasses the following species and subspecies: (1) *P. melanophrys* (*P. m. coahuilensis, P. m. consobrinus, P. m. melanophrys, P. m. micropus, P. m. xenurus,* and *P. m. zamorae*), which is distributed from the northern Central Mexican Plateau (Durango and Chihuahua states) to the states of Oaxaca and Chiapas in the south (Osgood, 1909); (2) *P. perfulvus* (*P. p. chrysopus* and *P. p. perfulvus*) is distributed along the Pacific coast from Jalisco to Guerrero and inland on the Balsas River Basin south of the Estado de México (Hooper, 1955); and (3) *P. mekisturus*, exclusively from a few sites in Puebla (Merriam, 1898; Hooper,1947) (Fig. 1).

Castañeda-Rico et al., (2014), evaluated the phylogeny of the *P. melanophrys* complex, using mitochondrial and nuclear DNA, and identified four main clades, one of which was structured within, forming three different groups. They proposed some taxonomic changes based on the fact that the clades and groups did not correspond with the species and subspecies described (the *Peromyscus melanophrys* complex is composed of at least four species –Clade I to Clade IV–). They also showed the *Peromyscus melanophrys* complex diverged during the Pliocene, while the main diversification events within the group occurred during the late Pliocene and throughout the Pleistocene.

Several authors have argued that Pliocene-Pleistocene vicariant events, and more recently Quaternary climatic shifts, have been prominent in shaping vertebrate biodiversity patterns in the Neartic-Neotropical transition zone (Glazier, 1980; Devitt, 2006; Castañeda-

Rico et al., 2014; Suárez-Atilano et al., 2014). In this regard, information about rodents inhabiting highlands is abundant (*e.g.* Sullivan et al., 1997, 2000; Tiemman-Boege et al., 2000; León-Paniagua et al., 2007), while studies of lowlands species are rather scarce. Hence, we aimed to test this with the *Peromyscus melanophrys* complex, specially by evaluating its phylogeographic patterns and demographic history. We consider that this group is an ideal model to try to explain what happened with rodents of lowlands during these periods of time, mainly due to characteristics of the group as endemicity, restriction to lowlands and the presence in different kinds of habitats.

2. Methods

2.1. Sample collection

We used samples from Castañeda-Rico et al. (2014) and we obtained additional samples from two more sources: 11 tissue samples collected from natural populations in the states of Coahuila, Zacatecas and Jalisco (fieldwork from 2009 and 2010), and 55 tissue, bone and skin samples from museum specimens (10 national and foreign mammal collections; see Acknowledgements). We provide collection localities of specimens examined in Appendix A and Fig. 1. We used sampling methods designed specifically for arboreal (see Castañeda-Rico et al., 2011 for details), semi-arboreal, and terrestrial rodent species. We placed Sherman live traps (7.6 x 8.9 x 22.9 cm; H. B. Sherman Traps, Tallahassee, Florida) on trees or on the ground, depending on the species. We took a tissue sample from an ear of each individual trapped at the Chamela Biological Station and stored it in 100% ethanol. For all other localities, voucher specimens were collected and deposited in the Mammal Collection of the Museo de Zoología ‘‘Alfonso L. Herrera’’, Facultad de Ciencias, UNAM (MZFC). Techniques we used are in compliance with

guidelines published by the American Society of Mammalogists for use of wild mammals in research (Kelt et al. 2010; Sikes et al. 2011).

2.2. DNA extraction, amplification and sequencing

We obtained DNA from tissue, fresh or dry skin and bone samples using the DNeasy Blood and Tissue Kit (Qiagen, Valencia, California). We amplified and sequenced mitochondrial (mtDNA) genes ND3, ND4L, arginine tRNA, and partial ND4 from all tissue and fresh skin samples using 6 pairs of specific primers designed by Castañeda-Rico et al. (2014) (Appendix A). We analyzed all mtDNA genes as a single locus because they evolve at the same rate and all are subunits of the same protein (Cao et al.1994; Russo et al., 1996; Engel et al., 1998, León-Paniagua et al., 2007, Castañeda-Rico et al., 2014). We used *Osgoodomys banderanus, Habromys simulatus, P. mexicanus* and *P. eremicus* as outgroups; their sequences were also obtained from Castañeda-Rico et al. (2014).

We amplified DNA in a 25 µL reaction volume containing the following: 25-50 ng of template DNA for mtDNA genes and 100 ng for the nuclear gene, 1 unit of Taq DNA polymerase (Vivantis), 1.5 mM of $MgCl_2$, 200 µM of each deoxynucleoside triphosphate, and 0.25 µM of each primer. Polymerase chain reaction (PCR) conditions for mitochondrial DNA (mtDNA) were as follows: Taq activation at 95°C for 10 min, initial 3 min denaturation at 95°C, followed by 40 cycles, each cycle consisting of 95°C denaturing for 30 s, 50°C annealing temperature for 30 s, and extension at 72°C for 30 s, with a final 72°C for 7 min. PCR parameters for mtDNA obtained from dry skin or bone were: denaturation at 95°C for 10 min, followed by 15 cycles at 95°C for 30 s, 50°C for 1 min, and 72°C for 1 min,

followed by 25 cycles at 95°C for 30 s, 55°C for 1 min, and 72°C for 1 min with a final extension at 72°C for 5 min. We used agarose (1.5%) gels stained with ethidium bromide to visualize amplified products. After amplification, PCR products were purified and run on an ABI3730xl DNA analyzer (Applied Biosystems, Carlsbad, California) by a sequencing service provider, High Throughput Sequencing (HTSeq), Washington, USA (http://www.htseq.org). To avoid contamination of samples, standard authentication criteria for ancient DNA studies (bone or skin) were followed. In particular, we included negative controls in all amplifications to check for contamination.

2.3. Phylogenetic and genetic structure analyses

We edited and cleaned sequences using BioEdit 7.0.9.0 (Hall, 1999) and performed multiple sequence alignment manually and using ClustalX2 (Thompson et al., 1997; Larkin et al., 2007). All sequences were deposited in GenBank (Appendix A). We analyzed populations of the group, as a complete data set. We did not consider taxonomic identity as *a priori* information. We estimated monomorphic, polymorphic, parsimony-informative sites, haplotype (h) and nucleotide diversity (π) values, number of segregating sites (S), and mean number of pairwise differences (k) for all data set with the program DNAsp 5.0 (Librado and Rozas, 2009).

Tajima´s D (Tajima, 1989), Fu and Li´s F (Fu and Li, 1993), and Fu´s F_s (Fu, 1997) indexes were used to examine if all mutations are selectively neutral for mitochondrial genes, statistical significance was determined using the coalescent simulator in DNAsp 5.0 (Librado and Rozas, 2009).

We analyzed evolutionary relationships among our sequence data using Bayesian Inference. We used jModeltest 0.1.1 (Guindon and Gascuel, 2003; Posada, 2008) to select

the best-fit model of evolution, based on the Akaike Information Criterion (AIC). The model selected was TIM1+I+G, with the following parameters: base frequencies = A (0.3795), C (0.2691), G (0.0623), T (0.2891); nst = 6; rates = gamma with shape parameter (α) = 0.7420, and proportion of invariant sites=0.4720.

The Bayesian Inference analysis was performed using MrBayes 3.1 (Ronquist and Huelsenbeck, 2003) and analyses consisted of four runs, each conducted with three heated and one cold Markov chains run for 20,000,000 generations, sampling every 1000 generations and initiating with random, unconstrained, starting trees. Heating temperature was set at 0.02 to facilitate greater movement between the four Markov chains (Braun et al., 2010). Output parameters were visualized using Tracer 1.5 (Rambaut and Drummond, 2007). We determined resulting burn-in values (the point at which the model parameters and tree scores became stationary) as 25% of all sampled trees (20,000 trees), and we obtained posterior probabilities from the remaining trees.

We performed a phylogenetic network using the Neighbor-Net algorithm implemented in SplitsTree 4.6 (Huson and Bryants, 2006) to investigate the relationship among haplotypes using the GTR+I+G model of evolution. This method is recommended using sequences of different species (Bryant and Moulton, 2003; Huson and Bryants, 2006).

We also used Structure 2.3.4 (Pritchard et al., 2000; Falush et al., 2003, 2007), which is a genetic program that implements a model-based clustering method for inferring population structure, to determine genetic clusters (*K*= number of clusters of individuals characterized by allele frequencies at each locus) within the *Peromyscus melanophrys* complex and to assign individuals to these clusters. We used the admixture model with correlated allele frequencies and clades obtained with Bayesian Inference as *a priori* information. In all simulations, we used a burn-in period of 100,000 followed by a run

length of 1,000,000 MCMC iterations, which were sufficient to give a stable α and estimate of the log probability of the data. For each value of *K*, 10 independent runs were computed. We first ran the whole data set with *K* ranging from 1 to 11. We selected the most likely *K* value, according to the Evanno method (ΔK value), performed in Structure Harvester (http://taylor0.biology.ucla.edu/structureHarvester/). Because two main clusters were identified (1 and 2), these were submitted separately to a second run of analysis, using *K*=1-4 (1A and1B) and *K*=1-5 (2A and 2B), respectively. Finally a third analysis was performed to cluster 2B, using *K*=1-6 (2Ba, 2Bb and 2Bc).

We analyzed divergence between clades as the average number of nucleotide substitutions per site (*Dxy*) and the net number of nucleotide substitutions per site (*Da*), under a Jukes and Cantor model using DNAsp 5.0 (Librado and Rozas, 2009).

2.4. Divergence time

We inferred divergence time (time to the most recent common ancestor, TMRCA) using a Yule tree prior and unique haplotypes (to avoid imbalances due to low sequence variation or phylogenies with short branch lengths; Sanderson, 2003; Fontanella et al., 2008; Guiher and Burbrink, 2008), using a Bayesian phylogenetic analysis and relaxed-clock dating implemented in BEAST 1.7.4. (Drummond et al., 2012). Dating molecular divergences requires *a priori* assumptions of ages of one or more clades to calibrate the relationship between age and molecular rate (Drummond et al., 2006). Because no adequate fossil dates, biogeographically-based dates, or known substitution rates were available, we used three node ages: the split of *Onychomys* (10.3 million years ago [Mya]), the separation between *Peromyscus* and *Habromys* (4.69 Mya), and the split between *H. simulatus* from Oaxaca and *H. simulatus* from Hidalgo (0.73 Mya), which were calibrated

by a fossil-based estimate (*Copemys russelli* 14.8 Mya) (León-Paniagua et al., 2007). These calibrations points were used in Castañeda-Rico et al. (2014). We ran the analysis 100,000,000 generations, sampling model parameters every 1,000 generations. We checked convergence statistics for effective sample sizes using Tracer 1.5 (Rambaut and Drummond, 2007). A consensus tree with node height distribution was generated using TreeAnnotator 1.7.4 (available in BEAST package), after elimination of 25% as burn-in, and visualized in FigTree 1.3.1 (Rambaut, 2009).

2.5. Genetic diversity and historical demography

We estimated, for each lineage identified using MrBayes, SplitsTree and Structure, the genetic diversity as haplotype (h) and nucleotide diversity (π) values, number of segregating sites (S), and mean number of pairwise differences (k), using DNAsp 5.0 (Librado and Rozas, 2009) (Table 2). Population history was assessed using an array statistics that were introduced as neutrality test but which are also used for detecting the genetic impacts of population growth, decline or stability (Ramos and Rozas, 2002; Nicolas et al., 2014): Tajima´s D (Tajima, 1989), Fu and Li´s F (Fu and Li, 1993), and Fu´s F_s test (Fu, 1997) (Table 2). These analyses were also performed with DNAsp 5.0 and for each lineage (Librado and Rozas, 2009).

We used the Bayesian skyline plot method to estimate a posterior distribution of effective population size through time from a sample of gene sequences given a nucleotide-substitution model at different points along the genealogical time scale (Drummond et al., 2005; Ho and Shapiro, 2011) using Markov Chain Monte Carlo (MCMC) in BEAST 1.7.4. (Drummond et al., 2012). Genealogies and model parameters were

sampled every 1,000 iterations along 400,000,000 generations. Demographic plots were visualized with Tracer 1.5 (Rambaut and Drummond, 2007).

We estimated the relationship between haplotypes, within each lineages, by constructing an unrooted minimum spanning network using the median-joining method in Network 4.612 (available at: http://www.fluxus-engineering.com/sharenet.htm).

3. Results

3.1. Sequence variation

We analyzed 1,312 base pairs (bp) of the mitochondrial genes (ND3, ND4L, arginine tRNA, and partial ND4) from 165 individuals and 68 unique localities of the *Peromyscus melanophrys* complex (133 *P. melanophrys*, 31 *P. perfulvus* and one *P. mekisturus*). Results showed 914 (72.65%) invariable sites and 344 (27.34%) variable sites, 313 (90.98) of which were parsimony informative and 31 (9.01%) singleton sites. We identified 121 unique haplotypes with the follow diversity values: h=0.994 ± 0.002, π=0.057 ± 0.003, S=344 and k=72.839. Sequences of mitochondrial genes, used in this work, did not deviate from neutrality under Tajima´s test (D=0.702, p>0.1) and Fu and Li´s test (F=0.576 p>0.1), contrary to results of Fu´s test (F_s=-17.315, p<0.05), which are also evidence of a sudden expansion in effective population size within the group.

3.2. Phylogenetic relationships and genetic lineages

Bayesian Inference analysis recovered a monophyletic *Peromyscus melanophrys* complex supported by high posterior probabilities (Fig. 2). We identified six main lineages, Lineage 1 and Lineage 2 including individuals of *P. perfulvus* from populations of the

states of Jalisco and Michoacán, respectively. Lineage 3, Lineage 4 and Lineage 5 including individuals of *P. melanophrys* from populations of the states of Chiapas-Oaxaca, Puebla-Oaxaca-Morelos and Nayarit-Durango, respectively. Finally, Lineage 6 including individuals of *P. melanophrys* and the sample of *P. mekisturus* from populations of the states of San Luis Potosí, Nuevo León, Coahuila, Durango, Querétaro, Michoacán, Jalisco, Zacatecas, Aguascalientes, Guanajuato, Estado de México, Hidalgo, Puebla and Oaxaca.

The network analysis showed the same pattern, where six different lineages are observed (Fig. 3). When Structure was run with the entire data set, two main genetic clusters (*K*=2, Clusters 1 and 2, *ΔK*=757.82) were determined (Fig. 4), which completely correspond with the recognized species *P. perfulvus* (Cluster 1) and *P. melanophrys-P. mekisturus* (Cluster 2). Because we identified little variation within each cluster, we performed the Structure analysis separately for each genetic cluster. In the second run we identified two genetic groups (*K*= 2, groups 1A and 1B, *ΔK*=5607.90) within the main genetic Cluster 1 and two more genetic groups (*K*=2, groups 2A and 2B, *ΔK*=866.49) within the main genetic Cluster 2. Finally, group 2B showed some structure in which a third run with Structure resulted in *K*=3 (groups 2Ba, 2Bb and 2Bc, *ΔK*=813.87).

According to the Bayesian, network (phylogenetic analysis) and Structure (populations structure analysis) results, the *Peromyscus melanophrys* complex is composed of six lineages or genetic clusters. Also, divergence values between lineages ranged from 4.1% to 12.4% for *Dxy* and from 3.2% to 11.8% for *Da* (Table 3). In accordance, all the following analyses were done based on these six lineages.

3.3. Divergence times

We analyzed 121 samples (unique haplotypes) to estimate divergence times (Table 1 and Fig. 5). According to these estimates, the *Peromyscus melanophrys* complex diverged from the other species 4.8 Mya (95% HDP: 4.2-5.3). The split between Lineage 1 and 2 with respect to Lineage 3, 4, 5 and 6 was dated 4.2 Mya (95% HDP: 3.5-4.8), while the separation between Lineage 3 from Lineage 4, 5 and 6 was dated 3.6 Mya (95% HDP: 2.8-4.3). This event was followed by the separation between Lineage 4 with respect to Lineage 5 and 6, 3.2 Mya (95% HDP: 2.4-4). Finally, the split between Lineage 5 and Lineage 6 happened 2.7 Mya (95% HDP: 1.8-3.6).

3.4. Genetic diversity and demographic history

Haplotype and nucleotide diversity values estimated for each lineage showed values of h=0.937-0.991 and π=0.003-0.033, respectively (Table 2). Values of segregating sites and mean number of pairwise differences range from S=12-140 and k=4.926-43.527, respectively. Results of Tajima´s test, Fu and Li´s test, and Fu´s test (Table 2) showed no deviations from neutrality in Lineage 2 and Lineage 3.

The Bayesian skyline plots for each lineage showed a pattern of population stability through time, from 1 Mya when we detected a starting point of population growth (Fig. 6). All lineages showed the maximum evidence of recent expansion in effective population size as follow: Lineage 1 starting at 0.2 Mya, Lineage 2 at 0.35 Mya, Lineage 3 at 0.35 Mya, Lineage 4 at 1 Mya, Lineage 5 at 0.55 Mya, and Lineage 6 at 0.2 Mya, approximately. This lineages population growth is supported by Fu´s F_s test for Lineages 4, 5 and 6, the latter is also supported by Tajima´s D and Fu and Li´s F test. Lineage 1 was significant for population decline with Fu and Li´s F test.

Minimum-distance networks for each lineage showed abundant haplotypes and numerous unique ones. Only the network of Lineage 6 showed evidence of population expansion, the rest did not show signals of demographic change or population stability.

4. Discussion

4.1. Dating and defining lineages within the Peromyscus melanophrys *complex*

Bradley et al. (2007) and Castañeda-Rico et al. (2014) showed that the *Peromyscus melanophrys* group is a monophyletic group. We also recovered a monophyletic group, relative to the outgroups included. The principal differences among this three works is the number of samples (4, 95, and 165 individuals, respectively), the mitochondrial genes used (Cyt b versus ND3, tRNA-Arg, ND4L and partial ND4), and the inclusion of the three species and eight subspecies (recognized for this group) in Castañeda-Rico et al. (2014) and in this work. Moreover, these works are in agreement with previous hypotheses based on morphological characters (Osgood, 1909; Hall and Kelson, 1959; Hooper and Musser, 1964; Hooper, 1968; Carleton, 1989).

Castañeda-Rico et al. (2014) recognized four clades (using mitochondrial and nuclear genes –Clade I to Clade IV–) and three groups (using mitochondrial genes –within Clade IV–) within the *Peromyscus melanophrys* complex. However, according to results presented herein (Bayesian Inference, network analysis and Structure) we were able to increase resolution and detect six lineages within the *Peromyscus melanophrys* complex, all with high values of support in the results. Lineage 1 (Pacific Coast –PC–) is restricted to the lowlands, near the coast, of Jalisco and possibly its distribution continues through Colima´s lowlands (we did not have samples of this state). Lineage 2 is restricted to the lowlands of Western Balsas Basin (WBB) in Michoacán. Lineage 3 is restricted to the inner

valleys of Oaxaca to Pacific lowlands in Chiapas and Oaxaca (Tehuantepec Coastal Plain-Sierra Madre de Chiapas lowlands –TCP-SMCh–). Populations of Lineage 4 are restricted to the lowlands of northern Oaxaca, Morelos and Puebla and one sample from the coastal sierras of southern Oaxaca (Eastern Balsas Basin –EBB–). Lineage 5 is restricted to lowlands of Durango and Nayarit along the Sierra Madre Occidental lowlands (SMOc) and Lineage 6 is widely distributed along the Mexican Plateau (MP).

Many authors have argued that the present day-distribution of the peromyscines has been impacted greatly by the climatic fluctuations since the appearance of the taxon in the mid-Pliocene to early Pleistocene (Hibbard, 1968; Dawson, 2005, Castañeda-Rico et al., 2014), and our results support this hypothesis. The *Peromyscus melanophrys* complex likely originated in the mid-Pliocene (4.8 Mya) and the first divergence event within the group was the split between Lineage 1-2 and Lineage 3-4-5-6 dated in the mid-Pliocene (4.2 Mya). The emergence of the Trans-Mexican Volcanic Belt (between 11 and 1.8 Mya; Ferrari et al., 2000), and the presence of the Sierra Madre del Sur to the south, isolated the Balsas Basin (Ferrusquía-Villafranca, 1998), which could explain this initial separation (Castañeda-Rico et al, 2014). Another explanation could be the climatic fluctuations during Pleistocene, that have been responsible for the most recent speciation events in vertebrates (Coyne and Orr, 2004; Weir and Schluter, 2004).

Four more events of cladogenesis occurred during mid and late Pliocene. One was between Lineage 1 (PC) and Lineage 2 (WBD) dated in 3.3 Myr, likely the mountains of western Michoacán may have provided some isolation between both lineages, as Castañeda-Rico et al. (2014) proposed. A second event was the split between Lineage 3 and Lineage 4-5-6 (3.6 Mya). It is possible that the highlands in Oaxaca (Sierra de Oaxaca) act as a geographical barrier to keep isolated Linage 3 (TCP-SMCh) from the rest. The third event was the split between Lineage 4 and Lineage 5-6 (3.2 Mya). Finally,

during the late Pliocene and early Pleistocene, one more event of cladogenesis took place within the *Peromyscus melanophrys* complex, the split between Lineage 5 (SMOc) and Lineage 6 (MP) dated in 2.7 Myr. The main diversification within each clade occurred during the Pleistocene.

We did not find any geographical barrier that explain the split among Lineage 4, 5 and 6. A possible explanation of speciation among these lineages is ecological speciation, which has not yet been tested and at least appears to be a rare phenomenon in vertebrates (Peterson, 2001; Castañeda-Rico, 2014). We consider that ecological events within the *Peromyscus melanophrys* complex have been shaping speciation in the group. Devitt (2006) and Glazier (1980), among others, argued that Pliocene-Pleistocene vicariant events and more recently Quaternary climatic shifts have been shaping biodiversity patterns in the Neartic-Neotropical transition zone, where the *Peromyscus melanophrys* complex is distributed (Castañeda-Rico et al., 2014).

The diversification pattern within these group was from south to north, which possible followed the advances and retreats of North American glaciers as has been reported for peromyscines. Dawson (2005) argued that during the interglacials, as species expanded their ranges northward and adapted to new habitats, dispersal biogeography was operative as vicariant evolution. These expansions generated waves of evolutionary novelty as they advanced into terrain modified by glaciation and associated vegetational changes.

Finally, in concordance with the previous analyses by Castañeda-Rico et al., (2014), the *Peromyscus melanophrys* complex diverged during the mid-Pliocene and the main diversification of the group occurring in late Pliocene and throughout the Pleistocene. Nevertheless our divergence times are a little more ancient than those previously obtained (Castañeda-Rico et al., 2014).

4.2. Demographic history of lineages

Knowledge of a population´s history can allow us to test hypotheses about the impact of climatic and anthropogenic factors (Ho and Shapiro, 2011). According with results of Tajima´s *D*, Fu and Li´s *F*, Fu´s F_s test, minimum-distance networks and skyline plots the six lineages identified within the *Peromyscus melanophrys* complex showed evidence of demographic stability until 1 Mya, when demographic growth started in some lineages. The lineage of the Mexican Plateau (Lineage 6) is the only one that shows evidence of recent population expansion according to all analyses. This lineage is the most recent within the complex, and clearly shows evidence of recent diversification (high number of haplotypes and numerous unique ones). Lineages from EBD and SMOc (Lineages 4 and 5) also show evidence of population growth, but not as high supported as Lineage 6, with high number of haplotypes and numerous unique ones. The evidence of a sudden expansion in effective population size within these lineages is dated approximately 0.5 Mya. Moreover, Lineage from the Pacific Coast shows evidence of population decreasing according to Fu and Li´s *F* test, but according to Skyline plots shows demographic stability. We argued that Lineage 1, 2 and 3 show population stability or according to our sample no signal of demographic change could be detected.

Our results suggest that after the main cladogenesis events that originated the six lineages within the complex, a period of population size stability follows. However, from approximately 1 Mya to present, populations within these lineages have showed demographic changes.

Pleistocene climatic oscillations are commonly invoked as the main factor driving recent diversification in peromyscine rodents (Glazier, 1980; Dawson, 2005) and in other vertebrates (*e.g*. Weir and Schluter, 2004; Weir, 2006; Barrera-Guzmán et al., 2012). Our results can be explained in light of these climatic changes. However, alternative

explanations cannot be discarded, specially due to wide confidence intervals in demographic changes through time and in divergence times. A rapid diversification over short evolutionary scales, coalescent stochasticity and calibration uncertainty can account for these large values (Arbogast et al., 2002; Ho et al., 2008; Weir and Schluter, 2008; Barrera-Guzmán et al., 2012).

Finally, we conclude that the *Peromyscus melanophrys* complex has a high level of genetic diversification, where possibly climatic and ecological barriers play an important role. We recognized six lineages within the group and consider that each one may be considered as a different species. Castañeda-Rico et al. (2014) proposed taxonomic changes but argued that more kind of information is necessary (ecological, morphological, etc.), in this work we contributed with more information and López et al. (*in prep*.) are supporting this hypothesis with morphological information.

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6. References

Antonelli, A., Quijada-Mascareñas, A., Crawford, A.J., Bates, J.M., Velazco, P., Wüster, W. 2010. Molecular studies and phylogeography of Amazonian tetrapods and their relation to geographical and climatic models, in Hoorn, L., Wesselingh, F.P. (Eds.), Amazonian, Landscape and Species Evolution: A look into the past. Blackwell Publishing.

Arbogast, B.S., Edwards, S.V., Wakeley, J., Beerli, P., Slowinski, J.B. 2002. Estimating divergence times from molecular data on phylogenetic and population genetic timescales. Ann. Rev. Ecol. Evol. Syst. 33. 707-740.

Avise, J.C. 1998. The history and purview of phylogeography: a personal reflection. Molecular Ecology. 7: 371-379.

Avise, J.C. 2000. Phylogeography: The history and formation of the species. Cambridge, MA, Harvard University Press.

Avise, J.C., Arnold, J., Ball, R.M., Bermingham, E., Lamb, T., Neigl, J.E., et al., 1987. Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. Annu. Rev. Ecol. 18, 489-522.

Barrera-Guzmán, A. O., Milá, B., Sánchez-González, L. A. and Navarro-Sigüenza, A. G. 2012. Speciation in an avian complex endemic to the mountains of Middle America (Ergaticus, Aves:Parulidae). Mol. Phylogenet. Evol. 62, 907-920.

Bradley, R.D., Durish, N., Rogers, D., Millar, J., Engstrom M., Kilpatrick, W., 2007. Toward a molecular Phylogeny for *Peromyscus*: Evidence from mitochondrial cytochrome-b sequences. J. Mammal. 88, 1146-1159.

Braun, J., Mares, A.M., Coyner, B.S., Van Den Bush, R.A., 2010. New species of *Akodon* (Rodentia: Cricetidae: Sigmodontinae) from central Argentina. J. Mammal. 91, 387-400.

Cao, Y., Adachi, J., Janke, A., Paabo, S., Hasegawa, M., 1994. Phylogenetic relationships among eutherian orders estimated from inferred sequences of mitochondrial proteins: instability of a tree based on a single gene. J. Mol. Evol. 39, 519-527.

Carleton, M. D., 1989. Systematics and Evolution, in: Kirkland, G.L., Layne, J. (Eds.) Advances in the study of *Peromyscus* (Rodentia). Texas Tech University Press., Lubbock. pp. 7-141.

Castañeda-Rico, S., León-Paniagua, L., Ruedas, L.A., Vázquez-Domínguez. E., 2011. High genetic diversity and extreme differentiation in the two remaining populations of *Habromys simulatus*. J. Mammal. 92, 963–973.

Castañeda-Rico , S., León-Paniagua, L., Vázquez-Domínguez, E., Navarro-Sigüenza, A.G. 2014. Evolutionary diversification and speciation in rodents of the Mexican lowlands: The *Peromyscus melanophrys* species group. Mol. Phyl. Evol. 70: 454-463.

Coyne, J.A. and Orr, H.A. 2004. Speciation. Sunderland, MA: Sinauer Associates, Inc.

Dawson, W., 2005. Peromyscine Biogeography, Mexican topography and Pleistocene climatology, in: Sánchez-Cordero, V., Medellín, R. (Eds.), Contribuciones Mastozoológicas en Homenaje a Bernardo Villa. UNAM-CONABIO. México, pp. 145-156

Devitt, T. J., 2006. Phylogeography of Western Lyresnake (*Trimorphodon biscutatus*): testing arid land biogeographical hypotheses across the Neartic-Neotropical transition. Mol. Ecol. 15, 4387-4407.

Drummond, A.J., Rambaut, A., Shapiro, B., et al. 2005. Bayesian coalescent inference of past population dynamics from molecular sequences. Mol. Biol. and Evol. 22, 1185-1192.

Drummond, A.J., Ho, S.Y.W., Phillips, M.J., Rambaut, A., 2006. Relaxed phylogenetics and dating with confidence. PloS Biol. 4, e88.

Drummond, A. J., Suchard, M. A., Xie, D., Rambaut, A. 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. Mol. Biol. Evol. 29, 1969-1973.

Engel, S.R., Hogan, K.M., Taylor, J.F. Davis, S.K., 1998. Molecular systematics and paleobiogeography of the South American Sigmodontine Rodents. Mol. Biol. Evol. 15, 35-49.

Ferrari, L., Cinticelli, S. Vaggelli, G., Peyrone, C.M., Manetti, P., 2000. Late Miocene volcanism and intera-arc tectonics during the early development of the Trans-Mexican Volcanic Belt. Tectonophysics. 318,161-185.

Ferrusquía-Villafranca, V.I., 1998. Geología de México: Una sinopsis, in: Ramamoorthy, T.P., Bye, R., Lot, A., Fa, J. (Eds.), Diversidad Biológica de México: Orígenes y Distribución. UNAM, México, pp. 3-108.

Finlay, E.K., Gaillard, C., Vahidi, S.M., et al. 2007. Bayesian inference of population expansion in domestic bovines. Biology Letters. 3, 449-452.

Fontanella, F., Feldman, C., Siddall., M., Burbrink, F., 2008. Phylogeography of *Diadophis punctatus*: Extensive lineage diversity and repeated patterns of historical demography in a trans-continental snake. Mol. Phylogenet. Evol. 46, 1049-1070.

Fu, X.Y. 1997. Statistical test of neutrality of mutations against population growth, hitchhiking and background selection. Genetics. 10, 564-567.

Fu, X.-Y. and Li, W.-H. 1993. Statistical test of neutrality of mutations. Genetics. 133,693-709.

Glazier, D.S., 1980. Ecological shifts and the evolution of geographically restricted species of North American *Peromyscus* (mice). J. Biogeogr. 7, 63-83.

Guiher, T., Burbrink, F., 2008. Demographic and phylogeographic histories of two venomous North American snakes of the genus *Agkistrodon*. Mol. Phylogenet. Evol. 48, 543-553.

Guindon, S., Gascuel, O., 2003. A simple, fast and accurate method to estimate large phylogenies by maximum likelihood. Syst. Biol. 52, 696–704.

Hall, T.A., 1999. BioEdit: a userfriendly biological sequence alignment editor and 886 analysis program for Windows 95/98/NT. Nucl. Acids Symp. 41, 95–98.

Hall, E.R., Kelson, K.R., 1952. Comments on the taxonomy and geographic distribution of some North American rodents. Univ. Kans. Publ. Mus. Nat. Hist. 5, 343-371.

Hibbard, C.W., 1968. Paleontology, in King, J.A. (Ed.), Biology of *Peromyscus* (Rodentia). American Society of Mammalogist Special Publication 2, USA, pp. 6-26.

Ho, S.Y.W., Saarma, U., Barret, R., et al. 2008. The effects of inappropriate calibration: three case studies in molecular ecology. PLos ONE. 3, e1615.

Ho, S. Y.-W., and Shapiro, B. 2011. Skyline-plot methods for estimating demographic history from nucleotide sequences. Molecular Ecology Resources. 11, 423-434.

Hooper, E.T., 1947. Notes on Mexican mammals. J. Mammal. 28, 40-57.

Hooper, E.T., 1955. Notes on mammals of western Mexico. Occas. Pap. Mus. Zool. Univ. Mich. 565, 1-26.

Hooper, E.T., 1968. Classification, in: King, J.A. (Ed), Biology of *Peromyscus* (Rodentia). American Society of Mammalogist Special Publication 2, USA, pp 27-74.

Hooper, E.T., Musser, G.G., 1964. Notes on classification of the rodents genus *Peromyscus*. Occas. Pap. Mus. Zool. Univ. Mich. 635, 1-13.

Kelt, D.A., Hafner, M.S., 2010. The American Society of Mammalogists' Ad Hoc Committee for Guidelines on Handling Rodents in the Field. Updated guidelines for protection of mammalogists and wildlife researchers from hantavirus pulmonary syndrome (HPS). J. Mammal. 91, 1524-1527.

Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, A., Lopez, R., Thompson, J.D., Gibson, T.J., Higgins, D.G., 2007. Clustal W and Clustal X version 2.0. Bioinformatics. 23, 2947-2948.

León-Paniagua, L., Navarro-Sigüenza, A., Hernández-Baños, B. Morales, J., 2007. Diversification of the arboreal mice of the genus *Habromys* (Rodentia: Cricetidae: Neotominae) in Mesoamerican Highlands. Mol. Phylogenet. Evol. 42, 653-664.

Librado, P., Rozas, J., 2009. DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. Bioinformatics. 25, 1451-1452.

Merriam, C.H., 1898. Description of twenty new species and a subgenus of *Peromyscus* from Mexico and Guatemala. P. Biol. Soc. Wash. 12, 115-125.

Milá, B., McCormack, J. E., Castañeda, G., Wayne, R., and Smith., T. 2007. Recent postglacial range expansion drives the rapid diversification of a songbird lineage in the genus *Junco*. Proceedings of the Royal Society B. 274, 2653-2660.

Osgood, W., 1909. Revision of the mice of the American genus *Peromyscus*. North American Fauna. 28, 1-285.

Peterson, T. A., 2011.Ecological niche conservatism: a time-structured review of evidence. J. Biogeogr. 38, 817-827.

Posada, D., 2008. JModelTest: phylogenetic model averaging. Mol. Biol. Evol. 25, 1253-1256.

Pritchard, J.K., Stephens, M., Donnelly, P. 2000. Inference of Population structure using multilocus genotype data. Genetics. 155: 945-959.

Rambaut, A., Drummond, A.J., 2007. Tracer v1.5. Available from: http://tree.bio.ed.ac.uk/software/tracer/

Rambaut, A., 2009. FigTree 1.3.1. http://tree.bio.ed.ac.uk/software/figtree/

Ramos-Onsins, S.E. and Rozas, J. 2002. Statistical properties of new neutrality test against population growth. Mol. Biol. Evol. 19, 2092-2100.

Ronquist, F., Huelsenbeck, J.P., 2003. Mr. Bayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics. 19, 1572-1574.

Russo, C.A., Takezaki, N., Nei, M., 1996. Efficiencies of different genes and different tree-building methods in recovering a known vertebrate phylogeny. Mol. Biol. Evol. 13, 525-536.

Sanderson, M.J., 2003. r8s; inferring absolute rates of evolution and divergence times in the absence of a molecular clock. Bioinformatics 19, 301–302.

Sikes, R.S., Gannon, W.L., the Animal Care and Use Committee of the American Society of Mammalogists., 2011. Guidelines of the American Society of Mammalogists for the use of wild mammals in research. J. Mammal. 92, 235-253.

Stiller, M., Baryshnikov, G., Bocherens, H., et al. 2010. Withering away- 25 000 years of genetic decline preceded caver bear extinction. Mol. Ecol. and Evol. 27, 975-978.

Sullivan, J., Market, A. Kilpatrick, C.W., 1997. Phylogeography and molecular systematics of *Peromyscus aztecus* group (Rodentia:Muridae) inferred using parsimony and likelihood. Syst. Biol. 46, 426-440.

Tajima, F. 1983. Evolutionary relationship of DNA sequences in finite populations. Genetics. 105, 437-460.

Thompson,J.D., Gibson,T.J., Plewniak,F., Jeanmougin, F., Higgins, D.G., 1997. The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic. Acids. Res. 25, 4876-4882.

Tiemann-Boege, I., Kilpatric, C.W., Schmidly, D.J., Bradley, R.D., 2000. Molecular phylogenetics of the *Peromyscus boylii* species group (Rodentia: Muridae) based on mictochodrial cytpcrome *b* sequences. Mol. Phylogenet. Evol.16, 366-378.

Weir, J.T. 2006. Divergent timing and patterns of species accumulation in lowland and highland Neotropical birds. Evolution. 60, 842-855.

Weir, J.T., and Schluter, D. 2008. Calibrating the avian molecular clock. Mol. Ecol. 17, 2321-2328.

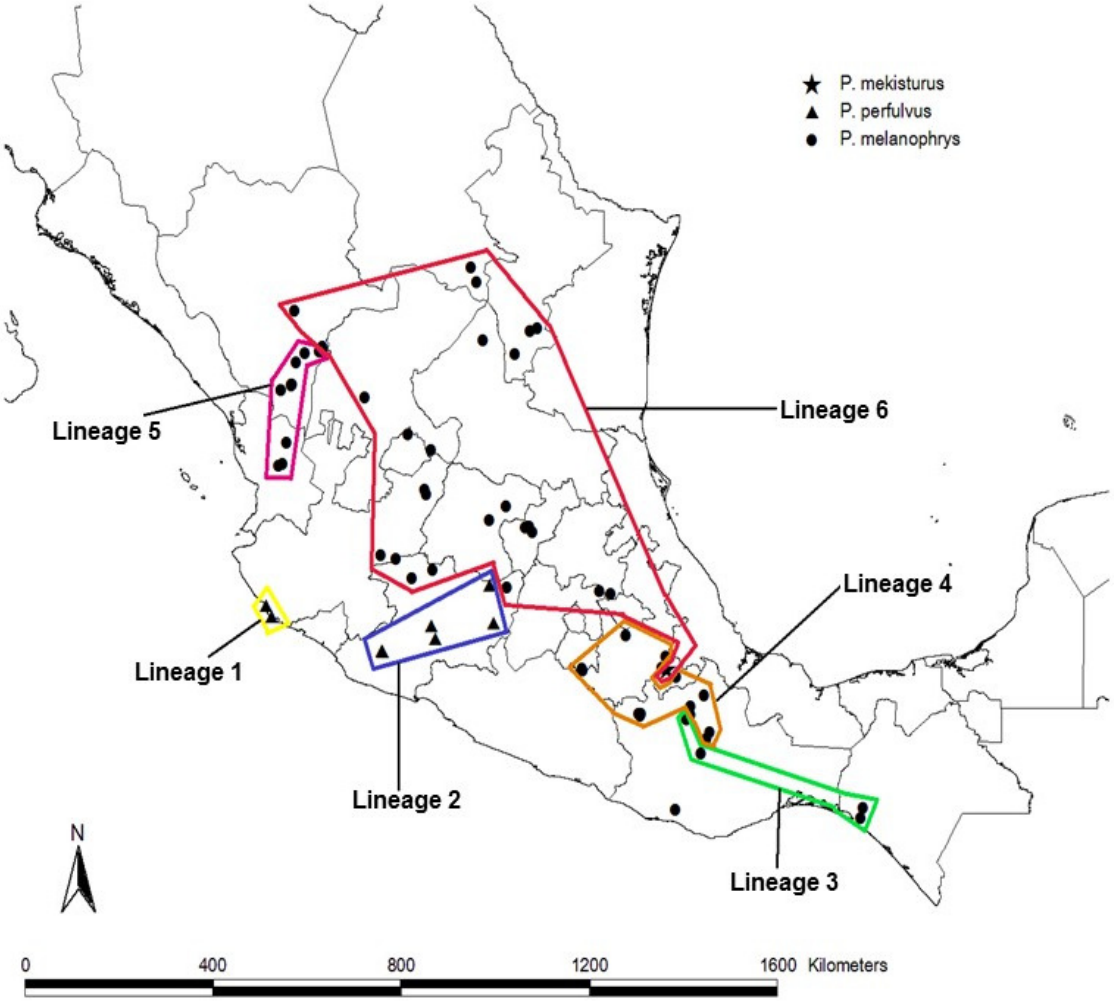

Fig. 1 Sampling localities for the *Peromyscus melanophrys* complex (*P. melanophrys*, *P. perfulvus* and *P. mekisturus*) in Mexico. Clades obtained from Bayesian Inference and Structure analyses based on mtDNA are show.

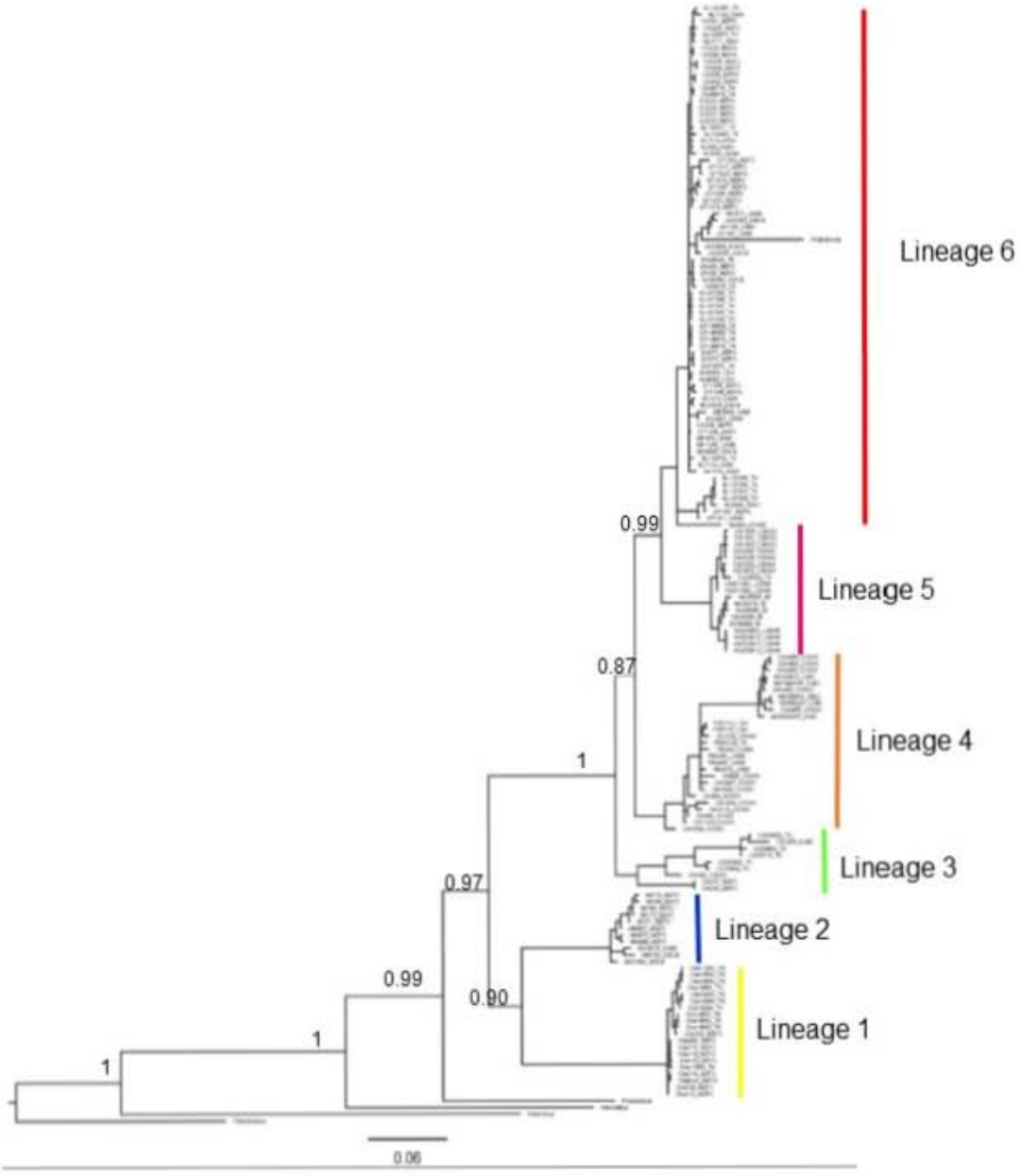

Fig. 2. Phylogenetic hypothesis for the *Peromyscus melanophrys* complex based on Bayesian Inference analysis of mtDNA. Numbers on nodes indicated posterior probabilities values.

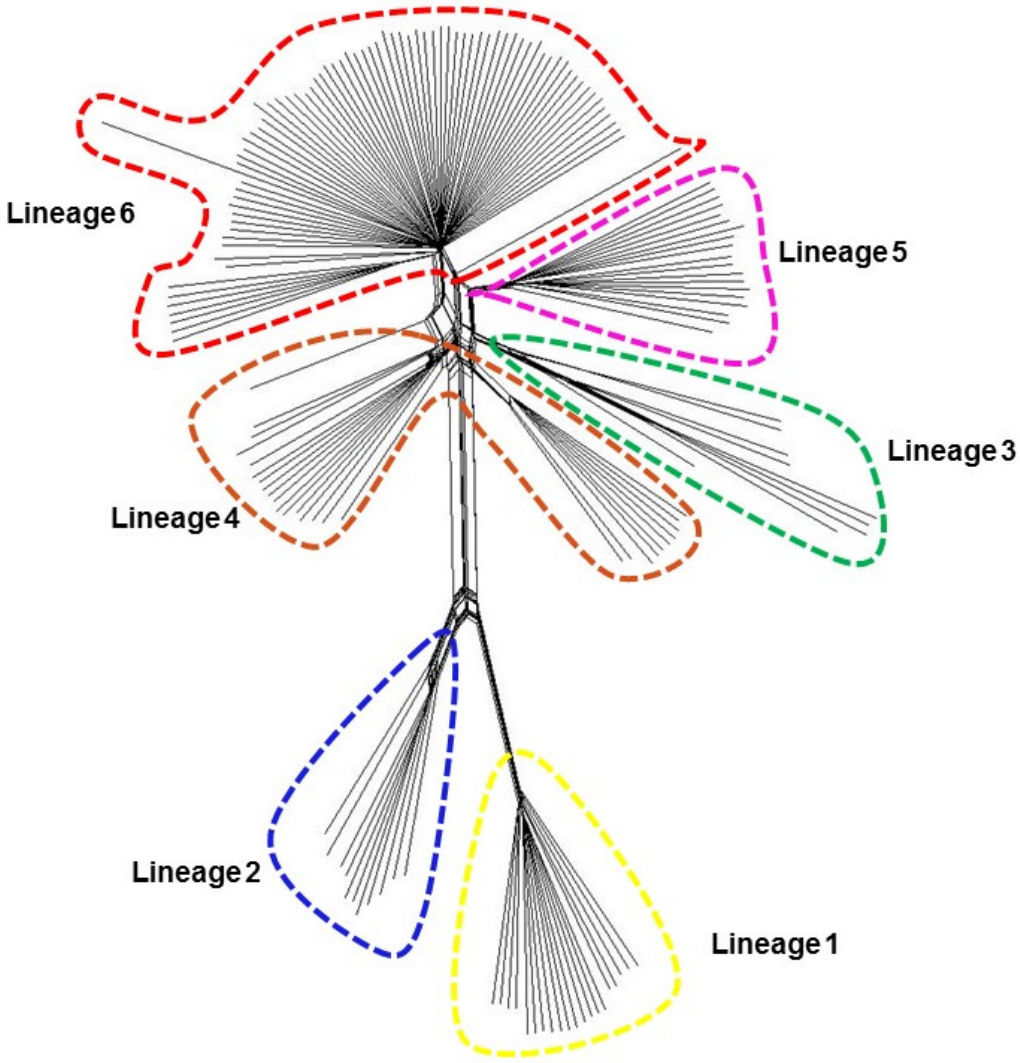

Fig. 3. Haplotype network obtained with Neighbor-Net algorithm, showing the six lineages identified within the *Peromyscus melanophrys* complex.

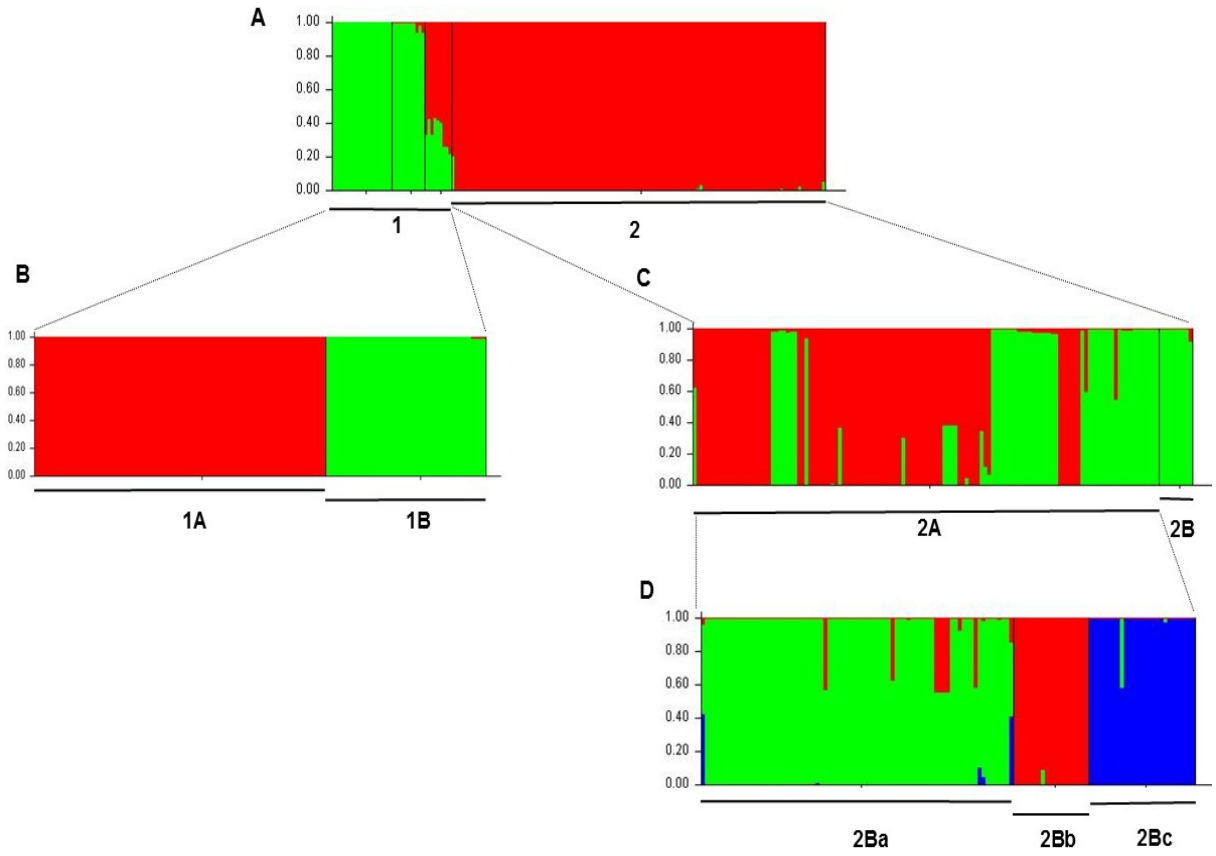

Fig. 4. Genetic clustering of the *Peromyscus melanophrys* complex samples are assessed by Structure simulation using sequences of mtDNA. A) Entire data set, B) Samples within cluster 1, C) Samples within cluster 2, D) Samples within cluster 2B.

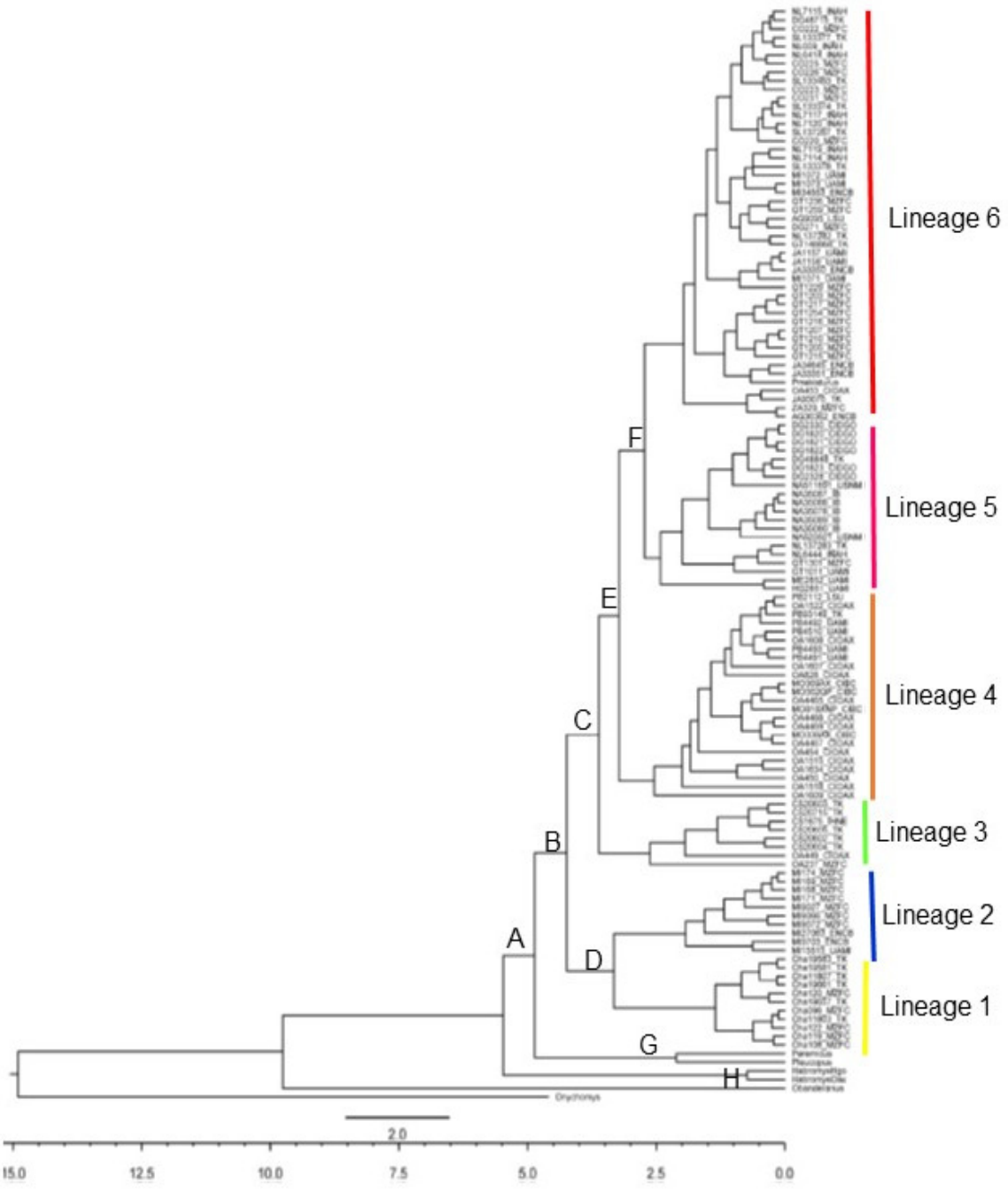

Fig. 5. Chronogram reconstructed from the mtDNA data set inferred from BEAST. Capital letters on nodes depict divergence times (see Table 1).

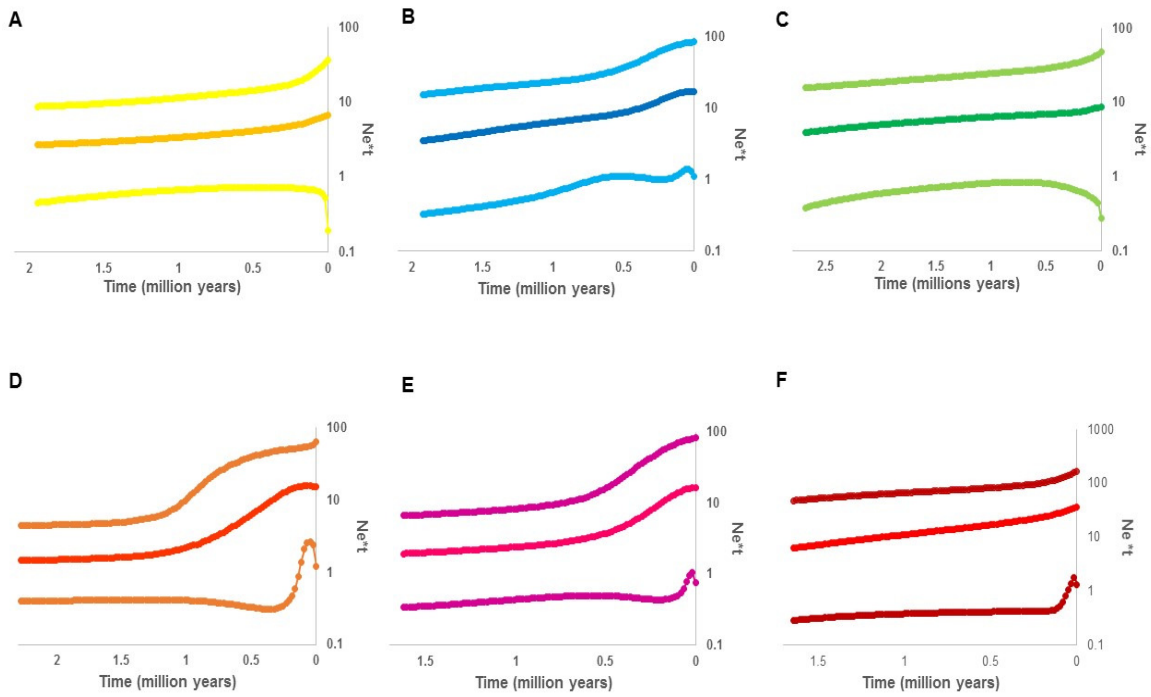

Fig. 6. Bayesian skyline plots for each lineage identified within the *Peromyscus melanophrys* complex. A) Lineage 1 (PC), B) Lineage 2 (WBD), C) Lineage 3 (TCP-SMCh), D) Lineage 4 (EBD), E) Lineage 5 (SMOc) and F) Lineage 6 (MP).

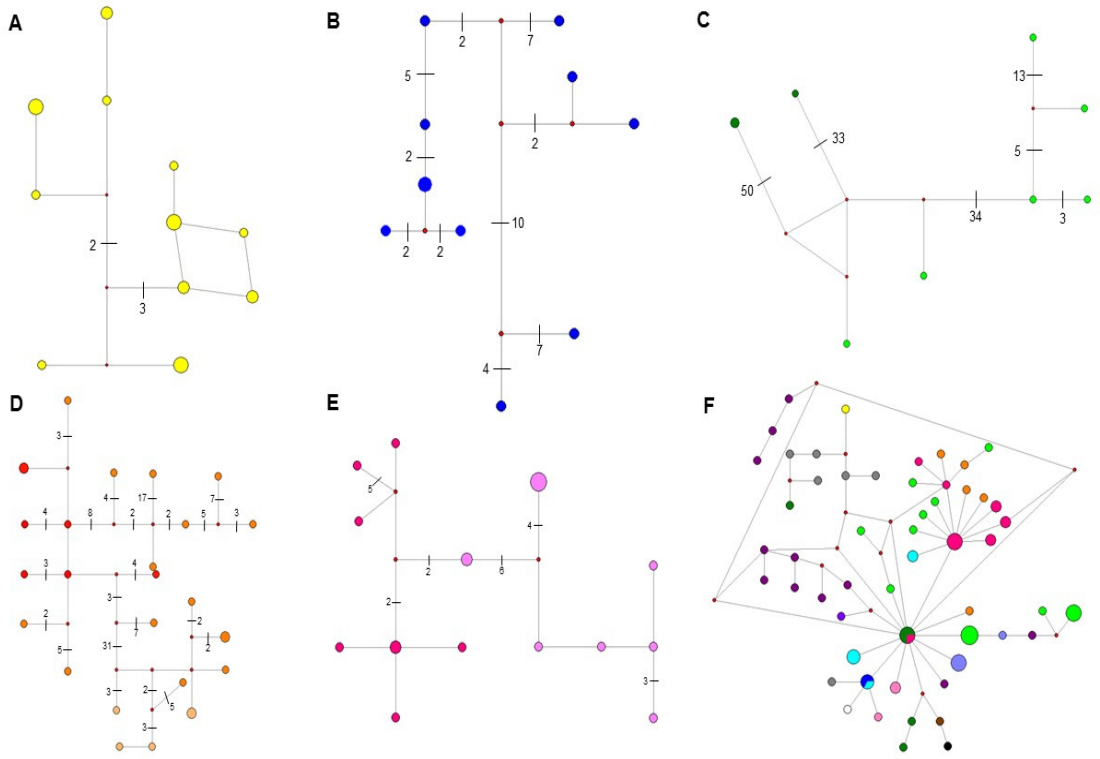

Fig. 7. Minimum spanning network using the median-joining method for each lineage identified within the *Peromyscus melanophrys* complex. A) Lineage 1 (PC), B) Lineage 2 (WBD), C) Lineage 3 (TCP-SMCh), D) Lineage 4 (EBD), E) Lineage 5 (SMOc) and F) Lineage 6 (MP).

Table 1. Estimated dates of divergence (time to the most recent common ancestor – TMRCA– and 95% confidence intervals in Mya) based on age calibrated in Castañeda-Rico et al. (2014).

Clade	TMRCA	95% HDP
A	4.8	4.4-5.3
B	4.2	3.5-4.8
C	3.6	2.8-4.3
D	3.3	2.1-4.3
E	3.2	2.4-4.0
F	2.7	1.8-3.6
G	2.1	0.8-3.9
H	0.7	0.6-0.8

Table 2. Genetic diversity values and neutrality tests for the six lineages identified for the *Peromyscus melanophrys* complex (Bayesian Inference and Structure analyses).

Group	*N*	*Hp*	*h (±SD)*	*π (±SD)*	*S*	*k*	*Tajima´s D (p value)*	*Fu and Li´s F (p value)*	*Fu´s (p value)*
Lineage 1									
Pacific Coast	20	11	0.937 (0.029)	0.003 (0.000)	12	4.926	1.638 (p>0.1)	1.753 (p<0.5)*	-1.774 (p>0.5)
Lineage 2									
Western Balsas Basin	11	10	0.982 (0.046)	0.009 (0.001)	40	12.763	-0.413 (p>0.1)	-0.351 (p>0.1)	-1.604 (p>0.1)
Lineage 3									
Tehuantepec Coastal Plain-Sierra Madre de Chiapas lowlands	9	8	0.972 (0.064)	0.033 (0.004)	103	43.527	0.545 (p>0.1)	0.922 (p>0.1)	1.653 (p>0.1)
Lineage 4									
Eastern Balsas Basin	27	24	0.991 (0.013)	0.021 (0.001)	100	27.381	0.135 (p>0.1)	-0.410 (p>0.1)	-3.980 (p<0.5)*
Lineage 5									
Sierra Madre Occidental lowlands	19	14	0.953 (0.036)	0.006 (0.000)	30	8.877	0.135 (p>0.1)	-0.541 (p>0.1)	-2.658 (p<0.05)*
Lineage 6									
Mexican Plateau	79	52	0.978 (0.008)	0.008 (0.001)	140	11.296	-2.115 (p<0.05)*	-3.164 (p<0.05)*	-27.376 (p<0.05)*

*p<0.05

Table 3. Divergence values, shown as percentage, between lineages within *Peromyscus melanophrys* complex. Number above the diagonal are *Dxy* and below are *Da*.

	Lineage 1	**Lineage 2**	**Lineage 3**	**Lineage 4**	**Lineage 5**	**Lineage 6**
Lineage 1		9.6	12.2	11.8	11.8	12.4
Lineage 2	9.1		11.4	10.6	11.3	11.4
Lineage 3	10.3	9.2		6.8	7.1	6.7
Lineage 4	10.5	9.1	4.1		5.8	5.2
Lineage 5	11.2	10.4	5.1	4.4		4.1
Lineage 6	11.8	10.5	4.5	3.7	3.2	

CAPÍTULO 4

Discusión y Conclusión General

DISCUSIÓN GENERAL

México ha sido foco de especial interés respecto a estudios biogeográficos debido al excepcional nivel de biodiversidad que existe en el país, y en particular en la zona de transición entre las regiones Neártica y Neotropical (Halfter, 1976; Lomolino et al., 2005; Devitt, 2006). Varios autores han sugerido que dicha zona incluye desde el suroeste de los Estados Unidos hasta México. Sin embargo, no existe una delimitación reconocida como única, a pesar de que se han realizado diversos estudios biogeográficos y filogeográficos con distintos taxa (*e.g.* Ortega y Arita, 1998; Marshall y Liebherr, 2000; Morrone y Márquez, 2001; Morrone, 2006). Estudios han permitido definir discontinuidades genéticas, zonas de hibridación y límites de especies en distintos taxa, los cuales tienen concordancia con barreras geográficas y en los que la divergencia o especiación dentro del grupo han sido explicadas bajo un modelo de vicarianza (*e.g.* Arbeláez-Cortés et al., Devitt, 2006; León-Paniagua et al., 2007).

Una de las característica de ésta zona de transición, en particular de México, es la excepcional topografía, que va desde las planicies áridas situadas entre la Sierra Madre Oriental y la Sierra Madre Occidental, combinado con las selvas húmedas de las costas del Pacífico y del Golfo de México hasta los bosques montanos del sureste del país que han funcionado como “incubadoras” para la formación de especies de peromicinos, desde el Plioceno medio hasta la fecha, siguiendo varios ciclos de especiación y expansión que corresponden con los avances y retrocesos glaciares en Norte América (Dawson, 2005). Durante estos periodos, diferentes eventos de vicariaza han tenido lugar en las dos Sierra Madre y las costas adyacentes, donde el Altiplano Mexicano ha funcionado como mecanismo de aislamiento. De igual forma, durante los interglaciales, las especies expanden su rango de distribución hacia el norte, donde son capaces de adaptarse a nuevos hábitats (Dawson, 2005).

El complejo *Peromyscus melanophrys*, es el único grupo dentro de los peromicinos que es endémico de México, por lo que resulta un excelente modelo para determinar patrones filogeográficos asociados a las tierras bajas y los tipos de vegetación que habitan estas especies y que han sido poco estudiados desde esta perspectiva (*e.g.* matorral xerófilo, selva baja y selva mediana), en comparación con las zonas altas (*e.g.* bosque mesófilo de montaña). Para lograr este objetivo fue necesario, sin embargo, antes determinar las relaciones filogenéticas dentro del grupo.

En este trabajo se confirmó la monofilia del grupo, ya que aunque ésta ya se había sugerido (Osgood, 1909; Hall and Kelson, 1959; Hooper and Musser, 1964; Hooper, 1968; Carleton, 1989, Bradley et al., 2007), ningún estudio previo había incluido a una de las especies, *P. mekisturus*. Sin embargo, el reconocimiento de las especies y subespecies descritas para el grupo no coincidió con los resultados de este trabajo. Se detectaron entre cuatro y seis linajes genéticos utilizando genes mitocondriales y un nuclear, así como varios análisis de agrupación y estructura genética. Cada linaje se comportó como una unidad evolutiva independiente. La falta de concordancia que existe entre las especies y subespecies descritas dentro los grupos de peromicinos al utilizar datos morfológicos *versus* datos moleculares es frecuente, tal es el caso de los grupos *Peromyscus eremicus, Peromyscus aztecus* y *Peromyscus boylii* (Riddle et al., 2000; Sullivan et al., 1997; Tiemman-Boege et al., 2000)

El género *Peromyscus* es un grupo relativamente reciente (data del Mioceno-Plioceno; Dawson, 2005), lo que complica la identificación de barreras geográficas que expliquen los eventos de especiación o diversificación, ya que los principales rasgos geomorfológicos (*i.e.* montañas, sierras, planicies y los principales sistemas de ríos) son más antiguos y no han presentado cambios significativos hasta la fecha (Ferrusquía-Villafranca et al., 2010). Sin embargo, en este trabajo se evidenció que tanto los eventos

vicariantes del Mioceno-Pleistoceno, como los cambios climáticos del Cuaternario son los que mejor explican la diversificación dentro del grupo. Dichos resultados concuerdan con una de las hipótesis más aceptadas dentro de los eventos de especiación, la que propone que los ciclos glaciales durante el Pleistoceno (2.5 to 0.01 Mya) incrementaron las tasas de especiación en los grupos Neárticos (Avise y Walker, 1998; Barraclough y Nee, 2001; Klicka y Zink, 1999). Es posible que el grupo *Peromyscus melanophrys* originalmente se distribuía en selva mediana y/o selva baja, pero debido a los cambios climáticos del Pleistoceno algunas de las poblaciones del grupo fueron capaces de adaptarse a ambientes más áridos y de esta forma diversificarse. Con el tiempo las poblaciones adquirieron cierto grado de aislamiento reproductivo, dando lugar a diferentes especies. Esto lo podemos corroborar, ya que hay evidencia de que parte del Altiplano Mexicano, cubierto ahora en su mayoría por vegetación xerófila, estuvo constituido por selva baja y/o mediana durante ciertos periodos del Pleistoceno (Ferrusquía-Villafranca et al., 2010). Por otro lado, durante el Plioceno tardío y el Pleistoceno temprano las posibles rutas de dispersión hacia el norte fueron a través de la Faja Volcánica Transmexicana y la Sierra Madre del Sur, aunque para algunas especies es posible que la primera haya constituido una barrera geográfica, propiciando su aislamiento, lo cual explicaría la separación entre *P. perfulvus* y *P. melanophrys*.

Se reconocieron algunos patrones filogeográficos de las especies de tierras bajas, así como la historia demográfica de los linajes identificados (Depresión del Balsas Oeste, Costa del Pacífico, tierras bajas de la Sierra Madre de Chiapas-Planicie Costera de Tehuantepec, Depresión del Balsas Este, tierras bajas de la Sierra Madre Occidental y Altiplano Mexicano), los cuales concuerdan con otros patrones encontrados en grupos de peromicinos (*e.g.* Riddle et al., 2000; Sullivan et al., 1997; Tiemman-Boege et al., 2000) en los que encontramos eventos de vicarianza y dispersión en poblaciones alopátridas.

La mayoría de las especies del orden Rodentia suelen estar adaptadas a condiciones ambientales particulares, facilitando así en algunos casos la especiación (Dawson, 2005; Ferrusquía-Villafranca et al., 2010). Por otro lado, responden fácilmente a cambios ambientales, por lo que podrían proporcionar información para las reconstrucciones climáticas o viceversa (Ferrusquía-Villafranca et al., 2010). Sin embargo, la información climática que se tiene del Pleistoceno no es muy completa (Ferrusquía-Villafranca et al., 2010; Habib et al., 1970), por lo que no ha permitido establecer detalles por periodos largos o continuos.

CONCLUSIÓN

Se proponen cambios taxonómicos que están fuertemente soportados por evidencia molecular. Se identificaron seis linajes genéticos dentro del complejo *Peromyscus melanophrys*, los cuales se propones que sean reconocidos como especies. Los posibles nombres serían: *Peromyscus perfulvus, P. chrysopus, P. melanophrys, P. mekisturus, P. micropus* y *P. sp. nov*. Sin embargo, es necesario otro tipo de estudios (*e.g* ecológicos, morfológicos, etc.), que corroboren estos resultados para elaborar una propuesta taxonómica formal. El origen del grupo, así como los eventos de cladogénesis que dieron lugar a los principales linajes, ocurrieron durante el Plioceno medio y la diversificación dentro de cada grupo fue durante el Plioceno tardío y el Pleistoceno. Dichos eventos posiblemente fueron resultado de eventos vicariantes y cambios ambientales y ecológicos. El complejo *Peromyscus melanophrys* es relativamente reciente, cuyos linajes se encuentran en plena diversificación.

REFERENCIAS

Arbeláez-Cortés, E., Nyári, A., Navarro-Sigüenza, A.G., 2010. The differential effect of lowlands on the phylogeographic pattern of a Mesoamerican montane species (*Lepidocolaptes affinis*, Aves: Furnariidae). Mol. Phylogenet. Evol. 57, 658-668.

Avise, J. y Walker, D. 1998. Pleistocene phylogeographic effects on avian population and the speciation process. Proc. R. Soc. London B Biol. Sci. 265: 457:463.

Barraclough, T. y Nee, S. 2001. Phylogenetics and speciation. Trends Ecol. Evol. 16 (7):391-399.

Bradley, R.D., Durish, N., Rogers, D., Millar, J., Engstrom M., Kilpatrick, W., 2007. Toward a molecular Phylogeny for *Peromyscus*: Evidence from mitochondrial cytochrome-b sequences. J. Mammal. 88, 1146-1159.

Carleton, M. D., 1989. Systematics and Evolution, in: Kirkland, G.L., Layne, J. (Eds.) Advances in the study of *Peromyscus* (Rodentia). Texas Tech University Press., Lubbock. pp. 7-141.

Dawson, W., 2005. Peromyscine Biogeography, Mexican topography and Pleistocene climatology, in: Sánchez-Cordero, V., Medellín, R. (Eds.), Contribuciones Mastozoológicas en Homenaje a Bernardo Villa. UNAM-CONABIO. México, pp. 145-156

Devitt, T. J., 2006. Phylogeography of Western Lyresnake (*Trimorphodon biscutatus*): testing arid land biogeographical hypotheses across the Neartic-Neotropical transition. Mol. Ecol. 15, 4387-4407.

Halfter, G. 1976. Distribución de los insectos en la zona de transición mexicana. Relaciones en la entomofauna de Norteamérica. Folia Entomológica Mexicana. 35:1-64.

Hall, E.R., Kelson, K.R., 1952. Comments on the taxonomy and geographic distribution of some North American rodents. Univ. Kans. Publ. Mus. Nat. Hist. 5, 343-371.

Hooper, E.T., 1968. Classification, in: King, J.A. (Ed), Biology of *Peromyscus* (Rodentia). American Society of Mammalogist Special Publication 2, USA, pp 27-74.

Hooper, E.T., Musser, G.G., 1964. Notes on classification of the rodents genus *Peromyscus*. Occas. Pap. Mus. Zool. Univ. Mich. 635, 1-13.

Klicka, J. y Zink, R. 1999. Pleistocene effects on North American songbird evolution. Proc. R. Soc. London B Biol. Sci. 266: 695-700.

León-Paniagua, L., Navarro-Sigüenza, A., Hernández-Baños, B. Morales, J., 2007. Diversification of the arboreal mice of the genus *Habromys* (Rodentia: Cricetidae: Neotominae) in Mesoamerican Highlands. Mol. Phylogenet. Evol. 42, 653-664.

Lomolino, M.V. Riddle, B. Brown, J.H. 2005. Biogeography, 3rd edn. Sinaur Associates, Sunderland, Massachussets.

Marshall, C.J., Liebherr, J.K. 2000. Cladistic biogeography of the Mexican Transition Zone. Journal of Biogeography. 27: 203-216.

Morrone, J.J. 2006. Biogeographic areas and transition zones of Latin American and the Caribbean Islands based on panbiogeographic and cladistics analyses of the entomofauna. Annual Review of Entomology. 51: 467-494.

Morrone, J.J., Márquez, J. 2001. Halfter´s Mexican Transition Zone, beetle generalized tracks, and geographical homology. Journal of Biogeography. 28: 635-650.

Ortega, J., Arita, H. 1998. Neotropical-Neartic limits in Middle America as determined by distribution of bats. Journal of Mammalogy. 79: 772-783.

Osgood, W., 1909. Revision of the mice of the American genus *Peromyscus*. North American Fauna. 28: 1-285.

Riddle, B., D. Hafner y L. Alexander. 2000. Phylogeography and Systematics of the *Peromyscus eremicus* species group and the historical biogeography of North American Warm Regional deserts. Molecular Phylogenetics and Evolution. 17(2):145-160.

Sullivan, J., A. Market y C. Kilpatrick. 1997. Phylogeography and molecular systematics of *Peromyscus aztecus* group (Rodentia:Muridae) inferred using parsimony and likelihood. Systematic Biology. 46:426-440.

Tiemman-Boege, I., Kilpatrick, W., Schmidly, D., Bradley, R. 2000. Molecular Phylogenetics of the Permyscus boylii species group (Rodentia:Muridae) based on mitochondrial cytochrome b sequences. Molecular Phylogenetics and Evolution. 16(3): 366-378.