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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

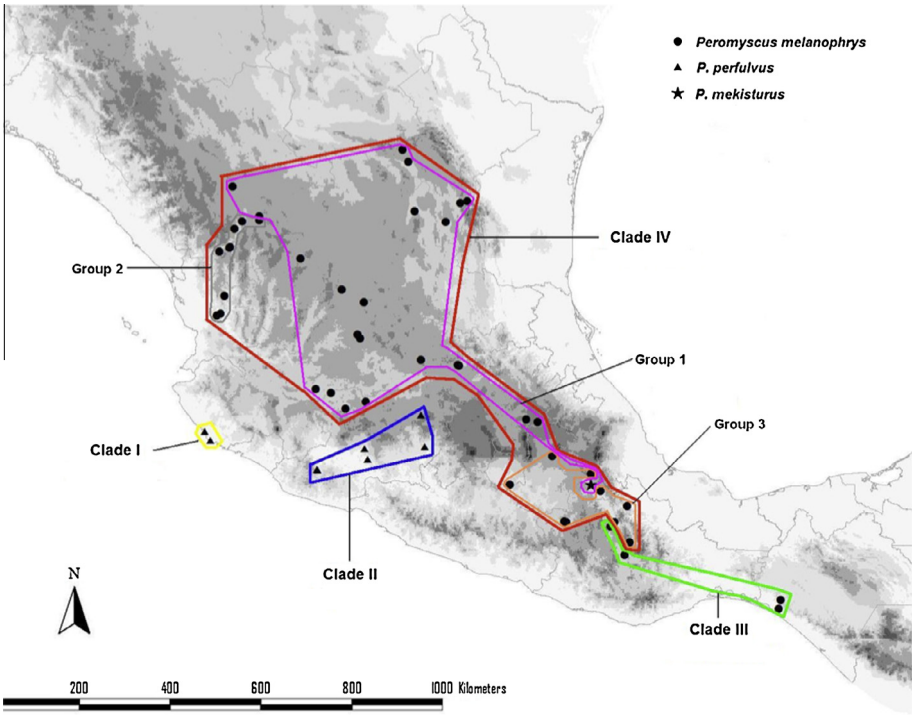

Fig. 1. Sampling localities for the *P. melanophrys* group (*Peromyscus melanophrys*, *P. perfulvus* and *P. mekisturus*) in Mexico. Clades and groups obtained from BI and ML analyses based on mitochondrial and nuclear genes are shown. Base map is the Digital Elevation Model from USGS (http://eros.usgs.gov/#/Find_Data/Products_and_Data_Available/gtopo30/hydro).