TAXONOMY AND PHYLOGENETICS OF THE PEROMYSCUS MANICULATUS SPECIES GROUP

Article · October 2019

CITATION
1

READS
809

3 authors, including:

Ira F Greenbaum
Texas A&M University
108 PUBLICATIONS **2,020** CITATIONS
SEE PROFILE

Rodney L Honeycutt
Pepperdine University
506 PUBLICATIONS **11,625** CITATIONS
SEE PROFILE

Taxonomy and Phylogenetics of the *Peromyscus maniculatus* Species Group

Ira F. Greenbaum, Rodney L. Honeycutt, and Scott E. Chirhart

Abstract

An overview is provided herein of advancements in the species composition and phylogenetics of the *Peromyscus maniculatus* species group since Carleton's 1989 review of the genus. These advancements primarily are the result of studies of chromosomal and nucleotide-sequence variation, with most sequence data derived from the mitochondrial genome. Evidence is summarized supporting the conclusion that variation in mitochondrial genes provides consistent and informative details relative to species-level identification and the phylogenetic relationships among major clades of deer mice. Based on studies of the mitochondrial cytochrome-*b* gene as well as previously published and new sequences of the mitochondrial ND3/ND4/ND4L genes, the number of species in the *P. maniculatus* group is increased to nine (*P. melanotis*, *P. polionotus*, *P. keeni*, *P. arcticus*, *P. gambelii*, *P. sejugis*, *P. sonoriensis*, *P. labecula*, and *P. maniculatus*). The newly identified species render *P. maniculatus* as being restricted to the northeastern United States and south-central Canada. The phylogenetic studies provide evidence of a sister-group relationship between the *P. leucopus* and *P. maniculatus* species groups and the placement of *P. melanotis* as basal to other members of the *P. maniculatus* group. The well-supported clade containing *P. keeni* and *P. gambelii*/*P. sejugis* appears to be best explained as having resulted from independent peripheral isolation. However, the available data fail to resolve the phylogenetic relationship of the clades corresponding to *P. maniculatus sensu stricto, P. sonoriensis*, *P. polionotus,* and *P. keeni*/*P. gambelii*/*P. sejugis*, and too little data exist to address the phylogenetic relationships of *P. arcticus* and *P. labecula* relative to the other species in the *P. maniculatus* species group. A more thorough resolution of the systematics of the species in the *P. maniculatus* group awaits broader and targeted geographic sampling and the inclusion of data from more rapidly evolving nucleotide sequences.

Key words: *Peromyscus maniculatus* species group, phylogenetics, systematics, taxonomy

Introduction

The genus *Peromyscus* represents the most ubiquitous genus of native North American rodents. One or more species of *Peromyscus* occur in nearly all habitats of North America from the Canadian taiga through central Mexico. In his revision of the genus, Osgood (1909) recognized the subgenus *Peromyscus* as a highly variable but distinct morphological group of species, and established species groups to reflect morphological discontinuities within the subgenus. Whereas some of Osgood's (1909) species groups and their constituent species have undergone substantial revision, his *Peromyscus maniculatus* group, containing *P. maniculatus*, *P. polionotus*, *P. melanotis*, and *P. sitkensis*, remained comparatively stable through Carleton's (1989) review of the genus. To Osgood's (1909) *P. maniculatus* group, Hooper (1968) added *P. sejugis* (Burt 1932) and tentatively *P. slevini* (Maillaird 1924), both from islands in the Gulf of California. Carleton (1989) retained the composition of Hooper's *P. maniculatus* group but followed Sheppe (1961), Gunn and Greenbaum (1986), and Allard et al. (1987) in recognizing and including *P. oreas* (Bangs 1897) as a distinct species in western Washington and southwestern British Columbia.

Traditional systematic studies of *Peromyscus* were based on evidence from morphology, pelage, geographic distribution, and ecology. Carleton (1989) noted that Hooper's (1968) bibliography contained no references to "nontraditional data as taxonomic evidence." As noted by Carleton (1989), the two decades between the review of Hooper (1968) and his review saw the inception and rapid growth of the application of chromosomal and biochemical techniques to studies of evolution, systematics, and taxonomy in general and to *Peromyscus* in particular. Carleton (1989) specified that of the more than 130 post-1968 references amassed for his review, nearly 70% reported results from karytoypic and/or biochemical studies. As such, Carleton (1989) provided a comprehensive review of both the traditional and nontraditional evidence relevant to the systematics of *Peromyscus* through 1987.

Subsequent to Carleton's (1989) review, most phylogenetic and systematic studies of the genus *Peromyscus* and the *P. maniculatus* group in particular have been based primarily on nucleotide sequence variation of mitochondrial DNA (mtDNA). The sole use of mtDNA for phylogenetic analyses and species delimitation has been criticized (for review see Galtier et al. 2009), and some authors (Yang and Kenagy 2009, 2011; Taylor and Hoffman 2012) have reported instances of mitonuclear discordance with respect to recent gene flow among populations of *P. maniculatus.* However, there is considerable evidence that mitochondrial sequence divergence of the cytochrome-*b* gene has proven useful for identifying species of *Peromyscus* and other mammals (Bradley and Baker 2001). Moreover, concordance between mitochondrial-sequence and nuclear-based evolution at the level of species differentiation within *Peromyscus* has been well documented. Hogan et al. (1993) found concordance among chromosomal, allozymic, and mtDNA variation in the recognition of *P. keeni* (formerly *P. oreas* and *P. sitkensis*). These characters also were concordant with the morphological cohesiveness of *P. keeni* relative to *P. maniculatus* (Allard et al. 1987; Allard and Greenbaum 1988; Sullivan et al. 1990). Analysis of microsatellite variation (Chirhart et al. 2005) recovered the same phylogenetic relationships among members of the *P. maniculatus* species group as obtained from analyses of mtDNA variation by Hogan et al. (1997). Combined analysis of mtDNA and nuclear sequences (Miller and Engstrom 2008; Platt et al. 2015) recovered the same phylogenetic relationships between and among species in the *P. leucopus* and *P. maniculatus* species groups as previously diagnosed from analyses of mtDNA sequence variation (Sullivan et al. 1995; Hogan et al. 1997; Engel et al. 1998; Durish et al. 2004; Bradley et al. 2007; Gering et al. 2009). Therefore, it is concluded that the available molecular data reflect reasonably accurate taxonomic inferences for the *P. maniculatus* species group.

The intent of this paper is three-fold. First, research since 1989 pertaining to the systematics of the *P. maniculatus* species group is reviewed and areas where new data have helped resolve systematic questions raised by Carleton (1989) are identified. Second, remaining taxonomic and phylogenetic issues that require further resolution are highlighted. Finally, new sequence data are introduced from the mitochondrial ND3/ND4/ND4L genes that are germane to defining the limits of *P. maniculatus sensu stricto*. Given the extraordinary popularity of deer mice as a research model for virtually all areas of organismal biology, discussion is limited to reports with direct relevance to the systematics and taxonomy of the *P. maniculatus* species group.

TAXONOMIC RESOLUTION SINCE 1989

Basal relation of the P. maniculatus *species group*.—Numerous molecular studies have included species of the *P. maniculatus* group as either outgroups or reference taxa in studies pertaining to either the systematics of the genus *Peromyscus* or as focal species for addressing questions other than the systematics of the *P. maniculatus* group (Table 1). However, studies that included species of both the *P. maniculatus* and *P. leucopus* species groups uniformly recovered these as highly supported and reciprocally monophyletic clades. Correspondingly, both Miller and Engstrom (2008) and Platt et al. (2015) entertained the notion that the clade containing the *maniculatus* + *leucopus* species groups will ultimately prove to constitute a distinct subgenus or genus.

Table 1. Studies since Carleton (1989) reporting molecular data for species of the *P. maniculatus* species group in which the data were used as outgroups or reference species and which were not designed as studies of the systematics of the *P. maniculatus* species group.

Reference	Data	Focus of study	Outgroup/reference species
Rogers and Engstrom 1992	allozymes	*P. mexicanus* group	*melanotis*
Sullivan et al. 1995	*Cyt*b, 12S rRNA	sigmodontine rodents	*melanotis*, *polionotus*, *keeni*
Engel et al. 1998	ND3/ND4/ND4L	sigmodontine rodents	*maniculatus*
Riddle et al. 2000	COIII	*P. eremicus* group	*maniculatus*
Tieman-Boege et al. 2000	*Cyt*b	*P. boylii* group	*melanotis*
Hafner et al. 2001	COIII	Sea of Cortez insular *Peromyscus*	*sejugis*, *maniculatus*
Bradley et al. 2004	*Cyt*b	neotomine-peromyscine rodents	*maniculatus*
Durish et al. 2004	*Cyt*b	*P. truei* group	*melanotis*, *maniculatus*
Dragoo et al. 2006	*Cyt*b	*P. maniculatus*	*melanotis*, *keeni*
Bradley et al. 2007	*Cyt*b	genus *Peromyscus*	*melanotis*, *keeni*, *polionotus, maniculatus*
Degener et al. 2007	*Cyt*b, microsatellites	*P. polionotus*	*melanotis*, *maniculatus*
Van Zant et al. 2007	*Cyt*b, D-loop	*P. polionotus*	*keeni*, *maniculatus*
Miller and Engstrom 2008	*Cyt*b, nuclear genes	genus *Peromyscus*	*melanotis*, *polionotus*, *maniculatus*
Gering et al. 2009	*Cyt*b	*Cyt*b evolution/adaptation	*melanotis*, *keeni*
Domingues et al. 2012	nuclear sequences	adaptation, *P. polionotus*	*maniculatus*
Kalkvik et al. 2012	*Cyt*b	phylogeography/niche modeling	*melanotis*, *polionotus, keeni*
Platt II et al. 2015	*Cyt*b, nuclear genes	genus *Peromyscus*	*melanotis*, *maniculatus*
Kingsley et al. 2017	SNPs, COIII–ND3	evolution of form/adaptation	*polionotus*, *keeni*
Cornejo-Latorre et al. 2017	*Cyt*b, COI, COIII	subgenus *Haplomylomys*	*sejugis*, *maniculatus*
Greenbaum et al. 2017	ND3/ND4/ND4L	*P. maniculatus*	*melanotis*, *keeni*
Kalkvik et al. 2018	*Cyt*b, microsatellites	*P. polionotus*	*melanotis*, *keeni*, *maniculatus*

Peromyscus slevini.—*P. slevini* is an island endemic restricted to Catalina Island in the Gulf of California (Álvarez-Castañeda and Cortés-Calva 2002). Although Carleton (1989) retained *P. slevini* in the *P. maniculatus* group, he noted that its "systematic position remains obscure." In their account of the species, Álvarez-Castañeda and Cortés-Calva (2002) suggested that *P. slevini* was derived from a *P. maniculatus* ancestral stock, but they failed to cite relevant molecular and chromosomal studies that clearly invalidate inclusion of *P. slevini* in the *P. maniculatus* group. Phylogenetic analyses of sequences of the mitochondrial ND3/ND4/ND4L genes (Hogan et al. 1997) grouped *P. slevini* outside a clade containing both the *P. leucopus* and *P. maniculatus* species groups. Smith et al. (2000) reported that *P. slevini* has an autosomally invariant chromosomal phenotype that lacks the chromosome 2 and chromosome 20 syapomorphies associated with members of the *P. maniculatus* group. The banded karyotype of *P. slevini* is unique among all banded karyotypes reported for *Peromyscus* but is similar to karyotypes reported for species in the *P. boylii* and *P. mexicanus* species groups. Based on analyses of craniofacial, exomorphological, and bacular variables, Carleton and Lawlor (2005) allocated *P. slevini* to the *P. melanophrys* species group.

Peromyscus melanotis.—Of the species in Carleton's (1989) *P. maniculatus* group (sans *P. slevini*), *P. melanotis*, distributed at higher elevations in south-

eastern Arizona (Bowers et al. 1973, Bowers 1974) and central Mexico (Fig. 1), has been the least affected by subsequent molecular phylogenetic studies. There is little morphological discontinuity among populations of *P. melanotis* (Martínez-Coronel et al. 1991), and chromosomal (Greenbaum et al. 1978; Robbins and Baker 1981; Stangl and Baker 1984) and most molecular data (Hogan et al. 1997; Chirhart et al. 2005; Walker et al. 2006; Gering et al. 2009; Kalkvik et al. 2012) identify it as the most basal member of the *maniculatus* group. That Dragoo et al. (2006) found *P. melanotis* to be weakly associated among clades of *P. maniculatus* is anomalous as three other studies (Gering et al. 2009; Kalkvik et al. 2012; Natarajan et al. 2015), using the same gene, recovered *P. melanotis* as the basal species of the group.

Peromyscus polionotus.—*Peromyscus polionotus* is restricted to sandy soils of the southeastern United States (Hall 1981) and is peripherally distributed relative to the other species in the *P. maniculatus* group (Fig. 1). Analysis of mtDNA variation (Van Zant et al. 2007; Kalkvik et al. 2018), microsatellites (Degener et al. 2007), and nuclear sequences (Domingues et al. 2012) supported *P. polionotus* as monophyletic with respect to other members of the *P. maniculatus* group, and cytosystematic analyses (Greenbaum et al. 1978; Robbins and Baker 1981; Stangl and Baker 1984) consistently identified *P. polionotus* as sister to *P. maniculatus*. Two studies of mtDNA sequence variation, however, recovered marginally supported associations of *P. polionotus* to clades within *P. maniculatus*. Kalkvik et al. (2012) obtained *P. polionotus* as sister to a clade of *P. maniculatus* from the central and western states, whereas Kingsley et al. (2017) obtained *P. polionotus* as sister to a clade (including *P. m. nubiterrae* and *P. m. gracilis*) from the eastern United States and Canada.

Peromyscus keeni (= *P. oreas* + *P. sitkensis*).—In support of the specific status of *P. oreas* and its inclusion in the *P. maniculatus* species group, Carleton (1989) cited its sympatry with (Sheppe 1961) and karytoypic (Gunn and Greenbaum 1986) and morphologic (Allard et al. 1987) distinction from *P. m. austerus*. *Peromyscus oreas* from Washington and coastal British Columbia are characterized by a largely biarmed karyotype (number of autosomal arms (FN) = 85–88), whereas *P. m. austerus* has karyotypes of FN = 74–76 (Gunn and Greenbaum 1986). This observation, however, left unanswered questions as to the range and island distribution of *P. oreas* and to its relationship with the chromosomally similar *P. sitkensis* (FN = 84–91; Thomas 1973; Pengilly et al. 1983). Gunn (1988) presented chromosomal homology data for a broad sampling of deer mice from Vancouver Island and islands in the Queen Charlotte Strait and Strait of Georgia and confirmed instances of sympatry without intermediates between the karytoypic groups representing *P. oreas* and *P. maniculatus,* respectively. Corresponding results were obtained for external, cranial, and mandibular (Allard and Greenbaum 1988) as well as genital (Sullivan et al. 1990) morphology. Hogan et al. (1993) incorporated results from previous studies, expanded the sampling to include additional localities from mainland British Columbia, the Queen Charlotte Islands, and southeastern Alaska, and analyzed chromosomal, allozymic, and mtDNA (ND3/ND4/ND4L) variation. Correspondingly, Hogan et al. (1993) subsumed *P. oreas*, *P. sitkensis*, and the *P. maniculatus* subspecies *algidus*, *hylaeus*, *macrorhinus*, and *prevostensis* under *P. keeni* (Fig. 1). With little data to clarify the taxonomic affinity of most of the insular subspecies of *P. maniculatus* in the Pacific Northwest (i.e., *beresfordi*, *crancrivorous*, *carli*, *doylei*, *maritimus*, *pluvialis*, *rubriventer*, *sartinensis*, and *triangularis*), Hogan et al. (1993) cited standard karytoypic data for *P. m. carli*, *P. m. doylei*, and *P. m. triangularis* (Thomas 1973) and morphological data presented by Cowan and Guiguet (1965) to suggest that all of these subspecies be referred to *P. keeni*. Chirhart et al. (2001) found that the mtDNA sequences of the deer mice from Triangle Island, British Columbia, confirmed that the subspecies *triangularis* is appropriately assigned to *P. keeni*. The classification by Musser and Carleton (2005) followed Hogan et al. (1993) in including *P. keeni* as a species in the *P. maniculatus* group.

Although inclusion of *P. keeni* in the *P. maniculatus* species group is uncontroversial, its phylogenetic relationship to *P. maniculatus* (*sensu* Musser and Carleton 2005) is incompletely resolved. Analyses including *P. keeni* reference sequences and widespread mtDNA variation in *P. maniculatus* generally have indicated a sister-group relationship between *P. keeni* and southwestern-most populations of *P. maniculatus* (Dragoo et al. 2006; Gering et al. 2009; Kalkvik et al. 2012; Natarajan 2015; Kingsley et al. 2017). More spe-

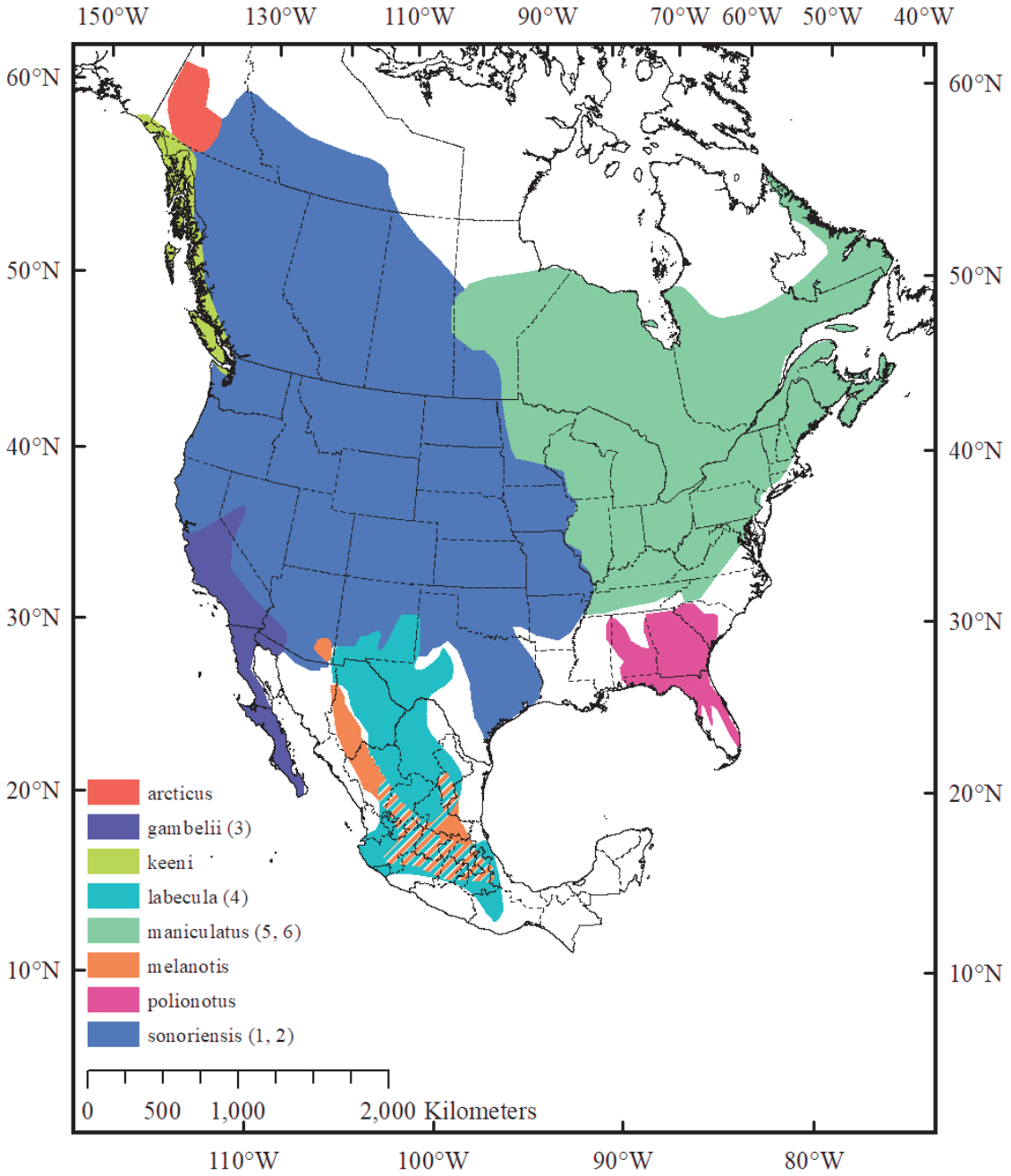

Figure 1. Map of the general distributions of species in the *P. maniculatus* group as discussed and recognized herein. *Peomyscus sejugis* is restricted to Isla Santa Cruz and Isla San Diego in the Gulf of California and is not figured. Numbers in parentheses refer to the DNA clades as designated by Dragoo et al. (2006), and cross hatching indicates distributional overlap of *P. melanotis* and *P. labecula*. The general distributions of species were determined by plotting the geographically marginal specimens reported in the following papers: Bowers et al. (1973), Allard and Greenbaum (1988), Hogan et al. (1993), Wike (1998), Zheng et al. (2003), Lucid and Cook (2004), Dragoo et al. (2006), Walker et al. (2006), Lucid and Cook (2007), Gering et al. (2009), Yang and Kenagy (2011), Domingues et al. (2012), Kalkvik et al. (2012), Natarajan et al. (2015), Greenbaum et al. (2017), Kingsley et al. (2017), Sawyer et al. (2017), and Kalkvik et al. (2018).

cifically, the combined results of Hogan et al. (1997), Chirhart et al. (2005) and Walker et al. (2006) indicated that *P. keeni* is sister to a clade including *P. sejugis* and *P. maniculatus* from Baja California (*P. m. coolidgei*) and southern California populations of *P. m. gambelii*; these *P. maniculatus* populations were elevated to species status as *P. gambelii* by Greenbaum et al. (2017). However, based on data from the mitochondrial ND3/ND4/ND4L genes, Greenbaum et al. (2017) found little difference in sequence divergence between *P. keeni* and *P. gambelii* (3.7%) and between *P. keeni* and *P. maniculatus* (3.8%) from eastern and northwestern California, Oregon, Washington, and Colorado (*P. m. austerus*, *artemisiae*, *rubidus*, *rufinus*, and *sonoriensis*). An analysis of single nucleotide polymorphisms (SNPs, Kingsley et al. 2017) provided no clarity, as it yielded a weakly supported association of *P. keeni* to a mixed variety of *P. maniculatus* subspecies from the western United States and Canada.

Peromyscus arcticus.—Phylogeographic analyses of deer mice from their northwestern-most range in the Yukon Territory, Canada (Wike 1998; Lucid and Cook 2007; Sawyer et al. 2017; Fig. 1) revealed the presence of a monophyletic clade distinct from *P. keeni* and *P. maniculatus*. Based on estimates of Kimura 2-parameter distances derived from cytochrome-*b* sequences, Lucid and Cook (2007) indicated that this clade was genetically equidistant from geographically proximate populations of *P. keeni* (4.32%) and *P. maniculatus* (4.56%). Wike (1998) and Lucid and Cook (2007) suggested that this third lineage represents an undescribed species for which the name *P. arcticus* (Wagner 1845) is available. Given the comparable sequence divergences among other species in this group, *P. arcticus* is recognized as a species in the *P. maniculatus* group.

Peromyscus sejugis.—*P. sejugis* is restricted to two small islands (Isla Santa Cruz and Isla San Diego) in the Gulf of California. Studies of mtDNA (Hogan et al. 1997; Hafner et al. 2001) and microsatellite (Chirhart et al. 2005) variation confirmed placement of *P. sejugis* in the *maniculatus* group but left the validity of this species open to question. Low mtDNA sequence divergence of *P. sejugis* relative to *P. maniculatus* from Baja California, as reported by Hogan et al. (1997), led Hafner et al. (2001) to speculate that additional sampling of *P. maniculatus* from Baja California would "demonstrate that *P. sejugis* should be included as a subspecies of *P. maniculatus*." Studies including *P. sejugis* and a geographic sampling of *P. maniculatus* from Baja California (Walker et al. 2006) and coastal western United States (Greenbaum et al. 2017) confirmed the close relationship between *P. sejugis* and deer mice from mainland Baja and southern California. Walker et al. (2006) considered a variety of factors in recommending retention of the specific status of *P. sejugis*. *Peromyscus sejugis* is larger than deer mice from mainland Baja California (Burt 1932), and both island populations of *P. sejugis* are fixed for a unique pericentric inversion of chromosome 13 (Smith et al. 2000). Despite a low level of mtDNA sequence divergence relative to deer mouse populations from mainland Baja California, *P. sejugis* populations have distinct mtDNA haplotypes (Walker et al. 2006; Greenbaum et al. 2017) and microsatellite alleles (Chirhart et al. 2005). Phylogenetic analyses of mtDNA and microsatellites consistently recover the two islands populations of *P. sejugis* as a single highly supported clade relative to deer-mouse populations from mainland Baja and southern California. Retention of the specific distinction of *P. sejugis* is further consistent with its restricted distribution and threatened status designated by the Government of Mexico (Alvarez-Castañeda 2001; Secretaría de Medio Amiente y Recursos Naturales SEMARNAT 2010).

Peromyscus gambelii.—Greenbaum et al. (2017) extended the work of Walker et al. (2006) in presenting a phylogeographic analysis of ND3/ND4/ND4L sequence variation for western deer mice from southern Baja California to Washington. These studies documented that deer mice from Baja California north to San Francisco Bay (corresponding to cytochrome-*b* clade 3, Fig. 1) comprise a phylogenetic lineage distinct from that including deer mice from eastern and northwestern California, Oregon, Washington, and Colorado. The mean nucleotide sequence divergence (based on p-distances) within these lineages was 0.8% and 0.9%, respectively, whereas that between these lineages was 3.7%. Considering that the San Francisco Bay and associated river drainages represent a physiographic boundary for numerous terrestrial genera and species and that these two lineages of deer mice occupy significantly different environmental spaces (Kalkvik et al. 2012), Greenbaum et al. (2017) referred the deer-mice from Baja and southern California to *P. gambelii*. As

such, *P. gambelii* includes all deer mouse populations previously recognized as *P. m. coolidgei* (Baja California) and those of *P. maniculatus gambelii* from south of the San Francisco Bay and west of the Sierra Nevada mountains (Fig. 1). The numerous insular subspecies of *P. maniculatus* (Hall 1981) along the Pacific coast of southern and Baja California are likely to prove to be representative of *P. gambelii.*

TAXONOMIC REVISIONS

The composite molecular data support the existence of paraphyly within *Peromyscus maniculatus* and call for a reevaluation of the specific integrity of the species. First noted by Lansman et al. (1983), patterns of variation of mtDNA restriction fragments identified five clonal assemblages representing the eastern states, northern Michigan, the central states, Texas-Mexico, and southern California. More recently, studies based on nucleotide sequences of the cytochrome-*b* gene (Dragoo et al. 2006; Gering et al. 2009; Kalkvik et al. 2012; Natarajan et al. 2015) identified six distinct clades distributed across the range of *P. maniculatus*. A study that included both cytochrome-*b* sequences and data from three nuclear genes recovered a corresponding pattern (Sawyer et al. 2017). These six clades (numbered according to Dragoo et al. 2006) and their general distributions (Fig. 1) are: 1) the Rocky Mountain states and including northern and central New Mexico, Washington, northern California and Michigan; 2) the Plains states; 3) the Pacific Coast including Southern and Baja California; 4) southern New Mexico and Mexico; 5) northeastern USA and eastern Canada; and 6) northeastern and north-central USA and south-central Canada.

Peromyscus labecula.—Several phylogeographic studies based on nucleotide sequences and single nucleotide polymorphisms or SNPs (Dragoo et al. 2006; Gering et al. 2009; Kalkvik et al. 2012; Natarajan et al. 2015; Kingsley et al. 2017) support the existence of an additional, geographically peripheral species within *P. maniculatus*. Each of these studies recovered a mtDNA lineage from southern New Mexico, southwestern Texas, and central Mexico to Oaxaca (clade 4, Fig. 1) as distinct and reciprocally monophyletic (posterior probabilities ranging between 92% and 100%) relative to the other mtDNA clades within *P. maniculatus* as well as to *P. keeni* and, when included in the phylogenetic analysis, to *P. melanotis*, *P. leucopus*, and *P. gossypinus*. The SNP analysis of Kingsley et al. (2017) recovered *P. m. blandus*, *P. m. fulvus*, and *P. m. labecula* as corresponding to mtDNA clade 4. Of these studies, only Gering et al. (2009) provided estimates of nucleotide divergence among the mtDNA clades within *P. maniculatus.* Nucleotide divergences (Kimura 2-parameter) estimated for cytochrome-*b* averaged 4.4% between the New Mexico/Mexico lineage (Clade 4) and the western/plains states lineages (clades 1 and 2). Clade 4 relative to the northeastern and north-central lineages (clades 5 and 6) averaged 3.5%. These values are consistent with estimates of ND3/ND4/ND4L gene divergence (Kimura 2-parameter) between *P. keeni* and western/central *P. maniculatus* (3.7%) and between *P. gambelii* and western/central *P. maniculatus* (3.8%, Greenbaum et al. 2017). Dragoo et al. (2006) suggested that the New Mexico/Mexico lineage (clade 4) might represent the formerly recognized species *P. blandus*. However, Kingsley et al. (2012) obtained a clade 4 cytochrome-*b* sequence for a specimen of *P. m. labecula* from Tepetitla, Tlaxcala, Mexico. The available data, both the phylogenetic and genetic species concepts (see review by Baker and Bradley 2006) and taxonomic priority, suggest that *P. labecula* (Elliot 1903, Fig. 1) represents a separate species that likely includes the subspecies *P. m. blandus*, *P. m. fulvus*, and *P. m. labecula*.

P. maniculatus and *P. sonoriensis*.—It is clear that the central issue for resolving the phylogeny and evolution of the *P. maniculatus* group requires addressing the residual paraphyly within *P. maniculatus*. Carleton (1989) recognized 67 subspecies of *P. maniculatus* with a composite distribution from the Atlantic to Pacific seaboards and from the Canadian taiga through south-central Mexico. Questions concerning the conspecificity of the various races of *P. maniculatus* date to Osgood (1909) and have historically centered on two distinctive morphological and ecological types, each of which comprises numerous subspecies (Blair 1950; Hooper 1968; Carleton 1989). Forest forms with long tails, large ears, and large hind feet range through the Appalachian Mountains, northeastern and boreal regions of Canada, and into the coastal forests of the western

United States. Grassland deer mice with short tails, small ears, and small feet generally occupy the prairies and grasslands of the continental interior and extend into the deserts of the western and southwestern United States. In regions where these forms come together, they generally maintain their morphological distinction and are assumed to not interbreed (Blair 1950; Hooper 1968). The genetic and morphological studies that resulted in the recognition of *P. keeni* (see Hogan et al. 1993 and references therein) confirmed the specific distinction of the northwestern-most forest form relative to *P. maniculatus,* and genome-wide SNP and mtDNA data partition the eastern and western forest forms of *P. maniculatus* into distinct and independently evolving clades (Kingsley et al. 2017). It does not appear, however, that the eastern forest forms are specifically distinct from eastern grassland forms. Nucleotide sequence divergence between the cytochrome-*b* lineages representing these forms was only 1.5% (Gering et al. 2009), and Kingsley et al. (2017) report successful reciprocal crossing (to the F2 generation) between corresponding short- and long-tailed forms of *P. maniculatus*. Carleton (1989) was apparently visionary when he wrote that "our tendency to pose the taxonomic dilemma of *maniculatus* as a species consisting of two contradistinctive sets of populations "the" long-tailed subspecies versus "the" short-tailed subspecies may mask the reticulate genealogical complexity of these organisms and hinder appreciation of the interrelationships and level of differentiation."

From their phylogeographic analysis, Dragoo et al. (2006) concluded that *P. maniculatus* (*sensu* Musser and Carleton 2005) is a complex of deeply divergent lineages and that the deepest genetic divergence is between the northeastern clades (5 and 6) and the central/western clades (1–4, Fig. 1). Each of the other sequence-based phylogeographic studies recovered the same strongly supported phylogenetic dichotomy between northeastern and central/western clades, and Gering et al. (2009) reported a mean nucleotide divergence of 3.9% between the two groups. Consistent with evolutionary independence of northeastern *P. maniculatus,* G- and C-banded karyotypes of deer mice representing five subspecies from the northeastern United States and eastern Canada (Myers Unice et al. 1998) indicated a unique (acrocentric or acrocentric with a heterochromatic short arm) condition of chromosome 10; all chromosomal homology analyses for central and western populations of *P. maniculatus* have reported the inverted and biarmed condition of chromosome 10 (Pathak et al. 1973; Murray and Kitchin 1976; Greenbaum et al. 1978a,b; Greenbaum and Reed 1984; Gunn and Greenbaum 1986; Gunn 1988; Hale and Greenbaum 1988a, b; Greenbaum et al. 1994; McAllister and Greenbaum 1997; Smith 1999).

Analysis of Northeastern versus Central/Western Clades of *P. maniculatus*

In an effort to contribute to the resolution of questions regarding the northeastern and central/western clades of *P. maniculatus,* mtDNA sequence variation (ND3/ND4/N4L) was analyzed from the populations reported by Myers Unice et al. (1998) and from a karyotypically characterized population from Kansas (McAllister and Greenbaum 1997). These sequences were compared to corresponding reference sequences from western/central populations of *P. maniculatus* and to reference sequences of *P. keeni*, *P. gambelii*, *P. sejugis*, *P. polionotus*, *P. melanotis*, and *P. leucopus.*

Materials and Methods

Specimens examined.—Specimens of northeastern *P. maniculatus* were live trapped from the following localities (Texas Cooperative Wildlife Collection (TCWC) accession and GenBank numbers in parentheses): CANADA: Ontario; 10 km N Moonbeam (*n*=3), 49.3432°N, 82.1541°W (56252–56254, MK122967); Quebec; 11.5 mi E of Havre-Saint-Pierre (*n*=3), 50.2418°N, 63.5986°W (59869, 59870, 59872, MK122965–MK122967). USA: Vermont;Washington Co., New Discovery Campground (*n*=10), 44.1987°N, 72.6973°W (56413,56415–56423, MK122965–MK122967, MK122971); Maine; Aroostook Co., Aroostook State Park (*n* =12), 46.6155°N, 68.0084°W (56385, 56398–56408, MK122966, MK122968–MK122970); Hancock Co., Mount Desert Island (*n* =1), 44.3924°N, 68.3021°W (56410, MK122970). Specimens representing central *P. maniculatus* (*n* =20) were live-trapped from 1 mi S, 2.3 mi W of Hayes, Ellis Co,

Kansas (56222, 56223, 56229–56232, 56234–56237, 56260, 56261, 56272–56279, 56328, and MK122972–MK122978). The capture and handling of animals followed the recommendations of Sikes et al. (2016).

Reference sequences were obtained from GenBank as follows: central/western *P. maniculatus*, Colorado, Gilpin Co. (U40250); California, Kern Co. (KC764393) and Humboldt Co. (KC764395); Oregon, Benton Co. (KC764399) and Harney Co. (KC764400); Washington, Gray's Harbor Co. (U40249) and Okanogan Co. (KC764408); *P. sejugis*, Isla San Diego, Baja California Sur (U40253); *P. gambelii*, Baja California, Mexico (DQ077697); *P. keeni*, Washington, Gray's Harbor Co. (U40062); *P. melanotis*, Durango, Mexico (U40247); and *P. polionotus*, South Carolina, Lexington Co. (U40254). A reference sequence for *P. leucopus*, Texas, Robertson Co. (U40252), was used as the outgroup in the phylogenetic analyses.

DNA isolation and sequencing.—The Sambrook et al. (1980) method was used to isolate total genomic DNA from liver and spleen tissues previously frozen at -80° C. A 1,439 base pair (bp) fragment containing the mitochondrial genes ND3/ND4L/ND4 as well as $tRNA^{Arg}$ and the 3' end of $tRNA^{Gly}$ were PCR (polymerase chain reaction) amplified following the techniques described in Arevalo et al. (1994). PCR primers included PI', Marg, ND4L, and Nap2, and amplifications were performed in a Perkin Elmer/Cetus DNA Thermal Cycler (Applied Biosystems, Foster City, California). Reaction conditions were as follows: 1 µL DNA (approximately 100 ng), 12.3 µL H_2O, 2.5 µL of 10X PCR Buffer II (PE Applied Biosystems), 2.5 µL of 25 mM $MgCl_2$, 0.5 µL BSA, 4 µL of 8 mM dNTPs (Amersham Pharmacia Biotech, Piscataway, New Jersey), 1.0 µL of forward and reverse primers, and 0.2 µL Takara *Taq* (TaKaRa, Japan). Conditions for PCR amplification were as follows: initial denaturation at 95° C for five min, followed by 35 cycles of 1 min each at 95° C (denaturation), 50° C (annealing), and 72° C (extension), and concluded with another extension cycle of 10 min at 72°C. Prior to sequencing, PCR amplification products were purified using Exonuclease I in combination with shrimp alkaline phosphatase (ExoSAP-IT, Affymetrix Inc., Santa Clara, California), and excess dye was removed using DyeEx spin columns (Qiagen, Germantown, Maryland).

Sequencing reactions were performed with a Big Dye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems) in a Perkin Elmer/Cetus DNA Thermal Cycler, following the protocol recommended by the supplier. Prior to sequencing, DyeEx 2.0 spin columns (Qiagen Valencia, California) were used to remove excess dye. Sequencing was performed in an Applied Biosystems 377 automated sequencer. All PCR fragments were sequenced in both directions, and sequence contigs were produced using Sequencher 4.1.1 (Gene Codes, Ann Arbor, Michigan). Sequences were aligned by eye.

Phylogenetic analyses.—PAUP* version 4.0b10 (Swofford 2002) was used to compute p-distances for estimation of nucleotide divergence among the major groups identified by the phylogenetic analyses. Maximum parsimony (MP) was conducted in PAUP* and Bayesian inference (BI) was performed in Mr. Bayes version 3.2.6 (Ronquist et al. 2012). Using the Akaike Information Criterion (Akaike 1974), both the jModel Test 2.1.10 (Darriba et al. 2012) and the model test in PAUP* identified K81uf + I + G as being the most suitable substitution model for the BI analysis.

Maximum parsimony employed the branch and bound search option with 5,000 bootstrap replications. All characters were unordered with equal weights, and there were 143 parsimony-informative characters. Bayesian inference involved two separate runs, each consisting of four chains (1 cold and 3 hot), 10 million generations sampled every 1,000 generations with a 25% burn-in. The standard split frequency was 0.00, and convergence to a stationarity distribution was observed based on the analysis in TRACER version 1.7.1 (Rambaut et al. 2018). Posterior probabilities of branch support were obtained from a 50% majority rule tree.

Results

Estimates of sequence divergences (uncorrected p-distances) for the analysis of ND3/ND4/N4L variation are presented in Table 2. Sequence divergence among northeastern samples was 0.5% and among the individuals from the central (Kansas) population was 0.9%. Divergence between the central and western reference sequences was 1.7%. These values are consistent with those reported (Greenbaum et al. 2017) for

Table 2. Mean sequence divergences (uncorrected p-distances) between the ND3/ND4/ND4L mtDNA haplotypes of the *Peromyscus* reference sequences (*P. gambelii*, *P. keeni*, *P. polionotus*, *P. melanotis*, and *P. leucopus*) and those of the eastern, central, and western populations sampled. Northeast refers to deer mice from Canada (Quebec and Ontario), Maine, and Vermont. Central refers to deer mice from Kansas, and western refers to deer mice from Colorado, California, Oregon, and Washington. Specific localities, collections, and GenBank numbers are listed in the Materials and Methods.

	sejugis	*gambelii*	*keeni*	western	central	northeast	*polionotus*	*melanotis*
gambelii	0.017							
keeni	0.036	0.038						
western	0.041	0.040	0.043	0.010				
central	0.043	0.042	0.046	0.017	0.009			
northeast	0.044	0.044	0.048	0.037	0.036	0.005		
polionotus	0.046	0.046	0.054	0.040	0.041	0.043		
melanotis	0.070	0.069	0.079	0.075	0.076	0.075	0.069	
leucopus	0.134	0.136	0.138	0.140	0.135	0.130	0.127	0.134

intraspecific variation of ND3/ND4/ND4L for western populations currently recognized as *P. maniculatus* (0.9%) and *P. gambelii* (0.8%). Mean sequence divergence between the northeast and central/western populations was 3.7%; this is consistent with that between *P. keeni* and *P. gambelii* (3.8%) as well as between *P. keeni* and western populations currently recognized as *P. maniculatus* (4.3%). Mean divergence between the central/western populations and *P. keeni*, *P. gambelii*, *P. sejugis*, and *P. polionotus* was 4.2% and between the northeastern population and these species was 4.5%. The western/central and northeastern populations were essentially equidistant from *P. melanotis* (7.6% and 7.5%, respectively).

Maximum parsimony (MP) and Bayesian Inference (BI) analyses of the data (Fig. 2) recovered several well-supported monophyletic groups including: 1) a clade containing *P. sejugis*, *P. gambelii*, and *P. keeni* (consistent with Greenbaum et al. 2017); 2) a western clade containing haplotypes from Colorado, Oregon, northern California, and Washington; 3) a central clade representing haplotypes from Kansas; 4) monophyly of a clade containing both the western and central lineages; and 5) a northeastern clade representing haplotypes from eastern Canada and the northeastern United States. These groups are similar to those previously identified by other phylogenetic studies (Dragoo et al. 2006; Gering et al. 2009; Kalkvik et al. 2012; Natarajan et al. 2015; Sawyer et al. 2017). Although the MP bootstrap and posterior probability are somewhat lower, these groups appear monophyletic relative to *P. polionotus* and *P. melanotis*. With exception of monophyly of a western/central clade, the phylogenetic relationships among these lineages were not well-resolved, resulting in a trichotomy. The analyses did provide strong support for the basal position of *P. melanotis* as well as placement of *P. polionotus* as sister to the other members of the *P. maniculatus* group.

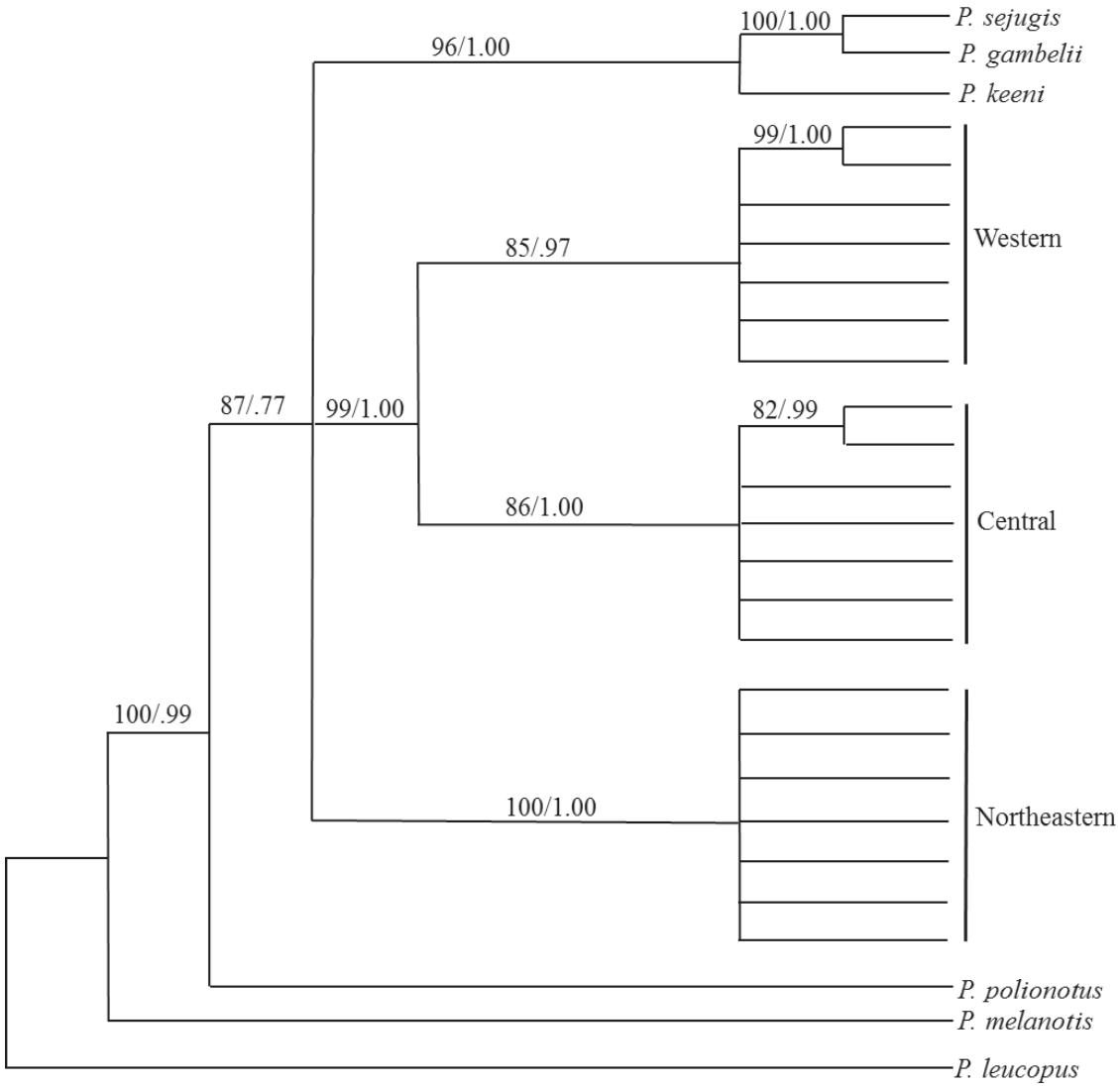

Figure 2. Maximum parsimony tree derived from sequence variation (ND3/ND4/ND4L) for the northeastern, central and western samples of *Peromyscus maniculatus* and reference sequences for *P. sejugis*, *P. gambelii*, *P. keeni*, *P. polionotus*, *P. melanotis*, and *P. leucopus*. Numbers associated with the branches are maximum parsimony bootstrap values and Bayesian posterior probabilities. Locality and GenBank references are given in the Materials and Methods.

DISCUSSION

Dragoo et al. (2006) noted that the type locality of *P. maniculatus* is from the Moravian Settlements in Labrador (Wagner 1845) and treated this taxon as restricted to eastern Canada and southward through the eastern United States. They correspondingly suggested that the central/western mtDNA clades of *P. maniculatus* might represent the previously described taxon *P. sonoriensis* (Le Conte 1853). Given the magnitude of sequence divergence (Table 2) and the consistent phylogenetic and apparent karytoypic dichotomy between the eastern and central/western mtDNA clades, the results of this study support the suggestions of Dragoo et al. (2006) and the recognition of *P. sonoriensis* as specifically distinct from *P. maniculatus* (Figs. 1 and 2). With

the other recommendations herein, the *P. maniculatus* species group is expanded to nine species (Table 3).

Table 3. Composition of the *P. maniculatus* species group as recognized by Carleton (1989) relative to changes in the number of species (recognized herein) resulting from the inclusion of chromosomal and molecular data. Carleton considered the conspecificity of all populations of *P. maniculatus* suspect and only tentatively included *P. slevini*. The indented species were formerly included as part of *P. maniculatus*.

Carleton 1989	This paper
P. slevini	
P. melanotis	*P. melanotis*
P. polionotus	*P. polionotus*
P. sejugis	*P. sejugis*
P. maniculatus	*P. maniculatus*
P. oreas	*P. keeni*
	P. articus
	P. gambelii
	P. labecula
	P. sonoriensis

Although the data supporting the specific recognition of *P. sonoriensis* and *P. maniculatus* include a broad sampling of deer mice from across the United States, large geographic expanses and many subspecies have not been correspondingly sampled. In addition to refining the distributional limits of *P. sonoriensis* and *P. maniculatus*, the specific affinity of many subspecies traditionally assigned to *P. maniculatus* (Hall 1981) will need to be investigated and evaluated. As with the mtDNA lineages within *P. maniculatus*, the genetic distances of the central and western lineages of *P. sonoriensis* (1.7% Gering et al. 2009; 1.2% Table 2) do not warrant taxonomic recognition. Based on the inferred geographic distributions (Fig. 1), it is suggested that the following subspecies formerly assigned to *P. maniculatus* (Hall 1981) be referred to *P. sonoriensis: alpinus*, *artemisiase*, *austerus*, *bairdii*, *borealis*, *gunnisoni*, *hollisteri*, *inclarus*, *luteus*, *nebrascensis*,*ozarkiarum, pallescens*, *rubidus*, *rufinus*, *saxamans*, *serratus*, and *sonoriensis.* Correspondingly, *P. maniculatus* would retain the subspecies: *abietorium*, *anticostiensis*, *argentatus*, *bairdii*, *eremus*, *gracilis*, *maniculatus*, *nubiterrae*, and *plumbeus.*

EVOLUTIONARY HISTORY

Previous studies concluded that the *P. maniculatus* species group evolved by peripheral isolation from a central stock (*maniculatus*-like ancestor) in response to effects of Pleistocene glaciation (Blair 1950; Bowers et al. 1973; Greenbaum et al. 1978; Carleton 1989). Given that the central and western range of deer mice corresponds to *P. sonoriensis*, it is most likely that *P. sonoriensis* is the modern-day remnant of the *P. maniculatus*-group central stock. Based on phylogenetic analyses of chromosomal banding data (Greenbaum et al. 1978; Robbins and Baker 1981; Stangl and Baker 1984), *P. melanotis* and *P. polionotus* are the most divergent lineages of the *P. maniculatus* species group, with the former being the most basal lineage. The basal position of *P. melanotis* has been widely supported by analyses of mtDNA sequences (Hogan et al. 1997; Walker et al. 2006; Gering et al. 2009; Kalkvik et al. 2012; Natarajan et al. 2015; Greenbaum et al. 2017; this paper; Fig. 2) and microsatellites (Chirhart et al. 2005). Overall the genetic data and the geographic distribution of *P. melanotis* (Fig. 1) support the hypothesis that this species was the earliest isolate off the *P. maniculatus*-group central stock.

The molecular data for *P. polionotus* are contradictory and fail to resolve its evolutionary derivation. Phylogenetic analysis of the ND3/ND4/ND4L sequences (Fig. 2) support the cytosystematic hypothesis (Greenbaum et al. 1978; Robbins and Baker 1981; Stangl and Baker 1984) in placing *P. polionotus* as the second most divergent lineage in the *P. maniculatus*-group. This hypothesis infers that *P. polionotus* diverged from the geographic central stock prior to the divergence of *P. sonoriensis* and *P. maniculatus*. Phylogeographic analysis of cytochrome-*b* sequences (Kalkvik et al. 2012; Natarajan et al. 2015) associated *P. polionotus* with samples referable to *P. sonoriensis*, and sequences of COIII-ND3 (Kingsley et al. 2017)

clustered *P. polionotus* to northeastern subspecies (*gracilis* and *nubiterrae*) of *P. maniculatus*. None of the above phylogenetic associations were strongly supported and each corresponds to a reasonable evolutionary hypothesis. It is noteworthy, however, that *P. polionotus*, *P. melanotis* (Greenbaum et al. 1978), and the northeastern populations of *P. maniculatus* (Myers-Unice et al. 1998) share the plesiomorphic condition of chromosome 10.

Although there can be little question that *P. sejugis* shares common ancestry with *P. gambelii*, the phylogeographic association of *P. keeni* and *P. gambelii* is less readily explained. Chirhart et al. (2005) postulated two alternative geographic scenarios relevant to the evolutionary history of *P. keeni* and *P. gambelii*. An "ancestral continuity" hypothesis proposes that these species diverged (north and south) from a common ancestor that occupied a Pacific coastal range after having been isolated from the geographic central stock. Alternatively, *P. gambelii* and *P. keeni* may have originated as independent peripheral isolates. In the latter case, the genetic similarities (Table 2) and apparent sister-group relationship between *P. gambelii* and *P. keeni* (Fig. 2) would be an artifact of coincidental founder effects and genetic drift. Despite the highly supported relationship between *P. keeni* and *P. gambelii*, the ancestral continuity hypothesis is unsupported by geography and the distributions of these species. Additionally, phylogeographic studies of *P. keeni* (Zheng et al. 2003; Lucid and Cook 2004; Sawyer et al. 2017) consistently support its isolation in Pleistocene refugia in coastal British Columbia and/or southeastern Alaska, and Greenbaum et al. (2017) cite physiographic and zoogeographic data that support the hypothesis that the San Francisco Bay and associated river drainages were the northern boundary of a southern California/Baja California refugium. As such, the results of this study support the conclusion of Greenbaum et al. (2017) that independent peripheral isolation is the more likely scenario for the evolution of *P. keeni* and *P. gambelii*. Although Sawyer et al. (2017) recovered sequences referable to *P. arcticus* as sister to those of *P. keeni*, too little else is known about the former to support its derivation as an isolate of the latter as opposed to its being an independent isolate of the geographic central stock.

All sequence analyses that included populations referable to *P. labecula* identified it as a distinct clade, but inferences of its phylogenetic association were inconsistent. Despite having included sequences from many of the same individuals, analyses of cytochrome-*b* variously but weakly linked *P. labecula* to a *keeni*, *sonoriensis*, *gambelii* clade (Dragoo et al. 2006); the northeast *maniculatus* clades (Gering et al. 2009); an unresolved trichotomy including *P. maniculatus*, *P. polionotus*, and *P. sonoriensis*/*gambelii* (Kalkvik et al. 2012); and as sister to *P. articus* (Natarajan et al. (2015). From sequences of COIII-ND3, Kinglsey et al. (2017) recovered *P. labecula* as basal to clades including *P. keeni*/*gambelii*, *P. polionotus*/*maniculatus*, and *P. sonoriensis*.

Most taxonomically significant, all sequence-based studies and the relevant chromosomal data support the specific and phylogenetic distinction of *P. sonoriensis* and *P. maniculatus* (Fig. 1). Absent inclusion of the complicating taxa *P. arcticus* and *P. labecula*, the ND3/ND4/ND4L analysis recovered *P. sonoriensis* and *P. maniculatus* as reciprocally monophyletic clades (Fig. 2). The level of sequence divergence (Gering et al. 2009, Table 2) and the distribution of *P. sonoriensis* and *P. maniculatus* support the hypothesis that during the Pleistocene glacial maximum the *P. maniculatus* group central stock was divided east and west by the Mississippi River. The modern distribution of these species would then have resulted from northward expansion following glacial recession. It is apparent that the inconsistency of inferences of the phylogeographic and phylogenetic history of the peripheral species *P. polionotus*, *P. arcticus*, and *P. labecula* reflects their recent and relatively contemporaneous divergence from the geographic central stock before or after divergence of *P. sonoriensis* and *P. maniculatus*. In particular, the phylogenetic inconsistency is apparently the result of the relative rate of the evolution of the mtDNA genes and the short internode branch lengths obtained. Detailed resolution of the evolutionary history of the *P. maniculatus* species group awaits greater sampling and analyses of characters with a more appropriate rate of evolution.

ACKNOWLEDGMENTS

The authors gratefully acknowledge E. A. Schall for producing the map figure and J. E. Light for assistance with the data. We also thank two anonymous reviewers for their helpful comments. Two of the authors (IFG and RLH) are former graduate students of R. J. Baker, and we wish to acknowledge his contributions to our academic growth. He remains an inspiration to both of us.

LITERATURE CITED

Akaike, H. 1974. A new look at the statistical model identification. IEEE. Transactions on Automatic Control 19:716–723.

Allard, M. W., and I. F. Greenbaum. 1988. Morphological variation and taxonomy of chromosomally differentiated *Peromyscus* from the Pacific Northwest. Canadian Journal of Zoology 66:2734–2739.

Allard, M. W., S. J. Gunn, and I. F. Greenbaum. 1987. Mensural discrimination of chromosomally characterized *Peromyscus oreas* and *P. maniculatus*. Journal of Mammalogy 68:402–406.

Alvarez-Castañeda, S. T. 2001. Peromyscus sejugis. Mammalian Species 658:1–2.

Álvarez-Castañeda, S. T., and P. C. Cortés-Calva. 2002. Peromyscus slevini. Mammalian Species 705:1–2.

Arevalo, E., S. K. Davis, and J. W. Sites, Jr. 1994. Mitochondrial DNA sequence divergence and phylogenetic relationships among eight chromosome races of the *Sceloporus grammicus* complex (Phrynosomatidae) in Central Mexico. Systematic Biology 43:387–418.

Baker, R. J., and R. D. Bradley. 2006. Speciation in mammals and the genetic species concept. Journal of Mammalogy 87:643–662.

Bangs, O. 1897. A new white-footed mouse from British Columbia. The American Naturalist 31:74–75.

Blair, W. F. 1950. Ecological factors in speciation of *Peromyscus*. Evolution 4:253–275.

Bowers, J. H. 1974. Genetic compatibility of *Peromyscus maniculatus* and *Peromyscus melanotis*, as indicated by breeding studies and morphometrics. Journal of Mammalogy 55:720–737.

Bowers, J. H., R. J. Baker, and M. H. Smith. 1973. Chromosomal, electrophoretic and breeding studies of selected populations of deer mice (*Peromyscus maniculatus*) and black-eared mice (*P. melanotis*). Evolution 27:378–386.

Bradley, R. D., and R. J. Baker. 2001. A test of the genetic species concept: cytochrome-*b* sequences and mammals. Journal of Mammalogy 82:960–973.

Bradley, R. D., N. D. Durish, D. S. Rogers, and J. R. Miller. 2007. Toward a molecular phylogeny for *Peromyscus*: evidence from mitochondria; cytochrome-*b* sequences. Journal of Mammalogy 88:1146–1159.

Bradley, R. D., C. W. Edwards, D. S. Carroll, and C. W. Kilpartick. 2004. Phylogenetic relationships of neotomine-peromyscine rodents: based on DNA sequences from the mitochondrial cytochrome-*b* gene. Journal of Mammalogy 85:389–395.

Burt, W. H. 1932. Description of heretofore unknown mammals from islands in the Gulf of California, Mexico. Transactions of the San Diego Society of Natural History 7:161–182.

Carleton, M. D. 1989. Systematics and evolution. Pp. 7–141 in Advances in the study of *Peromyscus* (Rodentia) (G. L. Kirkland Jr., and J. N. Layne, eds.). Texas Tech University Press, Lubbock.

Carleton, M. D., and T. E. Lawlor. 2005. *Peromyscus* from Santa Catalina Island, Sea of Cortez, Mexico: taxonomic identities and biogeographic implications. Journal of Mammalogy 86:814–825.

Chirhart, S. E., R. Arianpour, R. L. Honeycutt, and I. F. Greenbaum. 2001. Mitochondrial DNA sequence variation and the specific identification of deer mice (*Peromyscus*) from Triangle Island, British Columbia. Canadian Journal of Zoology 79:2257–2260.

Chirhart, S. E., R. L. Honeycutt, and I. F. Greenbaum. 2005. Microsatellite variation and evolution in the *Peromyscus maniculatus* species group. Molecular Phylogenetics and Evolution 34:408–415.

Cornejo-Latorre, C., P. Cortés-Calva, and S. T. Álvarez-Castañeda. 2017. The evolutionary history of the subgenus *Haplomylomys* (Cricetidae: *Peromyscus*). Journal of Mammalogy 98:1627–1640.

Cowan, I. M., and C. J. Guiget. 1965. The mammals of British Columbia. Handbook No. 11. British Columbia Provincial Museum, Victoria, British Columbia, Canada.

Darriba. D., G. L. Taboada. R. Doallo, and D. Posada. 2012. jModelTest 2: more models, new heuristics and parallel computing. Nature Methods 9:772

Degner, J. F., I. J. Stout, J. D. Roth, and C. L. Parkinson. 2007. Population genetics and conservation of the threatened southeastern beach mouse (*Peromyscus polionotus niveiventris*): subspecies and evolutionary units. Conservation Genetics 8:1441–1452.

Domingues, V. S., Y. Poh, B. K. Peterson, P. S. Pennings, J. D. Jensen, and H. E. Hoekstra. 2012. Evidence of adaptation

from ancestral variation in young populations of beach mice. Evolution 66:3209–3223.

Dragoo, J. W., J. A. Lackey, K. E. Moore, E. P. Lessa, J. A. Cook, and T. L. Yates. 2006. Phylogeography of the deer mouse (*Peromyscus maniculatus*) provides a predictive framework for research on hantaviruses. Journal of General Virology 87:1997–2003.

Durish, N. D., K. E. Halcomb, C. W. Kilpatrick, and R. D. Bradley. 2004. Molecular systematics of the *Peromyscus truei* species group. Journal of Mammalogy 85:1160–1169.

Elliot, D. G. 1903. A list of a collection of Mexican mammals with descriptions of some apparently new forms. Field Columbian Museum Publication 71, Zoology Series 3:141–149.

Engle, S. R., K. M. Hogan, J. F. Taylor, and S. K. Davis. 1998. Molecular systematics and paleobiogeography of South American sigmodontine rodents. Molecular Biology and Evolution 15:35–49.

Galtier, N., B. Nabholz, S. Glémin, and G. D. D. Hurst. 2009. Mitochondrial DNA as a marker of molecular diversity: a reappraisal. Molecular Ecology 18:4541–4550.

Gering, E. J., J. C. Opazo, and J. F. Storz. 2009. Molecular evolution of cytochrome *b* in high- and low-altitude deer mice (genus *Peromyscus*). Heredity 102:226–235.

Greenbaum, I. F., R. J. Baker, and J. H. Bowers. 1978a. Chromosomal homology and divergence between sibling species of deer mice: *Peromyscus maniculatus* and *Peromyscus melanotis* (Rodentia, Cricetidae). Evolution. 32:334–341.

Greenbaum, I. F., R. J. Baker, and P. R. Ramsey. 1978b. Chromosomal evolution and the mode of speciation in three species of *Peromyscus*. Evolution 32:646–654.

Greenbaum, I. F., S. E. Chirhart, M. L. Walker, and R. L. Honeycutt. 2017. Molecular phylogenetics of western deer mice (*Peromyscus*): taxonomic and biogeographic implications. The Southwestern Naturalist 62:126–134.

Greenbaum, I. F., et al. 1994. Cytogenetic nomenclature of deer mice, *Peromyscus* (Rodentia): revision and review of the standardized karyotype. Cytogenetics and Cell Genetics 6 :181–195.

Greenbaum, I. F., and M. J. Reed. 1984. Evidence for heterosynaptic pairing of the inverted segment in pericentric inversion heterozygotes of the deer mouse (*Peromyscus maniculatus*). Cytogenetics and Cell Genetics 38:106–111.

Gunn, S. J. 1988. Chromosomal variation and differentiation among insular populations of *Peromyscus* from the Pacific Northwest. Canadian Journal of Zoology 66:2726–2733.

Gunn, S. J., and I. F. Greenbaum. 1986. Systematic implications of karytoypic and morphological variation in mainland *Peromyscus* from the Pacific Northwest. Journal of Mammalogy 67:294–304.

Hafner, D. J., B. R. Riddle, and S. T. Álvarez-Castañeda. 2001. Evolutionary relationships of white-footed mice (*Peromyscus*) on islands in the Sea of Cortéz, Mexico. Journal of Mammalogy 82:775–790.

Hale, D.W., and I. F. Greenbaum. 1988a. Chromosomal pairing in deer mice heterozygous for the presence of heterochromatic short arms. Genome 30:44–47.

Hale, D. W., and I. F. Greenbaum. 1988b. Synapsis of a chromosomal pair heterozygous for a pericentric inversion and the presence of a heterochromatic short arm. Cytogenetics and Cell Genetics 48:55–57.

Hall, E. R. 1981. The mammals of North America, second edition. John Wiley and Sons, New York, New York.

Hogan, K. M., S. K. Davis, and I. F. Greenbaum. 1997. Mitochondrial-DNA analysis of the systematic relationships within the *Peromyscus maniculatus* species group. Journal of Mammalogy 78:733–743.

Hogan, K. M., M. C. Hedin, H. S. Koh, S. K. Davis, and I. F. Greenbaum. 1993. Systematic and taxonomic implications of karytoypic, electrophoretic, and mitochondrial-DNA variation in *Peromyscus* from the Pacific Northwest. Journal of Mammalogy 74:819–831.

Hooper, E. T. 1968. Classification. Pp. 27-74 in Biology of *Peromyscus* (Rodentia) (J. A. King, ed.). Special Publication of the American Society of Mammologists 2:1–593.

Kalkvik, H. M., I. J. Stout, T. J. Doonan, and C. L. Parkinson. 2012. Investigating niche and lineage diversification in widely distributed taxa: phylogeography and ecology niche modeling of the *Peromyscus maniculatus* species group. Ecography 35:54–64.

Kalkvik, H. M., I. J. Stout, E. A. Hoffman, and C. L. Parkinson. 2018. Colonization and divergence: phylogeography genetics of the Atlantic coast beach mouse. Systematics and Biodiversity DOI: 10.1080/14772000.2018.1486339.

Kingsley, E. P., K. M. Kozak, S. P. Pfeifer, D. Yang, and H. E. Hoekstra. 2017. The ultimate and proximate mechanisms driving the evolution of long tails in forest deer mice. Evolution 71(2):261–273.

Lansman, R. A., J. C. Avise, C. F. Aquadro, J. F. Shapiro, and S. W. Daniel. 1983. Extensive genetic variation in mitochondrial DNA's among geographic populations of the deer mouse, *Peromyscucs maniculatus*. Evolution 37:1–16.

Le Conte, J., C. Girard, W. H. Harvey, and J. W. Bailey. 1853. Description of three new species of American Arcicolae, with remarks upon some other American rodents. Proceedings of the Academy of Natural Sciences of Philadelphia 6:404-432.

Lucid, M. K., and J. A. Cook. 2004. Phylogeography of Keen's mouse (*Peromyscus keeni*) in a naturally fragmented landscape. Journal of Mammalogy 85:1149–1159.

Lucid, M. K., and J. A. Cook. 2007. Cytochrome-*b* haplotypes suggest an undescribed *Peromyscus* species from the Yukon. Canadian Journal of Zoology 85:916–919.

Maillaird, J. 1924. A new mouse (*Peromyscus slevini*) from the Gulf of California. Proceedings of the California Academy of Sciences 12:1219–1222.

Martínez-Coronel, M., J. Ramírez-Pulido, and T. Álvarez. 1991. Variación intrapoblacional de *Peromyscus melanotis* (Rodentia: Muridae) en el Eje Volcánico Transverso, México. Acta Zoológica Mexicana, nueva serie 47:1–51.

McAllister B. F., and I. F. Greenbaum. 1997. How common are common fragile sites: variation of aphidicolin-induced chromosomal fragile sites in a population of the deer mouse (*Peromyscus maniculatus*). Human Genetics 100:182–188.

Miller J. R., and M. D. Engstrom. 2008. The relationships of major lineages within peromyscine rodents: a molecular phylogenetic hypothesis and systematic reappraisal. Journal of Mammalogy 89:1279–1295.

Murray, J. D., and R. M. Kitchin. 1976. Chromosomal variation and heterochromatin polymorphisms in *Peromyscus maniculatus*. Experientia 32:307–309.

Musser, G. G., and M. D. Carleton. 2005. Superfamily Muroidea. Pp. 894–1531 in Mammal species of the world: a taxonomic and geographic reference, 3rd ed. (D. E. Wilson and D. M. Reeder, eds.). Johns Hopkins University Press, Baltimore, Maryland.

Myers Unice S. M., D. W. Hale, and I. F. Greenbaum. 1998. Karyotypic variation in populations of deer mice (*Peromyscus maniculatus*) from eastern Canada and the northeastern United States. Canadian Journal of Zoology 76:584–588.

Natarajan, C., et al. 2015. Intraspecific polymorphism, interspecific divergence and the origins of function-altering mutations in deer mouse hemoglobin. Molecular Biology and Evolution 32:978–997.

Osgood, W. H. 1909. Revision of the mice of the American genus *Peromyscus*. North American Fauna No. 28:1–285.

Pathak, S., T. C. Hsu, and F. E. Arrighi. 1973. Chromosomes of *Peromyscus* (Rodentia, Cricetidae) IV. The role of heterochromatin in karyotypic evolution. Cytogenetics and Cell Genetics 12:315–326.

Pengilly, D., G. H. Jarrell, and S. D. MacDonald. 1983. Banded karyotypes of *Peromyscus sitkensis* from Baranof Island, Alaska. Journal of Mammalogy 64:682–685.

Platt II, R. N., B. R. Amman, M. S. Keith, C. W. Thompson, and R. D. Bradley. 2015. What is *Peromyscus*? Evidence from nuclear and mitochondrial DNA sequences suggests the need for a new classification. Journal of Mammalogy 96:708–719.

Rambaut A., A. J. Drummond, D. Xie, G. Baele, and M. A. Suchard. 2018. Posterior summarisation in Bayesian phylogenetics using Tracer 1.7. Systematic Biology 67:901–904.

Riddle, B. R., D. J. Hafner, and L. F. 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 17:145-160.

Robbins, L. W., and R. J. Baker. 1981. An assessment of the nature of chromosomal rearrangements in 18 species of *Peromyscus* (Rodentia: Cricetidae). Cytogenetics and Cell Genetics 31:194–202.

Rogers, D. S., and M. D. Engstrom. 1992. Evolutionary implications of allozymic variation in tropical *Peromyscus* of the *mexicanus* group. Journal of Mammalogy 73:55–69.

Ronquist, F., et al. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61:539–542.

Sambrook, D. L., E. F. Fritsch, and T. Maniatis. 1989. Molecular cloning: a laboratory manual. Second edition. Cold Spring Harbor Press, Cold Spring Harbor, New York.

Sawyer, Y. E., M. J. Flamme, T. S. Jung, S. O. MacDonald, and J. A. Cook. 2017. Diversification of deermice (Rodentia: genus *Peromyscus*) at their north-western range limit: genetic consequences of refugial and island isolation. Journal of Biogeography 44:1572–1585.

Secretaría de Medio Ambiente y Recursos Naturales (SEMARNAT). 2010. Norma Oficial Mexicana NOM-059-ECOL-2010, Protección de especies nativas de México de flora y fauna silvestres-Categorías de riesgo y especificaciones para su inclusión, exclusión o cambio-Lista de especies en riesgo. Diario Oficial de la Federación, México, December 30th:1–78. http://www.profepa.gob.mx/innovaportal/file/435/1/NOM_059_SEMARNAT_2010.pdf.

Sheppe, W., Jr. 1961. Systematics and ecological relations of *Peromyscus oreas* and *P. maniculatus*. Proceedings of the American Philosophical Society 105:421–446.

Sikes, R. S., and the Animal Care and Use Committee of the American Society of Mammalogists. 2016. 2016 Guidelines of the American Society of Mammalogists for the use of wild mammals in research and education. Journal of Mammalogy 97:663−688.

Smith, L. R. 1999. Phenotypic and genetic heterogeneity between sympatric deer mice from Washington State. Ph.D. dissertation, Texas A&M University, College Station.

Smith, L. R., D. W. Hale, and I. F. Greenbaum. 2000. Systematic implications of chromosomal data from two insular species of *Peromyscus* from the Gulf of California. Journal of Heredity 91:162–165.

Stangl, F. B., and R. J. Baker. 1984. Evolutionary relationships in *Peromyscus*: congruence in chromosomal, genic and classical data sets. Journal of Mammalogy 65:643–654.

Sullivan, J., K. E. Holsinger, and C. Simon. 1995. Among-site rate variation and phylogenetic analysis of 12S rRNA in

sigmodontine rodents. Molecular Biology and Evolution 12:988-1001.

Sullivan, R. M., S. W. Calhoun, and I. F. Greenbaum. 1990. Geographic variation in genital morphology among insular and mainland populations of *Peromyscus maniculatus* and *Peromyscus oreas*. Journal of Mammalogy 71:48–58.

Swofford, D. L. 2002. PAUP*: phylogenetic analysis using parsimony and other methods. Version 4.0b10. Sinauer Associates, Sunderland, Massachusetts.

Taylor, Z. S., and M. G. Hoffman. 2012. Microsatellite genetic structure and cytonuclear discordance in naturally fragmented populations of deer mice (*Peromyscus maniculatus*). Journal of Heredity 103:71–79.

Tiemann-Boege, I., C. W. Kilpatrick, D. J. Schmidly, and R. D. Bradley. 2000. Molecular phylogenetics of the *Peromyscus boylii* species group (Rodentia: Muridae) based on mitochondrial cytochrome *b* sequences. Molecular Phylogenetics and Evolution 16:366–378.

Thomas, B. 1973. Evolutionary implications of karytoypic variation in some insular *Peromyscus* from British Columbia, Canada. Cytologia 38:485–495.

Van Zant, J. L., and M. C. Wooten. 2007. Old mice, young islands and competing biogeographical hypotheses. Molecular Ecology 16:5070–5083.

Wagner, A. 1845. Diagnosen einiger neuen Arten von Nagren und Handflüglern. Archiv für Naturgeschichte 11:148.

Walker, M. L., S. E. Chirhart, A. F. Moore, R. L. Honeycutt, and I. F. Greenbaum. 2006. Genealogical concordance and the specific status of *Peromyscus sejugis.* Journal of Heredity 97:340–345.

Wike, M. J. 1998. Mitochondrial-DNA variation among populations of *Peromyscus* from Yukon, Canada and southeastern Alaska. M.S. thesis. Texas A&M University, College Station.

Yang, D.-S., and G. J. Kenagy. 2009. Nuclear and mitochondrial DNA reveal contrasting evolutionary processes in populations of deer mice (*Peromyscus maniculatus*). Molecular Ecology 18:5115–5125.

Yang, D.-S., and G. J. Kenagy. 2011. Population delimitation across contrasting evolutionary clines in deer mice (*Peromyscus maniculatus*). Ecology and Evolution 1:26–36.

Zheng, X., B. S. Arbogast, and G. J. Kenagy. 2003. Historical demography and genetic structure of sister species: deermice (*Peromyscus*) in the North American temperate rain forest. Molecular Ecology 12:711–724.

Addresses of authors:

IRA F. GREENBAUM

Department of Biology
Texas A&M University
College Station, TX 77843-3258 USA
i-greenbaum@tamu.edu

RODNEY L. HONEYCUTT

Natural Science Division
Pepperdine University
Malibu, CA 90263 USA
rodney.honeycutt@pepperdine.edu

SCOTT E. CHIRHART

Department of Biology
Centenary College of Louisiana
2911Centenary Boulevard
Shreveport, LA 71134 USA
schirhar@centenary.edu

Suggested citation format:

Greenbaum, I. F., R. L. Honeycutt, and S. E. Chirhart. 2019. Taxonomy and phylogenetics of the *Peromyscus maniculatus* species group. Pp. 559–575 in From field to laboratory: a memorial volume in honor of Robert J. Baker (R. D. Bradley, H. H. Genoways, D. J. Schmidly, and L. C. Bradley, eds.). Special Publications, Museum of Texas Tech University 71:xi+1–911.