

Cytochrome-*b* haplotypes suggest an undescribed *Peromyscus* species from the Yukon

M.K. Lucid and J.A. Cook

Abstract: *Peromyscus maniculatus* (Wagner, 1845) and *Peromyscus keeni* Merriam, 1897 are two species of deer mouse currently recognized in the Yukon. Phylogenetic analyses (Kimura two-parameter and maximum parsimony) of cytochrome-*b* sequences (560 base pairs) from deer mouse specimens ($n = 4$) collected near Haines Junction, Yukon, resulted in a monophyletic clade genetically distant from the two currently recognized species. We suggest that the Haines Junction specimens may represent a previously undescribed peromyscine species. *Peromyscus arcticus* is an available name.

Résumé : *Peromyscus maniculatus* (Wagner, 1845) et *Peromyscus keeni* Merriam, 1897 sont deux espèces de souris à pattes blanches actuellement reconnues au Yukon. Des analyses phylogénétiques (distance à deux paramètres de Kimura et parcimonie maximale) des séquences du cytochrome-*b* de spécimens ($n = 4$) de souris à pattes blanches récoltés près de Haines Junction, Yukon, ont révélé l'existence d'un clade monophylétique à distance génétique égale des deux espèces actuellement reconnues. Nous croyons que les spécimens de Haines Junction représentent peut-être une espèce encore inédite de péromyscinés. *Peromyscus arcticus* est un nom disponible pour cette espèce.

[Traduit par la Rédaction]

Introduction

Taxonomic designations of peromyscine rodents have historically been complicated, particularly along the north Pacific coast (NPC) of North America. Recent molecular and morphological studies generally agree that at least two distinct species, *Peromyscus keeni* Merriam, 1897 and *Peromyscus maniculatus* (Wagner, 1845), occur in southeast Alaska, USA, and Yukon, Canada (Hogan et al. 1993; Wike 1998; Zheng et al. 2003; Lucid and Cook 2004; Walker 2005). Furthermore, *P. maniculatus* along the NPC may be genetically divergent from *P. maniculatus* in other parts of North America (Dragoo et al. 2006).

Wike (1998) sampled extensively throughout central Yukon, northern British Columbia, and northern southeast Alaska. Using sequencing and restriction fragment length polymorphisms of the ND3/ND4-L/ND4 mitochondrial DNA (mtDNA) region, she found a peromyscine lineage that did not associate phylogenetically with *P. maniculatus* or *P. keeni*, suggesting a previously unidentified species. Evidence of this unique lineage extended from Sulphur Lake, Yukon, southeast to Kluane National Park and Annie Lake, Yukon, Canada.

The objective of this study was to independently assess

the taxonomic status of deer mice from one of Wike's (1998) unpublished study localities using a different mtDNA region.

Materials and methods

Four specimens (independent of those analyzed by Wike 1998), collected near Haines Junction (60.83833°N, 137.32917°W), Yukon, were obtained from the University of Alaska mammal collection and sequenced. Mitochondrial DNA sequences from sister species *P. keeni* ($n = 7$) and *P. maniculatus* ($n = 13$) were collected and sequenced or obtained from GenBank. Sequencing of a 560 base pair region of the mtDNA cytochrome-*b* (*cytb*) gene followed protocols outlined in Lucid and Cook (2004).

Additionally, *cytb* sequences ($n = 16$) from nine other peromyscine species were obtained from GenBank. Sampling of *P. maniculatus* and *P. keeni* was designed to encompass a large portion of the species' ranges to account for geographic variability in sequences. Sampling of more distantly related species was designed to provide a comparison of genetic distance both within species and between sister species. Specimen numbers and collection localities are listed in Table A1 of Appendix A. American Society of

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Table 1. Average genetic distances for selected comparisons of *Peromyscus* taxa.

Comparison	Average genetic distance (%)
Within species	
Haines Junction	1.08
<i>P. keeni</i>	2.1
<i>P. maniculatus</i>	2.1
<i>P. melanotis</i>	3.88
<i>P. beatae</i>	2.75
<i>P. boylii</i>	0.36
<i>P. levipes</i>	2.81
<i>P. furvus</i>	0.72
Between sister species	
Haines Junction/ <i>P. keeni</i>	4.32
Haines Junction/ <i>P. maniculatus</i>	4.56
<i>P. keeni</i> / <i>P. maniculatus</i>	4.92
<i>P. beatae</i> / <i>P. levipes</i>	6.07
<i>P. boylii</i> / <i>P. simulus</i>	2.19
<i>P. gratus</i> / <i>P. truei</i>	9.47
Between Haines Junction, <i>P. keeni</i>, and <i>P. maniculatus</i> haplotypes, respectively	
<i>P. melanotis</i>	5.90, 4.97, 5.48
<i>P. beatae</i>	14.22, 12.64, 13.47
<i>P. boylii</i>	13.27, 12.31, 12.56
<i>P. levipes</i>	15.27, 14.24, 14.90
<i>P. simulus</i>	12.62, 12.19, 12.27
<i>P. gratus</i>	12.71, 12.73, 13.45
<i>P. truei</i>	14.79, 14.06, 14.74
<i>P. leucopus</i>	10.16, 10.10, 9.36
<i>P. furvus</i>	12.61, 10.66, 11.21

Note: Distances were estimated using the Kimura two-parameter model of evolution (Kimura 1980).

Mammalogists guidelines for animal treatment were adhered to (Animal Care and Use Committee 1998).

The program PAUP* (Swofford 2000) was used to generate unrooted neighbor-joining (NJ) and maximum parsimony (MP) trees. Bootstrap analyses (Felsenstein 1985) were used to evaluate nodal support.

The Kimura (1980) two-parameter model was selected to generate the NJ tree (bootstrapped 1000 iterations) so that distance values could be used to assess levels of genetic divergence among species of *Peromyscus* following the criteria outlined in Bradley and Baker (2001).

MP analysis consisted of a heuristic search with tree bisection–reconnection branch-swapping bootstrapped 1000 iterations. All phylogenetically uninformative characters were excluded and transitions and transversions were equally weighted.

Results

Cytb sequences from Haines Junction ($n = 4$) revealed three haplotypes that formed a monophyletic clade apart from other sequences in the analyses. The Haines Junction sequences exhibited a base composition expected for mammals (Irwin et al. 1991). Nucleotide composition of the Haines Junction sequences was 32% adenine, 30% thymine, 24% cytosine, and had an overall deficit of guanine (14%).

The average genetic distance value within the Haines Junction haplotypes was 1.08% and ranged from 0.36% to 2.81% within other species examined (Table 1). Distance

values between sister species ranged from 2.19% to 9.47% and were similar to those described by Bradley and Baker (2001) in a comparable analysis. The Haines Junction haplotypes diverged least from *P. keeni* (4.32%) and *P. maniculatus* (4.56%).

Both NJ and MP trees showed nearly identical topologies (Fig. 1). The Haines Junction haplotypes formed a monophyletic clade with strong bootstrap support in both analyses (NJ = 100, MP = 100). Strong bootstrap support was also shown for monophyletic clades of *P. keeni* (NJ = 99, MP = 97) and *P. maniculatus* (NJ = 99, MP = 94). The Haines Junction clade associated most closely to the *P. keeni* clade, but this was not supported by bootstrap values. An association between *P. maniculatus* and *P. keeni* was also not supported by bootstrap values.

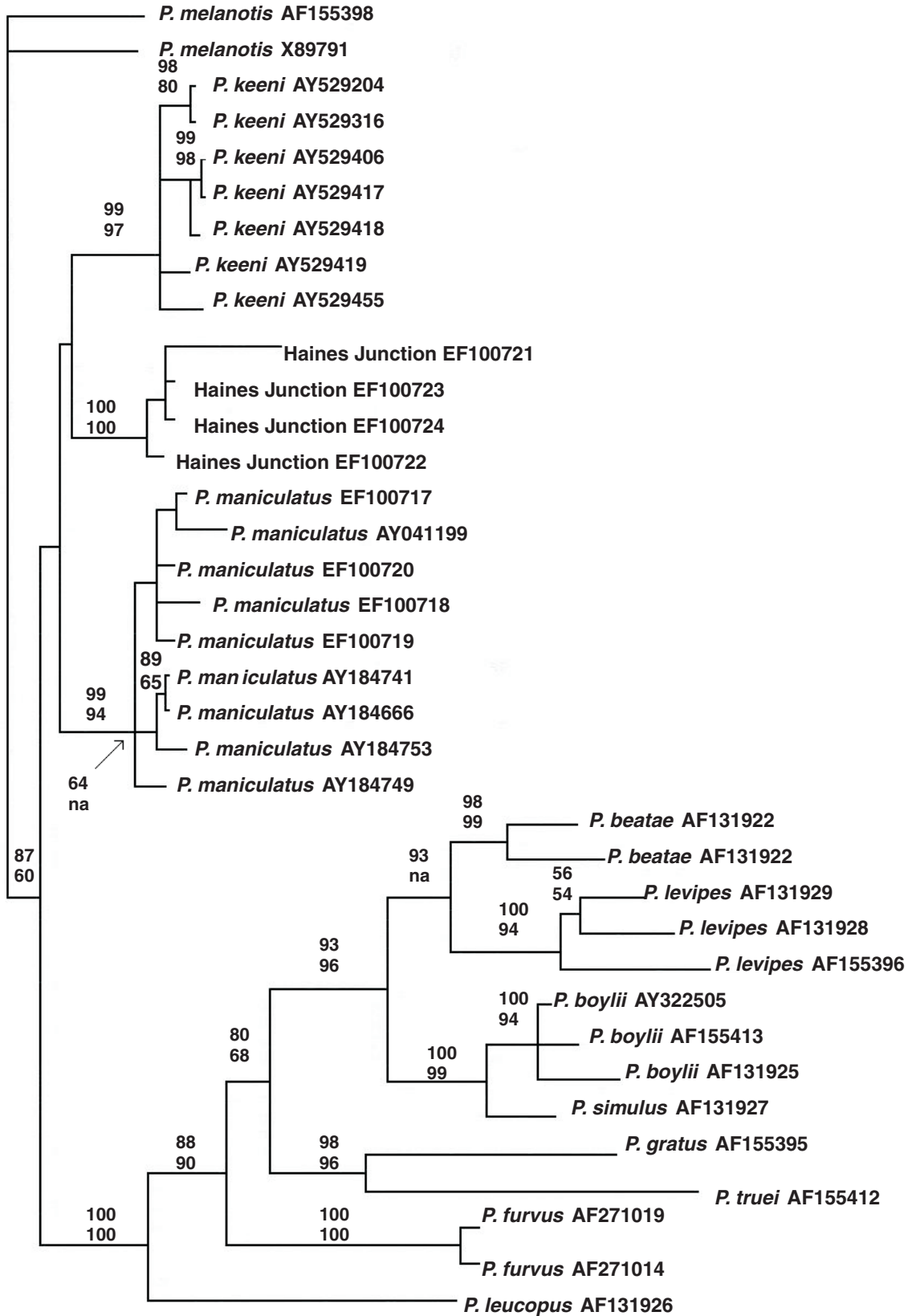
Discussion

The Haines Junction cytb haplotypes confirm Wike's (1998) unpublished findings by consistently forming strongly supported monophyletic clades in phylogenetic analyses. Additionally, distance values between the Haines Junction clade and *P. maniculatus* and *P. keeni* were similar to other sister species (Table 1). Thus, it may be appropriate to recognize these samples as a new taxon. Wike (1998) suggested *Peromyscus arcticus* as an available name.

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Fig. 1. Neighbor-joining (NJ) tree based on Kimura two-parameter model distances calculated for taxa included in this study. A nearly identical topology resulted when data were analyzed using maximum parsimony (MP) (heuristic search with tree bisection–reconnection branch-swapping) analysis. Numbers above branches indicate bootstrap values based on 1000 iterations (upper numbers for the NJ analysis and lower numbers for the MP analysis). MP nodes not supported by bootstrap analysis are noted with “na”. Reference numbers next to taxon names correspond with GenBank accession numbers found in Table A1 of Appendix A.



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Appendix A

Table A1. Specimens examined.

Specimen	Sample size (<i>n</i>)	Collection locality	GenBank accession Nos.
—	4	Haines Junction, Yukon	EF100721–EF100724; AF12937–AF12940
<i>Peromyscus maniculatus</i>	4	Atomic City, Idaho	EF100717–EF100720; IF396, IF398–IF400
	1	Castro County, Texas	AY041199
	1	Okanogan, Washington	AY184753
	1	Tillamook, Oregon	AY184749
	2	Atlin, British Columbia	AY184741, AY184666
<i>Peromyscus keeni</i>	1	Gravina Island, Southeast Alaska	AY529204
	1	Dall Island, Southeast Alaska	AY529316
	1	Skagway, Southeast Alaska	AY529455
	1	Stikine River, British Columbia	AY529406
	3	Whitehorse, Yukon	AY529417–AY529419
<i>Peromyscus beatae</i> Thomas, 1903	2		AF131916, AF131922
<i>Peromyscus boylii</i> (Baird, 1855)	3		AY322505, AY131925; AF155413
<i>Peromyscus furvus</i> J.A. Allen and Chapman, 1897	2		AF271014, AF271019
<i>Peromyscus gratus</i> Merriam, 1898	1		AF155395
<i>Peromyscus leucopus</i> (Rafinesque, 1818)	1		AF131926
<i>Peromyscus levipes</i> Merriam, 1898	3		AF131928, AF131929, AF155396
<i>Peromyscus melanotis</i> J.A. Allen and Chapman, 1897	2		AF155398; X89791
<i>Peromyscus simulus</i> Osgood, 1904	1		AF131927
<i>Peromyscus truei</i> (Shufeldt, 1885)	1		AF155412

Note: The 36 Haines Junction, *P. maniculatus*, and *P. keeni* sequences examined are listed below by collection locality and GenBank accession numbers. Sequences not previously published are followed by Alaska frozen tissue collection (AF) or Idaho State University frozen tissue collection (IF) numbers. Other *Peromyscus* sequences are listed by GenBank accession numbers only.