

Note

First evidence of White-footed Deer Mouse (*Peromyscus leucopus*) on mainland New Brunswick, Canada

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Abstract

White-footed Deer Mouse (*Peromyscus leucopus*) and the closely related, and more northerly ranging, Deer Mouse (*Peromyscus maniculatus*) broadly overlap in distribution and are often difficult to distinguish from each other. Based on molecular genetic data (cytochrome *b* gene), we report two new distribution records for *P. leucopus* for New Brunswick, Canada, the first mainland localities for this species in the province. Previous sampling of *Peromyscus* in New Brunswick may have overlooked the presence of *P. leucopus*, possibly because the specimens collected were all assumed to be *P. maniculatus*. However, current detection in New Brunswick may be part of a broader recent northward range expansion documented to be underway in *P. leucopus*. Although our use of a single mitochondrial gene to identify *P. leucopus* does not eliminate the possibility that the New Brunswick specimens are of hybrid origin, our results support the presence of *P. leucopus* in New Brunswick and suggest more detailed analyses will be required to determine the nature of any genetic interaction between *P. leucopus* and *P. maniculatus* in the province. Recognition of morphologically cryptic *Peromyscus* in southern New Brunswick also emphasizes the need to incorporate comprehensive methods to ensure the correct identification of specimens of this genus in Maritime Canada. We also note the potential implications of this discovery with respect to the incidence of Lyme disease in New Brunswick.

Key words: Distributional range; *Peromyscus*; White-footed Mouse; New Brunswick distribution; Lyme disease

White-footed Deer Mouse (*Peromyscus leucopus*) is one of several species of Nearctic rodents in the speciose genus *Peromyscus*. The species tolerates variable environmental conditions, but is most abundant in warm, wooded-shrubby habitats (Kaufman *et al.* 1983). Compared with the closely related Deer Mouse (*Peromyscus maniculatus*), *P. leucopus* has a less northward-ranging distribution in eastern Canada; the extent of the species' northern range is believed to occur across southern Ontario and Quebec, with a disjunct Maritime population confined to Nova Scotia (Hall 1981; Forbes *et al.* 2010).

Recent studies have documented *P. leucopus* in new localities in northeastern North America, which suggests the species is undergoing a northward range expansion, perhaps in response to climate warming (Roy-Dufresne *et al.* 2013; Fiset *et al.* 2015; Garcia-Elfring *et al.* 2017). Huynh *et al.* (2021) recently

documented the presence of *P. leucopus* on Grand Manan Island, based on specimens taken in 2011 and identified via molecular genetic methods. Those vouchers represented the first New Brunswick reports and emphasized the need to establish whether the species was present on the adjacent mainland. Here we report the first evidence for *P. leucopus* on mainland New Brunswick, likewise supported by molecular genetic data, and discuss wildlife management implications of this information.

In 2013–2014, *Peromyscus* spp. were collected from various localities throughout New Brunswick using museum special snap traps (Woodstream Corporation, Lititz, Pennsylvania, purchased from Forestry Suppliers, Inc., Jackson, Mississippi, USA) and Sherman live traps (BioQuip Products, Inc., Rancho Dominguez, California, USA). Mice were collected from several trap lines of 100–125 traps deployed

at ~5-m intervals in microhabitats (e.g., entrance to burrows, runways) that appeared suitable for *Peromyscus*. All specimens ($n = 92$) were prepared as traditional museum vouchers (skin and skull), with tissues extracted and preserved in 95% ethanol and archived in the New Brunswick Museum frozen tissue collection.

Genomic DNA was extracted from subsamples of frozen tissues at the Canadian Rivers Institute Genomics Laboratory using an OMEGA DNA extraction kit (Omega Bio-tek, Inc., Norcross, Georgia, USA). DNA samples were subsequently stored in elution buffer (Tris) and archived at -80°C . The entire cytochrome *b* gene (1143 base pairs) for almost all specimens was amplified via polymerase chain reaction (PCR) using primers MVZ05 (Smith and Patton 1993) and PERO3' (Tiemann-Boege *et al.* 2000). The PCR thermal profile consisted of the following: initial denaturation at 95°C for 2 min, followed by 35 cycles of denaturation at 95°C for 1 min, annealing at 51°C for 1 min, and extension at 72°C for 2 min, with a final extension at 72°C for 7 min. PCR products were subjected to electrophoresis on a 1% agarose gel and then viewed on a molecular imager (ChemiDoc XRS+ Gel Imaging System, Bio-Rad Laboratories, Montréal, Quebec, Canada) to confirm successful amplification of the target gene.

The PCR products were then shipped to Genome Quebec for Sanger sequencing. Resulting sequences were aligned (using ClustalW, a standard general purpose software program for aligning nucleotide sequences) and proofed using the program MEGAX (Kumar *et al.* 2018); chromatograms were examined to verify all base changes. Sequences were then input into BLAST (Basic Local Alignment Search Tool, developed by the National Center for Biotechnology Information) to ascertain species identity (i.e., *P. maniculatus* or *P. leucopus*) and to compare with other *Peromyscus* sequences. Among the 85 samples sequenced (seven of the original 92 did not produce suitable PCR product), two specimens were identified as *P. leucopus*: an adult, lactating, female, 172 mm in total length, trapped 28 May 2014 at Blacks Harbour, Charlotte County (45.059°N , 66.785°W ; NBM-MA-13000) and an adult male with testes 9 mm and total length 159 mm, trapped 6 August 2014 at Lake Utopia, Charlotte County (45.170°N , 66.794°W ; NBM-MA-14183; Figure 1). NBM-MA-13000 was collected concurrently with *P. maniculatus*, Red-backed Vole (*Myodes gapperi*), and Masked Shrew (*Sorex cinereus*), while NBM-MA-14183 was the sole specimen collected at the Lake Utopia site. The remaining 83 specimens were determined to be *P. maniculatus*. Sequences for the two vouchers of *P.*

leucopus were deposited in GenBank: OK263085 and OK263086, respectively.

Range expansion of *P. leucopus* at the species' northeastern range limit has been reported in the northern Great Lakes (Myers *et al.* 2009; Moscarella 2011), in southern Quebec (Garcia-Elfring *et al.* 2017), and in adjacent Maine (Bennett 2020). Such expansion has been attributed mainly to anthropogenic activity, including habitat modification and climate change (Roy-Dufresne *et al.* 2013; Leo and Milien 2017).

The Blacks Harbour and Lake Utopia specimens are the first evidence that *P. leucopus* is present on mainland New Brunswick. Lake Utopia is ~14.5 km north of Blacks Harbour, suggesting that the species is established, at minimum, in the southwestern region of New Brunswick. Mainland New Brunswick records are about 90 km northeast of coastal Maine reports from Mount Desert Island (Bennett 2020) and about 56 km northeast of Great Wass Island (Rich 1993). Mount Desert Island is just 300 m offshore and connected to the mainland by a causeway; Great Wass Island, although about 5 km offshore, is likewise connected to the mainland by a series of causeways that link adjacent islands. New Brunswick records are about 485 km east of the nearest confirmed Quebec records (Fiset *et al.* 2015) and about 350 km west by land to the nearest Nova Scotia occurrences for *P. leucopus* (Naughton 2012).

Huynh *et al.* (2021) reported *P. leucopus* on Grand Manan Island, but it is unclear how or when the species colonized and established itself there, i.e., historical natural dispersal and (or) recent human transport. Blacks Harbour is the northern terminus for ferries that serve as a daily connection between Grand Manan Island and mainland New Brunswick, ferrying passengers, vehicles, and goods year-round. It is feasible that the ferries are an accessible vector for point of dispersal for *Peromyscus*. However, it is possible that *P. leucopus* has been present on both Grand Manan and the adjacent mainland for some time but has been previously undetected. This could be because the species occurs at very low densities in the region and has not been collected in the past (Rich 1993) or, more likely, because it has been assumed that all specimens encountered are *P. maniculatus* and appropriate methods to identify *P. leucopus* have not been used (see Rich *et al.* 1996). It is also possible that *P. leucopus* has been present on Grand Manan, as in Nova Scotia, as a relict population and has only recently recolonized southwestern mainland New Brunswick as part of an apparently recent and now well-documented northward range expansion (Fiset *et al.* 2015).

Garcia-Elfring *et al.* (2017) noted gene flow between *P. leucopus* and *P. maniculatus* via secondary

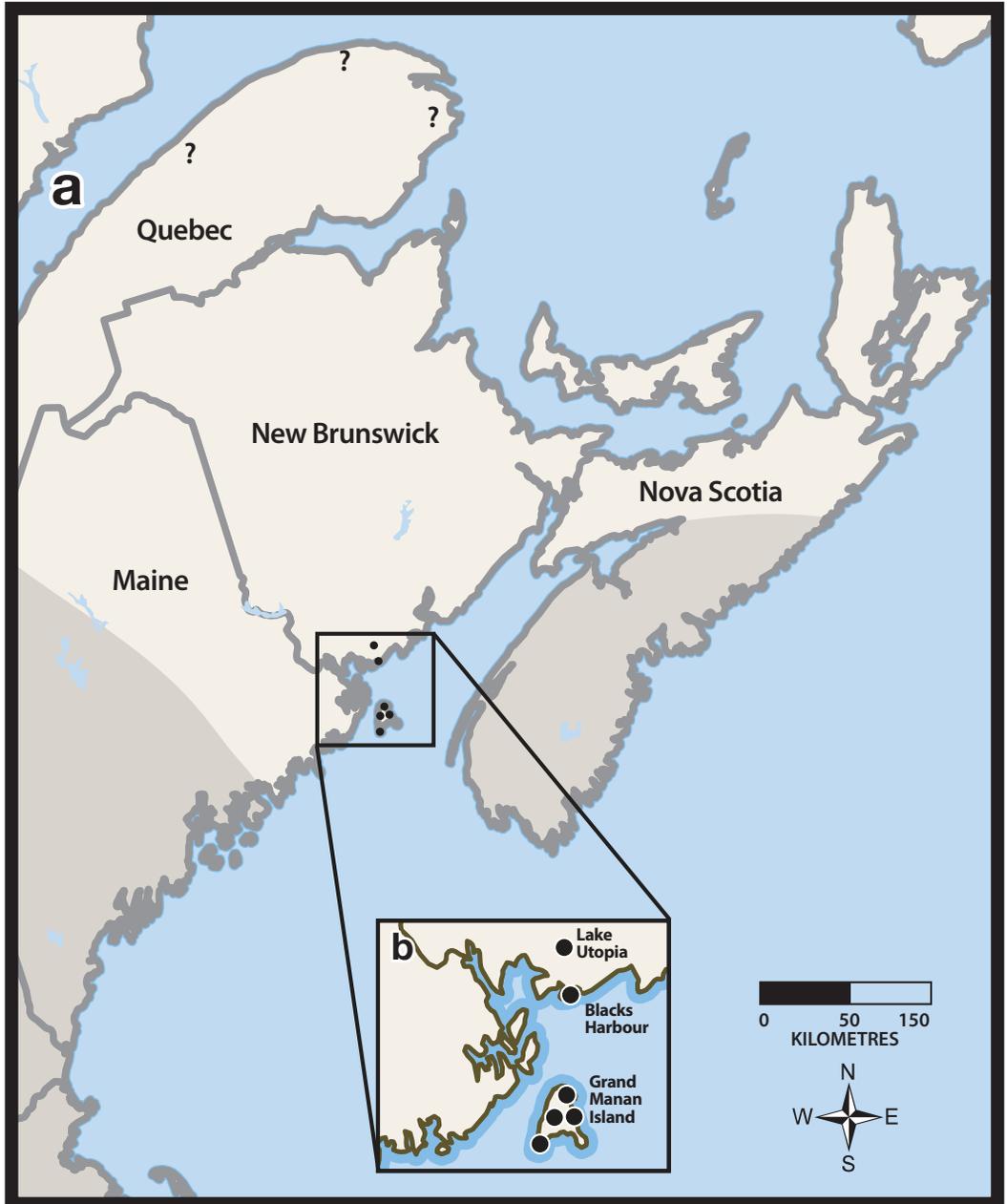


FIGURE 1. a. Range of White-footed Deer Mouse (*Peromyscus leucopus*) in Quebec (?) and Nova Scotia and Maine (shaded area). b. Closed circles mark recent localities for *P. leucopus* in New Brunswick: Grand Manan Island (Huynh *et al.* 2021), Blacks Harbour, and Lake Utopia. Unconfirmed Gaspé localities are from Desrosiers *et al.* (2002).

contact in some populations in southern Quebec, resulting in apparent hybridization and introgression at extremely low frequencies ($n = 5$ in a sample of 238). Likewise, working in the same region, Leo and Millien (2017) report low frequencies of apparent

hybridization ($n = 5-8$ out of 153, depending on method of analysis) among *P. leucopus* and *P. maniculatus*. Vrla (2019) used genetic (including sequencing of the cytochrome *b* gene) and morphometric methods to separate *P. leucopus* and *P. maniculatus* in

western Oklahoma, identifying a series of *Peromyscus* that are putative hybrids.

Although our use of a single mitochondrial gene to identify *P. leucopus* does not eliminate the possibility that the New Brunswick specimens are of hybrid origin, we believe the probability is low. Previous studies suggest that both pre- and post-zygotic mechanisms ensure that these species are normally well isolated reproductively (e.g., see Leo and Millien 2017 and references therein). Leo and Millien (2017) concluded that the low rate of apparent hybridization appeared to justify their use of the mtDNA COIII gene to separate *P. leucopus* and *P. maniculatus*, but they noted that recorded natural hybridization between these two congeners may warrant more comprehensive identification methods. Evidence is accumulating that where *P. leucopus* is undergoing range expansion (perhaps associated with climate change), pre-zygotic barriers with *P. maniculatus* may be altered, and hybridization at low rates may occur (Garcia-Elfring *et al.* 2017; Vrla 2019). Although our results support the presence of *P. leucopus* in New Brunswick, more detailed analyses will be required to determine the true nature of any genetic interaction between these species in the province.

Regardless of when *P. leucopus* became established in New Brunswick, the occurrence of the species in the province may have relevance to wildlife management and human health. Although both *P. leucopus* and *P. maniculatus* are considered competent host reservoirs for *Borrelia burgdorferi*, the spirochete bacterium that causes Lyme disease, there is evidence that *P. leucopus* may be the more competent of the two (Donahue *et al.* 1987; Garman *et al.* 1994; Fiset *et al.* 2015). *Peromyscus leucopus* also appears to be the preferred host species among rodents for ticks (*Ixodes scapularis*) that transmit *B. burgdorferi* (Schmidt *et al.* 1999). Thus, the apparent geographic expansion of *P. leucopus*, alongside the concurrent range expansion of Lyme disease in Canada (Ogden *et al.* 2008), may have an impact on the health of human communities in New Brunswick, as has been suggested for southern Quebec (Fiset *et al.* 2015).

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