

# A reclassification of red squirrels, *Tamiasciurus hudsonicus* (Rodentia: Sciuridae), on Isle Royale

ALEANA R. SAVAGE<sup>1</sup>, PHILIP J. MANLICK<sup>1\*</sup>, MARK C. ROMANSKI<sup>2</sup> and JONATHAN N. PAULI<sup>1</sup>

<sup>1</sup>Department of Forest and Wildlife Ecology, University of Wisconsin – Madison, 1630 Linden Drive, Madison, WI 53706, USA

<sup>2</sup>Isle Royale National Park, National Park Service, 800 East Lakeshore Drive, Houghton, MI 49931, USA

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Isle Royale (western Lake Superior, USA) is a geographically isolated archipelago with the potential to sustain endemic populations. The island has served as an ecological model for predator–prey interactions between wolves and moose, but little is known about the 17 other mammalian inhabitants. Previous studies of locally abundant red squirrels (*Tamiasciurus hudsonicus*) described phenotypic differences in pelage colour and skull size, resulting in the classification of an endemic Isle Royale subspecies (*T. h. regalis*). However, the phylogenetics of this putative subspecies have not been explored. We sequenced mitochondrial DNA to compare haplotypes and genetic variation of seven red squirrels from Isle Royale to 42 other individuals sampled across North America. We detected 25 haplotypes in total, and four haplotypes within Isle Royale. Sequences obtained from red squirrels inhabiting Isle Royale were not unique and were shared with individuals from across the Great Lakes Region and north-eastern North America. Our results indicate that extant *T. hudsonicus* on Isle Royale are polyphyletic and should not be considered a unique subspecies. These findings suggest that Isle Royale is less insular and more connected than previously assumed and highlights the need to investigate the origins of other taxa in putatively isolated systems, including Isle Royale.

ADDITIONAL KEYWORDS: ecological conservation – endemic – island colonization – polyphyly – subspeciation.

## INTRODUCTION

Isle Royale National Park is a remote and isolated archipelago in western Lake Superior, USA. It is located approximately 24 km from mainland in Ontario, 35 km from Minnesota and 80 km from the Keweenaw Peninsula of Michigan (Romanski & Belant, 2008). The archipelago has been isolated for >10 000 years, resulting in phylogenetically unique plant and animal taxa (Kowal *et al.*, 2011). While the vast majority of the Isle Royale archipelago (hereafter Isle Royale) is currently designated as wilderness and widely viewed as insulated from anthropogenic change (Radeloff *et al.*, 2015), Isle Royale was historically subject to numerous human disturbances. For instance, copper mining, commercial logging and unregulated harvest led to the extirpation of multiple species, including the Canada lynx (*Lynx canadensis*) and woodland caribou (*Rangifer tarandus*) (Mech, 1966; Bick *et al.*, 1985;

Licht *et al.*, 2015). There are currently 19 extant species of mammals on Isle Royale and there is heightened interest in restoring its communities and ecosystem function (National Park Service, 2018). Nevertheless, little is actually known about the phylogenetic history of these extant taxa, limiting our ability to develop ecologically informed targets for conservation (Manlick *et al.*, 2018).

The red squirrel (*Tamiasciurus hudsonicus*) is one of the most abundant mammals on Isle Royale and is presumed to be an isolated and naturally colonized population (Kramm *et al.*, 1975). Initially described as its own species (*Tamiasciurus regalis*) based on body size and coloration (Howell, 1936), it was later recognized as one of the 25 subspecies of *T. hudsonicus* (*T. h. regalis*; Kramm *et al.*, 1975; Steele, 1998). Kramm *et al.* (1975) noted that red squirrels on Isle Royale had smaller cranial measurements than those of Michigan and Ontario, in addition to different dorsal tail coloration. Similarly, the Mount Graham red squirrel (*T. h. grahamensis*), an

\*Corresponding author. E-mail: [pmanlick@wisc.edu](mailto:pmanlick@wisc.edu)

endangered subspecies in the sky islands of Arizona, was initially identified via pelage characteristics and later confirmed as a subspecies via mitochondrial DNA analysis (Fitak *et al.*, 2013). The phylogenetics of red squirrels on Isle Royale, however, have not been assessed, and it is increasingly recognized that subspecific designation requires morphological differences supported by genetic data (Sackett *et al.*, 2014; Collinson *et al.*, 2017). Moreover, previous morphological analyses only compared red squirrels on Isle Royale to populations in Ontario, Canada, and the Upper Peninsula of Michigan, USA, despite the strong dispersal power of red squirrels (Pauli, 2005; Hope *et al.*, 2016). Ultimately, characterizing the dynamics and genetic diversity of insular or geographically isolated populations such as Isle Royale or Mount Graham red squirrels is essential to understanding and managing biodiversity (Lomolino, 1986; Fitak *et al.*, 2013).

To determine whether red squirrels on Isle Royale represent a unique taxonomic group, we tested the monophyly of red squirrels on Isle Royale by comparing their phylogenetic relatedness to red squirrels across North America. We hypothesized that red squirrels on Isle Royale were an endemic lineage and predicted that Isle Royale individuals would exhibit reciprocal monophyly with mainland populations. Furthermore, red squirrels, like many North American species, are broadly represented by eastern and western clades (Arbogast *et al.*, 2001), and we predicted that Isle Royale red squirrels would fall within the eastern clade.

## METHODS

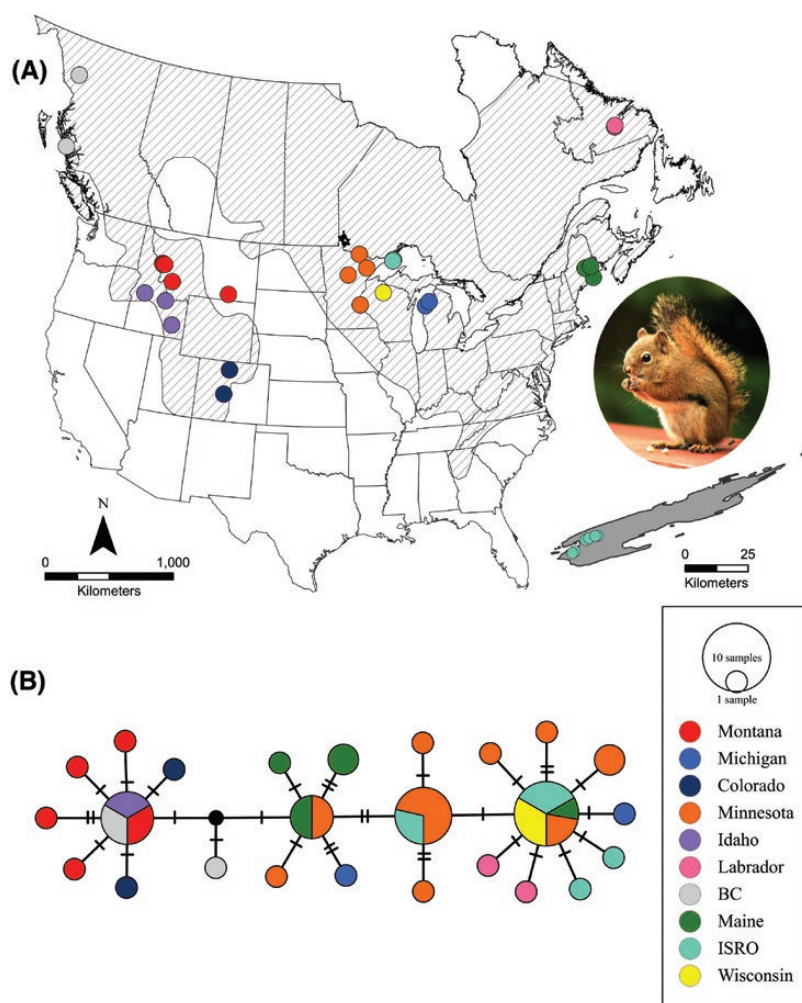
We analysed red squirrel samples collected opportunistically on the main island of Isle Royale National Park from 2017 to 2018, combined with samples collected by the Minnesota Department of Natural Resources and by Carlson *et al.* (2014). We also supplemented our sampling with archived sequences, all from the continental clade identified by Hope *et al.* (2016) (Table 1; Appendix). All sampling was approved by the National Park Service and University of Wisconsin Animal Care and Use Committees (MWR\_ISRO\_Romanski\_Marten\_2017.A3, A01486) and conducted under the guidelines established by the American Society of Mammalogists (Sikes, 2016). We isolated DNA from high-quality tissue from the inner thigh or tail of all samples following standard, commercially available kits (Qiagen, Valencia, CA, USA). We amplified 812-bp fragments (minimum 764 bp) of the mitochondrial gene cytochrome *b* (hereafter, *CytB*) following Hope *et al.* (2016). We used the primers MVZ05 and MVZ16,

**Table 1.** Statistical analysis of haplotypes within each state or province. Number of sequences within each boundary ( $N$ ), nucleotide diversity ( $\pi$ ), number of segregating sites ( $SS$ ) and Tajima's  $D$  ( $TD$ )

Location	$N$	$\pi$	$SS$	$TD$
British Columbia	3	0.002	2	–
Colorado	2	0.003	2	–
Idaho	2	0.000	0	–
Isle Royale	7	0.002	3	–0.654
Labrador	2	0.003	2	–
Maine	6	0.004	9	–0.818
Michigan	2	0	0	–
Minnesota	16	0.291	11	–1.204
Montana	6	0.002	5	–1.337
Wisconsin	3	0	0	–

and reactions included 0.16  $\mu\text{M}$  of 10  $\mu\text{M}$  primers, 0.16  $\mu\text{M}$  bovine serum albumin, 0.66  $\mu\text{L}$  magnesium chloride, 200  $\mu\text{M}$  deoxyribonucleotide triphosphate, 200  $\mu\text{M}$  10 $\times$  Qiagen PCR buffer, Taq polymerase at 5 units/ $\mu\text{L}$ , and 2  $\mu\text{L}$  of template DNA. The 15- $\mu\text{L}$  reaction was run with the following conditions: 94  $^{\circ}\text{C}$  for 3 min, 35 cycles of 94  $^{\circ}\text{C}$  for 30 s, 48  $^{\circ}\text{C}$  for 30 s and 72  $^{\circ}\text{C}$  for 46 s, followed by a final elongation of 72  $^{\circ}\text{C}$  for 10 min. PCR products were confirmed via gel electrophoresis.

We visualized and cleaned both forward and reverse sequences using MEGA 7.0 (Kumar *et al.*, 2016) and the resulting sequences were aligned using the ClustalW algorithm (Larkin *et al.*, 2007). Forward and reverse sequences were confirmed against one another to assess sequence quality and only matching pairs were used in downstream analyses. We confirmed all sequences as representing red squirrels via GenBank nucleotide BLAST searches. We aligned sequences from Isle Royale squirrels with 42 red squirrel sequences from Montana, Michigan, Colorado, Minnesota, Idaho, Labrador, British Columbia, Maine and Wisconsin (Fig. 1; Appendix). We assessed the relatedness of squirrel populations via the program PopART (Leigh & Bryant, 2015) and created a median-joining haplotype network to test for reciprocal monophyly in red squirrels from Isle Royale. Genetic diversity analyses of the samples were performed for each state or province separately. Each group was assessed for nucleotide diversity and the number of segregating sites within PopART. Sites with sample sizes greater than three and exhibiting at least one segregating site were analysed for Tajima's  $D$  ( $TD$ ) in MEGA to estimate demographic history and the observed variation in the sample group compared to that expected given the population size.



**Figure 1.** A, map of red squirrel (*Tamiasciurus hudsonicus*) species (hatching) and sample distribution (coloured dots) across North America. Inset: map of Isle Royale and sample locations. B, median joining networks for *CytB* sequences. Dashed lines represent single nucleotide mutations and colours correspond to collection sites. Photo credit: Ray Dumas (<https://www.flickr.com/photos/rtphotography/2827518825>).

## RESULTS

We sequenced seven individuals from Isle Royale, ten from Minnesota and three from Wisconsin and compared *CytB* fragments to existing sequences from 29 individuals across seven North American locations (Hope *et al.*, 2016). *CytB* exhibited moderate nucleotide diversity, with 40 segregating sites across populations. As expected, the haplotype network was divided into eastern and western clades (Fig. 1B). The eastern clade consisted of Isle Royale, Labrador, Maine, Minnesota, Ohio and Wisconsin, while the western clade consisted of British Columbia, Colorado, Idaho and Montana. Isle Royale haplotypes were most similar to those from Labrador, Maine, Michigan, Minnesota and Wisconsin (Fig. 1B). Red squirrels on Isle Royale displayed four haplotypes with three segregating sites, and shared haplotypes with Wisconsin, Maine and Minnesota samples (Table 1;

Fig. 1B). Of the ten states and provinces sampled, red squirrels from Isle Royale exhibited the second lowest nucleotide diversity and a negative *TD* (Table 1).

## DISCUSSION

Red squirrels on Isle Royale are currently classified as a distinct subspecies (*T. h. regalis*) based on phenotypic traits. Our analysis of mitochondrial DNA, however, did not support this hypothesis and revealed that Isle Royale squirrels are not genetically distinct from other mainland populations across temperate North America. Indeed, red squirrels on Isle Royale did not exhibit reciprocal monophyly and were closely related to multiple populations that formed an eastern continental clade. Consequently, our results indicate

that red squirrels within Isle Royale National Park should not be classified as an endemic island subspecies.

These findings also suggest that red squirrels on Isle Royale are not geographically isolated from mainland populations. Squirrels possess strong dispersal power in relation to individual mass and may shift home territories considerably under environmental pressures (Haughland & Larsen, 2004), even across an inhospitable matrix (Bakker & Van Vuren, 2004). Indeed, squirrels have been documented to traverse ice and snow (Pruitt & Lucier, 1958) and have strong swimming ability across open water (Pauli, 2005). Thus, we suspect that recent, and possibly continued, connectivity between mainland populations and red squirrels on Isle Royale is plausible, probably via barrier islands and ice bridges across Lake Superior. Other mammal community members on Isle Royale have been similarly influenced by dispersal and colonization from mainland populations (Mech, 1966; Licht *et al.*, 2017). For example, American martens (*Martes americana*) recently recolonized Isle Royale from neighbouring Canada nearly a century after initial extirpation (Manlick *et al.*, 2018), and grey wolves (*Canis lupus*) have immigrated and emigrated from Isle Royale on at least three occasions since 1948 (Vucetich *et al.*, 2012). Therefore, it is possible that red squirrels on Isle Royale exhibit similar connectivity to the mainland as martens and wolves.

In addition to natural dispersal and colonization, Isle Royale has an important history of mammalian introductions. White-tailed deer (*Odocoileus virginianus*) were introduced for hunting in 1912 and red foxes (*Vulpes vulpes*) were brought for fur farming around 1925 (Mech, 1966; Scarpino, 2011). Consequently, it is also possible that red squirrels were translocated to Isle Royale. Historical surveys indicate that red squirrels were abundant on Isle Royale at the turn of the 20<sup>th</sup> century (Adams, 1909; Mech, 1966), but early explorers and indigenous peoples frequented Isle Royale for centuries and could have also introduced red squirrels. Given the slow mutation rate of *CytB* in mammals (Bradley & Baker, 2001), Isle Royale red squirrels could also have been isolated from mainland populations following an introduction within the last several hundred years. Nevertheless, the lack of structure of Isle Royale red squirrels and their similarity to mainland populations adds to the growing body of literature revealing how dynamic and connected – via natural dispersal events or human assistance – this island is with the mainland (Licht *et al.*, 2015, 2017; Manlick *et al.*, 2018).

Evidence of connectivity among putatively insular squirrel populations has also been detected in previous studies. For example, Eurasian red squirrels (*Sciurus vulgaris*) have been characterized into 40 subspecies based on phenotypic variation, but there is little

genetic divergence across their range (Grill *et al.*, 2009) and isolated populations in the UK share haplotypes with mainland Europe populations (Barratt *et al.*, 1999). Similarly, northern flying squirrels (*Glaucomys sabrinus*) on nearshore islands of the Alexander Archipelago in south-east Alaska, USA, cluster with mainland haplotypes (Demboski *et al.*, 1998), and isolated populations of red and Douglas squirrels (*Tamiasciurus douglasii*) on Vancouver Island, Canada, exhibit evidence of historical gene flow (Chavez *et al.*, 2014). Our results join this literature illustrating the connectivity and underappreciated dispersal power of tree squirrels.

Isle Royale is an isolated wilderness area that has been largely devoid of human impact over the last century. Consequently, the uniqueness of Isle Royale taxa has been a topic of interest to taxonomists and conservationists alike (Kramm *et al.*, 1975; Wayne *et al.*, 1991; Kowal *et al.*, 2011). Recent evidence suggests that Isle Royale is more connected than previously thought (Manlick *et al.*, 2018) and our results support this hypothesis. We found that red squirrels on Isle Royale, an assumed island endemic, are not genetically distinct from mainland red squirrel populations, although morphological divergences suggest gene flow may be reduced (Kramm *et al.*, 1975). Consequently, red squirrels on Isle Royale should not be considered a distinct subspecies, and our results indicate that Isle Royale individuals share haplotypes with at least two other recognized subspecies (*T. h. minnesota*, *T. h. hudsonicus*; Steele, 1998). Future research is needed to further elucidate the relationships between *T. hudsonicus* subspecies within the eastern clade, while regional efforts should consider additional genetic markers (e.g. microsatellites, single nucleotide polymorphisms) to examine the colonization history and rate of gene flow in Isle Royale squirrels.

## ACKNOWLEDGMENTS

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Appendix. A Reclassification of Red Squirrels, *Tamiasciurus Hudsonicus* (Rodentia: Scuridae), on Isle Royale

Species	Catalogue number	Museum	mtDNA lineage	<i>Cytb</i> haplotype	State/Province	Locality	<i>Cytb</i> GenBank accession number	Source
<i>Tamiasciurus hudsonicus</i>	FKB:Michigan	n/a	Continental	6	Michigan	?	FJ200686	Hope et al. (2016)
<i>Tamiasciurus hudsonicus</i>	MMNH:Mamm:18640	Midwest Museum of Natural History	Continental	7	Montana	Bitterroot range, 15 km W Lolo	FJ200676	Hope et al. (2016)
<i>Tamiasciurus hudsonicus</i>	MMNH:Mamm:18642	Midwest Museum of Natural History	Continental	8	Montana	Bitterroot range, 15 km W Lolo	FJ200680	Hope et al. (2016)
<i>Tamiasciurus hudsonicus</i>	MMNH:Mamm:18648	Midwest Museum of Natural History	Continental	4	Montana	Bitterroot range, 15 km W Lolo	FJ200674	Hope et al. (2016)
<i>Tamiasciurus hudsonicus</i>	MMNH:Mamm:18649	Midwest Museum of Natural History	Continental	9	Montana	Bitterroot range, 15 km W Lolo	FJ200675	Hope et al. (2016)
<i>Tamiasciurus hudsonicus</i>	MMNH:Mamm:18961	Midwest Museum of Natural History	Continental	19	Colorado	Collegiate Mountain	FJ200679	Hope et al. (2016)
<i>Tamiasciurus hudsonicus</i>	MSB:Mamm:76659	Museum of Southwestern Biology	Continental	33	Colorado	Jackson	KU977225	Hope et al. (2016)
<i>Tamiasciurus hudsonicus</i>	MMNH:Mamm:18963	Midwest Museum of Natural History	Continental	20	Minnesota	Grant TWP	FJ200687	Hope et al. (2016)
<i>Tamiasciurus hudsonicus</i>	MMNH:Mamm:18964	Midwest Museum of Natural History	Continental	21	Minnesota	Grant TWP	FJ200688	Hope et al. (2016)
<i>Tamiasciurus hudsonicus</i>	MMNH:Mamm:18965	Midwest Museum of Natural History	Continental	22	Minnesota	Grant TWP	FJ200689	Hope et al. (2016)
<i>Tamiasciurus hudsonicus</i>	MMNH:Mamm:18966	Midwest Museum of Natural History	Continental	23	Minnesota	Grant TWP	FJ200690	Hope et al. (2016)
<i>Tamiasciurus hudsonicus</i>	MSB:Mamm:100407	Museum of Southwestern Biology	Continental	4	Montana	S Busby	KU977146	Hope et al. (2016)
<i>Tamiasciurus hudsonicus</i>	MSB:Mamm:155276	Museum of Southwestern Biology	Continental	4	Idaho	Stolle Meadows; 2 km. S. Warm Lake	KU977195	Hope et al. (2016)

Appendix. *Continued*

Species	Catalogue number	Museum	mtDNA lineage	<i>Cytb</i> haplotype	State/Province	Locality	<i>Cytb</i> GenBank accession number	Source
<i>Tamiasciurus hudsonicus</i>	MSB:Mamm:155691	Museum of Southwestern Biology	Continental	4	British Columbia	Cassiar Highway, Rescue Creek	KF823939	<a href="#">Hope et al. (2016)</a>
<i>Tamiasciurus hudsonicus</i>	MSB:Mamm:155872	Museum of Southwestern Biology	Continental	4	British Columbia	Crossing Cassiar Highway, Rescue Creek	KF823940	<a href="#">Hope et al. (2016)</a>
<i>Tamiasciurus hudsonicus</i>	MSB:Mamm:156537	Museum of Southwestern Biology	Continental	4	Montana	Crossing Pettengill Creek	KU977207	<a href="#">Hope et al. (2016)</a>
<i>Tamiasciurus hudsonicus</i>	MSB:Mamm:228884	Museum of Southwestern Biology	Continental	26	Labrador	5 km N Gosling Park	KU977213	<a href="#">Hope et al. (2016)</a>
<i>Tamiasciurus hudsonicus</i>	MSB:Mamm:228885	Museum of Southwestern Biology	Continental	27	Labrador	No specific locality	KU977214	<a href="#">Hope et al. (2016)</a>
<i>Tamiasciurus hudsonicus</i>	MSB:Mamm:262283	Museum of Southwestern Biology	Continental	4	Idaho	Lemhi Range, Iron Creek, NNE of Bear Mountain	KU977216	<a href="#">Hope et al. (2016)</a>
<i>Tamiasciurus hudsonicus</i>	MSB:Mamm:70594	Museum of Southwestern Biology	Continental	28	Maine	Acadia National Park	KU977226	<a href="#">Hope et al. (2016)</a>
<i>Tamiasciurus hudsonicus</i>	MSB:Mamm:73416	Museum of Southwestern Biology	Continental	31	Michigan	Sutton's Bay	KU977229	<a href="#">Hope et al. (2016)</a>
<i>Tamiasciurus hudsonicus</i>	MSB:Mamm:73420	Museum of Southwestern Biology	Continental	32	Minnesota	Ash River Ranger Station	KU977230	<a href="#">Hope et al. (2016)</a>
<i>Tamiasciurus hudsonicus</i>	MSB:Mamm:73422	Museum of Southwestern Biology	Continental	32	Minnesota	Ash River Ranger Station	KU977231	<a href="#">Hope et al. (2016)</a>



## Appendix. Continued

Species	Catalogue number	Museum	mtDNA lineage	<i>Cytb</i> haplotype	State/Province	Locality	<i>Cytb</i> GenBank accession number	Source
<i>Tamiasciurus hudsonicus</i>	RBCM:Mamm:002900	Royal British Columbia Museum and Archives	Continental	34	British Columbia	Swindle Island; Meyers Passage South Lagrange	KU977252	Hope et al. (2016)
<i>Tamiasciurus hudsonicus</i>	YPM:Mamm:5227	Yale Peabody Museum of Natural History	Continental	35	Maine	South Lagrange	FJ200681	Hope et al. (2016)
<i>Tamiasciurus hudsonicus</i>	YPM:Mamm:5228	Yale Peabody Museum of Natural History	Continental	35	Maine	South Lagrange	FJ200685	Hope et al. (2016)
<i>Tamiasciurus hudsonicus</i>	YPM:Mamm:5229	Yale Peabody Museum of Natural History	Continental	36	Maine	Nicatous Lake	FJ200682	Hope et al. (2016)
<i>Tamiasciurus hudsonicus</i>	YPM:Mamm:5230	Yale Peabody Museum of Natural History	Continental	28	Maine	West Lake	FJ200683	Hope et al. (2016)
<i>Tamiasciurus hudsonicus</i>	YPM:Mamm:5231	Yale Peabody Museum of Natural History	Continental	37	Maine	West Lake	FJ200684	Hope et al. (2016)
<i>Tamiasciurus hudsonicus</i>	MN-11R	Pauli Lab, University of Wisconsin-Madison	Continental	1	Minnesota	Superior National Forest	MK493348	This study
<i>Tamiasciurus hudsonicus</i>	MN-10F	Pauli Lab, University of Wisconsin-Madison	Continental	23	Minnesota	Superior National Forest	MK493349	This study
<i>Tamiasciurus hudsonicus</i>	MN-9F	Pauli Lab, University of Wisconsin-Madison	Continental	36	Minnesota	Superior National Forest	MK493350	This study
<i>Tamiasciurus hudsonicus</i>	MN-8F	Pauli Lab, University of Wisconsin-Madison	Continental	MN-8F	Minnesota	Superior National Forest	MK493351	This study
<i>Tamiasciurus hudsonicus</i>	MN-6R	Pauli Lab, University of Wisconsin-Madison	Continental	MN-6R	Minnesota	Superior National Forest	MK493352	This study
<i>Tamiasciurus hudsonicus</i>	MN-5F	Pauli Lab, University of Wisconsin-Madison	Continental	21	Minnesota	Superior National Forest	MK493353	This study

## Appendix. Continued

Species	Catalogue number	Museum	mtDNA lineage	<i>Cytb</i> haplotype	State/Province	Locality	<i>Cytb</i> GenBank accession number	Source
<i>Tamiasciurus hudsonicus</i>	MN-4F	Pauli Lab, University of Wisconsin-Madison	Continental	23	Minnesota	Superior National Forest	MK493354	This study
<i>Tamiasciurus hudsonicus</i>	MN-3F	Pauli Lab, University of Wisconsin-Madison	Continental	MN-3F	Minnesota	Superior National Forest	MK493355	This study
<i>Tamiasciurus hudsonicus</i>	MN-2F	Pauli Lab, University of Wisconsin-Madison	Continental	23	Minnesota	Superior National Forest	MK493356	This study
<i>Tamiasciurus hudsonicus</i>	MN-1F	Pauli Lab, University of Wisconsin-Madison	Continental	23	Minnesota	Superior National Forest	MK493357	This study
<i>Tamiasciurus hudsonicus</i>	WI-3F	Pauli Lab, University of Wisconsin-Madison	Continental	36	Wisconsin	Chequamegon-Nicolet National Forest	MK493358	This study
<i>Tamiasciurus hudsonicus</i>	WI-2F	Pauli Lab, University of Wisconsin-Madison	Continental	36	Wisconsin	Chequamegon-Nicolet National Forest	MK493359	This study
<i>Tamiasciurus hudsonicus</i>	WI-1F	Pauli Lab, University of Wisconsin-Madison	Continental	36	Wisconsin	Chequamegon-Nicolet National Forest	MK493360	This study
<i>Tamiasciurus hudsonicus</i>	TAHU_ISRO1	Pauli Lab, University of Wisconsin-Madison	Continental	TAHU_ISRO1	Michigan	Isle Royale National Park	MK493361	This study
<i>Tamiasciurus hudsonicus</i>	TAHU_ISRO2	Pauli Lab, University of Wisconsin-Madison	Continental	36	Michigan	Isle Royale National Park	MK493362	This study
<i>Tamiasciurus hudsonicus</i>	TAHU_ISRO4	Pauli Lab, University of Wisconsin-Madison	Continental	36	Michigan	Isle Royale National Park	MK493363	This study
<i>Tamiasciurus hudsonicus</i>	TAHU_ISRO3	Pauli Lab, University of Wisconsin-Madison	Continental	36	Michigan	Isle Royale National Park	MK493364	This study
<i>Tamiasciurus hudsonicus</i>	ISRO-3F	Pauli Lab, University of Wisconsin-Madison	Continental	23	Michigan	Isle Royale National Park	MK493365	This study

## Appendix. Continued

Species	Catalogue number	Museum	mtDNA lineage	<i>Cytb</i> haplotype	State/Province	Locality	<i>Cytb</i> GenBank accession number	Source
<i>Tamiasciurus hudsonicus</i>	ISRO-2R	Pauli Lab, University of Wisconsin-Madison	Continental	23	Michigan	Isle Royale National Park	MK493366	This study
<i>Tamiasciurus hudsonicus</i>	ISRO-1F	Pauli Lab, University of Wisconsin-Madison	Continental	ISRO_1F	Michigan	Isle Royale National Park	MK493367	This study