First Record of the Holarctic Least Shrew (*Sorex minutissimus*) and Associated Helminths from Canada: New Light on Northern Pleistocene Refugia

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Manuscripts

1 **First Record of the Holarctic Least Shrew (*Sorex minutissimus*) and Associated**
2 **Helminths from Canada: New Light on Northern Pleistocene Refugia**

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Abstract: We report the first Canadian record of the Holarctic least shrew (*Sorex minutissimus* Zimmermann, 1780) and associated helminth worms, collected along the Dempster Highway in central Yukon in 2014. We identify the specimen based on morphological characters, characterize the habitat, report other mammals and helminth species associated with this specimen, and use mitochondrial DNA sequences to place the specimen within a phylogenetic context and address Pleistocene refugial hypotheses. Although long considered an Asian endemic, the diminutive least shrew was first reported from Alaska in 1994. Our new record for Canada indicates that the species may occur at least as far east as the MacKenzie River and DNA variation suggests this species persisted only in the Beringian refugium in North America during the Last Glacial Maximum. The discovery of a new mammal and associated parasites for Canada points to the urgent need for more detailed information on high-latitude biotas in North America, data that are best obtained through museum-based field surveys, particularly for small, cryptic species.

Key words: distribution, helminth, museum collections, *Sorex minutissimus*, Holarctic least shrew, Yukon

Introduction

From a continental perspective, shrew (Soricomorpha: Soricidae) assemblages at high latitudes in northwestern North America are relatively species rich (Berman et al. 2007) and these assemblages have yielded a wealth of information on the complex historical biogeography and dynamic climatic change that has occurred in the region, particularly Beringia (Demboski and Cook 2003; Hope et al. 2011, 2012). For example, Hope (2012) documented six species of *Sorex* in syntopy on Alaska’s Seward Peninsula and similar diversity is expected elsewhere in the region. However, despite relatively high species richness, the shrew fauna of northwestern Canada has rarely been the focus of research efforts and remains poorly sampled (Jung et al. 2007). Because several *Sorex* species are morphologically cryptic or infrequently encountered in small mammal inventories, distributions remain poorly delineated (Slough and Jung 2007) and cryptic diversity within shrew assemblages of northwestern North America may still exist (e.g. Dokuchaev 1997; Hope et al. 2014). Moreover, very little is currently known regarding helminthes of *Sorex* in Canada, with no records from Yukon. Only one cestode species, *Cladotaenia globifera* Batsch 1786 (from Ontario) and 11 species of nematodes (from Ontario, Alberta, Manitoba, Quebec, British Columbia, and Nova Scotia) are reported from Canada (Kinsella and Tkach 2009).

Sorex yukonicus Dokuchaev, 1997, the Alaskan tiny shrew, was originally described based on Alaskan specimens. Hope et al. (2010) later showed that Alaska populations of this shrew were minimally distinctive from populations of the Eurasian least shrew (*S. minutissimus* Zimmermann, 1780) in eastern Siberia and suggested that they be synonymized. We agree with the synonymy of Hope et al. (2010) and suggest

that the common name for this single wide-ranging species, *Sorex minutissimus*, should now be Holarctic least shrew. Still, this species remains poorly known in North America and north-central Asia, and additional morphological and genetic investigations based on increased spatial sampling are needed. The paucity of museum records (only two North American specimens as of 1994) suggests that Holarctic least shrews are either not easily collected or relatively scarce compared to other species of *Sorex* at northern latitudes (MacDonald and Cook 2009). Intensive field efforts over the past two decades have documented this species in a range of forested and non-forested habitats including riparian areas, open spruce forest, tussock tundra, and willow and alder shrub-land. However, despite apparently wide habitat niche breadth, only 54 specimens of *S. minutissimus* (34 University of Alaska Museum of North, 16 Museum of Southwestern Biology, 3 University of Washington Burke Museum, 1 Moscow State University) are known from North America, all from Alaska (Peirce and Peirce 2000; MacDonald and Cook 2009; Baltensperger et al. 2015, Arctos 2015).

We report the first record of *S. minutissimus* and associated endoparasites from Canada. Using DNA sequences, we further test whether this new easternmost record is genetically distinctive, possibly reflecting diversification of this species in multiple high latitude refugia (Macpherson 1965; Fedorov et al. 2003) in North America through the Late Pleistocene.

Methods

In summer 2014 as part of an international effort to document and understand the distribution and evolutionary history of high-latitude mammalian hosts and associated

parasites in the Beringian region (Cook et al. 2005), we sampled small mammal communities in a variety of habitats following methods of Cook and MacDonald (2004) along a broad north-south transect in Yukon, Canada. We followed acceptable field methods for specimen collection (Sikes et al. 2011). On 15 July, approximately 100 m east of the Dempster Highway (66.88678°N, 136.33443°W; 565 m asl; Figure 1), we captured this extremely small male shrew (MSB:Mamm:277743) in a pitfall trap. Based on size, external morphology and dentition, this specimen was tentatively identified as *S. minutissimus*. The specimen was brushed for ectoparasites, necropsied for endoparasites, sex was determined by external examination and confirmed by microscopic internal examination of testes, prepared as a holistic voucher specimen (skin, skeleton) with multiple tissues (heart, liver, kidney, spleen) frozen in nitrogen (Yates et al., 1996; Dunnum and Cook 2013), and archived in the Museum of Southwestern Biology (Albuquerque, New Mexico, USA).

Weighing less than 2 g, *S. minutissimus* is among the smallest mammalian species on Earth. Of the other shrews occurring in northwestern North America, only *S. hoyi* approaches *S. minutissimus* in size; however, *S. hoyi* is easily distinguished by its diminutive third and fifth unicuspid giving the appearance of only three unicuspid in the lateral view. External morphological measurements of MSB:Mamm:277743 were compared to those of 43 specimens of *S. minutissimus* previously collected in Alaska.

We used DNA sequences to provide phylogeographic context for this farthest east record of *S. minutissimus* in North America. DNA was extracted from frozen heart tissue through standard salt extraction with 1118 bp of sequence obtained for the mitochondrial cytochrome b (Cytb) locus through PCR and cycle sequencing methods using primers

(MSB05/MSB14) and experimental methods outlined in Hope et al. (2010). The Cytb sequence was aligned with Cytb sequences downloaded from GenBank of *S. minutissimus* (and *S. yukonicus*), *S. arcticus*, and *S. minutus* using ClustalW as implemented in the BioEdit program, version 7.0.1 (Hall 1999). Phylogenetic analysis was carried out using Bayesian inference (BI) in MrBayes version 2.01 (Huelsenbeck and Ronquist 2001). The following nucleotide substitution parameters were used: lset nst=6, rates=gamma, prset (prior assumptions) pinvarpr=fixed (0) shapepr=fixed (0.2250), that correspond to the transition (TIM2+G) model including rate variation among sites with 4 rate categories (G). The analysis was run for over 3,200,000 generations and log-likelihood scores were examined to ensure convergence. The final 75% of trees were used to produce the consensus tree. The TIM2+G model was used for the analysis based on results obtained from jModelTest, version 0.1.1 (Posada, 2009). *Sorex minutus* was designated as the outgroup based on the phylogenetic analysis of Hope et al. (2010).

Results

The pelage of MSB:Mamm:277743 is slightly tricolored with the dorsum being nearest fuscous, the flanks near gray, and the ventrum pale smoke gray (Figure 2a, b). The tail is bicolored with the dorsal side being the same color as the dorsum, and the ventral side whiter than the ventrum. External measurements (total length (mm)-tail length (mm)-hindfoot length (mm) $\equiv$ mass (g)) for this specimen (67-22-8 $\equiv$ 1.5) were within the range of measurements of other North American specimens of *S. minutissimus* (average (range) -- 70.4(59-74)-24.2(19-27)-8.6(8-10) $\equiv$ 1.8(1-2.5), $n = 48$). The upper unicuspid teeth lack a pigmented ridge on the lingual side from apex to the cingulum,

with the first unicuspid largest, the second and third are, generally, equal and smaller than the first, the fourth unicuspid is smaller than the third, and the fifth is the smallest (Figure 3a, b, c). The post-mandibular canal on the jaw (Figure 3d, e) is well developed. Pelage, dentition, and morphological measurements of MSB:Mamm:277743 are aligned with those described for *S. minutissimus* (Dokuchaev 1997). Bayesian analysis placed the specimen in a highly supported clade with the Alaskan and Russian specimens and closely allied with representatives of the species collected nearby in Alaska (Figure 4).

Vegetation in the vicinity of the collection site was characterized as low shrub tundra with willow (*Salix reticulata* L.), dwarf birch (*Betula nana* L.), cloudberry (*Rubus chamaemorus* L.), lingonberry (*Vaccinium vitis-idaea* L.), Labrador tea (*Rhododendron tomentosum* Harmaja) and crowberry (*Empetrum nigrum* L.) present. Other small mammals collected at this locality were masked shrew (*S. cinereus* Kerr, 1792), northern red-backed vole (*Myodes rutilus* Pallas, 1779), and red squirrel (*Tamiasciurus hudsonicus* Erxleben, 1777).

The 1118 base pair segment of the Cytb sequence from this new Canadian specimen (GenBank KU568471) is identical to a haplotype from an Alaska specimen (GenBank GU223653). These sequences followed the patterns of compositional bias expected for a functional mitochondrial Cytb in mammals (Ma et al. 1993).

No ectoparasites were found on MSB:Mamm:277743, but we recovered six helminths from the small intestine that represented two genera of cestodes. Five belonged to *Lineolepis parva* Rausch & Kuns, 1950 and one incomplete specimen belonged to *Staphylocystoides* sp.

Discussion

This first record of *S. minutissimus* in Canada represents a significant range extension (Figure 1), being approximately 270 km east of the closest record in Alaska (Dokuchaev 1997; MacDonald and Cook 2009). *Sorex minutissimus* is now known to have a widespread distribution extending from Europe eastward across the Palearctic and Alaska and into Canada at least as far east as central Yukon. Analyses of ecological niche models (ENMs) previously predicted a high probability for the distribution of *S. minutissimus* to extend into the Yukon as far east as the MacKenzie Mountains (Hope et al 2010). Based on their range-wide genetic footprint, Hope et al (2010) hypothesized that *S. minutissimus* persisted in North America only in Beringia during the Last Glacial Maximum (Alaska and now Canada). Because the sequence from the new Canadian specimen is identical to a specimen from Alaska, these new data corroborate the hypothesis that this boreal species persisted in a single refugium (Beringia) in North America during the LGM. Overall low genetic divergence between Asian and North American tiny shrews reflects the recent isolation (<12,000 years bp) of Asian from North American populations with inundation of the Bering Land Bridge by rising sea levels.

We have no reason to believe that *S. minutissimus* is a new addition to the mammalian fauna of Yukon, Canada; rather, it has been present but previously undetected due to its apparent rarity and morphological similarity to other *Sorex*. Given the limited sampling of *Sorex* in northwestern Canada (Jung et al. 2007; Slough and Jung 2008), targeted field sampling will likely yield significant new information on the diversity and distribution of this and other shrew species.

Similarly, this is the first report of *L. parva* from *S. minutissimus* and from Canada, and *Staphylocystoides* sp. is the first representative of the genus reported from Canada. These 2 genera of cestodes are Holarctic in distribution. *Lineolepis parva*, a North American endemic, is widespread and has been isolated from 7 species of *Sorex* in North America (Alaska, California, Iowa, Montana, Wisconsin and Wyoming). The genus *Staphylocystoides* is the most species-rich of North American shrew cestodes, with 7 species, and is also widespread throughout North America, from Alaska to California and eastward to Pennsylvania and Tennessee (Kinsella and Tkach 2009). Further parasitological examination of shrews from northwestern Canada and North America will likely result in greater helminth diversity and phylogeographic studies are needed to understand their historical biogeography (Hoberg et al. 2012). Documenting the diversity, distribution, and phylogeographic history of small mammals and associated parasites in northwestern Canada and Alaska is particularly important in light of climatic change occurring in the region and its anticipated impact on faunas (Baltensperger and Huettmann 2015; Hope et al. 2015; Lanier et al. 2015).

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List of Figures:

Figure 1. North American distribution of *Sorex minutissimus* Zimmermann, 1780 based on museum records and the new Canadian specimen (star). Maximum extent of terrestrial and continental shelf glaciation during the Last Glacial Maximum is shown by overlay (reconstruction from Ehlers et al. 2011). Potential locations of nearest high Canadian Arctic refugia are indicated (after Macpherson 1965, Andersen and Borns 1997).

Figure 2. First specimen of *Sorex minutissimus* Zimmermann, 1780 (MSB:Mamm:277743) from Canada showing a) dorsal and b) ventral views of study skin.

Figure 3. First specimen of *Sorex minutissimus* Zimmermann, 1780 (MSB:Mamm:277743) from Canada showing a) dorsal, b) ventral and c) lateral views of skull; and d) dorsal and e) lingual (zoomed) views of mandible. Arrows indicate b) lack of pigmented ridge on the lingual side of unicuspid from apex to the cingulum, c) all 5 unicuspid visible in lateral view, and e) the post-mandibular canal. Scale bar equals 5mm.

Figure 4. Majority rule consensus tree of selected *Sorex minutissimus* Zimmermann, 1780 specimens based on the mitochondrial DNA locus Cytochrome b. Taxon labels for North American specimens follow synonymy of *S. minutissimus* and *S. yukonicus* Dokuchaev, 1997 (Hope et al. 2010).

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