

High-latitude diversification within Eurasian least shrews and Alaska tiny shrews (Soricidae)

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A novel shrew was discovered recently in Alaska and described based on morphological characters as *Sorex yukonicus*. This species is closely allied to *Sorex minutissimus*, a widespread shrew ranging through Eurasia. Together their distribution spans Beringia, a large Pleistocene nonglaciated area that connected Asia and North America. Beringia was repeatedly divided due to raised sea levels during Pleistocene interglacials and subsequently reconnected during glacials. We tested predictions related to the influence of large-scale geologic events on genetic variability through a phylogeographic analysis of both species of shrew using evidence from 3 independent genetic loci. We found low genetic divergence between *S. minutissimus* and *S. yukonicus* across continents. However, major phylogeographic breaks were found for Eurasian and Maritime Northeast Asia populations. Neither species is reciprocally monophyletic for any of the loci examined. Coalescence times for all pairwise population comparisons within both species fall within the Wisconsin–Weichselian glacial (<130 thousand years ago), and significant population expansion estimates date to the Holocene suggesting that divergence between these taxa is minimal and may not warrant recognition of 2 distinct species. Phylogeographic relationships and sequence divergence estimates place populations of North American *S. yukonicus* and Siberian *S. minutissimus* as most closely related, and together they are sister to European *S. minutissimus*. We conclude that populations east and west of the Bering Strait represent a single Holarctic species, *S. minutissimus*. Temporal changes in range based on ecological niche predictions and a comparative assessment of other codistributed taxa provide a preliminary view of potential Last Glacial Maximum refugia in northern Asia. DOI: 10.1644/09-MAMM-A-402.1.

Key words: Bayesian skyline plot, Beringia, ecological niche model, multiple loci, phylogeography, Pleistocene refugia, *Sorex minutissimus*, *Sorex yukonicus*

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Climatic variability during the Pleistocene had a major impact on the evolution and distribution of high-latitude species (Hewitt 1996, 2004), with >20 significant glacial cycles recorded during this epoch (Williams et al. 1998). Northern species experienced major range shifts involving contractions into discrete refugia and subsequent expansions as they recolonized deglaciated areas (Schmitt 2007). The record of these demographic changes through repeated

climatic cycles often is obscured by the actions of the most-recent glacial cycle, effectively wiping the historical slate clean (Avice 2000); however, sometimes the signature of past glaciations persists through multiple glacial events (Lu et al.



2001; Runck et al. 2009). Fossil remains are independent evidence of previous occupation but provide only a minimum time estimate of geographic occupation. For many small mammals, fossils often are not available or are difficult to identify (Near and Sanderson 2004; Smith and Peterson 2002). Geographic distributions coupled with knowledge of geologic and climate change through time can be compared with morphologic or genetic information from extant populations to infer the history of diversification and spatiotemporal change within and among species (Lister 2004).

In Europe, species occupied putative southern refugia during glacial maxima, and many recolonized northward following retreat of the Fennoscandian ice sheet in response to a warming climate (Hewitt 2001; Schmitt 2007). In addition, genetic signatures of other newly recognized refugia situated further east in Europe (e.g., Carpathians—Provan and Bennett 2008) also have been identified and implicated as sources for westward recolonization (Fedorov et al. 2008; Hewitt 2004). Likewise, most of northern North America was covered by the Cordilleran and Laurentide ice sheets. Following deglaciation, species that were pushed south subsequently expanded north to occupy newly exposed land (Runck and Cook 2005). In contrast, much of eastern Europe, Siberia, and northwestern North America remained virtually ice-free from the Taimyr Peninsula eastward across Beringia to the Mackenzie Mountains in the Yukon (Hopkins 1967; Svendsen et al. 2004), and yet phylogeographic patterns for high-latitude species occupying North America and East Asia remain poorly understood. Heterogeneous topography including mountain ranges, large rivers, and lakes, coupled with variable climatic cycles, fragmented this immense region into discrete xeric or mesic refugia (Ehrich et al. 2008; Fedorov et al. 2008; Mangerud et al. 2004).

Beringia is perhaps the most recognized ice-free refuge at high latitudes (Abbott and Brochmann 2003; Hultén 1937). During glacial maxima lowered sea levels exposed a land bridge between Asia and North America effectively extending Asia eastward to the western limits of the North American ice sheets and allowing for continental exchange of terrestrial biota (DeChaine 2008; Elias and Crocker 2008; Sher 1999). During glacial episodes species from Asia could pass into eastern Beringia (central Alaska and northwestern Yukon), whereas North American species were restricted to lower latitudes south of continental ice sheets. Consequently, most known intercontinental movements of species across Beringia are eastward, although notable exceptions include some shrews (Waltari et al. 2007b).

Species and complexes that span the nexus between Asia and North America provide a window into the historical biogeography of faunal exchanges. The Alaska tiny shrew, *Sorex yukonicus* (Dokuchaev, 1997), was described from previously misidentified museum specimens. Originally thought to be the Eurasian least shrew, *S. minutissimus* (Dokuchaev 1994), these specimens were later elevated to a new species based on morphological differences (Dokuchaev 1997). Dokuchaev (1997) suggested that *S. minutissimus* invaded Alaska prior to

the Illinoian glacial and then diverged within Beringia as *S. yukonicus* during the Illinoian (0.21–0.13 million years ago [mya]). Although morphological differences were detected with principal component analysis, the study by Dokuchaev (1997) was based on a limited number of samples (6 individuals). Since that study an additional 27 specimens have been archived, all within the confines of Alaska. The geographic range of *S. minutissimus* is broad, extending from Scandinavia eastward across Asia to the Bering Strait and as far south as Mongolia and southeast to Primorsky Krai and Japan (Ohdachi et al. 2001; Sheftel 2005). The least shrew inhabits forest–tundra, taiga, forest–steppe, and dry steppe habitats (Yudin 1971).

Other taxa (e.g., arthropods, birds, fish, parasites, and plants) also exhibit broad Holarctic distributions (Fedorov et al. 2008; Haukisalme et al. 2004, 2009; Hewitt 2004; Waltari et al. 2007b). Distinctive phylogeographic breaks are common among high-latitude mammals including an east–west split in the vicinity of the Ural Mountains of western Russia (Brunhoff et al. 2003; Deffontaine et al. 2005; Fedorov et al. 1999a, 1999b); splits between the major river basins of the Ob, Yenisei, Lena, and Kolyma (Fedorov et al. 2003; Galbreath and Cook 2004; Fedorov et al. 2008); the Beringian refugium (Eddingsaas et al. 2004; Galbreath and Cook 2004; Waltari et al. 2004); and parts of Maritime Northeast Asia including Primorsky Krai, Japan, and the Korean Peninsula (Ehrich et al. 2008; Fedorov et al. 2008; Lee et al. 2008; Ohdachi et al. 2001; Yasuda et al. 2005). Other species exhibit a distinct barrier at the Bering Strait (Elias and Crocker 2008; Fedorov and Goropashnaya 1999). Dokuchaev (1997) suggested that *S. yukonicus* is an autochthon of Beringia, although the distribution of this species is limited to Alaska (eastern Beringia only). At least 3 alternative hypotheses have been proposed for the distribution of *S. minutissimus* and *S. yukonicus*: 2 distinct species divided at the Bering Strait (i.e., the current taxonomic arrangement); a single Beringian autochthon, *S. yukonicus*, with a distribution that spans the Bering Strait; or a single Holarctic species that subsumes the recently described *S. yukonicus* within *S. minutissimus*. Given the wide distribution (Europe through Siberia to Alaska) inherent in the “single species” hypothesis, good potential of significant phylogeographic structure exists.

We sequenced 3 independent genetic loci to investigate phylogeographic patterns within the *minutissimus*–*yukonicus* group and also develop distributional models based on ecological niche predictions to assess population-level demography and test spatial and temporal components for historical diversification. Finally, we place this study within the context of phylogeographic research on other Arctic species and discuss emerging evidence for the persistence of high-latitude species in multiple discrete refugia within largely ice-free areas of Eurasia during glacial periods.

MATERIALS AND METHODS

Sampling and laboratory techniques.—*Sorex yukonicus* in North America and *S. minutissimus* in Eurasia are compara-

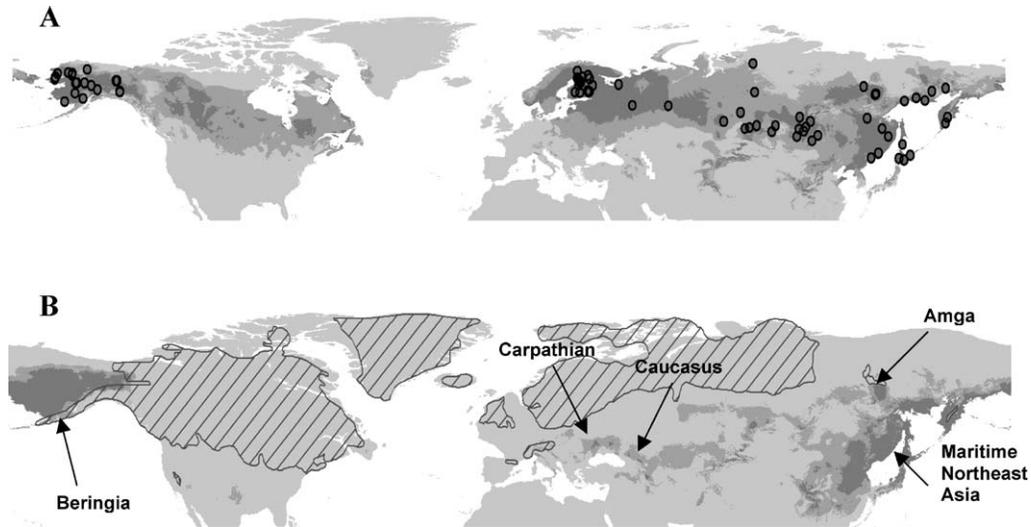


FIG. 1.—Ecological niche models (ENMs) depicting potential distribution for the *Sorex minutissimus*–*yukonicus* group during A) the present and B) the Last Glacial Maximum (LGM) dated to 18 kya. Circles indicate specimen locality records used to produce ENMs. Dark shading indicates strong distributional prediction and light shading a weak prediction. Some potential refugia are labeled for the LGM. Slanted lines indicate extent of major ice sheets at the LGM.

tively uncommon shrews through their present range (Fig. 1A). Few specimens of *S. minutissimus* from Eurasia exist, and most are preserved as study skins or formalin fluid samples. We obtained samples from 29 *S. yukonicus* (21 localities) and 66 *S. minutissimus* for this study (Appendix I). Samples were collected in conjunction with the Beringian Coevolution Project ($n = 44$ —Cook et al. 2005), or received on loan from Europe and northern Asia ($n = 41$; Appendix I). We added 10 (mitochondrial DNA [mtDNA]: cytochrome-*b*) partial sequences of *S. minutissimus* obtained from GenBank resulting in 61 total localities spanning northern Europe, through Asia to Alaska (Fig. 2).

Heart, liver, or skin were obtained from frozen (stored at -80°C), ethanol-preserved, or dried samples. Purified total genomic DNA was obtained through standard salt extraction, followed by polymerase chain reaction and cycle sequencing (Fleming and Cook 2002). We amplified 3 independent loci, the mtDNA cytochrome-*b* gene (*Cytb*: 561–1,140 base pairs [bp]), and 2 nuclear loci, the apolipoprotein B (ApoB: 514 bp—Dubey et al. 2007) and breast cancer susceptibility 1 (BRCA1: 722 bp—Dubey et al. 2006) genes. Primers used for double-stranded amplifications and sequencing of *Cytb* were modified from Irwin et al. (1991—MSB14, 5′-CCC ATC TCY GGT TTA CAA GAC-3′) and Anderson and Yates (2000—MSB05, 5′-GAC ATG AAA AAT CAT TGT TGT AAT TC-3′). For highly fragmented DNA the internal primers L15135 and H15392 (Ohdachi et al. 2001) were used. Primer pairs ApoBF–ApoBR and BRCA1F–BRCA1R (Dubey et al. 2006, 2007) were used to amplify and sequence ApoB and BRCA1 genes, respectively. For all targeted regions polymerase chain reaction reagents and conditions were: 1 μl of DNA template (variable concentration); 1.5 μl each of deoxynucleoside triphosphates (10 mM), MgCl (25 mM), 10 \times PCR buffer, and bovine serum albumin (1%); 0.5 μl of each primer (10 mM); 0.08 μl of AmpiTaq DNA polymerase (Applied

Biosystems, Foster City, California); and 6.92 μl of double-distilled H_2O to total 15- μl reactions. Polymerase chain reaction was performed in a PTC 200 thermocycler (MJ Research, Waltham, Massachusetts) with initial denaturation at 94°C for 5 min, followed by 40 cycles of denaturation at 94°C for 15 s, annealing at 50°C for 20 s, extension at 72°C for 1 min, and final extension at 72°C for 5 min, with cooling at 15°C for 10 min. Polymerase chain reaction products were cleaned using polyethylene glycol 30% precipitation (Bernstein and Abbot 1987) and cycle sequenced using the ABI BigDye version 3.1 Sequencing Kit (Applied Biosystems, Foster City, California). Reagents for each 12- μl cycle sequencing reaction included 1.25 μl of H_2O , 2 μl of BigDye buffer (Applied Biosystems), 2 μl of primer (10 mM), 0.75 μl of ABI BigDye version 3.1 (Applied Biosystems), and 5 μl of DNA (variable concentration). Conditions for cycle sequencing followed Platt et al. (2007) and reactions were cleaned using a 125 mM ethylenediaminetetraacetic acid–ethanol protocol. Automated sequencing of complimentary strands was conducted using the Applied Biosystems 3110 DNA sequencer of the molecular biological facility at the University of New Mexico. Complimentary strands of DNA were compared and contiguous sequences were then deposited in GenBank (Appendix I).

Phylogeny reconstruction.—Degraded DNA from study skins ($n = 37$) coupled with availability of partial *Cytb* sequences from GenBank resulted in a mitochondrial phylogeny based on a range of gene length for *Cytb* (561–1,140 bp; $n = 100$). Phylogenies were estimated with and without a designated outgroup to explore the possibility of long-branch attraction. The purported sister species for the *minutissimus*–*yukonicus* group is *Sorex hosonoi*, an endemic to Honshu Island, Japan (Ohdachi et al. 1997, 2001), and 5 sequences were obtained from GenBank (AB028593–AB028597) for the *Cytb* genealogy. In addition, the *Cytb*

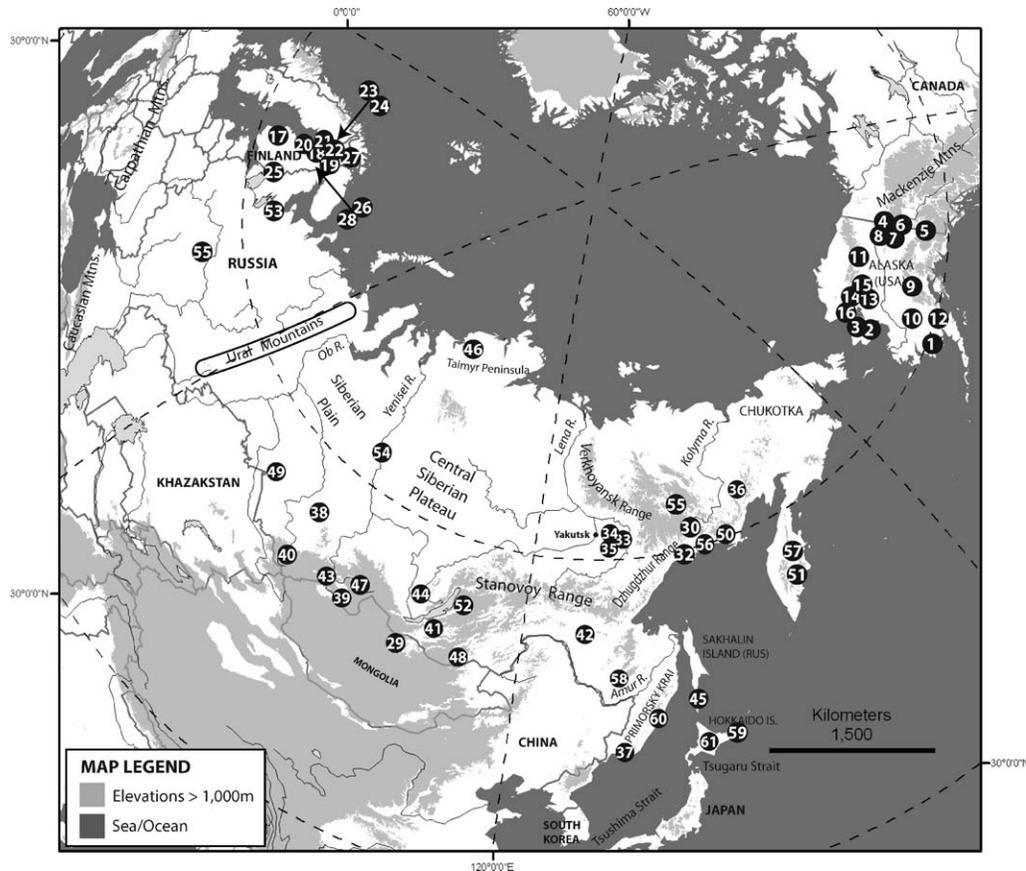


FIG. 2.—Map of the study area illustrating major geographic features within the Holarctic that reflect potential phylogeographic breaks. Specimen localities are indicated by number corresponding with Appendix I and are associated with designated clades from Bayesian cytochrome-*b* phylogeny reconstruction (Alaska: 1–16; Siberia: 29, 30–36, 39, 44, 47, 50, 51, 54, 56–58; SE Siberia: 41, 42, 48, 52, 59; MNA: 37, 45, 60, 61; Europe-to-Yenisei: 25, 28, 38, 40, 43, 49, 53, 55; Finland: 17–24, 26, 27).

data set was pruned to haplotypes excluding incomplete and repeat sequences and standardizing gene length (836 bp; 22 *S. minutissimus*; 8 *S. yukonicus*) to control for population bias. Nuclear data sets for ApoB (514 bp) and BRCA1 (722 bp) include 30 specimens from the *Cytb* pruned haplotype data set that are represented by all 3 genetic loci. Nuclear sequences for *S. hosonoi* were unavailable so 1 specimen each of *Sorex arcticus*, *Sorex caecutiens*, *Sorex daphaenodon*, *Sorex minutus*, and *Sorex roboratus* was included for subsequent nuclear analyses of multiple loci. These species were chosen based on their recognized taxonomic affiliations with *S. minutissimus* (all within the subgenus *Sorex*—Hutterer 2005).

Sequences were edited with SEQUENCHER 4.8 (GeneCodes, Ann Arbor, Michigan), aligned in BioEdit 7.0.9 (Ibis Biosciences, Carlsbad, California) using ClustalW, and checked by eye. Sequences were translated to amino acids and examined for internal stop codons that might uncover pseudogenes. Alleles of nuclear heterozygotes were inferred using PHASE (Stephens et al. 2001; Stephens and Scheet 2005). The program PHASE implements a Bayesian statistical method for reconstructing haplotypes from population nuclear genotype data that includes multiple heterozygous base sites within individuals. PHASE was run 5 times, and results from the run with best goodness-of-fit to an approximate coalescent

model were retained, resulting in 2 haplotype sequences per individual to obtain accurate estimates of allele frequencies. For Bayesian phylogenies a hierarchical likelihood ratio test was run on each sequence data set to determine the best model of DNA substitution using MrModeltest version 2.3 (Nylander 2004). The Markov chain Monte Carlo procedure was performed in MrBayes version 3.1 (Ronquist and Huelsenbeck 2003) to estimate posterior probabilities of phylogenetic trees for each sequence data set. Two separate runs were conducted for each data set, with sequences partitioned by codon position to allow for variable substitution rates among different codon positions. Each run computed 5,000,000 generations, sampling every 100 generations, with 4 independent chains, and the first 5,000 trees discarded as burn-in. The resulting phylograms and posterior probabilities for each locus were visualized in FigTree version 1.2.2 (Rambaut 2009).

Species tree estimation from multiple loci.—Evidence from multiple independent genes is critical to inferring relationships within and among species (Edwards and Beerli 2000). Gene tree–species tree discord can result from gene duplication, introgression, sorting of ancestral polymorphism, or rapid fixation of linked loci through selective sweeps (Carstens and Knowles 2007). Multiple independent loci allow for the potential detection of these phenomena. For species tree

estimation we used the minimizing deep coalescences method (Maddison and Knowles 2006) implemented in Mesquite version 2.6 (Maddison and Maddison 2009). The minimizing deep coalescences method is preferred over concatenation because of the independent (unlinked) nature of our 3 loci (Edwards et al. 2007; Kubatko and Degnan 2007). This topology-based method estimates species trees based on the minimum number of deep coalescences over all independent genealogies. Data were phased for all 3 loci (including the haploid mitochondrial gene for consistency) to estimate accurately alleles from nuclear gene sequences with multiple heterozygous sites and produce 2 haplotypes for each individual, including the 5 outgroup taxa ($n_{\text{tot}} = 70$). The same specimens were included in all 3 phylogeny reconstructions using independent loci. The minimizing deep coalescences reconstruction was run with 100 iterations for a robust estimate.

Ecological niche modeling.—For development of ecological niche models (ENMs) we used current and Last Glacial Maximum (LGM) monthly climate data at 2.5-min (4×4 km) spatial resolution. Waltari et al. (2007a) and Peterson and Nyári (2008) discussed the process of layer development more fully. To summarize, LGM climate data were based on 2 general circulation model simulations, the community climate system model (Collins et al. 2004) and the Model for Interdisciplinary Research on Climate (version 3.2—Hasumi and Emori 2004; <http://www.pmip2.cnrs-gif.fr>). Each model was generated at a spatial resolution of 2.8° , or roughly 300×300 km, and model outputs were processed by interpolating differences between LGM and recent (preindustrial) conditions based on the WorldClim (<http://www.worldclim.org/>) data set to create monthly climate surfaces at 2.5-min spatial resolution.

The ENMs for both the present and LGM were based on the 19 bioclimatic variables in the WorldClim data set (Hijmans et al. 2005). These variables represent summaries of means and variation in temperature and precipitation and characterize dimensions of climate considered particularly relevant in determining species distributions. Present-day ENMs were developed within a mask consisting only of land north of 30°N .

From biodiversity information systems of natural history collection data (e.g., ARCTOS [<http://arctos.database.uaf.edu>] and MaNIS [<http://manisnet.org/>])—Stein and Wiczorek 2004) we collated georeferenced occurrence points of both *S. minutissimus* and *S. yukonicus*. We refined these records by removing duplicate records, falsely georeferenced locations (e.g., locations over ocean), and records with low precision taken from atlas grid centroids, resulting in 78 occurrence points used for analysis.

We used Maxent version 3.3 (Phillips et al. 2006; Phillips and Dudik 2008) to construct ENMs. Maxent generates ENMs using only presence records, contrasting them with pseudo-absence or background data resampled from the remainder of the study area. In each case we developed present-day ENMs and then projected the ENM to LGM conditions. We used the

default convergence threshold (10^{-5} ; change in log loss per iteration) and maximum number of iterations (1,000) values. We allowed the program to select both suitable regularization values and functions of environmental variables automatically, which it achieves based on considerations of sample size. Maxent can run multiple analyses of a data set under a jackknife approach. We ran 5 replicates using the cross-validate option, in which the localities are divided into 5 bins, with each bin used as a subset for model testing (equivalent to 20% testing).

Maxent outputs a logistic probability value as an indicator of relative suitability for the species, based on the principle of maximum entropy, as constrained by the input occurrence data. We chose a low and high threshold for consideration of “suitable” habitat based upon the Maxent outputs using present-day climate data. The low threshold was the median value over the 5 replicates of the lowest presence threshold (Pearson et al. 2007), which is the threshold at which all occurrences in a training data set fall into suitable habitat, or a 0% omission rate. These values are more conservative than those used in recent studies (Pearson et al. 2007; Waltari et al. 2007a). The high threshold was the median value over the 5 replicates of the Maxent-generated value “Equal training sensitivity and specificity,” which we found to have the least variance of all standard thresholds generated by Maxent (E. Waltari, pers. obs.). This threshold identified smaller areas than a lowest presence threshold that yielded 0 omission error, resulting in a more restricted picture of potential LGM distributions. We then generated summary maps in ArcGIS 9.3 that show Maxent predictions for present day and LGM.

Population genetic inference.—Population parameters were calculated for the mtDNA *Cytb* data set to include the maximum number of specimens with a common gene length (549 bp; $n = 84$). Populations were grouped based on the reconstructed phylogenies for the 2 species, on our present knowledge of the geologic and phylogeographic history of the Holarctic, and on predicted potential distribution through ENMs. We grouped *S. yukonicus* as a single population (Alaska) based on a shallow phylogeny (Fig. 3) and a discrete distribution. We split *S. minutissimus* into 5 groups: a population consisting of individuals from Finland (Finland), a population consisting of individuals between Finland and the Yenisei River (Europe-to-Yenisei), a Siberian population (Siberia), a population consisting of individuals south of the Stanovoy Range and north of the Amur River (SE Siberia), and a Maritime Northeast Asia population (MNA) consisting of individuals from Primorsky Krai south of the Amur River, northern Japan, and the Kurile Islands (Fig. 2). As an alternative we grouped all *S. yukonicus* and the 2 Siberian populations of *S. minutissimus* together to form a single population (Beringia) and similarly grouped the 2 European populations together (Europe). Summary statistics were calculated for each population to assess genetic diversity and patterns of population demographics: segregating sites (S), haplotype diversity (Hd), nucleotide diversity (π), and Watterson’s theta (θ —Watterson 1975). For a potential signal

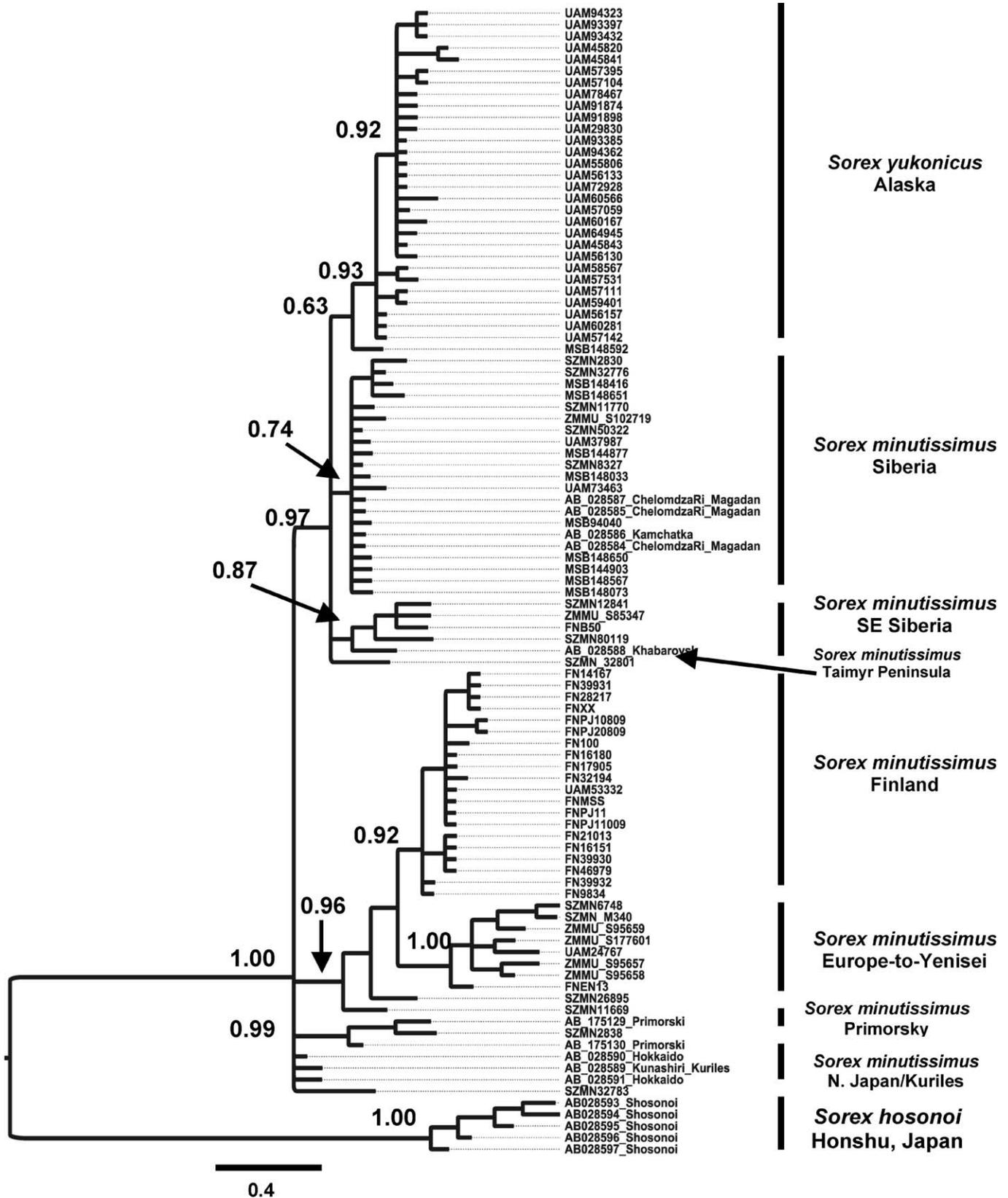


FIG. 3.—Bayesian phylogeny representing a midpoint-rooted genealogy for the *Sorex minutissimus*–*yukonicus* group based on cytochrome-*b* gene sequences (561–1,140 bp). Posterior probabilities for major nodes are illustrated. *Sorex hosonoi*, a Honshu Island endemic, is included as the sister species.

of demographic expansion we used DnaSP (Librado and Rozas 2009) to calculate Tajima's D (Tajima 1989), Fu's F_s (Fu 1997), and R_2 (Ramos-Onsins and Rozas 2002) and assessed significance with 10,000 coalescent simulations.

Estimation of mutation rate and population size change.—Estimations of mutation rate and effective population size are generally difficult. For example, estimates of mitochondrial *Cytb* mutation rates for mammals range from 0.7% to 60% per million years (Nabholz et al. 2008), although an established universal mammalian mitochondrial rate of 2.5% per million years often is used for phylogeographic investigations (Avice 2000). Statistical methods for analyzing population histories based on the coalescent rely on robust estimates of parameters for inferring historical demographics. We can calculate theta (θ) as a measure of effective population size proportional to the mutation rate ($\theta = 4$ effective population size $[N_e]\mu$ for diploid loci; $\theta = 2$ effective female population size $[N_f]\mu$ for haploid loci; $\mu =$ population size). However, for a single locus such as *Cytb*, theta has broad confidence limits and so point estimates are potentially inaccurate (Edwards and Beerli 2000). Using the program BEAST (Drummond and Rambaut 2007), we included the entire set of mitochondrial sequences for the *minutissimus*–*yukonicus* group ($n = 95$) and 5 sequences of the sister species, *S. hosonoi*, retrieved from GenBank (AB028593–AB028597). We grouped individuals into populations as described above and designated monophyly for all *S. minutissimus* and *S. yukonicus* and all *S. hosonoi*. Under a strict molecular clock and HKY + G model of nucleotide substitution (obtained from Modeltest—Posada and Crandall 1998), 2 partitions into codon positions ((1 + 2)3), and a Bayesian skyline tree prior, we ran independent Markov chain Monte Carlo analyses under a range of mutation rates (2%, 4%, and 6% per million years). All 3 estimates placed a most recent common ancestor (MRCA) in the region of 1 mya. *S. hosonoi* is endemic to Honshu Island (Ohdachi et al. 1997). The isolation history of Honshu Island includes a number of hypothesized isolation–reconnection events via land bridges through the Quaternary, with Hokkaido at the Tsugaru Strait, and with mainland Korea at the Tsushima Strait (Dobson and Kawamura 1998; Sota and Hayashi 2007; Van den Bergh et al. 2001; Yoshikawa et al. 2007; Fig. 2). However, late-Pliocene to early-Pleistocene events that coincide with a divergence estimate of ~ 1 mya consist of only 2 possible bridges of ~ 2.5 mya (Dobson and Kawamura 1998; Van den Bergh et al. 2001) and ~ 1 mya. The latter date is estimated by Yoshikawa et al. (2007) at 1.2 mya, by Dobson and Kawamura (1998) at 1 mya, and by Van den Bergh et al. (2001) at 1–0.8 mya. All estimates are consistent with evidence from oxygen isotope ratios and records of historical sea-level change. Younger bridges are inferred at ~ 0.6 mya, ~ 0.4 mya, 130 thousand years ago (kya), and finally the LGM at 20 kya. All of these latter ages are outside the 95% bound of our 1-my divergence estimate, although other constituents of the mammalian fauna are considered to have used 1 or more of these recent potential bridges (Inoue et al. 2007; Kawamura 2007; Millien-Parra and Jaeger 1999; Sota and Hayashi 2007;

Yasuda et al. 2005). We set a prior in BEAST of 1 mya for the MRCA of *S. hosonoi* and the *S. minutissimus* clade with upper and lower bounds of 1.2 mya and 0.8 mya, respectively, unfixed the substitution rate, and set the molecular clock model to a relaxed clock: uncorrelated lognormal. We reran the Markov chain Monte Carlo algorithm with a length of chain of 30,000,000, logging trees every 3,000 to obtain an estimate for mutation rate of 5.5% per million years. Two further Markov chain Monte Carlo iterations were performed by fixing the substitution rate to 0.055 and applying a strict clock and then a relaxed clock to check for consistency among these 2 assumptions. These final iterations also provided divergence estimates for the time to MRCA (T_{MRCA}) of each designated population under our specified mutation rate.

Bayesian inference also can be used to assess change in population size through time. For Alaska, Siberia, and Europe clades individually we reran the Bayesian skyline estimation using our calculated mutation rate under similar model parameters but using all tree priors and constraining the tree root height to the T_{MRCA} for each group. Because of small sample sizes only these groups were analyzed. This provided us with Bayesian skyline plots for population size change in each designated group. The x-axis of a Bayesian skyline plot is reported as time in millions of years, and the y-axis is reported as $N_e\tau$ where N_e is the effective population size and τ is the generation time in the units ascribed to model parameters. Estimates of ancestral and contemporary N_e were calculated from Bayesian skyline plot estimation for all specimens according to the equation $N_e = g/\tau$, where g is expressed as generation time in millions of years. Shrews (*Sorex*) are known to produce up to 3 litters per year with the potential of 1st-litter females themselves reproducing in the same year (Dokuchaev 2005). However, survival rate is very low for shrews, and most newborn individuals that survive do not breed until the following spring (Churchfield 1990). We therefore maintained a generation time of 1 year.

RESULTS

Mitochondrial DNA.—We sequenced 561–1,140 bp of *Cytb* from 29 individuals of *S. yukonicus* and 56 individuals of *S. minutissimus*, with an additional 10 sequences retrieved from GenBank. No sequences contained insertions, deletions, or internal stop codons. There were no 2nd-position mutations. The Bayesian phylogeny illustrated well-supported and reciprocally monophyletic clades for *S. hosonoi* as the sister taxon and for the *minutissimus*–*yukonicus* group (Fig. 3) with uncorrected sequence divergence between sister taxa of 8.6%. Uncorrected sequence divergence within the *minutissimus*–*yukonicus* group varied from 0.6% to 2.2% (Table 1). We obtained high support for a monophyletic clade consisting of all *S. yukonicus* (posterior probability = 0.93; Fig. 3), but *S. minutissimus* was paraphyletic with respect to *S. yukonicus*. Considering a potential Beringian autochthon, high support existed for a clade consisting of all *S. yukonicus* (eastern Beringia) and all *S. minutissimus* from Siberia (western

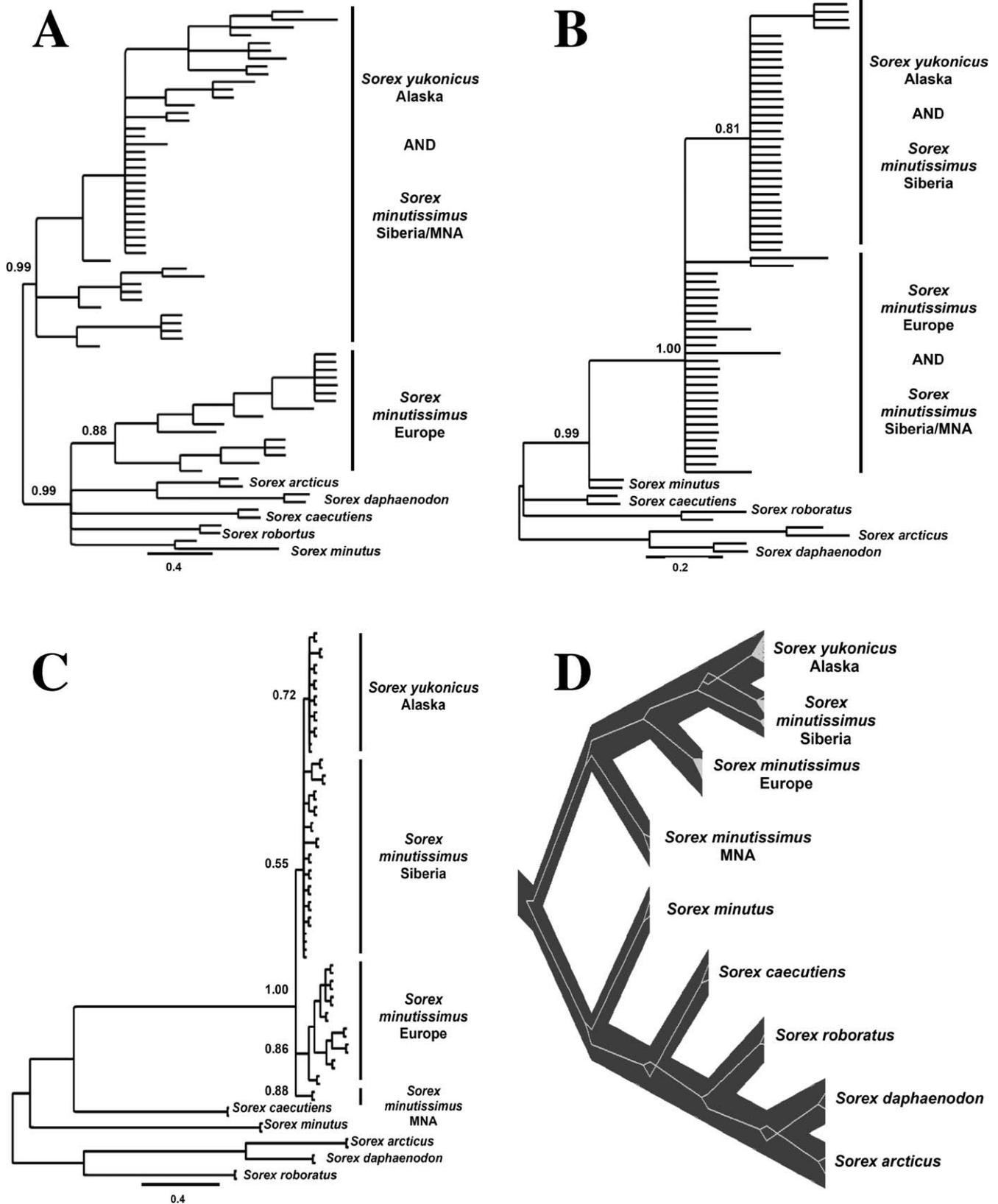


FIG. 4.—Gene tree and species tree reconstructions for the *Sorex minutissimus*–*yukonicus* group. Bayesian estimation was used for all midpoint-rooted genealogies using independent loci: A = apolipoprotein B (ApoB); B = breast cancer susceptibility 1 (BRCA1); C = cytochrome *b* (*Cytb*). Posterior probabilities for major nodes are illustrated (≥ 0.95 is considered significant) along with major clade relationships. Species tree D was produced based on topologies of all genealogies using the minimizing deep coalescences method (100 iterations). The line within species tree D indicates a single genealogy (here BRCA1); branch lengths are not proportional to genetic distance.

TABLE 1.—Uncorrected sequence divergence between major clades within the *Sorex minutissimus*–*yukonicus* group using the cytochrome-*b* gene. Beringia consists of 2, 3, and 4; Europe consists of both 7 and 8. *Sorex hosonoi* is the recognized sister taxa to the *S. minutissimus*–*yukonicus* group.

Group	1	2	3	4	5	6	7	8	9
1. Beringia	—	—	—	—	0.010	0.014	0.013	0.018	0.086
2. <i>S. yukonicus</i> —Alaska	—	—	0.006	0.009	0.010	0.014	0.013	0.018	0.086
3. <i>S. minutissimus</i> —Siberia	—	—	—	0.009	0.010	0.013	0.012	0.018	0.086
4. <i>S. minutissimus</i> —SE Siberia	—	—	—	—	0.014	0.017	0.016	0.022	0.086
5. <i>S. minutissimus</i> —MNA	—	—	—	—	—	0.011	0.010	0.016	0.082
6. Europe	—	—	—	—	—	—	—	—	0.086
7. <i>S. minutissimus</i> —Finland	—	—	—	—	—	—	—	0.006	0.086
8. <i>S. minutissimus</i> —Europe-to-Yenisei	—	—	—	—	—	—	—	—	0.086
9. <i>Sorex hosonoi</i>	—	—	—	—	—	—	—	—	—

Beringia; posterior probability = 0.97), although specimens within this clade include geographic localities west of the recognized limits of Beringia (e.g., Taimyr Peninsula; Fig. 3). A well-supported clade consisting of *S. minutissimus* from Europe and western Russia (including both Finland and Europe-to-Yenisei groups) represents the most divergent clade within the *minutissimus*–*yukonicus* group, followed by another well-supported clade from the Primorsky Krai area, south of the Amur River (Fig. 3; Table 1). Specimens from Finland form a distinct clade from other individuals occurring in Europe and Russia east to the Yenisei River. A reduced *Cytb* data set consisting of 30 haplotypes exhibits the same relationships as for the full data set, although relative support values for clades are weaker (Fig. 4C).

Species tree estimation and multiple loci.—We reduced the data set to 30 individuals, including specimens from Finland, Europe-to-Yenisei, Siberia, MNA, and Alaska clades of the *minutissimus*–*yukonicus* group. All 3 genealogies exhibited similar major patterns of divergence within the *minutissimus*–*yukonicus* group. We found consistently low support or lack of reciprocal monophyly between *S. minutissimus* and *S. yukonicus* despite all 3 loci being phylogenetically informative (Figs. 4A–C). Numbers of segregating sites (*S*) for the *minutissimus*–*yukonicus* group among the 3 loci are: *ApoB* = 26, *BRCA1* = 7, *Cytb* = 57. European *S. minutissimus* were consistently separated from the remainder of the group, although they did not form a monophyletic clade in the *BRCA1* phylogeny (Fig. 4B). Relationships among the outgroup taxa were largely unresolved over the 3 genealogies. A consistent sister relationship between *S. arcticus* and *S. daphaenodon* was observed in all genealogies, but the closest taxon to the *minutissimus*–*yukonicus* group was ambiguous. A species tree estimated using the minimizing deep coalescences method based on all 3 genealogies (Fig. 4D) placed *S. yukonicus* and Siberian *S. minutissimus* as sister taxa, European *S. minutissimus* sister to these, and MNA *S.*

minutissimus as basal to the rest of the group. Higher-level relationships among outgroup taxa were resolved with *S. minutus* closest to the *minutissimus*–*yukonicus* group, although elsewhere *S. roboratus* has been considered a closer relative (Ohdachi et al. 2006), and more often relationships are unresolved. Ancestral polymorphism between *S. yukonicus* and Siberian *S. minutissimus* is sorted only for the *Cytb* locus (not shown within the species tree) and clearly remains unsorted in the 2 nuclear genealogies (e.g., *BRCA1*; Fig. 4D). In contrast, European specimens of *S. minutissimus* are sorted from the remainder of the *minutissimus*–*yukonicus* group in all 3 genealogies.

Ecological niche models.—The predicted current distribution of *S. minutissimus* and *S. yukonicus* is generally consistent with the known range of these species (Fig. 1A). Notable exceptions to this are an absence of *S. yukonicus* in suitable habitat predicted for Canada. Likewise, *S. minutissimus* is not known to occur in central Europe. A projection of potential distribution back to conditions at 18 kya at the LGM indicates several potential refugia (Fig. 1B). Most notably, a large proportion of Alaska and the Bering Land Bridge are predicted as suitable at 18 kya, according to the ENM, as well as much of MNA. More discrete refugia are predicted further west, including high-suitability areas (dark shading) in the vicinity of the Carpathian Mountains, Black Sea, the Caucasus, and northwestern China. In Europe and Central Asia a shift of potential distribution southward occurs during the LGM, coupled with significant range contraction. However, MNA and discrete areas within Beringia are predicted as potential habitat during both glacial and interglacial maxima, providing the potential for species persistence in these areas. In addition, the vicinity of the Amga River basin between the Lena River and Verkhoyansk Range in central Siberia is strongly predicted during both time frames.

Population genetic statistics.—European *S. minutissimus* are consistently most divergent from other populations at

←

Notice incomplete lineage sorting based on current taxonomic designations within the *Sorex minutissimus*–*yukonicus* group for this genealogy. Outgroup includes 5 species all belonging to the subgenus *Sorex*.

TABLE 2.—Population genetic statistics for all major clades within the *Sorex minutissimus*–*yukonicus* group. Summary statistics are n = sample size; S = segregating sites; h = number of haplotypes; Hd = haplotype diversity; π = nucleotide diversity. Population growth statistics are Tajima's D , Fu's F_s , and Ramos-Onsins and Rozas' R_2 . Significance of growth statistics are indicated by asterisks (* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$). NA = not applicable.

Group	n	S	h	Hd	π	D	F_s	R_2
All	84	51	37	0.917	0.009	NA	NA	NA
Beringia	56	26	22	0.843	0.005	–1.819*	–14.525***	0.043**
<i>S. yukonicus</i> —Alaska	29	8	8	0.571	0.001	–1.848**	–5.027***	0.054***
<i>S. minutissimus</i> —Siberia	20	9	8	0.647	0.002	–1.869**	–4.361***	0.069***
<i>S. minutissimus</i> —SE Siberia	5	7	4	0.9	0.005	–0.747	–0.332	0.172
<i>S. minutissimus</i> —MNA	6	11	6	1	0.007	–0.943	–2.587*	0.145
Europe	22	14	9	0.797	0.004	–1.552	–2.67	0.069***
<i>S. minutissimus</i> —Finland	14	2	3	0.582	0.001	0.036	–0.04	0.179
<i>S. minutissimus</i> —Europe-to-Yenisei	5	9	4	0.9	0.008	0.132	0.286	0.166

~1.5%, followed by MNA *S. minutissimus* at ~1.0% uncorrected sequence divergence (Table 1). Siberian *S. minutissimus* are minimally divergent from Alaska *S. yukonicus* at 0.6%. Among populations, haplotype and nucleotide diversity are high for SE Siberia, MNA, and Europe-to-Yenisei (Table 2) and comparatively low for Alaska, Siberia, and Finland. In particular, Alaska *S. yukonicus* and Siberia *S. minutissimus* exhibit minimal genetic diversity among all individuals, and all but 1 haplotype is unique to only 1 or 2 individuals. However, most Finland haplotypes are shared among multiple individuals. Specimens of *S. minutissimus* from Amga River collectively exhibit relatively high nucleotide and haplotype diversity ($\pi = 0.004$; $Hd = 0.933$). Population growth statistics (Table 2) are significant for Alaska and Siberia but not significant for SE Siberia, MNA, Finland, or Europe-to-Yenisei.

Temporal divergence and effective population size change.—With an estimated mutation rate for *S. minutissimus* at 5.5%/million years, the time to coalescence of lineages within the *minutissimus*–*yukonicus* group for the *Cytb* locus was calculated using posterior probabilities. The T_{MRCA} can be considered a minimum estimate for divergence, considering times related to the glacial periodicity of the middle Pleistocene to present. The T_{MRCA} values for all lineages within the *minutissimus*–*yukonicus* group were dated from an earliest date in the previous (Sangamon) interglacial at 0.13 mya to most recently near the close of the Wisconsinan–Weichselian glacial at 0.04 mya (mean values; Table 3). The 95% confidence interval (95% CI) includes times coincident with the Illinoian glacial (up to 0.22 mya) for the T_{MRCA} of the entire *minutissimus*–*yukonicus* group and for T_{MRCA} of MNA and European populations, but T_{MRCA} for all Beringian populations and Finland fall strictly within the Wisconsinan glacial. Bayesian skyline plots give a visual interpretation of change in N_e through time. Alaska *S. yukonicus* (eastern Beringia), Siberia *S. minutissimus* (western Beringia excluding SE Siberia), and Europe all indicate an increase in N_e during the late stages of the Wisconsinan glacial followed by a leveling off toward the present (Fig. 5). Population growth was estimated earliest within Europe (from

~0.045 mya) and latest in Alaska (~0.020 mya). For the entire *minutissimus*–*yukonicus* group ancestral N_e was calculated to be 120,000 and contemporary N_e to be 2,000,000.

DISCUSSION

During Pleistocene glacial episodes the Nearctic and Palearctic were connected multiple times as a single continental landmass so genetic associations of the contemporary biota across the Holarctic are a result of this dynamic history. The *minutissimus*–*yukonicus* group has a current distribution that reflects the reach of Eurasia into North America across Beringia during glacial maxima. The close relationship of *S. minutissimus* and *S. yukonicus* provides an opportunity to investigate the dynamics of northern intercontinental exchange and diversification. Although 2 species in this group are recognized (Hutterer 2005), the asymmetric distribution on either side of the Bering Strait and low divergence across loci suggests a relatively recent colonization of these shrews into North America.

This 1st investigation of genetic relationships within the *minutissimus*/*yukonicus* group reveals that although strong support exists for both an Alaska clade and a combined Beringia clade based on only *Cytb* (Fig. 3), these relationships are supported only weakly across multiple loci (Fig. 4). Instead, the most distinct evolutionary lineages within the *minutissimus*–*yukonicus* group consist of European *S. minutissimus* and MNA *S. minutissimus* (Figs. 3 and 4). European *S. minutissimus* are distinct in all genealogies, separately and combined. A lack of genetic distinction of *S. yukonicus* over multiple loci is consistent with recent isolation, and T_{MRCA} places divergence of the Alaskan population from Asian populations in the late Wisconsinan glacial (Table 3).

Population genetics and demographics.—Ohdachi et al. (2001) recognize a sister relationship between *S. minutissimus* and *S. hosonoi*. We found that these species were reciprocally monophyletic but closely related. Uncorrected sequence divergence between the *minutissimus*–*yukonicus* group and *S. hosonoi* of 8.6% (*Cytb*) is consistent with levels of differentiation observed in other sister species of shrews

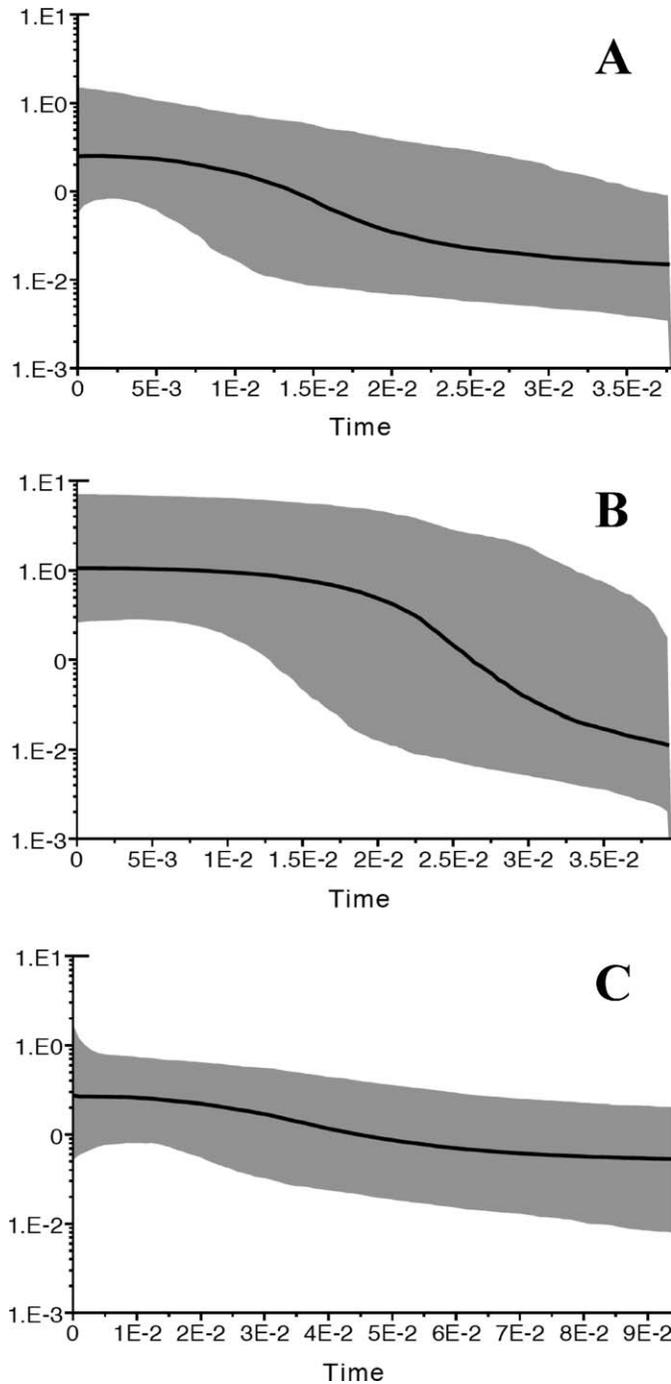


FIG. 5.—Bayesian skyline plots for A) Alaska *Sorex yukonicus*, B) Siberia *S. minutissimus*, and C) Europe *S. minutissimus*, created in BEAST from the cytochrome-*b* data set. Central line indicates the Bayesian skyline plot and change in effective population size through time. Shading defines the 95% CI. Bayesian skyline plots here extend right-to-left from past to present scaled in millions of years. Vertical axis represents τ as a function of generation time and effective population size.

(Demboski and Cook 2001; Fumagalli et al. 1999). Levels of mtDNA sequence differentiation of $\leq 2.2\%$ for all *S. minutissimus* and *S. yukonicus* comparisons are typical of intraspecific differentiation in shrews (e.g., *Sorex cinereus*—Demboski and Cook 2003). Sequence divergence between

TABLE 3.—Divergence estimates using BEAST (Drummond and Rambaut 2007) showing the sample size (n) and the mean for time to most recent common ancestor (\bar{X}_{TMRCA} , in million years) for major groups, with 95% CI within the *Sorex minutissimus*–*yukonicus* group and including the sister species *S. hosonoi*.

Group	n	\bar{X}_{TMRCA}	95% CI
<i>S. minutissimus</i> – <i>S. hosonoi</i>	99	0.98	0.79–1.19
<i>S. minutissimus</i> – <i>S. yukonicus</i>	51	0.13	0.07–0.20
Beringia	57	0.08	0.04–0.13
<i>S. yukonicus</i> —Alaska	29	0.04	0.02–0.07
<i>S. minutissimus</i> —Siberia	21	0.04	0.02–0.07
<i>S. minutissimus</i> —SE Siberia	5	0.05	0.02–0.09
MNA	7	0.12	0.03–0.22
<i>S. minutissimus</i> —Primorski	3	0.04	0.01–0.08
Europe	30	0.10	0.05–0.16
<i>S. minutissimus</i> —Finland	20	0.04	0.02–0.08
<i>S. minutissimus</i> —Europe-to-Yenisei	8	0.09	0.04–0.14

Siberian *S. minutissimus* and Alaskan *S. yukonicus* of 0.6% suggests that these populations have not been isolated for an extended period (Table 1). Haplotype and nucleotide diversity are low (Table 2), and we interpret these patterns as reflecting recent population expansion, a conclusion supported by significant values over multiple measures of expansion (Table 2). Significance over multiple expansion statistics suggests that expansion, and not selection, is the major influence producing this genetic signature (Lessa et al. 2003).

Biogeographic perspectives.—European and MNA clades within the *minutissimus*–*yukonicus* group likely reflect distinct geographic partitioning and temporal divergence. Environmental associations in the form of ENMs provide a framework for generating hypotheses of spatiotemporal divergence within a species group. The limited distribution of *S. yukonicus* in North America to only Alaska, despite a predicted potential distribution that spans northern Canada (Fig. 1A), raises several possibilities: this species is competitively excluded from some areas of potential habitat in North America, it is physically constrained to Alaska by some geographic barrier, or it has not had the time to disperse into available habitat across Canada. A competitive exclusion hypothesis has not been investigated, and little is known of interspecific ecological relationships for *S. yukonicus*, although it is sympatric with 5 other species of *Sorex* (MacDonald and Cook 2009). A physical barrier such as the north–south orientation of the Mackenzie Mountains at the border of Yukon and Northwest Territories delimits the eastern edge of Beringia (i.e., the western extent of the Laurentide Ice Sheet at the LGM) and could pose a significant barrier to dispersal of *S. yukonicus* eastward (Fig. 2). If the Mackenzie Mountains constituted a barrier to *S. yukonicus* over multiple glacial cycles, we would expect high genetic diversity within *S. yukonicus* combined with statistics reflecting population stability. Instead, the molecular signal suggests that *S. yukonicus* is a recent occupant of Alaska. Estimates of T_{MRCA} coincide with the most recent glacial period (Table 3), and Bayesian skyline plots indicate that population expansion took place in the Holocene.

In Eurasia *S. minutissimus* exhibits 2 major phylogeographic breaks, 1 separating European individuals (east to the Yenisei River) from Siberian animals (Ohdachi et al. 2001) and 1 separating MNA individuals (Primorsky Krai and Insular) from all others. All 3 groups are consistent with diversification in ENM-predicted refugia (Fig. 1B). In addition, these breaks are shared across other taxonomic groups. Among mammals, a phylogeographic break at the Ural Mountains and again at the western edge of Beringia has been identified for collared lemmings (*Dicrostonyx groenlandicus*—Fedorov and Goropashnaya 1999; Fedorov and Stenseth 2002) and root or tundra voles (*Microtus oeconomus*—Brunhoff et al. 2003). A split between a clade centered on northern Eurasia and 1 centered on MNA (Primorsky Krai near the Amur River) has been suggested for wood lemmings (*Myopus schisticolor*—Fedorov et al. 2008), harvest mice (*Micromys minutus*—Yasuda et al. 2005) and red deer (*Cervus elaphus*—Ludt et al. 2004). Although much of Siberia remained ice-free through Pleistocene glacial periods, much of northern Europe was covered by the Fennoscandian ice sheet. *S. minutissimus* likely persisted in 1 or more refugia south of the ice sheet such as the Carpathian, Black Sea, or Caucasian refugia, as predicted by our ENM (Figs. 1B and 2) and as has been predicted for other species (Flojgaard et al. 2009). The exact location of refugia for each clade within *S. minutissimus* is unknown, but evidence from fossils shows that *S. minutissimus* occurred from the Caucasus to north of the Black Sea during the late Pleistocene (Zaitsev and Osipova 2005). In addition, a fossil attributable to *S. minutissimus* was found in South Korea dating to the middle to late Pleistocene (Ohdachi et al. 1997). Structure within Europe uncovers 2 distinct clades, 1 consisting of Finland individuals and another of individuals distributed from southeast Finland eastward to the Yenisei River. This indicates potential contact between these clades within Finland. The most basal individuals from the Europe-to-Yenisei group are easternmost in distribution opening the possibility of an origin of this group in south-central Russia (Fig. 2). Considering that no individuals belonging to the Siberian clade occur west of the Yenisei River, the geographic area known as the Siberian Plain between the Ural Mountains east to the Yenisei River likely represents the geographic border between European and Siberian groups. The Siberian Plain constituted extensive pluvial lakes during glacial maxima (Mangerud et al. 2004) and currently constitutes extensive swamplands and formidable rivers, providing a potentially permanent barrier to dispersal of taxa. Il'yashenko and Onishchenko (2003) report a morphological break between west and east Siberian *S. minutissimus*, and a phylogeographic break in the vicinity of the Yenisei River also is shown for moose (*Alces*—Boyeskorov 1999; Grubb 2005; Hundertmark et al. 2002).

In Siberia and MNA, environmental predictions from ENMs suggest potential persistence of *S. minutissimus* in large refugial areas (Fig. 1), although LGM environments in Beringia were considered to have been harsh (DeChaine 2008; Elias and Crocker 2008). Within MNA, population

stability is reflected in expansion statistics (Table 3). Other populations (Alaska and Siberia) that might have persisted within Beringia all show significant expansion signals dated from the LGM (Table 2; Fig. 5). We suggest that ancestors of Alaskan and Siberian populations persisted in discrete areas within Beringia in low numbers through the Wisconsinan, followed by subsequent range expansion and population growth. Ancestral population size is greater than an order of magnitude lower than contemporary size estimates, suggesting emergence from a population bottleneck. Evidence for another refugium within Eurasia consists of a clade centered on southeastern Siberia that is bounded by the Dzhugdzhur Range and Stanovoy Range to the north and the Amur River to the south (Fig. 2). Additionally, high genetic diversity exists within the 6 individuals collected from the Amga Basin of central Siberia that is indicative of persistence. The Amga Basin is geographically isolated in central Siberia and surrounded by the Verkhoyansk Range to the north, Dzhugdzhur Range to the east, Stanovoy Range to the south, and Lena River to the west (Fig. 2).

Taxonomy.—We tested hypotheses pertaining to the taxonomic validity of *S. minutissimus* and *S. yukonicus*. Three distinct biogeographic regions for these shrews were identified: Europe, MNA, and Beringia. All are recently divergent at levels coincident with isolation through the Wisconsinan–Weichselian glacial period. Minimal sequence divergence across Beringia (0.6%; *Cytb*) is inconsistent with species-level molecular differentiation within *Sorex*. This pattern of minimal differentiation across the Bering Strait is comparable to levels of divergence among several nominal species within the *S. cinereus* group in Beringia (Demboski and Cook 2003) and suggests the need for careful reevaluation of a number of shrew complexes at these high latitudes. Low genetic divergence that reflects recent isolation in Alaska and lack of reciprocal monophyly between the 2 nominal species suggests that they represent a single species, *S. minutissimus*, as originally proposed (Dokuchaev 1994). We hypothesize that Alaskan populations were recently isolated from Siberian *S. minutissimus* by rising sea levels that inundated the Bering Land Bridge.

Evidence from multiple independent loci shows that European *S. minutissimus* is consistently diagnosable (e.g., genetic distance of 1.5%; *Cytb*). Recent coalescence of *Cytb* within *S. minutissimus* is at odds with fossil evidence of this species occurring in Europe from the early Pleistocene (Rzebik-Kowalska 1995, 2005). However, Rzebik-Kowalska (1995) suggested marked movement of these shrews in response to climate change that would be consistent with ENM predictions within Europe (Fig. 1). Recognized karyotypic differences between European and Siberian *S. minutissimus* (diploid number of 38 versus 42, respectively—Hutterer 2005) further corroborate the genetic distinction between the shrews of these regions. The type specimen for *S. minutissimus* is described from the vicinity of the Yenisei River (Hutterer 2005) corresponding with our hypothesized phylogeographic break between European and Siberian clades; therefore, taxonomic revision should wait for additional sampling from this region.

Future directions.—In light of our conclusions, *S. minutissimus* provides a contemporary snapshot into the evolutionary processes acting on mammals at the nexus between the northern continents. Alaskan populations of *S. minutissimus* are now on a unique evolutionary trajectory through physical isolation from Eurasia at the Bering Strait, although relatively short interglacial isolation could be followed by reconnection and population admixture before reproductive isolation is achieved. In addition, physical boundaries coupled with dynamic climate shifts in Eurasia have produced distinct genetic lineages within *S. minutissimus*, suggesting the presence of previously undiscovered refugial areas. We stress the relative paucity of information pertaining to spatiotemporal evolution of the biota within Siberia considering potential persistence in ice-free, high-latitude areas through extended periods of glacial cycling. The 77 extant species of *Sorex* occur in either the Palearctic or Nearctic (Hutterer 2005), with the exception of *Sorex tundrensis* and now *S. minutissimus*, which are the only species recognized as Holarctic. Across its broad range *S. minutissimus* is sympatric or parapatric with up to 18 other species of *Sorex* (MacDonald and Cook 2009; Sheftel 2005). A comparative phylogeographic approach should be implemented that aims to explore the response of multiple related species as a precursor to understanding how members of this diverse genus will respond to future environmental scenarios (Carnaval et al. 2009). Understanding how the constituents of these high-latitude communities responded to past climatic cycling can provide insight into community change through time and pinpoint cold-adapted species that could be of future conservation concern (Lister 2004). Finally, further investigation of phylogenetic relationships within the genus *Sorex* throughout the Holarctic will help to clarify evolutionary processes within this widespread, diverse, and enigmatic genus of mammals.

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- Kathul Mountain: UAM57059 (GU223656, NA, NA); UAM57104 (GU223654, GU223695, GU223727); UAM57111 (GU223655, HM002724, HM002743); UAM57142 (GU223652, GU223693, GU223725); UAM57395 (GU223653, GU223694, GU223726); UAM59401 (HM002706, NA, NA); (5) United States, Alaska: Carden Hills: UAM57531 (GU223657, GU223696, GU223728); (6) United States, Alaska: Glenn Creek Cabin: UAM58567 (GU223650, HM002723, HM002742); (7) United States, Alaska: Kandik Cabin: UAM60167 (GU223651, GU223692, GU223724); (8) United States, Alaska: across Yukon River from Glenn Creek Cabin: UAM60281 (GU223648, GU223691, GU223723); UAM60566 (GU223649, NA, NA); (9) United States, Alaska: Chilchukabena Lake: UAM64945 (GU223658, GU223697, GU223729); (10) United States, Alaska: 7 miles SW Red Devil: UAM72928 (GU223643, GU223687, GU223719); (11) United States, Alaska: Fortress Mountain: UAM78467 (GU223659, HM002725, HM002744); (12) United States, Alaska: Chulitna River and Turner Bay confluence: UAM91874 (GU223660, GU223682, GU223714); UAM91898 (GU223661, GU223683, GU223715); (13) United States, Alaska: Waring Mountains: UAM93385 (GU223685, GU223717); (14) United States, Alaska: Baird Mountains, Salmon River: UAM93397 (GU223663, NA, NA); (15) United States, Alaska: Baird Mountains, headwaters of Akillik River: UAM93432 (GU223664, NA, NA); (16) United States, Alaska: Kakagrak Hills: UAM94323 (HM002707, NA, NA); UAM94362 (GU223622, GU223648, GU223716); (no specific locality recorded) United States, Alaska: UAM29830 (GU223639, GU223681, GU223713).
- Sorex minutissimus*.—(17) Finland: Haapavesi, Mieluskylä: FN 9834 (HM002675, NA, NA); (18) Finland: Utajärvi, Pälli: FN 14167 (HM002664, NA, NA); FN 16151 (HM002665, HM002708, HM002727); FN 16180 (HM002666, NA, NA); FN XX (HM002676, HM002711, HM002730); (19) Finland: Kuusamo: FN 17905 (HM002667, NA, NA); (20) Finland: Oulu, Kaukovaio: FN 21013 (HM002668, NA, NA); (21) Finland: Kiiminki: FN 28217 (HM002669, NA, NA); (22) Finland: Rovaniemi, Kaihua: FN 32194 (HM002670, HM002709, HM002728); (23) Finland: Kuivaniemi, Korkiakangas: FN 39930 (HM002671, NA, NA); Finland: Kuivaniemi, Näsiö: FN 39931 (HM002672, NA, NA); FN 39932 (HM002673, NA, NA); (24) Finland: Tervola, Karsikkokangas: FN 46979 (HM002674, NA, NA); (25) Finland: Enonkoski: FN EN13 (HM002676, HM002710, HM002729); (26) Finland: Salla: FN MSS (GU223627, GU223678, GU223710); (27) Finland: Pallasjärvi: FN PJ11 (GU223628, GU223679, GU223711); FN PJ10809 (HM002696, NA, NA); FN PJ11009 (HM002697, HM002726, HM002745); FN 20809 (HM002698, NA, NA); (28) Finland: Kuhmo: UAM24767 (HM002693, HM002720, HM002739); (no specific locality recorded) Finland: FN 100 (HM002663, NA, NA); Finland: UAM53332 (HM002695, NA, NA); (29) Mongolia: Hangal Soum: MSB94040 (GU223629, NA, NA); (30) Russia, Magadanskaya Oblast: Stokovo Station: MSB144877 (HM002704, HM002712, HM002731); (31) Russia, Republic of Sakha: Delyankir River: MSB144903 (GU223631, GU223671, GU223703); (32) Russia, Khabarovskiy Krai: Okhotsk Village: MSB148033 (GU223632, GU223672, GU223704); MSB148072 (GU223633, GU223673, GU223705); (33) Russia, Republic of Sakha: Amga River Basin, 7 km N Sulgachi: MSB148416 (HM002705, HM002721, HM002740); MSB148592 (GU223634, GU223674, GU223706); MSB148593 (GU223635, NA, NA); (34) Russia, Republic of Sakha: Amga River, 10 km NE Sulgachi: MSB148567 (HM002694, HM002713, HM002732); (35) Russia, Republic of Sakha: Amga River, 8 km ENE Mikhaylovka: MSB148650 (GU223636, GU223675,

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APPENDIX I

Specimens examined are listed by scientific name, specific locality number (in boldface type, corresponding to Fig. 2), specific locality, specimen number (museum catalog number or tissue number, corresponding to Fig. 3), and corresponding GenBank accession numbers (mitochondrial DNA cytochrome-*b*, nuclear DNA apolipoprotein B, nuclear DNA breast cancer susceptibility 1 [NA = not applicable]). Acronyms for museum or tissue numbers are FN = Finland tissues (provided by H. Henttonen); MSB = Museum of Southwestern Biology; SZMN = Siberian Zoological Museum, Novosibirsk; UAM = University of Alaska Museum of the North, Fairbanks; ZMMU = Zoological Museum of Moscow University.

Sorex yukonicus.—(1) United States, Alaska: Goodnews River: UAM45820 (GU223640, HM002722, HM002741); UAM45841 (GU223642, GU223686, GU223718); UAM45843 (GU223641, NA, NA); (2) United States, Alaska: Serpentine Hot Springs: UAM55806 (GU223647, GU223690, 223722); (3) United States, Alaska: Devil Mountain Lakes: UAM56130 (GU223644, GU223688, GU223720); UAM56133 (GU223645, GU223689, GU223721); UAM56157 (GU223646, NA, NA); (4) United States, Alaska: 12.5 km ENE of

UAM57059 (GU223656, NA, NA); UAM57104 (GU223654, GU223695, GU223727); UAM57111 (GU223655, HM002724, HM002743); UAM57142 (GU223652, GU223693, GU223725); UAM57395 (GU223653, GU223694, GU223726); UAM59401 (HM002706, NA, NA); (5) United States, Alaska: Carden Hills: UAM57531 (GU223657, GU223696, GU223728); (6) United States, Alaska: Glenn Creek Cabin: UAM58567 (GU223650, HM002723, HM002742); (7) United States, Alaska: Kandik Cabin: UAM60167 (GU223651, GU223692, GU223724); (8) United States, Alaska: across Yukon River from Glenn Creek Cabin: UAM60281 (GU223648, GU223691, GU223723); UAM60566 (GU223649, NA, NA); (9) United States, Alaska: Chilchukabena Lake: UAM64945 (GU223658, GU223697, GU223729); (10) United States, Alaska: 7 miles SW Red Devil: UAM72928 (GU223643, GU223687, GU223719); (11) United States, Alaska: Fortress Mountain: UAM78467 (GU223659, HM002725, HM002744); (12) United States, Alaska: Chulitna River and Turner Bay confluence: UAM91874 (GU223660, GU223682, GU223714); UAM91898 (GU223661, GU223683, GU223715); (13) United States, Alaska: Waring Mountains: UAM93385 (GU223685, GU223717); (14) United States, Alaska: Baird Mountains, Salmon River: UAM93397 (GU223663, NA, NA); (15) United States, Alaska: Baird Mountains, headwaters of Akillik River: UAM93432 (GU223664, NA, NA); (16) United States, Alaska: Kakagrak Hills: UAM94323 (HM002707, NA, NA); UAM94362 (GU223622, GU223648, GU223716); (no specific locality recorded) United States, Alaska: UAM29830 (GU223639, GU223681, GU223713).

Sorex minutissimus.—(17) Finland: Haapavesi, Mieluskylä: FN 9834 (HM002675, NA, NA); (18) Finland: Utajärvi, Pälli: FN 14167 (HM002664, NA, NA); FN 16151 (HM002665, HM002708, HM002727); FN 16180 (HM002666, NA, NA); FN XX (HM002676, HM002711, HM002730); (19) Finland: Kuusamo: FN 17905 (HM002667, NA, NA); (20) Finland: Oulu, Kaukovaio: FN 21013 (HM002668, NA, NA); (21) Finland: Kiiminki: FN 28217 (HM002669, NA, NA); (22) Finland: Rovaniemi, Kaihua: FN 32194 (HM002670, HM002709, HM002728); (23) Finland: Kuivaniemi, Korkiakangas: FN 39930 (HM002671, NA, NA); Finland: Kuivaniemi, Näsiö: FN 39931 (HM002672, NA, NA); FN 39932 (HM002673, NA, NA); (24) Finland: Tervola, Karsikkokangas: FN 46979 (HM002674, NA, NA); (25) Finland: Enonkoski: FN EN13 (HM002676, HM002710, HM002729); (26) Finland: Salla: FN MSS (GU223627, GU223678, GU223710); (27) Finland: Pallasjärvi: FN PJ11 (GU223628, GU223679, GU223711); FN PJ10809 (HM002696, NA, NA); FN PJ11009 (HM002697, HM002726, HM002745); FN 20809 (HM002698, NA, NA); (28) Finland: Kuhmo: UAM24767 (HM002693, HM002720, HM002739); (no specific locality recorded) Finland: FN 100 (HM002663, NA, NA); Finland: UAM53332 (HM002695, NA, NA); (29) Mongolia: Hangal Soum: MSB94040 (GU223629, NA, NA); (30) Russia, Magadanskaya Oblast: Stokovo Station: MSB144877 (HM002704, HM002712, HM002731); (31) Russia, Republic of Sakha: Delyankir River: MSB144903 (GU223631, GU223671, GU223703); (32) Russia, Khabarovskiy Krai: Okhotsk Village: MSB148033 (GU223632, GU223672, GU223704); MSB148072 (GU223633, GU223673, GU223705); (33) Russia, Republic of Sakha: Amga River Basin, 7 km N Sulgachi: MSB148416 (HM002705, HM002721, HM002740); MSB148592 (GU223634, GU223674, GU223706); MSB148593 (GU223635, NA, NA); (34) Russia, Republic of Sakha: Amga River, 10 km NE Sulgachi: MSB148567 (HM002694, HM002713, HM002732); (35) Russia, Republic of Sakha: Amga River, 8 km ENE Mikhaylovka: MSB148650 (GU223636, GU223675,

- GU223707); MSB148651 (GU223637, GU223676, GU223708); **(36)** Russia, Magadanskaya Oblast: Omolon: SZMN 2830 (HM002680, NA, NA); **(37)** Russia, Primorskyi Krai: Kedrovaya Pad Reserve: SZMN 2838 (HM002681, NA, NA); **(38)** Russia, Tomskaya Oblast: Kozhevnikovo Village: SZMN 6748 (HM002684, NA, NA); **(39)** Russia, Republic of Tuva: Totginskyi District, Lake Azas: SZMN 8327 (HM002686, NA, NA); **(40)** Russia, Republic of Altai: Cherga: SZMN 11669 (HM002699, NA, NA); **(41)** Russia, Republic of Buryatia: Muhurshibir: FN B50 (GU223626, GU223677, GU223709); **(42)** Russia, Amurskaya Oblast: Zeya: SZMN 12841 (HM002679, NA, NA); **(43)** Russia, Krasnoyarskyi Krai: Western Sayan, Abaza: SZMN 26895 (HM002700, HM002714, HM002733); **(44)** Russia, Irkutskaya Oblast: Zhigalovskyi District, Yakimovka: SZMN 32776 (HM002682, NA, NA); **(45)** Russia, Sakhalinskaya Oblast: Novoaleksandrovsk Village: SZMN 32783 (HM002701, HM002715, HM002734); **(46)** Russia, Krasnoyarskyi Krai: Taimyr, Pyasina River: SZMN 32801 (GU223638, HM002719, HM002738); **(47)** Russia, Republic of Tuva: Totginskyi District, Shurmak Village: SZMN 50322 (HM002683, HM002716, HM002735); **(48)** Russia, Zabaykalskyi Krai: Kyra, Sochondinskyi Reserve: SZMN 80119 (HM002685, HM002717, HM002736); **(49)** Russia, Novosibirskaya Oblast: Karasukskyi: SZMN M340 (GU223630, GU223680, GU223712); **(50)** Russia, Magadanskaya Oblast: near Magadan: UAM37987 (HM002702, NA, NA); **(51)** Russia, Kamchatskyi Krai: 22 km SW of Elizovo: UAM73463 (GU223625, NA, NA); **(52)** Russia, Republic of Buryatia: NE lakeside, Baikal: ZMMU S85347 (HM002689, NA, NA); **(53)** Russia, Republic of Karelia: Pudoch Region: ZMMU S95657 (HM002690, NA, NA); ZMMU S95658 (HM002691, NA, NA); ZMMU S95659 (HM002692, NA, NA); **(54)** Russia, Krasnoyarskyi Krai: Turukhanskyi District, Village Mirnoe: ZMMU S102719 (HM002687, HM002718, HM002737); **(55)** Russia, Kostroma Oblast: Manchurovskia: ZMMU S177601 (HM002688, NA, NA); **(no specific locality recorded)** Russia: SZMN 11770 (HM002678, NA, NA); **(56)** Russia, Magadanskaya Oblast: Chelomdzha River: (AB028584, NA, NA; AB028585, NA, NA; AB028587, NA, NA); **(57)** Russia, Kamchatskyi Krai: (AB028586, NA, NA); **(58)** Russia, Khabarovskiy Krai: (AB028588, NA, NA); **(59)** Russia Sakhalinskaya Oblast: Kuriles, Kunashir Island: (AB028589, NA, NA); **(60)** Russia, Primorskyi Krai: (AB175129, NA, NA; AB175130, NA, NA); **(61)** Japan: Hokkaido Island: (AB028590, NA, NA; AB028591, NA, NA).
- Sorex hosonoi* (all retrieved from GenBank; *Cytb* only).—Japan: Honshu Island: AB028593; AB028594; AB028595; AB028596; AB028597.
- Sorex arcticus*.—Canada, British Columbia: Horn Lake: UAM77955 (GU223666, GU223698, GU223730).
- Sorex caecutiens*.—Russia, Republic of Sakha: Lena River, 2 km NW Tochtur: MSB146478 (GU223668, GU223699, GU223731).
- Sorex daphaenodon*.—Russia, Republic of Sakha: Amga River, 8 km ENE Mikhaylovka: MSB148704 (GU223669, GU223700, GU223732).
- Sorex minutus*.—Hungary: Osli, Kiraly-to: MSB95476 (GU223667, GU223702, GU223733).
- Sorex roboratus*.—Russia, Republic of Sakha: Kenkeme River, 40 km W Yakutsk: MSB148679 (GU223670, GU223701, GU223734).