

Mitochondrial DNA variation in water shrews (*Sorex palustris*, *Sorex bendirii*) from western North America: implications for taxonomy and phylogeography

M.B. O'Neill, D.W. Nagorsen, and R.J. Baker

Abstract: Inter- and intra-specific variations in cytochrome *b* (*Cytb*) sequence were assessed in 22 specimens of *Sorex palustris* Richardson, 1828 and 6 specimens of *Sorex bendirii* (Merriam, 1884) from 20 locations in western North America. Phylogenetic analyses revealed three distinct clades: Boreal (*S. p. palustris*), Cordilleran (*S. p. brooksi*, *S. p. navigator*), and Coastal (*S. b. palmeri*, *S. b. bendirii*). Sequence divergence between the Boreal and the Coastal–Cordilleran lineages was 6.9%, while the divergence between the Coastal and the Cordilleran clades was 3.1%. *Sorex palustris brooksi*, a subspecies endemic to Vancouver Island, showed minor divergence from mainland samples of *S. p. navigator*. The results suggest that *S. palustris* may consist of two species: a boreal eastern form (*S. palustris*) and a Cordilleran form (*S. navigator*). The taxonomic validity of *S. p. brooksi* is unresolved. Distribution of the three clades are consistent with vicariance and isolation in coastal, Cordilleran, and eastern refugia in the Late Pliocene or Pleistocene. The Vancouver Island subspecies *S. p. brooksi* is probably derived from postglacial colonization in the Late Pleistocene.

Résumé : Nous avons évalué les variations interspécifique et intraspécifique de la séquence du cytochrome *b* (*Cytb*) dans 22 spécimens de *Sorex palustris* Richardson, 1828 et 6 spécimens de *Sorex bendirii* (Merriam, 1884) provenant de 20 sites de l'ouest de l'Amérique du Nord. Les analyses phylogénétiques révèlent l'existence de trois clades distincts, un du nord (*S. p. palustris*), un autre de la cordillère (*S. p. brooksi*, *S. p. navigator*) et un troisième de la côte (*S. b. palmeri*, *S. b. bendirii*). La divergence des séquences entre la lignée du nord et celles de la cordillère et de la côte est de 6,9 %, alors que la divergence entre les clades de la côte et de la cordillère est de 3,1 %. *Sorex palustris brooksi*, une sous-espèce endémique de l'île de Vancouver, montre une divergence mineure d'avec les échantillons de *S. p. navigator* du continent. Ces résultats laissent croire que *S. palustris* comprend peut-être deux espèces, une forme orientale et nordique (*S. palustris*) et une forme de la cordillère (*S. navigator*). La validité taxonomique de *S. p. brooksi* demeure problématique. La répartition des trois clades s'accorde avec la vicariance et l'isolation dans des refuges sur la côte, dans la cordillère et dans l'est à la fin du pliocène ou au pléistocène. La sous-espèce *S. p. brooksi* de l'île de Vancouver provient probablement d'une colonisation post-glaciaire à la fin du pléistocène.

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Introduction

Two species of North American shrews, *Sorex palustris* Richardson, 1828 and *Sorex bendirii* (Merriam, 1884), share similar morphological and physiological adaptations for a semi-aquatic lifestyle. *Sorex bendirii* is restricted to the Pacific coast region where it inhabits the coastal lowlands and lower slopes of the coastal mountain ranges from northern

California to extreme southwestern British Columbia (Hall 1981). *Sorex palustris* is widely distributed across the Cordillera of western North America and the boreal regions of Canada and the eastern United States (Hall 1981; van Zyll de Jong 1983). Their distributional areas are largely allopatric, but narrow zones of parapatry evidently occur in coastal Oregon, Washington, and British Columbia, where the two species are segregated by elevation (Nagorsen 1996; Johnson and Cassidy 1997).

Although Merriam (1895) and Jackson (1928) assigned *S. bendirii* and *S. palustris* to separate subgenera (*Atophyrax*, *Neosorex*), Findley (1955), based on dental morphology, considered *S. bendirii* and *S. palustris* to be closely related sister species in the *Otisorex* subgenus, an arrangement supported by allozymes (George 1988) and the mitochondrial cytochrome *b* (*Cytb*) gene (Demboski and Cook 2001). There has been no genetic analysis of geographic variation within these species. At least nine subspecies are recognized in *S. palustris* (Hall 1981; van Zyll de Jong 1983). Three (*S. p. brooksi*, *S. p. navigator*, *S. p. palustris*) occur in west-

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ern North America, with *S. p. brooksi* endemic to Vancouver Island, *S. p. navigator* inhabiting the Cordillera from California and New Mexico north to Alaska and Yukon, and *S. p. palustris* ranging from the foothills of the Rocky Mountains east to the Great Lakes region. A morphologically distinct population known from only three historical museum specimens taken at Glacier Bay, Alaska (MacDonald and Cook 1996), has been treated as either a distinct species, *Sorex alakanus* Merriam, 1900, by some authorities (Baker et al. 2003a) or a subspecies (*S. p. alakanus*) of *S. palustris* by others (e.g., Junge and Hoffmann 1981). Despite its limited distributional area, three subspecies are recognized in *S. bendirii*: *S. b. albiventer* on the Olympic Peninsula of Washington, *S. b. bendirii* of the Cascades and southwestern British Columbia, and *S. b. palmeri* of coastal Oregon (Hall 1981). All are based on minor differences in pelage colour and descriptive skull or dental traits (Jackson 1928; Hoffmann 1971; van Zyll de Jong 1983); their taxonomic validity is unresolved.

To unravel inter- and intra-specific phylogenetic relationships in the *S. palustris* – *S. bendirii* complex, we examined sequence variation in the *Cytb* gene of the mitochondrial genome. We selected this gene because its level of sequence divergence is useful for delimiting genetic species (Bradley and Baker 2001) and phylogeographic patterns (Avice 2000), and it has been used in phylogenetic studies of shrews (Fumagalli et al. 1999; Demboski and Cook 2001, 2003). Our objectives were to evaluate geographic variation and its implications for the current taxonomy and biogeography of the *S. bendirii* – *S. palustris* complex.

Materials and methods

We generated complete *Cytb* sequence from 18 specimens of *S. palustris* and 4 specimens of *S. bendirii*, which were then combined with 6 additional sequences archived on GenBank from Fumagalli et al. (1999) and Demboski and Cook (2001, 2003). Two specimens retrieved from GenBank had complete sequence data, 1140 base pairs (bp), while the remaining 8 specimens varied in length from 801 to 1011 bp. Specimen, GenBank accession numbers, and collection localities are in Fig. 1 and Table 1.

The *Cytb* sequence from GenBank of *Sorex ornatus* Merriam, 1895 (AF238035, 238036) and *Sorex vagrans* Baird, 1857 (AF154551, 238037), members of the *Otisorax* subgenus, were used as outgroups for their basal relationship to the *S. palustris* – *S. bendirii* complex (Fumagalli et al. 1999; Demboski and Cook 2001, 2003).

Genomic DNA was extracted from frozen liver (0.1–0.5 g) following procedures described in Longmire et al. (1997). We performed double-stranded amplification of the *Cytb* gene via polymerase chain reaction (PCR) using universal primers L14724 and H15915 (Table 2) to amplify the 1140 bp of the cytochrome *b* gene (Irwin et al. 1991). The thermal profile for the PCR included 35 cycles of 96 °C for 50 s, 50 °C for 45 s, and 72 °C for 2 min. The 35 cycles were then followed by a 7 min extension period at 72 °C. PCR products were purified using the Qiaquick PCR Purification kit (Qiagen Inc., Chatsworth, California) following the manufacturer's instructions. Six oligos were used in sequence reactions of the *Cytb* gene (Table 2): MBO3L, MBO4L,

MBO5H, MBO6H, and H15149 (Irwin et al. 1991; O'Neill 2000). Forward and reverse strands of DNA were sequenced using an ABI dRhodamine Dye Terminator Ready Reaction Mix (Applied Biosystems, Foster City, California) on an ABI Prism® 310 Genetic Analyzer (PE Applied Biosystems, Foster City, California).

Sequences generated were edited and aligned using SEQUENCHER (version 3.01; Gene Codes Corp., Ann Arbor, Michigan). Hypotheses of phylogenetic relationships of taxa were determined using likelihood, parsimony, and Bayesian methods. Sequence data were treated as unordered, discrete characters with four possible states: A, C, G, or T.

Parsimony analyses were performed using PAUP* version 4.0b10 (Swofford 2001) with an unweighted character scheme. Genetic distance values were determined using MEGA version 2.1 (Kumar et al. 2001). Bootstrap analysis (Felsenstein 1985) was performed using 1000 bootstrap iterations and Bremer support values (Bremer 1994) testing node robustness were calculated using AutoDecay version 3.0.3 (Eriksson 1997).

A Bayesian analysis, using MrBayes version 2.01 (Huelsenbeck and Ronquist 2001), was used for a comparison to the maximum-likelihood method and developed the clade probabilities, or support values. This analysis used the GTR+I+G model with no prior assignments of parameters. The following options were used: four Markov chains ran simultaneously for 2×10^6 generations with trees being sampled every 100th generation. After visual inspection of the likelihood scores, the first 100 trees were discarded to allow for tree construction based on stabilized scores. A consensus tree (50% majority rule) was constructed from the remaining trees (Fig. 2).

Pairwise genetic distance analysis was based on Kimura's two-parameter model of evolution (Kimura 1980). This model was chosen so that resulting values could be compared with those from other studies involving water shrews with *Cytb* data sets (Fumagalli et al. 1999; Demboski and Cook 2001, 2003). Mean pairwise genetic distance values were created for comparison within and among species (Table 3).

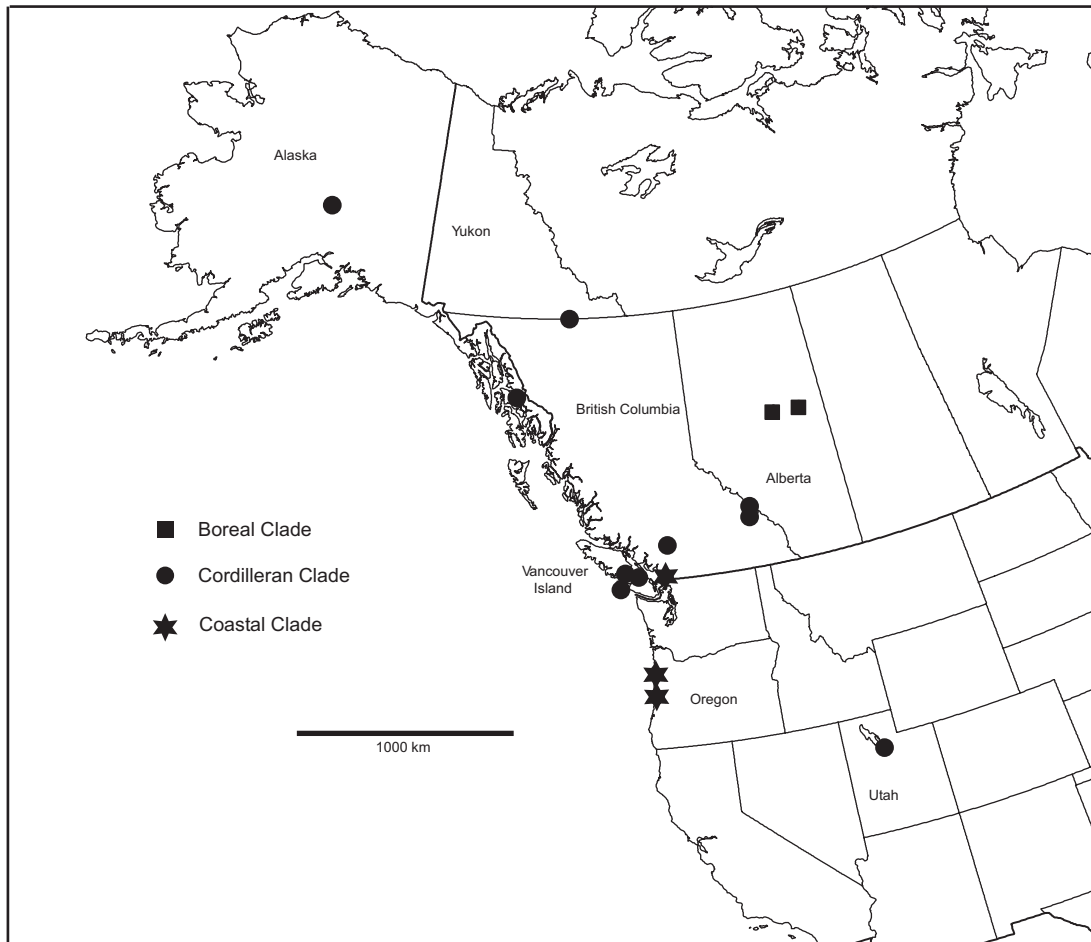
Results

For the 22 sequences obtained from tissue samples, the complete cytochrome *b* gene consisted of 1140 bp representing 380 codons. No insertions or deletions were detected. Of the 10 sequences from GenBank, 2 *S. palustris* sequences were complete. Specimens retrieved from GenBank with partial sequence data had gaps filled with noninformative characters and were treated as missing characters. No insertions or deletions were detected.

Of the 178 variable positions, 119 were parsimony informative, with 19 (17.6%) at the first position, 5 (4.6%) at the second position, and 84 (77.8%) occurring at the third position. We identified 25 unique haplotypes in the 32 samples.

Sequence divergence values (Table 3) between *S. bendirii* and *S. palustris* and the outgroups *S. vagrans* and *S. ornatus* ranged from 9.0% to 10.9%. Divergence levels between *S. bendirii* and *S. palustris* subspecies was <4.0%, except for *S. p. palustris* where divergence was 7.3%. Divergence between *S. b. bendirii* and *S. b. palmeri* was 0.7%. Variation

Fig. 1. Map of western North America showing sampling locations of *Sorex bendirii* and *Sorex palustris* listed in Table 1 and the distribution of the three clades. The Coastal clade consists of *S. bendirii*, the Cordilleran clade consists of *S. p. brooksi* and *S. p. navigator*, and the Boreal clade consists of *S. p. palustris*.



among the three subspecies of *S. palustris* ranged from 0.8% to 8.0%. *Sorex palustris palustris* was highly divergent at 6.9%–8.0%. *Sorex palustris brooksi*, a subspecies endemic to Vancouver Island, showed minor divergence (<1%) from mainland samples of *S. p. navigator*.

Results from the phylogenetic analyses (parsimony, likelihood, and Bayesian) produced nearly identical trees with similar support values for most clades. Given the similarity in the tree topology of the three analyses, only the Bayesian topology with clade probability values is shown and discussed (Fig. 2). Three major clades were apparent. A “Boreal” clade basal to the other clades comprised *S. p. palustris* from northern Alberta. A “Coastal” clade corresponded to *S. bendirii*; a “Cordilleran” clade consisted of samples of *S. p. brooksi* and *S. p. navigator*. This clade is clearly more closely related to the Coastal clade (*S. bendirii*) than to the Boreal clade (*S. p. palustris*). Bayesian support values were 100 for all three clades. The trees show conflicting evidence for *S. p. brooksi* forming a monophyletic group. In the maximum-likelihood analysis (Fig. 2), *S. p. brooksi* samples formed a weakly defined subclade that was distinct from *S. p. navigator* samples. But, in the neighbour-joining tree (not shown), there was no clear separation of *S. p. brooksi* from *S. p. navigator*. Although the Coastal lineage consists

of samples representing two subspecies of *S. bendirii* (*S. b. bendirii*, *S. b. palmeri*), none of the phylogenetic trees showed evidence for subclades within this lineage.

Discussion

Taxonomy

Our results suggest that *S. palustris* may consist of two species: one boreal eastern form (*S. palustris*) and the other (*S. navigator*) associated with the western Cordillera. *Sorex palustris palustris* separates as a monophyletic clade and its divergence levels from *S. p. navigator* (6.9%) were similar to the genetic distances that separate it from *S. bendirii*, *S. ornatus*, and *S. vagrans*. The *Cytb* gene data demonstrate that *S. p. brooksi* and *S. p. navigator* are more closely related to *S. bendirii* than to *S. p. palustris*. These genetic distances in the *Cytb* gene that separate *S. p. palustris* from other western populations are at the upper end of divergence levels observed by Bradley and Baker (2001) for intraspecific populations and within the range of sister-species divergence for species of bats and rodents. No comparable review of genetic distances has been done for shrew species, but Fumagalli et al. (1999) concluded that intraspecific genetic distances based on mitochondrial DNA sequences were

Table 1. Location data, specimen/tissue numbers, and GenBank accession numbers for 4 samples of *Sorex bendirii* and 24 samples of *Sorex palustris* used in the analyses.

| Taxonomy | Clade | Location | Coordinates | Specimen/tissue No. | GenBank No. | | | |
|------------------------|---|--|-------------------|---------------------|--------------------------------------|-------------------|-------------------|----------|
| <i>S. p. brooksi</i> | Cordilleran | Vancouver Island, Hamilton Creek, B.C. | 49°19'N, 124°26'W | RBCM19493/TK55978 | AY954926 | | | |
| | | | | RBCM19495/TK55979 | AY954927 | | | |
| | Cordilleran | Vancouver Island, Lowry Lake, B.C. | 49°24'N, 125°08'W | RBCM19496/TK55980 | AY954928 | | | |
| | | | | RBCM19497/TK55981 | AY954929 | | | |
| | | | | RBCM19498/TK55982 | AY954930 | | | |
| Cordilleran | Vancouver Island, Lower Lost Shoe Creek, B.C. | 49°00'N, 125°35'W | RBCM19803/TK75001 | AY954931 | | | | |
| <i>S. p. navigator</i> | Cordilleran | Yoho National Park, Yoho River Valley, B.C. | 51°29'N, 116°28'W | RBCM19354/TK55961 | AY954932 | | | |
| | Cordilleran | Yoho National Park, Natural Bridge, B.C. | 51°23'N, 116°32'W | RBCM19388/TK55969 | AY954933 | | | |
| | Cordilleran | Yoho National Park, Leancoil, B.C. | 51°13'N, 116°35'W | RBCM19400/TK75015 | AY954934 | | | |
| | Cordilleran | Yoho National Park, Sink Lake, B.C. | 51°27'N, 116°18'W | RBCM199401/TK55974 | AY954935 | | | |
| | Cordilleran | Cayoosh Creek, B.C. | 50°30'N, 122°18'W | RBCM19956/TK75012 | AY954936 | | | |
| | | | | RBCM19957/TK75013 | AY954937 | | | |
| | Cordilleran | Big Creek, Watson Lake, Y.T. | 60°04'N, 128°29'W | — | AJ000449 | | | |
| | Cordilleran | Little Cottonwood Canyon, Salt Lake County, Utah | 40°34'N, 111°42'W | — | AJ000448 | | | |
| | Cordilleran | Stikine River, Alaska | 56°38'N, 132°25'W | UAM20787/AFTC2806 | AF238033 | | | |
| | Cordilleran | Moose Creek, Alaska | 63°30'N, 150°35'W | UAM763602/AFTC5786 | AF238034 | | | |
| <i>S. p. palustris</i> | Boreal | Calling Lake, Alta. | 55°11'N, 113°39'W | RBCM19830/TK75002 | AY954938 | | | |
| | | | | RBCM19829/TK75003 | AY954939 | | | |
| | | | | RBCM 19831/TK75005 | AY954940 | | | |
| | | | | RBCM19832/TK75006 | AY954941 | | | |
| | | | | RBCM19833/TK75007 | AY954942 | | | |
| | | | | RBCM19834/TK75008 | AY954943 | | | |
| | | | | RBCM19434/TK55975 | AY954944 | | | |
| | | | | Boreal | Lac La Biche, Alta. | 55°08'N, 111°45'W | RBCM19435/TK55976 | AY954945 |
| | | | | Coastal | Sumas Mountain, Clayburn Creek, B.C. | 49°05'N, 122°13'W | RBCM19435/TK55976 | AY954945 |
| | | | | Coastal | Sumas Mountain, McKee Creek, B.C. | 49°04'N, 122°17'W | RBCM19435/TK55976 | AY954945 |
| Coastal | Seymour River, B.C. | 49°21'N, 123°00'W | RBCM19527/TK75011 | AY954946 | | | | |
| Coastal | Aldergrove, B.C. | 49°05'N, 122°29'W | RBCM19436/TK75014 | AY954947 | | | | |
| <i>S. b. palmeri</i> | Coastal | Tillamook County, Oreg. | 45°16'N, 123°53'W | UAM52163/AFTC18979 | AF238031 | | | |
| | Coastal | Lane County, Oreg. | 44°14'N, 123°49'W | UAM52161/AFTC18980 | AF238032 | | | |

Note: AFTC, tissue sample from the University of Alaska Museum; RBCM, specimen from the Royal British Columbia Museum; TK, tissue sample from the Museum of Texas Tech University; UAM, specimen from the University of Alaska Museum.

Table 2. Primers used for PCR amplification and sequencing reactions.

| Primer name | Primer sequence 5'→3' | Source |
|-------------|-----------------------------------|---------------------|
| L14724 | CGAAGCTTGATATGAAAAACCATCGTTG | Irwin et al. (1991) |
| H15915 | AACTGCAGTCATCTCCGGTTTACAAGAC | Irwin et al. (1991) |
| MBO3L | TTACAAACCTACTATCAGCAATCCC | Design Primer, IDT |
| MBO4L | CCCGAATGATATTTCTATTTGCCTATG | Design Primer, IDT |
| MBO5H | RCTRAATGGTCGGAATA | Design Primer, IDT |
| MBO6H | GTGTAGTAGGGGTGGAATG | Design Primer, IDT |
| H15149 | AAACTGCAGCCCCTCAGAATGATATTTGCCTCA | Irwin et al. (1991) |

less than 5.2% for species. Within the genus *Notiosorex*, *Cytb* intraspecific distance values ranged from 0% to 1.3%, while interspecific divergence values were 12.96% and were used to describe a new biological species (Baker et al.

2003b). Nevertheless, mitochondrial DNA sequences from additional locations on the eastern slopes of the Rocky Mountains in British Columbia and Alberta and from representative specimens of the six eastern subspecies not as-

Fig. 2. Phylogenetic tree from the Bayesian analyses of six taxa of *Sorex*. *Sorex ornatus* and *Sorex vagrans* were used as the outgroup taxa. Clade probability values are listed above branches.

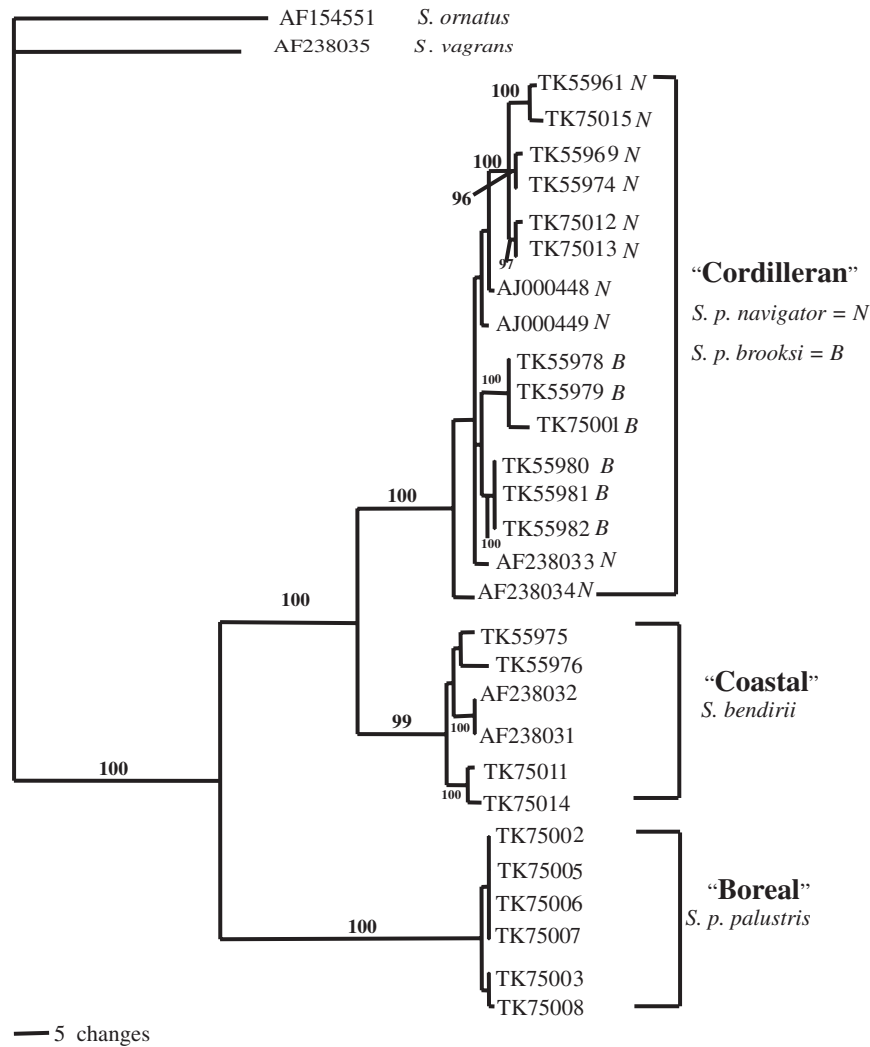


Table 3. Percent divergence in cytochrome *b* sequences between *Sorex ornatus*, *Sorex vagrans*, four subspecies of *Sorex palustris*, and two subspecies of *Sorex bendirii*.

| | <i>S. p. brooksi</i> | <i>S. p. navigator</i> | <i>S. p. palustris</i> | <i>S. b. bendirii</i> | <i>S. b. palmeri</i> | <i>S. ornatus</i> | <i>S. vagrans</i> |
|------------------------|----------------------|------------------------|------------------------|-----------------------|----------------------|-------------------|-------------------|
| <i>S. p. brooksi</i> | 0.4 | | | | | | |
| <i>S. p. navigator</i> | 0.8 | 0.6 | | | | | |
| <i>S. p. palustris</i> | 8.0 | 6.9 | 1.0 | | | | |
| <i>S. b. bendirii</i> | 3.4 | 3.1 | 7.3 | 0.7 | | | |
| <i>S. b. palmeri</i> | 3.5 | 3.3 | 7.3 | 0.7 | 0.0 | | |
| <i>S. ornatus</i> | 9.6 | 9.1 | 10.9 | 9.1 | 9.0 | — | |
| <i>S. vagrans</i> | 9.6 | 9.1 | 10.9 | 9.1 | 9.0 | 8.2 | — |

Note: Genetic distances among taxa based on Kimura's two-parameter estimates.

essed in this study should be analyzed before the taxonomy of *S. palustris* is revised. Our results also suggest that *S. palustris* could profit from a modern morphometric study with multivariate techniques. Soper (1964) observed size and shape differences in the skulls of Alberta populations of *S. p. navigator* and *S. p. palustris*.

The Vancouver Island subspecies *S. p. brooksi* was described by Anderson (1934) from only a single specimen.

Nagorsen (1996) noted that it was distinguished from mainland populations of *S. palustris* by only minor pelage and size differences and suggested a modern taxonomic study was needed to verify its systematic status. Our phylogenetic analyses demonstrate that *S. p. brooksi* is most closely related to adjacent mainland populations of *S. p. navigator*. Divergence levels from samples of *S. p. navigator* from Cayoosh Creek on the eastern slopes of the Coast Mountains

in British Columbia were generally less than 1%. Genetic distance from the Stikine River, Alaska, specimen, the only *S. palustris* sample in our study from lowlands of the coastal mainland, was only 0.6%. This divergence is well within the range of intraspecific variation (Fumagalli et al. 1999; Baker et al. 2003b) and typical of the values within a population. A comprehensive study using samples of *Cytb* gene sequences from coastal mainland populations adjacent to Vancouver Island, other genetic markers such as microsatellite DNA, and morphometric traits is required to resolve the taxonomic status of *S. p. brooksi*.

The genetic distances between *S. bendirii* and *S. p. navigator* are only 3.1% and within the range expected for intraspecific divergence in shrews. Nevertheless, *S. bendirii* forms a monophyletic clade, although the *Cytb* data suggest that it and *S. p. navigator* shared a common ancestry after diverging from *S. p. palustris*. Morphologically, the two species differ in a suite of pelage, cranial, and dental traits (Junge and Hoffmann 1981; Carraway 1995). No morphological intergradation has been reported from areas of parapatry, but Jackson (1928) noted that some specimens of *S. b. bendirii* from British Columbia approached *S. p. navigator* in "all essential features". A genetic study assessing introgression in zones of parapatry in the Olympic Mountains and lower slopes of the Cascade Mountains or Coast Mountains would be revealing. Our analyses included *S. b. bendirii* and *S. b. palmeri*, two of three subspecies recognized in *S. bendirii*. The three phylogenetic trees and the genetic divergence values revealed no evidence for two distinct subclades or groups concordant with these taxa. *Cytb* sequences should be assessed in *S. b. albiventer*, a distinctive subspecies with a whitish ventral pelage (Jackson 1928) that is associated with the Olympic Peninsula.

Phylogeography

Speciation among North American *Sorex* has been generally attributed to vicariance events associated with environmental changes during the Late Pleistocene (Findley 1955; George 1988), although mitochondrial DNA data suggest that some of this divergence is older, possibly Late Pliocene (Demboski and Cook 2001, 2003). The three major lineages in North American water shrews show a strong concordance with Hoffmann's (1981) western coastal, western montane, and eastern boreal taiga refugia. The major bifurcation in the *S. bendirii* – *S. palustris* complex involves the Boreal and Cordilleran–Coastal clades and probably reflects the initial isolation of water shrews in separate eastern and Rocky Mountain refugia. With a sequence divergence of 6.9%, and assuming a sequence divergence rate in the *Cytb* gene of shrews of $1.36\% \pm 0.2\%$ /million years (Fumagalli et al. 1999), this split may date to the Late Pliocene. Stewart et al. (2003) described a similar east–west dichotomy in *Sorex hoyi* Baird, 1857.

In western North America, this initial divergence was followed by the bifurcation of Rocky Mountain populations into separate Coastal and Cordilleran lineages that represent *S. bendirii* and *S. p. navigator*. Findley (1955) and George (1988) hypothesized a Late Pleistocene origin for their divergence. However, their sequence divergence of 3.1% may date to the Early Pleistocene. Whatever the timing, the *S. bendirii* – *S. palustris* complex is one of a number of

mammalian species or species complexes with a distinct genetic lineage that is associated with the Pacific coast of North America (Stone and Cook 2000; Arbogast and Kenagy 2001). The origin of these Pacific coast lineages is contentious, with various southern mainland and coastal island refugia proposed (Demboski et al. 1999). Given its restricted distributional area in the Pacific Northwest, *S. bendirii* probably originated in a southern coastal refugium in Oregon or California.

Although our samples of the Cordilleran clade span a large geographic area (Fig. 1), they show little geographic structure. More sequence samples from coastal regions of Alaska and British Columbia are required to determine if a shallow lineage is associated with this region. The low genetic divergence of the Vancouver Island population from mainland populations of *S. palustris* is consistent with a postglacial origin for this insular endemic as proposed by Nagorsen (1996). During the last glaciation of the Late Wisconsin, Vancouver Island was inundated by the Cordilleran Ice Sheet by 15 500 years before present (BP); de-glaciation on the island began before 14 000 years BP (Ward et al. 2003). With the Cordilleran Ice Sheet more than 1300 m thick, only small nunataks and a few elevated ridges escaped glaciation during glacial maximum, and it is likely that the modern mammalian fauna of Vancouver Island is derived entirely from postglacial immigrants that first colonized the island 12 000 – 13 000 years BP (Nagorsen and Keddie 2000).

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