

# Phylogeography of the dusky shrew, *Sorex monticolus* (Insectivora, Soricidae): insight into deep and shallow history in northwestern North America

JOHN R. DEMBOSKI\* and JOSEPH A. COOK†

University of Alaska Museum and Biochemistry and Molecular Biology Program, University of Alaska Fairbanks, 907 Yukon Drive, Fairbanks, Alaska 99775–6960, USA

## Abstract

Phylogenetic relationships among the dusky shrew (*Sorex monticolus*) and eight related species (*S. bairdi*, *S. bendirii*, *S. neomexicanus*, *S. ornatus*, *S. pacificus*, *S. palustris*, *S. sonomae* and *S. vagrans*) were assessed using sequences from the mitochondrial cytochrome *b* gene (801 bp). Analyses using parsimony and maximum likelihood revealed significant molecular variation not reflected in previous morphological studies of these species. Conversely, three morphologically defined species (*S. bairdi*, *S. neomexicanus* and *S. pacificus*) were poorly differentiated. *Sorex ornatus* and *S. vagrans* represented basal taxa for a more inclusive group that included: (i) a widespread Continental clade containing *S. monticolus* (Arizona to Alaska, including *S. neomexicanus*); (ii) a Coastal clade containing *S. monticolus* (Oregon to south-east Alaska, including *S. bairdi* and *S. pacificus*); (iii) the semiaquatic species (*S. bendirii* and *S. palustris*); and (iv) *S. sonomae*. Additional subdivision was observed within the Continental clade corresponding to populations from the northern and southern Rocky Mountains. Average uncorrected sequence divergence between the Coastal and Continental clades was 5.3% (range 4.5–6.2%), which exceeds many interspecific comparisons within this species complex and within the genus *Sorex*. Lack of resolution of internal nodes within topologies suggests a deep history of rapid diversification within this group. Late Pleistocene/Holocene glacial perturbations are reflected in the shallow phylogeographic structure within these clades in western North America. Our results suggest also that *S. monticolus* is not monophyletic under current taxonomic nomenclature. This perspective on phylogeographic history was developed within a growing comparative framework for other organisms in western North America.

**Keywords:** cytochrome *b*, maximum likelihood, parametric bootstrap, phylogeography, shrews, *Sorex*

Received 8 May 2000; revision received 30 November 2000; accepted 30 November 2000

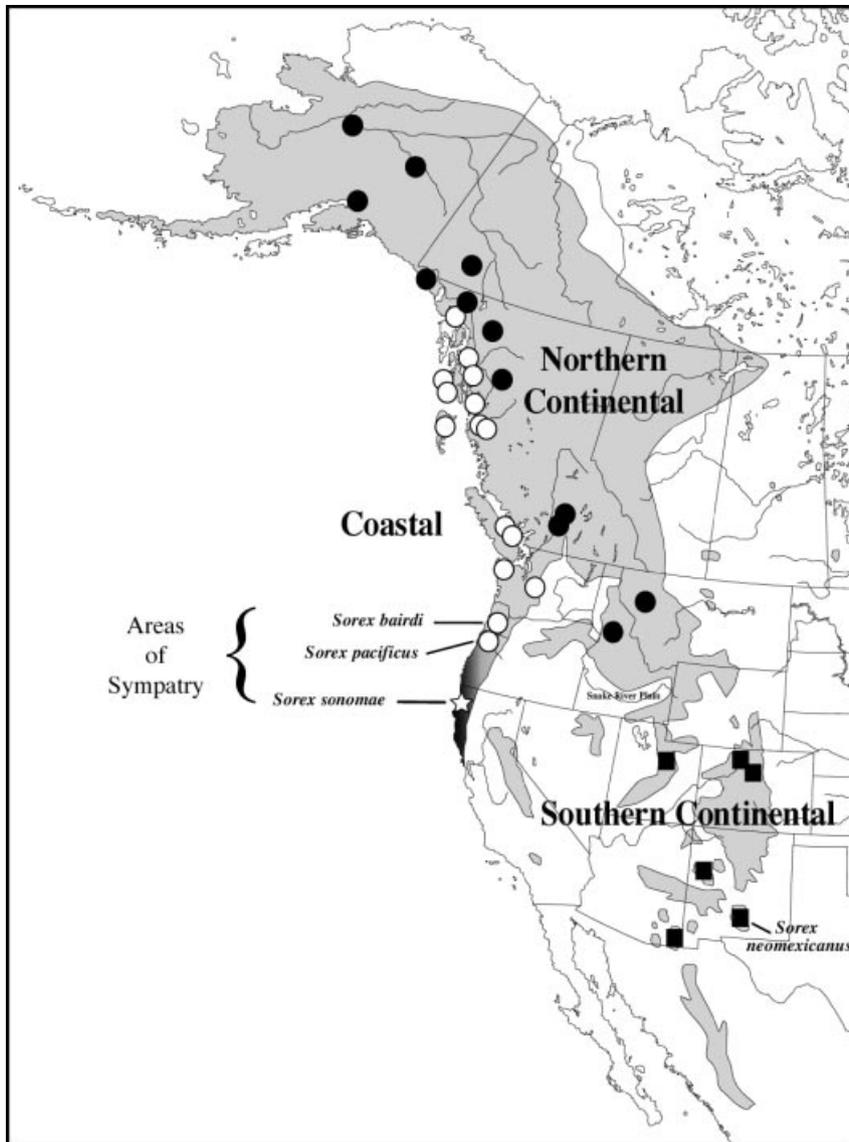
## Introduction

A primary factor shaping the biogeographic and evolutionary histories of species has been past climatic fluctuations (e.g. Graham & Grimm 1990; Vrba 1992; Roy *et al.* 1996)

that resulted in shifts in habitats and colonization corridors (Webb & Bartlein 1992). Prevailing dogma has embraced recent Pleistocene glacial/interglacial cycles as an important series of vicariance events that subdivided ancestral populations and led to genetic divergence. In particular, the most recent interglacial/glacial cycle in North America and Eurasia has been invoked to explain alpha-level taxonomic diversity (Hewitt 1996). A growing body of molecular evidence has re-evaluated the role of Late Pleistocene events on speciation. Many studies have shifted the focus to earlier events in the Pliocene/Early Pleistocene (Zink & Slowinski 1995; Klicka & Zink 1997) or Miocene

Correspondence: J. R. Demboski. \*Present address: Department of Biological Sciences, PO Box 443051, University of Idaho, Moscow, Idaho 83844–3051, USA. Fax: (208) 885–7905; E-mail: demboski@uidaho.edu

†Present address: Biological Sciences, Idaho State University, Pocatello, Idaho 83209–8007, USA.



**Fig. 1** Map of western North America depicting sampling locations of the major clades containing *Sorex monticolus* and other species (○), Coastal, including *S. bairdi* and *S. pacificus*, Continental; ●, Northern Continental and ■, Southern Continental (including *S. neomexicanus*) overlaid on the distribution of these species (adapted from Hennings & Hoffmann 1977; Carraway 1990). The sampling location of *S. sonomae* (☆) and the area of sympatry with *S. bairdi* and *S. pacificus* are also shown. Sampling locations and distribution of the semiaquatic species (*S. bendirii* and *S. palustris*) and outgroups are not shown.

(Riddle 1995; Nielson *et al.* 2001). Although some of these conclusions have generated debate regarding molecular clock estimates (Arbogast & Slowinski 1998; Avise *et al.* 1998), it seems likely that high levels of inter- and intra-specific divergence cannot be adequately explained by Late Pleistocene events.

Molecular studies have also provided novel perspectives on morphological evolution. In many cases, taxonomy based on morphological characters has not adequately reflected the deeper phylogeographic structure (e.g. Avise 1992; Riddle 1996). Conversely, lack of reciprocal monophyly between morphologically defined species may represent incomplete lineage sorting (Avise 1994) or indicate episodes of rapid morphological diversification.

In northwestern North America, molecular studies of select plants and animals have suggested complex phylogenetic and phylogeographic structure (Zink 1996; Soltis *et al.* 1997; Demboski *et al.* 1999). Hypotheses concerning the biogeography of flora and fauna in this region (Rand 1954; Hoffmann 1981; Rogers *et al.* 1991) have focused on the mechanistic role of glacial refugia in promoting species diversification. Indeed, mammalian (and nonmammalian) taxa in western North America have been broadly classified as either Beringian or Southern, reflecting their presumed origins in refugia (Youngman 1975; Hoffmann 1981). Possible refugia along the north Pacific Coast have also been proposed (Scudder & Gessler 1989; Rogers *et al.* 1991; MacDonald & Cook 1996). Similar patterns of genetic

divergence across diverse taxa in western North America indicate that common historical events may be tractable with comparative phylogeographic approaches (Soltis *et al.* 1997; Arbogast 1999; Demboski *et al.* 1999; Cook *et al.* 2001).

One widespread species of mammal on which the climatic fluctuations during the Pleistocene had an impact is the dusky or montane shrew, *Sorex monticolus* (Findley 1955; Hennings & Hoffmann 1977). The northern range of the dusky shrew spans the boreal and temperate rain forest regions of western North America, which were previously covered by the Cordilleran and Laurentide ice sheets (Fig. 1). Dusky shrews (and other fauna and flora) colonized these regions after the retreat of the continental ice sheets about 12 000 years ago. Further south, in the montane forests of the southern Rocky Mountains, the distribution of *S. monticolus* largely reflects historic range retraction into forested sky islands (Hennings & Hoffmann 1977; Alexander 1996). The systematics, taxonomy and biogeography of *S. monticolus* and suspected sister taxa have been the subjects of a series of reviews (Merriam 1895; Jackson 1928; Findley 1955; Hennings & Hoffmann 1977; Carraway 1990; Alexander 1996). Species thought to be closely related to *S. monticolus* were placed in the '*Sorex vagrans* complex' (Merriam 1895) and have included *S. bairdi*, *S. neomexicanus*, *S. pacificus*, *S. sonomae* and *S. vagrans* (Carraway 1990; Alexander 1996). In western North America, some of these morphologically similar species are widely sympatric in many regions contributing to additional taxonomic confusion (Fig. 1, *S. bairdi*, *S. pacificus* and *S. sonomae*, Carraway 1990; *S. vagrans* and *S. monticolus*, George & Smith 1991).

Demboski *et al.* (1999) reported high values of uncorrected sequence divergence within *S. monticolus* (cytochrome *b*, 4.7–5.7%) for a small data set ( $n = 12$ ) that also included *S. bairdi* and *S. vagrans*. Those results prompted this expanded view of molecular variation throughout the range of *S. monticolus*, including six additional species that are considered to be closely related, *S. neomexicanus*, *S. ornatus*, *S. pacificus*, the semiaquatic species (*S. bendirii* and *S. palustris*), and *S. sonomae* (George 1988; Carraway 1990; Alexander 1996; Fumagalli *et al.* 1999). Results of parsimony and maximum likelihood analyses provide new insight into the phylogenetic complexity of this group.

## Materials and methods

Seventy shrews were examined (Table 1) including six GenBank sequences of *Sorex monticolus*, *S. pacificus* and *S. palustris* from Fumagalli *et al.* (1999). Due to the lack of an unambiguous outgroup for the '*S. vagrans* complex' (George 1988; Fumagalli *et al.* 1999), *S. cinereus*, *S. fumeus* and *S. hoyi* were designated as paraphyletic outgroups in initial phylogenetic analyses. Genomic DNA was isolated from heart, kidney, or liver from frozen (–70 °C) tissue or

tissues preserved in ethanol. Methods for DNA extraction, polymerase chain reaction (PCR) amplification, and cycle sequencing followed those summarized in Lessa & Cook (1998) and Halanych *et al.* (1999). Three primer pairs were used for amplification and sequencing of cytochrome *b*: MVZ4/5, MVZ14/23 (Smith & Patton 1993) and SOREX16/SOREX37 (Demboski *et al.* 1999). PCR products were sequenced in both directions using an Applied Biosystems 373 DNA sequencer. Sequences were assembled and aligned with SEQUENCE NAVIGATOR, Version 1.01 (ABI). Sequence statistics and phylogenetic trees were generated using PAUP\*, version 4.0b2a (Swofford 1999) and conducted under frameworks of parsimony and maximum likelihood (ML).

Initially, a heuristic parsimony search [equal weights, 100 random addition replicates, tree bisection–reconnection (TBR) branch swapping] with the complete data set ( $n = 70$ ) was conducted. Nodal support was estimated via bootstrapping (Felsenstein 1985) with 500 parsimony replicates (TBR branch swapping, MAXTREES set to 100). These analyses guided pruning of the data set to 31 sequences to ease the computational constraints associated with subsequent ML analyses. Sequences pruned included three initial outgroups (*S. cinereus*, *S. fumeus* and *S. hoyi*), redundant sequences and partial sequences (< 801 base pairs). A subsequent heuristic parsimony search (equal weights, 100 random addition replicates, TBR branch swapping) of the pruned data set provided a starting tree for an iterative-search strategy using ML (e.g. Sullivan *et al.* 1997). This approach relied on evaluation of the pruned parsimony tree under four models of nucleotide substitution: Jukes–Cantor (JC; Jukes & Cantor 1969); K2P (Kimura 1980); HKY85 (Hasegawa *et al.* 1985); and the general time-reversible (GTR; Yang 1994a). In addition, the rate-heterogeneity parameters,  $\alpha$  (rates among all sites assumed to vary following a gamma distribution; Yang 1994b) and  $p_{inv}$  (a proportion of sites assumed to be invariable; Hasegawa *et al.* 1985), were also estimated, allowing an evaluation of 16 models of substitution as summarized in Sullivan *et al.* (1997).

The best-fit model of substitution was determined by the likelihood-ratio test (Yang *et al.* 1995) and a heuristic ML search under this model was then conducted (TBR branch swapping). Model parameters were re-optimized on the first ML tree recovered and the search was repeated until the likelihood score did not change. Both ML (100 replicates) and parsimony (500 replicates) bootstrap support was estimated. A maximum of one tree per replicate (MAXTREES = 1) was fixed during the ML bootstrap analysis to ease computational constraints.

We tested monophyly of *S. monticolus*, an observation not apparent in previous work (Demboski *et al.* 1999) or in our parsimony and ML topologies, using the parametric bootstrap (Huelsenbeck *et al.* 1996). An optimal ML tree constrained to support monophyly of *S. monticolus* (the

**Table 1** Collection localities, specimen numbers and GenBank numbers for specimens examined

Species	Location	Specimen no.	GenBank No.
<i>S. bairdi</i>	Oregon, Lincoln County	AFTC18982*	AF238022
<i>S. bairdi</i>	Oregon, Tillamook County	AFTC18983	AF154550†
<i>S. bairdi</i>	Oregon, Tillamook County	AFTC18984	AF238023
<i>S. bairdi</i>	Oregon, Yamhill County	AFTC18985	AF238024
<i>S. bendirii</i>	Oregon, Tillamook County	AFTC18979	AF238031
<i>S. bendirii</i>	Oregon, Lane County	AFTC18980	AF238032
<i>S. monticolus</i>	Alaska, Anchorage	AFTC11500	AF238013
<i>S. monticolus</i>	Alaska, Coronation Island	AFTC5205	AF237996
<i>S. monticolus</i>	Alaska, Fairbanks	AFTC5929	AF154539†
<i>S. monticolus</i>	Alaska, Forrester Island	AFTC16733	AF238000
<i>S. monticolus</i>	Alaska, Forrester Island	AFTC16740	AF238001
<i>S. monticolus</i>	Alaska, Galena	AFTC3502	AF238042
<i>S. monticolus</i>	Alaska, Gustavus	AFTC5217	AF237997
<i>S. monticolus</i>	Alaska, Haines	AFTC4588	AF238009
<i>S. monticolus</i>	Alaska, Misty Fiords	AFTC4259	AF237998
<i>S. monticolus</i>	Alaska, Mitkof Island	AFTC2498	AF154541†
<i>S. monticolus</i>	Alaska, Stikine River	AFTC2639	AF237995
<i>S. monticolus</i>	Alaska, Yakutat	AFTC6396	AF238008
<i>S. monticolus</i>	Arizona, Cochise County	NK8001‡	AF154548†
<i>S. monticolus</i>	Arizona, Cochise County	NK8002	AF238016
<i>S. monticolus</i>	Arizona, Cochise County	NK8003	AF238017
<i>S. monticolus</i>	British Columbia, Barriere	WK10185	AF238015
<i>S. monticolus</i>	British Columbia, Barriere	WK10201	AF238010
<i>S. monticolus</i>	British Columbia, Dease Lake	AFTC12777	AF154540†
<i>S. monticolus</i>	British Columbia, Denman Island	AFTC24280	AF154543†
<i>S. monticolus</i>	British Columbia, Denman Island	Denman I-2	AF238002
<i>S. monticolus</i>	British Columbia, Graham Island	AFTC30714	AF237994
<i>S. monticolus</i>	British Columbia, Iskut River	AFTC12773	AF238007
<i>S. monticolus</i>	British Columbia, Lakelse Lake	AFTC12677	AF154542†
<i>S. monticolus</i>	British Columbia, Nass River	AFTC12687	AF238005
<i>S. monticolus</i>	British Columbia, Opax Mountain	WK3348	AF238011
<i>S. monticolus</i>	British Columbia, Opax Mountain	WK7113	AF238012
<i>S. monticolus</i>	British Columbia, Quadra Island	Quadra I-1	AF238003
<i>S. monticolus</i>	British Columbia, Quadra Island	Quadra I-2	AF238004
<i>S. monticolus</i>	British Columbia, Stewart	AFTC12708	AF238006
<i>S. monticolus</i>	Colorado, Boulder County	NK1042	AF154547†
<i>S. monticolus</i>	Colorado, Jackson County	NK56586	AF238019
<i>S. monticolus</i>	Colorado, Mineral County	BJH9863	AF238043
<i>S. monticolus</i>	Idaho, Idaho County	JRD016	AF237993
<i>S. monticolus</i>	Montana, Lake County	AFTC24262	AF154544†
<i>S. monticolus</i>	New Mexico, Cibola County	NK11015	AF154549†
<i>S. monticolus</i>	New Mexico, Cibola County	NK11019	AF238018
<i>S. monticolus</i>	Oregon, Tillamook County		AJ000450§
<i>S. monticolus</i>	Oregon, Tillamook County		AJ000451§
<i>S. monticolus</i>	Utah, Wasatch County	NK55578	AF154546†
<i>S. monticolus</i>	Washington, Grays Harbor County	NK11005	AF238041
<i>S. monticolus</i>	Washington, Kittitas County	AFTC14014	AF237999
<i>S. monticolus</i>	Washington, Kittitas County	AFTC14460	AF154545†
<i>S. monticolus</i>	Yukon Territory, Whitehorse	AFTC10905	AF238014
<i>S. neomexicanus</i>	New Mexico, Otero County	NK867	AF238028
<i>S. neomexicanus</i>	New Mexico, Otero County	NK868	AF238029
<i>S. neomexicanus</i>	New Mexico, Otero County	NK869	AF238030
<i>S. pacificus</i>	Oregon, Benton County	NK3240	AF238020
<i>S. pacificus</i>	Oregon, Benton County	AFTC18981	AF238021
<i>S. pacificus</i>	Oregon, Lane County		AJ000452§
<i>S. pacificus</i>	Oregon, Lane County		AJ000453§

Table 1 Continued

Species	Location	Specimen no.	GenBank No.
<i>S. palustris</i>	Alaska, Petersburg Quad.	AFTC2806	AF238033
<i>S. palustris</i>	Alaska, Healy Quad.	AFTC5786	AF238034
<i>S. palustris</i>	Utah, Salt Lake County		AJ000448§
<i>S. palustris</i>	Yukon Territory, Watson Lake		AJ000449§
<i>S. sonomae</i>	California, Humboldt County	NK3313	AF238025
<i>S. sonomae</i>	California, Humboldt County	NK3324	AF238026
<i>S. sonomae</i>	California, Humboldt County	NK3325	AF238027
Outgroups			
<i>S. cinereus</i>	Montana, Carbon Co.	AFTC14893	AF238038
<i>S. fumeus</i>	Pennsylvania, Westmoreland County	AFTC22614	AF238039
<i>S. hoyi</i>	Alaska, Hughes Quad.	AFTC7982	AF238040
<i>S. ornatus</i>	California, San Diego County	SD4	AF238035
<i>S. ornatus</i>	California, San Diego County	SD9	AF238036
<i>S. vagrans</i>	Montana, Lake County	AFTC24263	AF154551†
<i>S. vagrans</i>	British Columbia, Sicamous	WK14126	AF238037

\*AFTC, Alaska Frozen Tissue Collection, University of Alaska Museum.

†GenBank accession numbers from Demboski *et al.* (1999).

‡NK, Museum of Southwestern Biology, University of New Mexico.

§GenBank accession numbers from Fumagalli *et al.* (1999).

null hypothesis) was estimated using the HKY85 +  $\Gamma$  model (see Results section). SEQ-GEN, version 1.1 (Rambaut & Grassly 1997) was used to simulate 500 data sets under the assumed model and the null hypothesis. PAUP\* was then used to calculate ln likelihood scores under the null hypothesis and the optimal tree (nonmonophyly, no constraints) for each of the 500 simulated data sets. The difference between the constrained and unconstrained ln likelihood scores ( $\delta$ ) for all 500 simulations was used to create the null distribution for the test. The actual difference in ln likelihood scores based on the real data set was then compared to the simulated null distribution.

## Results

### Mitochondrial DNA (mtDNA) sequences

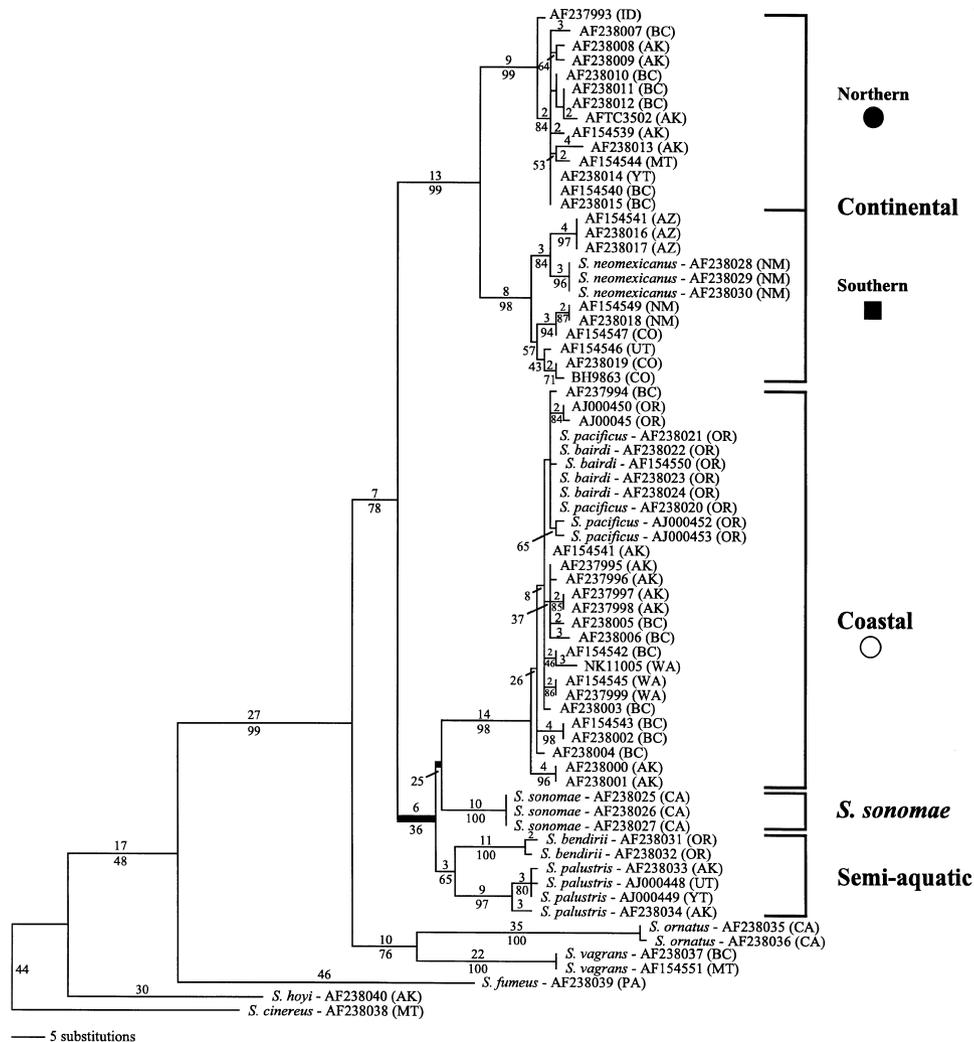
Cytochrome *b* sequences were aligned unambiguously and exhibited the expected codon biases reported for other vertebrate cytochrome *b* gene sequences (e.g. Irwin *et al.* 1991) and for *Sorex* (Demboski *et al.* 1999; Fumagalli *et al.* 1999). The complete data set ( $n = 70$ ) had an overall deficit of guanines (13.9%), most notable at the third position of codons (2.7%). The null hypothesis of stationary base frequencies across sequences (as determined in PAUP\*) was not violated ( $\chi^2_{[207]} = 16.61$ ,  $P = 1.0$ , ignoring correlation due to phylogenetic structure). Nucleotide substitutions were observed at 209 sites (26.1%) and 164 of these were phylogenetically informative. Substitutions at the third position of codons accounted for 62.5% of the overall

variation. Sequences in the pruned data set ( $n = 31$ ) used for ML analysis exhibited similar patterns.

### Phylogenetic analyses

The initial equal weight parsimony search with the complete data set ( $n = 70$ ) recovered 1628 shortest trees of 414 steps (consistency index = 0.5676; rescaled consistency index = 0.4988). These topologies were characterized by five major clades; semiaquatic species, *Sorex sonomae*, Coastal, Continental and *S. ornatus*/*S. vagrans* (Fig. 2). Designation of Coastal and Continental clades follow the nomenclature of Demboski *et al.* (1999). Areas of incongruence between Fig. 2 and the strict consensus of 1628 trees (not shown) were characterized by the collapse of the two nodes depicting sister relationships between the Coastal, *S. sonomae* and semiaquatic clades. Shallow relationships between terminal taxa within several of these clades also differed among the 1628 trees. Strong bootstrap support (99%, Fig. 2) for monophyly of the five major clades led us to designate *S. ornatus* and *S. vagrans* as monophyletic outgroups for subsequent parsimony and ML analyses.

An equal weight parsimony search with the pruned data set ( $n = 31$ ) recovered 13 shortest trees (229 steps; consistency index = 0.6609; rescaled consistency index = 0.5658). One tree (no. 1) was evaluated under the 12 models of nucleotide substitution described above. The best ln likelihood score was obtained under the GTR + I +  $\Gamma$  model (lnL = -2370.7646); however, the score obtained under the HKY85



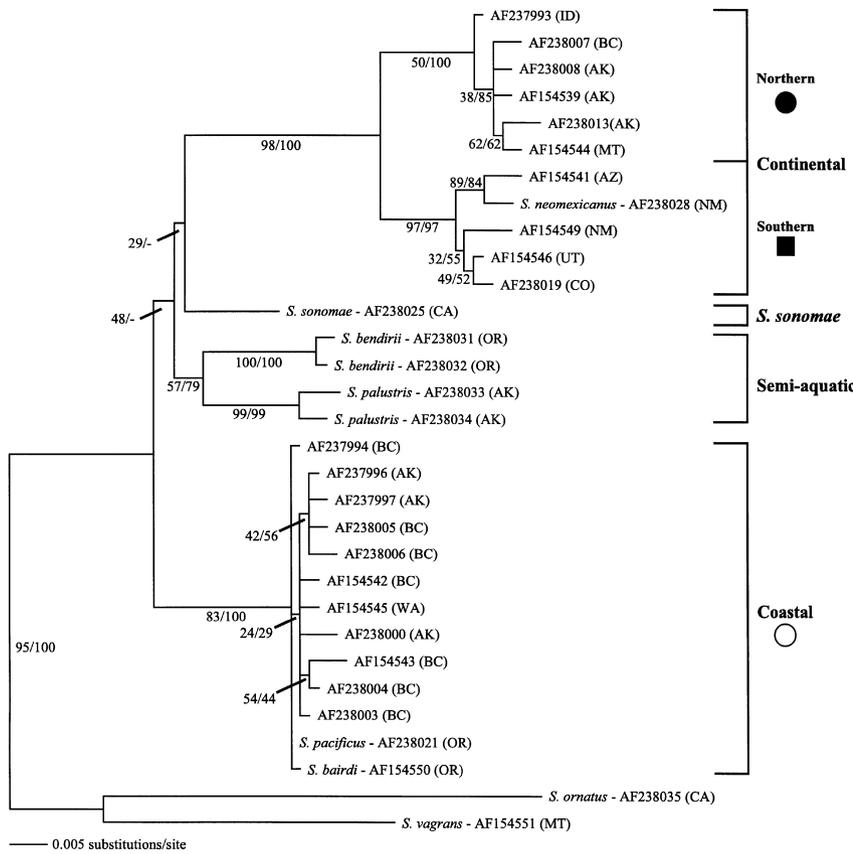
**Fig. 2** Equal weight parsimony tree (tree 1267 of 1628 trees, consistency index = 0.5676; rescaled consistency index = 0.4988) recovered from the full data set ( $n = 70$ ). Samples are *Sorex monticolus* unless otherwise noted and are denoted by GenBank accession numbers and state or province of collection (Table 1). *Sorex cinereus*, *S. fumeus* and *S. hoyi* were designated as paraphyletic outgroups. Number of substitutions  $\geq 2$  (inferred using ACCTRAN) are shown above the branches with parsimony bootstrap values (500 replicates) shown below. Areas of incongruence between this topology and the strict consensus topology are reflected by the collapse of two nodes (heavy branch lengths) depicting a sister relationship between the Coastal, *S. sonomae* and semiaquatic clades.

+  $\Gamma$  model was not significantly different ( $\ln L = -2374.5564$ ;  $\chi^2_{[5]} = 7.584$ ,  $0.5 < P < 0.1$ ). This simpler model, with less associated variance, was used for subsequent heuristic ML searches using TBR branch swapping. After successive parameter optimization (transition to transversion ratio = 10.3749,  $\alpha = 0.11465$ ), a single ML topology was recovered ( $\ln L = -2369.3308$ , Fig. 3). The topology of the ML tree differed from the parsimony tree with regard to the relationships between the major clades (Figs 2 and 3). However, as in the parsimony tree, these internal nodes were not strongly supported (ML bootstrap, 29% and 48%).

In both parsimony and ML topologies, the Coastal clade was composed of haplotypes of *S. monticolus* extending

from Washington to southeast Alaska and the Oregon species *S. bairdi* and *S. pacificus* (Fig. 1). Bifurcation within the Continental clade corresponded to distinct northern and southern Rocky Mountain haplotypes. The Northern Continental clade was distributed from Idaho/Montana to western Alaska (Fig. 1). The Southern Continental clade was represented by haplotypes of *S. monticolus* from Arizona, Colorado, New Mexico and Utah, including *S. neomexicanus* from southcentral New Mexico. The northern California species, *S. sonomae* (Carraway 1991) and the semiaquatic species, *S. bendirii* and *S. palustris*, represented the remaining ingroup clades.

Many of the genetic distances (uncorrected and HKY85



**Fig. 3** Maximum-likelihood tree,  $\ln L = -2369.33078$ , for the pruned cytochrome *b* data set ( $n = 31$ ) under the best-fit HKY85 +  $\Gamma$  model (transitions to transversions ratio = 10.3749,  $\alpha = 0.11465$ ). Nodal support from ML (HKY85 +  $\Gamma$  model 100 replicates) and parsimony (500 replicates) bootstrap analyses are shown below the branches (ML/parimony). Samples are *Sorex monticolus* unless otherwise noted and are denoted by GenBank accession numbers and state or province of collection (Table 1). *Sorex ornatus* and *S. vagrans* were designated as monophyletic outgroups.

**Table 2** Range of corrected and uncorrected genetic distances between major named clades (Figs 2 and 3, including two outgroups, *Sorex ornatus* and *S. vagrans*). Uncorrected values (p) are in the upper-right of the table and corrected values (HKY85 +  $\Gamma$ ) are in the lower-left of the table

	Coastal	Ncont*	Scont†	<i>S. sonomae</i>	<i>S. bendirii</i>	<i>S. palustris</i>	<i>S. vagrans</i>	<i>S. ornatus</i>
Coastal	—	0.046–0.062	0.045–0.059	0.031–0.036	0.036–0.043	0.035–0.041	0.064–0.070	0.077–0.086
NCont	0.071–0.111	—	0.025–0.035	0.025–0.035	0.049–0.056	0.050–0.059	0.076–0.082	0.089–0.092
SCont	0.070–0.111	0.031–0.050	—	0.040–0.049	0.050–0.056	0.050–0.055	0.065–0.071	0.080–0.090
<i>S. sonomae</i>	0.042–0.050	0.069–0.081	0.060–0.084	—	0.031–0.033	0.025–0.034	0.061	0.074–0.072
<i>S. bendirii</i>	0.050–0.061	0.077–0.088	0.081–0.100	0.041–0.044	—	0.030–0.033	0.066–0.067	0.076–0.079
<i>S. palustris</i>	0.047–0.059	0.081–0.100	0.081–0.094	0.031–0.045	0.039–0.043	—	0.069–0.072	0.080–0.082
<i>S. vagrans</i>	0.120–0.136	0.164–0.194	0.136–0.177	0.117	0.133–0.132	0.137–0.148	—	0.071–0.072
<i>S. ornatus</i>	0.150–0.176	0.198–0.225	0.173–0.212	0.140–0.144	0.149–0.160	0.160–0.168	0.134–0.138	—

\*Ncont, Northern Continental clade includes *S. monticolus* and *S. neomexicanus*.

†Scont, Southern Continental clade includes *S. monticolus*, *S. bairdi* and *S. pacificus*.

+  $\Gamma$ , Table 2) between major clades were comparable to interspecific distances reported for other sister species within *Sorex* (Fumagalli *et al.* 1999). For example, average uncorrected genetic distances within *S. monticolus* (5.3%) were greater than interspecific comparisons between *S. palustris* and *S. bendirii* (3.1%).

Monophyly of *S. monticolus* was rejected ( $P < 0.01$ ) based on the results of the parametric bootstrap test (Fig. 4). The observed difference ( $\delta$ ) between the constrained (monophyly of *S. monticolus*) and the unconstrained topologies was 97.12, a value much greater than the greatest difference in  $\ln$  likelihood scores ( $< 18$ ) in the simulated null distribution.

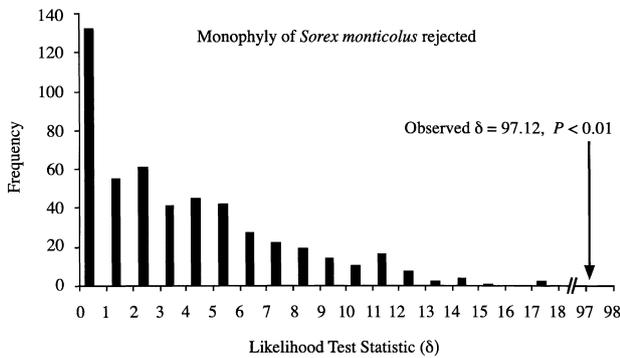


Fig. 4 The null distribution based on the parametric bootstrap test comparing the difference between the best trees supporting monophyly (constrained) and nonmonophyly (unconstrained best tree) of *Sorex monticolus*. Five hundred replicate data sets were simulated using ML under the HKY85 +  $\Gamma$  model. Monophyly of *S. monticolus* was rejected as the observed likelihood ratio test statistic for the real data set was much higher ( $\delta = 97.12$ ) than values sampled from the simulated data sets ( $\delta < 18$ ,  $P < 0.01$ ).

## Discussion

Molecular data often provide an alternative view of diversity and can reveal underlying phylogenetic structure at a scale of resolution not possible with other characters (Avice 1994). This new perspective on phylogenetic relationships within the dusky shrew complex suggests: (i) deep phylogenetic differentiation within *Sorex monticolus* coupled with a lack of support for monophyly of the species based on current taxonomy; (ii) minimal differentiation of three morphologically recognized species (*S. bairdi*, *S. pacificus*, *S. neomexicanus*); (iii) support for the distinctness of *S. sonomae*; and (iv) inclusion of the semiaquatic shrews, *S. palustris* and *S. bendirii*, within the 'dusky shrew complex'. While high levels of intraspecific divergence suggest that diversification events are deeper than previously hypothesized (Findley 1955; George 1988), the morphologically distinct forms, *S. bairdi*, *S. pacificus* and *S. neomexicanus* have probably responded to more recent Late Pleistocene/Holocene events.

### Phylogenetic considerations

Our observations suggest an early and rapid diversification within this group of shrews based on the polytomous structuring of deep nodes in the parsimony and ML topologies (Figs 2 and 3). This phenomenon, a 'star phylogeny', has been observed across diverse groups of mammals with different mtDNA markers (e.g. Lara *et al.* 1996; Lessa & Cook 1998; Conroy & Cook 1999; Halanych & Robinson 1999). If reasonable estimates of divergence times across these studies and colleagues are tenable in the future, independent tests of major diversification events related to earth history may be possible.

Many aspects of our results are supported by previous work. George (1988) characterized a trichotomous clade composed of *S. monticolus*, *S. pacificus* and *S. vagrans*, with each species defined by fixed allozyme differences. We examined these same specimens, designated *S. pacificus* (California, Humboldt County) in George's study (but later reclassified as *S. sonomae* by Carraway 1990), and as in the allozyme data set these individuals constitute a distinct clade in our analyses. George (1988) also indicated that *S. bendirii* and *S. palustris* were sister taxa; however, did not find them allied with *S. pacificus*, *S. monticolus* and *S. vagrans* as our mtDNA sequences suggest. Control region (Stewart & Baker 1994) and cytochrome *b* (Fumagalli *et al.* 1999) sequences also indicate close relationships of the semiaquatic *S. palustris* with *S. monticolus* and *S. pacificus*. Cytogenetic data, although generally problematic for determining phylogenetic relationships within *Sorex* (Dannelid 1991; Ivanitskaya 1994), are also consistent with a common evolutionary history for the group. *Sorex bendirii*, *S. monticolus*, *S. pacificus* and *S. vagrans* possess 2N complements of 53–54 (Brown 1974; Brown & Rudd 1981) which is distinct when compared with other species of *Sorex* (Dannelid 1991).

Morphological data have formed the basis for three monographs on the *S. vagrans* complex. However, ascertaining phylogenetic relationships and determining levels of divergence between purported species has been problematic. For example, Findley (1955) grouped taxa previously designated as *S. obscurus* (= *monticolus*) and *S. pacificus* as subspecies of *S. vagrans*. Subsequent studies recognized the specific status of *S. bairdi*, *S. monticolus*, *S. neomexicanus*, *S. pacificus* and *S. sonomae* based on morphological and ecological differences (Carraway 1990; Alexander 1996). Our data set was unable to provide resolution of relationships among the three recognized species within the Coastal clade, *S. bairdi*, *S. monticolus* and *S. pacificus*, suggesting that the measurable morphological divergence between these taxa may represent rapid ecological responses during the Holocene. *Sorex neomexicanus* (Alexander 1996) represents another minimally differentiated species nested within the Southern Continental clade (Figs 2 and 3). Although these morphologically differentiated species are not supported by our mtDNA data, hypotheses regarding some relationships are supported by previous work. For example, Carraway (1990) speculated that *S. bairdi*, *S. monticolus* and *S. pacificus* were more closely related to each other than to *S. sonomae*, an observation supported by our data set. The semiaquatic species, *S. bendirii* and *S. palustris*, also have derived morphological and physiological adaptations to aquatic lifestyles (e.g. Schmid 1976), and represent reciprocally monophyletic clades in our cytochrome *b* trees.

The levels of divergence (5.3% average uncorrected) observed between the Coastal and Continental clades, coupled with the results of the parametric bootstrap test

(i.e. rejection of monophyly for *Sorex monticolus* — Fig. 4), are strongly indicative of species-level differentiation for these clades. However, because our phylogenies are based on single gene estimations, genealogical concordance (e.g. Baum & Shaw 1995) with other markers should be examined before suggesting taxonomic revisions.

#### Deep phylogeographic history

Given the relatively high levels of uncorrected sequence divergence observed between the major clades (2.50–6.24%, Table 2), initial diversification events within the '*S. vagrans* complex' probably precede the most recent glacial maximum. Fossil remains have been assigned to *S. monticolus*, *S. ornatus* and *S. palustris* from the Late Pleistocene and Holocene (Miller 1971; Kurtén & Anderson 1980; Bonnicksen *et al.* 1986; Mullican & Carraway 1990), however, fossil data are not available from earlier periods. Although the cytochrome *b* sequences are evolving in a clock-like manner ( $\text{HK85} + \Gamma_{\text{clock}}$ ,  $\ln L = -2389.9123$ ,  $\chi^2_{[29]} = 41.1630$ ,  $0.1 > P > 0.05$ ), the lack of multiple calibration points (i.e. limited fossil record), coupled with morphologically cryptic clades render estimates of the timing of divergence difficult. Divergence dates, based on biochemical and molecular data, have been reported for *Sorex* but may suffer from the above concerns, as well as possible rate increases within these small insectivores (Martin & Palumbi 1993; but see Fumagalli *et al.* 1999). In the absence of reliable calibration points, we have opted to forego estimation of absolute divergence dates within this group of shrews. However, we speculate that some diversification events may have coincided with the onset of glacial/interglacial cycles beginning at the Late Pliocene/Early Pleistocene interface (~2 Myr ago). This remains to be tested.

With the exception of *S. palustris*, which occurs across northern North America, this complex of shrews is geographically centred in western North America, suggesting that the isolation of ancestral populations in putative coastal (western) and continental (eastern) refugia may be responsible for the phylogeographic patterns we observed. Early diversification in multiple coastal refugia might have produced ancestral stocks leading to the Coastal clade, plus *S. bendirii* and *S. sonomae* (Fig. 3). A phylogeographic break near the California and Oregon border, similar to that between *S. sonomae* and the Coastal clade, has also been observed in some restricted coastal plant taxa (Soltis *et al.* 1997).

Similarly, continental refugia may have promoted isolation of ancestral populations of *S. palustris* and the Continental clades. Subsequently, *S. palustris* may have spread east from a Rocky Mountain refugium, or conversely, spread west from an eastern North America refugium (see Arbogast 1999); however, more extensive sampling throughout this species' widespread North American distribution is

obviously needed. Further bifurcation within the Continental clade may have occurred later, as reflected by lower levels of sequence divergence (2.5–3.5%, uncorrected) between the Northern and Southern Continental clades. The phylogeographic split between the northern and southern Rocky Mountain clades may centre on the arid Snake River Plain (noted on Fig. 1), which bisects the general north–south distribution of these clades in the southern Idaho, western Wyoming and northern Utah region. Although the distribution of *S. monticolus* in this region is shown as continuous on Fig. 1 (adapted from Hennings & Hoffmann 1977), appropriate habitat, and thus the occurrence of the montane shrew, is more fragmented than depicted. Obviously, additional sampling in this region is required to document the occurrence of Northern and Southern Continental haplotypes and the possibility of contact zones.

The concept that coastal and continental refugia contributed to diversification within the '*S. vagrans* complex' and the semiaquatic species was initially proposed by Findley (1955), who expanded on the ideas of Dalquest (1948). Findley (1955), suggested that the observed morphological differences between Pacific Coast and Rocky Mountain shrews were the result of Late Pleistocene divergence. He speculated that with the onset of the Sangamonian interglacial, arid habitat spread in the Great Basin and initiated the east–west split of widespread ancestral populations of '*S. vagrans*' (Findley 1955). Following the expansion of continental ice sheets during the Wisconsin glaciation, these ancestral populations were forced south and further isolated in Coastal and Continental refugia. With the onset of the Holocene, populations were again able to colonize northward. We agree that a similar mechanistic scenario might explain the phylogeographic patterns observed, but suggest that initial divergence probably preceded the Late Pleistocene and was maintained across multiple glacial events. In particular, the continued expansion and retraction of Great Basin arid habitats (presently found in central Oregon, Washington and Nevada) would have reinforced the east–west isolation of Coastal and Continental ancestral populations over the course of the Pleistocene.

#### Shallow phylogeographic history

Although deep biogeographic events within this complex are difficult to establish, present distributions of the major clades have been profoundly shaped by the most recent glacial/interglacial cycle. Much of the current distribution of *S. monticolus* was covered by the Cordilleran and Laurentide ice sheets during the Wisconsin, such that current distributions of the Coastal and Continental clades are a result of postglacial expansion in the north (and range retraction in the south). We focus on northern clades because of our limited geographical sampling elsewhere.

During the height of the Wisconsin glaciation, cooler conditions occurred throughout North America allowing the southerly expansion of boreal forest across the continent (Davis 1983). However, southerly extensions of the continental ice sheets and piedmont glaciers in the Rocky and Cascade Mountains (Wright & Porter 1983; Whitlock 1992) enforced continued isolation of the Coastal and Continental clades. At this time, the Southern Continental clade may have included more contiguous populations of shrews since boreal forest was present at lower elevations. With the onset of warming, approximately 12 000 years ago, the ice sheets began to recede and northward or southward colonization of previously glaciated habitat by flora and fauna proceeded (Mandryk 1996). We suggest that the Coastal and Northern Continental clades represent postglacial migrants following the northward expansion of forest from refugia south of the ice sheets. Colonization may have coincided with the initial establishment of boreal and coastal rain forests in Alaska and Canada as early as 10 500 years ago (Peteet 1991; Mann & Hamilton 1995; Hu *et al.* 1996).

Conversely, others have suggested that mammalian colonization (including *S. monticolus*) of newly deglaciated mainland regions or islands may have originated from glacial refugia along the coasts of British Columbia and Alaska (Foster 1965; Scudder & Gessler 1989; Byun *et al.* 1997, 1999). Molecular work with bears (black bear, *Ursus americanus*, Byun *et al.* 1997, 1999; brown bear, *U. arctos*, Heaton *et al.* 1996) has been interpreted to support the scenario of different north Pacific Coast refugia. Demboski *et al.* (1999) and Stone & Cook (2000) recently challenged those conclusions because they were based on incomplete geographical sampling. Leonard *et al.* (2000) reported the historical distribution 37 000 years ago of the contemporary Alaska coastal clade (= ABC, Talbot & Shields 1996) of *U. arctos* in Yukon Territory, thus further complicating coastal refugia hypotheses (Heaton *et al.* 1996). The existence of refugia along the north Pacific Coast remains an intriguing area of research, however, lack of persistent coastal refugia throughout the glacial maximum does not preclude the possible role of an early Holocene coastal corridor for colonization that may now be submerged (Josenhans *et al.* 1995; Fedje & Josenhans 2000).

Based on our sampling, the distributions of the Coastal and Northern Continental clades parallel each other along the Coast Range of British Columbia and southeast Alaska (Fig. 1), suggesting the possibility of repeated areas of secondary contact between these populations. At higher latitudes (56–60°N), the mountains are a formidable barrier between coastal and interior regions, with contact possible only through river corridors and mountain passes of lower elevation. Our sampling in the southeast Alaska region suggests that contact zones may occur based on the close geographical distance between Coastal and Northern

Continental haplotypes in some areas (Fig. 1). It is possible that more intensive sampling along the Pacific Coast, particularly in southeast Alaska, will reveal contact zones, the dynamics of which can be characterized. In this same region, several mammalian studies have documented the occurrence of haplotypes from different mammalian clades in close proximity (marten, *Martes americana*, Demboski *et al.* 1999; *U. americanus*, Stone & Cook 2000) or in the same trap line (long-tailed voles, *Microtus longicaudus*, Conroy & Cook 2000). In the southern Rocky Mountains, range expansion–retraction events occurred on multiple occasions throughout the Pleistocene when boreal forests were fragmented (e.g. Patterson 1982; Lomolino *et al.* 1989). The Southern Continental clade has relatively higher levels of intraclade divergence, perhaps indicative of longer persistence in the southern Rocky Mountains, in contrast to recent expansion into deglaciated areas by the less variable Northern Continental and Coastal clades (Rogers 1995; Conroy & Cook 2000). Our sampling, albeit limited, also indicates a phylogeographic break (Fig. 1) between shrews from the north (Colorado, Utah, northern New Mexico) and the south (Arizona, *S. neomexicanus*). A similar disjunction was observed in *M. longicaudus* (Conroy & Cook 2000).

#### Comparative phylogeography

The ability to compare molecular data from multiple co-distributed taxa has allowed a geographical region of interest to be characterized with respect to possible shared histories (e.g. Avise 1992; Zink 1996; Moritz & Faith 1998; Taberlét *et al.* 1998). The phylogeographic patterns observed within *S. monticolus* may not be a taxon-specific response to historical episodes of vicariance (Demboski *et al.* 1999). Rather, because similarly distributed Coastal and Continental clades have been observed for other mammalian taxa (Table 1, Demboski *et al.* 1999), shared histories may be evidence for the cohesion of large-scale biotic assemblages in the Pacific Northwest. Contrary to a scenario of individualistic (Gleasonian) responses by flora and fauna (Graham *et al.* 1996), these biotic assemblages may be the result of concerted responses to earth history changes (Riddle 1998). This is supported by an increasing number of studies that suggest phylogeographic concordance for many taxa in northwestern North America (e.g. Green *et al.* 1996; Soltis *et al.* 1997; Arbogast 1999; Demboski *et al.* 1999; Cook *et al.* 2001; Nielson *et al.* 2001).

Discrepancies between divergence values of mammalian Coastal and Continental clades for different taxa (Demboski *et al.* 1999) may be the result of various processes, including different phylogeographic histories, variable rates of mtDNA evolution (Martin & Palumbi 1993) and the effect of random variation on coalescence and mutation events (Hudson 1991). The geographical extent of mtDNA clades also varies along the north Pacific Coast for different

taxa. Coastal haplotypes of *S. monticolus*, *M. americana* (Demboski *et al.* 1999) and *U. americanus* (Stone & Cook, 2000) occur as far north as southeast Alaska, however, coastal haplotypes of the northern flying squirrel, *Glaucomys sabrinus*, are restricted south of British Columbia (Arbogast 1999). *Sorex bendirii* is also similarly restricted to southern British Columbia and south along the Pacific Coast to California. Other possible examples of restricted Coastal taxa (Hall 1980; Demboski *et al.* 1999) include the Western red-backed vole (*Clethrionomys californicus*), the Douglas squirrel (*Tamiasciurus douglasii*) and the Pacific jumping mouse (*Zapus trinotatus*). Disparity between these species' distributions may be the result of differential colonization abilities following Late Pleistocene/Early Holocene glacial recession or factors such as dispersal from different glacial refugia, habitat specificity, competition and/or latitudinal constraints (e.g. bats, *Myotis*, Parker *et al.* 1997). With the accumulation of diverse molecular data from northwestern North America for mammals, and other fauna and flora (e.g. Soltis *et al.* 1997; Nielson *et al.* 2001), direct tests of phylogeographic congruence may be possible (Sullivan *et al.* 2000).

## Conclusions

Dusky shrews and closely related species have represented an evolutionary, taxonomic and biogeographic conundrum since the complex was first recognized (Merriam 1895). We have expanded on our finding of previously undetected phylogenetic and biogeographic diversity (Demboski *et al.* 1999). Although the Coastal and Continental clades are highly divergent, they may form secondary contact zones throughout western North America. For example, the Southern Continental and Northern Continental clades may contact in the central Rocky Mountains. The dynamics of these contact zones should be carefully characterized with morphological, ecological and genetic investigations, including nuclear DNA. The morphologically distinct, yet genetically similar, Oregon species (*Sorex bairdi* and *S. pacificus*) also raise questions regarding rapid morphological evolution in shrews and microhabitat partitioning of these widely sympatric taxa with *S. sonomae* (Carraway 1990). These species are also sympatric with *S. sonomae* in Oregon (Fig. 1). Intensive sampling in this region may clarify these issues.

Our phylogeographic analysis of this widely distributed complex of shrews provides an opportunity to address conservation issues in western North America. For example, the Southern Continental clade is geographically restricted in the southern Rocky Mountains. These populations represent continental islands that have been formed by recession of boreal forests with warmer climates. Comparative molecular studies of other southwestern montane taxa should assess levels of endemism and the possibility

of shared phylogeographic histories. The Coastal clade is geographically restricted along the north Pacific Coast where logging of old-growth timber has impacted the temperate rain forest (Cook and MacDonald 2001). The observation that other vertebrates (e.g. *Ursus americanus*, Stone & Cook 2000) also are represented by unique coastal haplotypes supports the idea that the region possesses an endemic regional fauna (Swarth 1936; Scudder & Gessler 1989; MacDonald & Cook 1996). Recent molecular work indicates that many of these mtDNA clades may be older than previously suspected (Wooding & Ward 1997; Arbogast 1999), increasing the urgency for revised conservation priorities (Vane-Wright *et al.* 1991; Avise 1996; Cook *et al.* 2001). Other taxa along the north Pacific Coast are represented by multiple clades that show similar geographical patterns (Cook *et al.* 2001), perhaps indicating that particular areas along the north Pacific Coast are either experiencing ongoing colonization or represent historical associations (Conroy *et al.* 1999). In any case, the discovery of substantial levels of geographically structured genetic variation should be incorporated into conservation strategies in western North America.

## Acknowledgements

We are grateful to J. Balke, H. Chen, C. Conroy, K. Foresman, B. Hayward, W. Klenner, M. MacDonald, S. MacDonald, K. Martin, J. Merritt and C. T. Seaton for their assistance in collecting specimens used in this study. Additional tissue loans were graciously provided by the Museum of South-western Biology, University of New Mexico (T. Yates and W. Gannon). We thank A. Schmiechen and T. LeCroy for automated sequencing support. Funding for this project was provided by the US Fish and Wildlife Service (J.A.C.), USDA Forest Service (J.A.C.), the National Science Foundation (J.A.C.), Center for Global Change and Arctic System Research (J.R.D.), Sigma Xi (J.R.D.) and the Arctic Institute of North America (J.R.D.). We thank E. Lessa, G. Shields, J. Sullivan, R. Wayne and an anonymous reviewer for comments on earlier versions of this manuscript. We also extend additional gratitude to J. Sullivan for excellent suggestions concerning phylogenetic analyses.

## References

- Alexander LF (1996) A morphometric analysis of geographic variation within *Sorex monticolus* (Insectivora: Soricidae). *University of Kansas Natural History Museum, Miscellaneous Publication*, **88**, 1–54.
- Arbogast BS (1999) Mitochondrial DNA phylogeography of the New World flying squirrels (*Glaucomys*): implications for Pleistocene biogeography. *Journal of Mammalogy*, **80**, 142–155.
- Arbogast BS, Slowinski JB (1998) Pleistocene speciation and the mitochondrial DNA clock. *Science*, **282**, 1955a.
- Avise JC (1992) Molecular population structure and the biogeographic history of a regional fauna: a case history with lessons for conservation biology. *Oikos*, **63**, 62–76.
- Avise JC (1994) *Molecular Markers, Natural History and Evolution*. Chapman & Hall, New York.
- Avise JC (1996) Toward a regional conservation genetics perspective:

- phylogeography of faunas in the southeastern United States. In: *Conservation Genetics: Case Histories from Nature* (eds Avise JC, Hamrick JL), pp. 431–470. Chapman & Hall, New York.
- Avise JC, Walker D, Johns GC (1998) Speciation durations and Pleistocene effects on vertebrate phylogeography. *Proceedings of the Royal Society of London*, **265**, 1707–1712.
- Baum DA, Shaw KL (1995) Genealogical perspectives on the species problem. In: *Experimental Approaches to Plant Biosystematics* (eds Hoch PC, Stephenson AG), pp. 289–302. Monographs in Systematic Botany from the Missouri Botanical Garden, St. Louis, MO.
- Bonnichsen R, Graham RW, Geppert JS *et al.* (1986) False Cougar and Shield Trap Caves, Pryor Mountains, Montana. *National Geographic Research*, **2**, 276–290.
- Brown RJ (1974) A comparative study of the chromosomes of three species of shrews, *Sorex bendirii*, *Sorex trowbridgii*, and *Sorex vagrans*. *Wasman Journal of Biology*, **32**, 303–326.
- Brown RJ, Rudd RL (1981) Chromosomal comparisons within the *Sorex ornatus*–*S. vagrans* complex. *Wasman Journal of Biology*, **39**, 30–35.
- Byun SA, Koop BF, Reimchen TE (1997) North American black bear mtDNA phylogeography: implications for morphology and the Haida Gwaii glacial refugium controversy. *Evolution*, **51**, 1647–1653.
- Byun SA, Koop BF, Reimchen TE (1999) Coastal refugia and postglacial recolonization routes: a reply to Demboski, Stone, and Cook. *Evolution*, **56**, 2013–2015.
- Carraway LN (1990) A morphologic and morphometric analysis of the 'Sorex Vagrans species complex' in the Pacific Coast region. *Special Publications of The Museum, Texas Technical University*, **32**, 1–76.
- Conroy CJ, Cook JA (1999) mtDNA evidence for repeated pulses of speciation with Arvicolina and Murid Rodents. *Journal of Mammalian Evolution*, **6**, 221–246.
- Conroy CJ, Cook JA (2000) Phylogeography of a post-glacial colonizer: *Microtus longicaudus* (Muridae: Rodentia). *Molecular Ecology*, **9**, 165–176.
- Conroy CJ, Demboski JR, Cook JA (1999) Mammalian biogeography of the Alexander Archipelago of Alaska: a north temperate nested fauna. *Journal of Biogeography*, **26**, 343–352.
- Cook JA, MacDonald SO (2001) The need for historical perspectives on endemism: the mammals of the North Pacific Coast of North America. *Biological Conservation*, **97**, 207–213.
- Cook JA, Bidlack AL, Conroy CJ *et al.* (2001) A, phylogeographic perspective on endemism in the Alexander Archipelago. *Biological Conservation*, **97**, 215–227.
- Dalquest WW (1948) *Mammals of Washington*, University of Kansas Publications. Museum of Natural History, Lawrence, KA.
- Dannelid E (1991) The genus *Sorex* (Mammalia, Soricidae) — distribution and evolutionary aspects of Eurasian species. *Mammalian Review*, **21**, 1–20.
- Davis MB (1983) Quaternary history of deciduous forests of eastern North America and Europe. *Annals of the Missouri Botanical Garden*, **70**, 550–563.
- Demboski JR, Stone KD, Cook JA (1999) Further perspectives on the Haida Gwaii glacial refugium. *Evolution*, **56**, 2008–2012.
- Fedje DW, Josenhans H (2000) Drowned forests and archaeology on the continental shelf of British Columbia, Canada. *Geology*, **28**, 99–102.
- Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. *Evolution*, **39**, 783–791.
- Findley JS (1955) *Speciation of the Wandering Shrew*. University of Kansas Publications, Vol. 9, pp. 1–68. Museum of Natural History, Lawrence, KA.
- Foster JB (1965) Evolution of mammals on the Queen Charlotte Islands, British Columbia. *British Columbia Provincial Museum Occasional Papers*, **14**, 1–30.
- Fumagalli L, Taberlét P, Stewart DT *et al.* (1999) Molecular phylogeny and evolution of *Sorex* shrews (Soricidae: Insectivora) inferred from mitochondrial DNA sequence data. *Molecular Phylogenetics and Evolution*, **11**, 222–235.
- George SB (1988) Systematics, historical biogeography and evolution of the genus *Sorex*. *Journal of Mammalogy*, **69**, 443–461.
- George SB, Smith JD (1991) Inter- and intraspecific variation among coastal and island populations of *Sorex monticolus* and *Sorex vagrans* in the Pacific Northwest. In: *The Biology of Soricidae* (eds Findley JS, Yates TL), pp. 75–91. Special Publication, The Museum of Southwestern Biology, Albuquerque, NM.
- Graham RW, Grimm EC (1990) Effects of global climate change on the patterns of terrestrial biological communities. *Trends in Ecology and Evolution*, **5**, 289–292.
- Graham RW, Lundelius EL Jr, Graham MA *et al.* (1996) Spatial response of mammals to Late Quaternary environmental fluctuations. *Science*, **272**, 1601–1606.
- Green DM, Sharbel TF, Kearsley J, Kaiser H (1996) Postglacial range fluctuation, genetic subdivision and speciation in the western North American spotted frog complex, *Rana pretiosa*. *Evolution*, **50**, 373–390.
- Halanych KM, Demboski JR, van Vuuren BJ *et al.* (1999) Cytochrome *b* phylogeny of North American hares and jackrabbits (*Lepus*, Lagomorpha) and the effects of mutational saturation in outgroup taxa. *Molecular Phylogenetics and Evolution*, **11**, 213–221.
- Halanych KM, Robinson TJ (1999) Multiple substitutions affect the phylogenetic utility of cytochrome *b* and 12S rDNA data: examining a rapid radiation in Leporidae (Lagomorpha) evolution. *Journal of Molecular Evolution*, **48**, 369–379.
- Hall ER (1980) *Mammals of North America*. John Wiley and Sons, New York.
- Hasegawa M, Kishino M, Yano T (1985) Dating the human-ape split by a molecular clock of mitochondrial DNA. *Journal of Molecular Evolution*, **22**, 160–174.
- Heaton TH, Talbot SL, Shields GF (1996) An ice age refugium for large mammals in the Alexander Archipelago, southeastern Alaska. *Quaternary Research*, **46**, 186–192.
- Hennings D, Hoffmann RS (1977) A review of the taxonomy of the *Sorex vagrans* species complex from western North America. *Occasional Papers, Museum of Natural History of the University of Kansas*, **68**, 1–35.
- Hewitt GM (1996) Some genetic consequences of ice ages, and their role in divergence and speciation. *Biological Journal of the Linnean Society*, **58**, 247–276.
- Hoffmann RS (1981) Different voles for different holes: environmental restrictions on refugial survival of mammals. In: *Evolution Today: Proceedings of the Second International Congress of Systematic and Evolutionary Biology, Vancouver, British Columbia*. (eds Scudder GGE, Reveal JL), pp. 25–45.
- Hu SH, Brubaker LB, Anderson PM (1996) Boreal ecosystem development in the northwestern Alaska Range since 11,000 B.P. *Quaternary Research*, **45**, 188–201.
- Hudson RR (1991) Gene genealogies and the coalescent process. In: *Oxford Surveys in Evolutionary Biology* (eds Futuyma D, Antonovics J), pp. 1–44. Oxford University Press, Oxford.
- Huelsenbeck JP, Hillis DM, Jones R (1996) Parametric bootstrapping in molecular phylogenetics: applications and performance. In: *Molecular Zoology: Advances, Strategies and Protocols* (eds Ferraris JD, Palumbi SR), pp. 19–45. Wiley-Liss, New York.

- Irwin DM, Kocher TD, Wilson AC (1991) Evolution of the cytochrome *b* gene of mammals. *Journal of Molecular Evolution*, **32**, 128–144.
- Ivanitskaya EY (1994) Comparative cytogenetics and systematics of *Sorex*: a cladistic approach. In: *Advances in the Biology of Shrews* (eds Merritt JF, Kirkland GL, Rose RK), pp. 313–323. Carnegie Museum of Natural History Special Publication, Pittsburgh, PA.
- Jackson HHT (1928) A taxonomic revision of the American long-tailed shrews (genera *Sorex* and *Microsorex*). *North American Fauna*, **51**, 1–238.
- Josenhans HW, Fedje DW, Conway KW, Barrie JV (1995) Postglacial sea levels on the western Canadian continental shelf: evidence for rapid change, extensive subaerial exposure, and early human habitation. *Marine Geology*, **125**, 73–94.
- Jukes TH, Cantor CR (1969) Evolution of protein molecules. In: *Mammalian Protein Metabolism* (ed. Munro HN), pp. 21–132. Academic Press, New York.
- Kimura M (1980) A simple method for estimating evolutionary rate of base substitution through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, **10**, 111–120.
- Klicka J, Zink RM (1997) The importance of recent ice ages in speciation: a failed paradigm. *Science*, **277**, 1666–1669.
- Kurtén B, Anderson E (1980) *Pleistocene Mammals of North America*. Columbia University Press, New York.
- Lara MC, Patton JL, da Silva MNF (1996) The simultaneous diversification of South American echimyd rodents (*Hystricognathi*) based on complete cytochrome *b* sequences. *Molecular Phylogenetics and Evolution*, **5**, 403–413.
- Leonard JA, Wayne RK, Cooper A (2000) Population genetics of ice age brown bears. *Proceedings of the National Academy of Sciences of the USA*, **97**, 1651–1654.
- Lessa EP, Cook JA (1998) The molecular phylogenetics of tuco-tucos (genus *Ctenomys*, Rodentia: Octodontidae) suggests an early burst of speciation. *Molecular Phylogenetics and Evolution*, **9**, 88–99.
- Lomolino MV, Brown JH, Davis R (1989) Island biogeography of montane forest mammals in the American Southwest. *Ecology*, **70**, 180–194.
- MacDonald SO, Cook JA (1996) The land mammal fauna of Southeast Alaska. *Canadian Field-Naturalist*, **110**, 571–599.
- Mandryk CAS (1996) Late Wisconsinan deglaciation of Alberta: processes and paleogeography. *Quaternary International*, **32**, 79–85.
- Mann DH, Hamilton TD (1995) Late Pleistocene and Holocene paleoenvironments of the North Pacific coast. *Quaternary Science Reviews*, **14**, 449–471.
- Martin AP, Palumbi SR (1993) Body size, metabolic rate, generation time, and the molecular clock. *Proceedings of the National Academy of Sciences of the USA*, **90**, 4087–4091.
- Merriam CH (1895) Synopsis of the American shrews of the genus *Sorex*. *North American Fauna*, **10**, 57–98.
- Miller WE (1971) Pleistocene vertebrates of the Los Angeles Basin and vicinity (exclusive of Rancho Le Brea). *Scientific Bulletin, Los Angeles County Museum of Natural History*, **10**, 1–124.
- Moritz C, Faith DP (1998) Comparative phylogeography and the identification of genetically divergent areas for conservation. *Molecular Ecology*, **7**, 419–430.
- Mullican TR, Carraway LN (1990) Shrew remains from Moonshiner and Middle Butte Caves. *Journal of Mammalogy*, **71**, 351–356.
- Nielson N, Lohman K, Sullivan J (2001) Phylogeography of the tailed frog (*Ascaphus truei*): Implications for the biogeography of the Pacific Northwest disjuncts. *Evolution*, in press.
- Parker DI, Lawhead BE, Cook JA (1997) Distributional limits of bats in Alaska. *Arctic*, **50**, 256–265.
- Patterson BD (1982) Pleistocene vicariance, montane islands, and the evolutionary divergence of some chipmunks (genus *Eutamias*). *Journal of Mammalogy*, **71**, 180–194.
- Peteet DM (1991) Postglacial migration history of lodgepole pine near Yakutat, Alaska. *Canadian Journal of Botany*, **69**, 786–796.
- Rambaut A, Grassly NC (1997) Seq-Gen: an application for the Monte Carlo simulation of DNA sequence evolution along phylogenetic trees, Version 1.1. *Computer Applications in the Biosciences*, **13**, 235–238.
- Rand AL (1954) The ice age and mammal speciation in North America. *Arctic*, **7**, 31–35.
- Riddle BR (1995) Molecular biogeography in the pocket mice (*Perognathus* and *Chaetodipus*) and grasshopper mice (*Onychomys*): the late Cenozoic development of a North American aridlands rodent guild. *Journal of Mammalogy*, **76**, 283–301.
- Riddle BR (1996) The molecular phylogeographic bridge between deep and shallow history in continental biotas. *Trends in Ecology and Evolution*, **11**, 207–212.
- Riddle BR (1998) The historical assembly of continental biotas: Late Quaternary range-shifting, areas of endemism, and biogeographic structure in the North American mammal fauna. *Ecography*, **21**, 437–446.
- Rogers RA, Rogers LA, Hoffmann RS, Martin LD (1991) Native American biological diversity and the biogeographic influence of ice age refugia. *Journal of Biogeography*, **18**, 623–630.
- Rogers RA (1995) Genetic evidence for a Pleistocene population explosion. *Evolution*, **49**, 608–615.
- Roy K, Valentine JW, Jablonski D, Kidwell SM (1996) Scales of climatic variability and time averaging in Pleistocene biotas: implications for ecology and evolution. *Trends in Ecology and Evolution*, **11**, 458–563.
- Schmid WD (1976) Temperature gradients in the nasal passage of some small mammals. *Comparative Biochemistry and Physiology*, **54A**, 305–308.
- Scudder GGE, Gessler N (1989) *The Outer Shores*. Queen Charlotte Islands Museum Press, Skidegate, BC, Canada.
- Smith MF, Patton JL (1993) The diversification of South American murid rodents: evidence from mitochondrial DNA sequence data for the akodontine tribe. *Biological Journal of the Linnean Society*, **50**, 149–177.
- Soltis DE, Gitzendanner MA, Strenge DD, Soltis PE (1997) Chloroplast DNA intraspecific phylogeography of plants from the Pacific Northwest of North America. *Plant Systematics and Evolution*, **206**, 353–373.
- Stewart DT, Baker AJ (1994) Evolution of mtDNA *d*-loop sequences and their use in phylogenetic studies of shrews in the subgenus *Otisorex* (*Sorex*: Soricidae: Insectivora). *Molecular Phylogenetics and Evolution*, **3**, 38–46.
- Stone KD, Cook JA (2000) Phylogeography of black bears (*Ursus americanus*) of the Pacific Northwest. *Canadian Journal of Zoology*, **78**, 1218–1223.
- Sullivan J, Markert J, Kilpatrick CW (1997) Phylogeography and molecular systematics of the *Peromyscus aztecus* species group (Rodentia: Muridae) inferred using parsimony and likelihood. *Systematic Biology*, **46**, 426–440.
- Sullivan J, Arellano E, Rogers DS (2000) Comparative phylogeography of Mesoamerican highland rodents: concerted versus independent response to past climatic fluctuations. *American Naturalist*, **155**, 755–768.

- Swarth HS (1936) Origins of the fauna of the Sitkan District, Alaska. *Proceedings of the California Academy of Sciences*, **223**, 59–78.
- Swofford DL (1999) *PAUP\*: Phylogenetic Analysis Using Parsimony (\*And Other Methods)*, Version 4.0. Sinauer Associates, Sunderland, MA.
- Taberlét P, Fumagalli L, Wust-Saucy AG, Cosson JF (1998) Comparative phylogeography and postglacial colonization routes in Europe. *Molecular Ecology*, **7**, 453–464.
- Talbot SL, Shields GF (1996) Phylogeography of brown bears (*Ursus arctos*) of Alaska and parapatry within the Ursidae. *Molecular Phylogenetics and Evolution*, **5**, 477–494.
- Vane-Wright RI, Humphries CJ, Williams PH (1991) What to protect?: systematics and the agony of choice. *Biological Conservation*, **55**, 235–254.
- Vrba ES (1992) Mammals as key to evolutionary theory. *Journal of Mammalogy*, **73**, 1–28.
- Webb TI, Bartlein PJ (1992) Global changes during the last 3 million years: climatic controls and biotic responses. *Annual Review of Ecology and Systematics*, **23**, 141–173.
- Whitlock C (1992) Vegetational and climatic history of the Pacific Northwest during the last 20,000 years: implications for understanding present-day biodiversity. *Northwest Environmental Journal*, **8**, 5–28.
- Wooding S, Ward R (1997) Phylogeography and Pleistocene evolution in the North American black bear. *Molecular Biology and Evolution*, **14**, 1096–1105.
- Wright HEJ, Porter SC (1983) *Late-Quaternary Environments of the United States*. University of Minnesota Press, Minneapolis, MN.
- Yang Z (1994a) Estimating the pattern of nucleotide substitution. *Journal of Molecular Evolution*, **39**, 105–111.
- Yang Z (1994b) Maximum likelihood phylogenetic estimation from DNA sequences with variable rates over sites; approximate methods. *Journal of Molecular Evolution*, **39**, 306–314.
- Yang Z, Goldman N, Friday A (1995) Maximum likelihood trees from DNA sequences: a peculiar statistical problem. *Systematic Biology*, **44**, 384–399.
- Youngman PM (1975) Mammals of the Yukon Territory. *National Museums of Canada Publications in Zoology*, **10**, 1–192.
- Zink RM (1996) Comparative phylogeography in North American birds. *Evolution*, **50**, 308–317.
- Zink RM, Slowinski JB (1995) Evidence from molecular systematics for decreased avian diversification in the Pleistocene Epoch. *Proceedings of the National Academy of Sciences of the USA*, **92**, 5832–5835.

---

This paper represents part of J.R.D.'s doctoral work at the University of Alaska Fairbanks on the molecular systematics and phylogeography of long-tailed shrews, hares and northern flying squirrels. J.R.D. is currently a postdoctoral fellow at the University of Idaho examining the comparative phylogeography of highland rodents in north-western United States. J.A.C. is Professor of Biology at Idaho State University. J.A.C.'s research centres on the evolution and conservation of mammals. His laboratory is addressing similar questions related to mammals and associated parasites in northern latitudes.

---