

# Are the western water shrew (*Sorex navigator*) and American water shrew (*Sorex palustris*) morphologically distinct?

David W. Nagorsen, Nick Panter, and Andrew G. Hope

**Abstract:** Recent phylogenetic studies applying the mitochondrial cytochrome *b* (*Cytb*) gene and nuclear genes revealed divergent cordilleran and boreal lineages in western populations of the *Sorex palustris* complex that are now recognized as distinct species: *Sorex navigator* (Baird, 1858) (western water shrew) and *Sorex palustris* Richardson, 1828 (American water shrew). Their distributions in western Canada are parapatric with a potential contact zone in the foothills of the Rocky Mountains. Using 80 specimens including 69 of known genotype, we assessed morphological differentiation in metric and nonmetric traits among *S. navigator*, *S. palustris*, and *Sorex bendirii* (Merriam, 1894) (marsh shrew), a sister species of *S. navigator*. Multivariate analyses revealed discrete morpho groups concordant with their genetic lineages and species-level divergence. Three individuals from northwestern British Columbia and one from southwestern Alberta with discordant mitochondrial and nuclear DNA aligned with the *S. navigator* morpho group showing no evidence for morphological intergradation with *S. palustris*. Although differentiated in genotype, *S. navigator* and *S. palustris* are cryptic species that can only be discriminated from morphology with metric cranio-dental data. We developed diagnostic morphometric criteria to identify specimens lacking genetic data.

**Key words:** *Sorex navigator*, *Sorex palustris*, *Sorex bendirii*, western water shrew, American water shrew, genotypes, morphometric differentiation, identification.

**Résumé :** Des études phylogénétiques récentes qui appliquent le gène mitochondrial du cytochrome *b* (*Cytb*) et des gènes nucléaires ont révélé des lignées cordillérienne et boréale divergentes dans les populations de l'Ouest du complexe de *Sorex palustris* qui sont aujourd'hui reconnues comme étant des espèces distinctes, à savoir : *Sorex navigator* (Baird, 1858) et *Sorex palustris* Richardson, 1828 (musaraigne palustre). Les deux espèces ont des répartitions parapatricues dans l'Ouest canadien avec une zone de contact possible dans le contrefort des montagnes Rocheuses. En utilisant 80 spécimens dont 69 de génotype connu, nous avons évalué la différenciation morphologique de caractères mesurables et non mesurables chez *S. navigator*, *S. palustris* et *Sorex bendirii* (Merriam, 1884) (musaraigne de Bendire), une espèce-sœur de *S. navigator*. Des analyses multivariées ont révélé des morpho-groupes distincts qui concordent avec leurs lignées génétiques et une divergence au niveau de l'espèce. Trois individus du nord-ouest de la Colombie-Britannique et un du sud-ouest de l'Alberta présentant de l'ADN mitochondrial et nucléaire discordant s'alignent avec le morpho-groupe de *S. navigator*, ne présentant aucun indice de chevauchement morphologique avec *S. palustris*. Bien que leurs génotypes soient différenciés, *S. navigator* et *S. palustris* sont des espèces cryptiques qui ne peuvent être distinguées morphologiquement que sur la base de mesures craniodentaires. Nous établissons des critères morphométriques diagnostiques permettant d'identifier les spécimens pour lesquels des données génétiques ne sont pas disponibles. [Traduit par la Rédaction]

**Mots-clés :** *Sorex navigator*, *Sorex palustris*, *Sorex bendirii*, musaraigne palustre, génotypes, différenciation morphométrique, identification.

## Introduction

A challenge for resolving the alpha taxonomy of mammals is reconciling discordant patterns of phenotypic and genetic differentiation in closely related taxa. Phenotypic modification from environmental or biotic factors may obscure genetic relationships (Rychlik et al. 2006). Species defined by morphology may show little genetic differentiation, or molecular data may reveal genetically divergent cryptic species indistinguishable from morphology. Most shrew (Eulipotyphla: Soricidae) species were defined by anatomical or morphometric criteria associated primarily with skulls and dentition (Junge and Hoffmann 1981; Dannelid 1991). Despite the somewhat arcane diagnostic traits distinguishing shrew species, results from recent molecular data often support existing taxonomies developed from morphology. Among the

*Sorex cinereus* Kerr, 1792 (cinereus shrew) group of species, a number of species originally defined by morphology (van Zyll de Jong 1982, 1991) are supported by phylogenetic data based on mitochondrial (mtDNA) or nuclear (nuDNA) genes (Demboski and Cook 2003; Hope et al. 2012). van Zyll de Jong's (1983a) proposal that the maritime subspecies of *Sorex arcticus* Kerr, 1792 (arctic shrew) be classified as a distinct species *Sorex maritimensis* Smith, 1939 (maritime shrew) based on cranial mandibular morphology was substantiated by subsequent chromosome (Volobouev and van Zyll de Jong 1988) and mitochondrial cytochrome *b* (*Cytb*) (Stewart et al. 2002) data. However, molecular and morphological data sets may disagree. *Sorex bairdii* Merriam, 1895 (Baird's shrew) and *Sorex pacificus* Coues, 1877 (Pacific shrew), two morphologically distinct species endemic to Oregon (Carraway 1990), are minimally divergent from *Sorex monticolus* Merriam, 1890 (montane

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shrew) in their *Cytb* genome (Demboski and Cook 2001). Within a species, mitochondrial clades may differ from patterns of geographic variation in morphology (Maldonado et al. 2004). Mitochondrial and nuclear genetic data have also revealed unrecognized cryptic species evidently distinguishable only from their genotypes (Baker et al. 2003).

A series of phylogenetic studies applying mitochondrial *Cytb* and nuclear genes revealed divergent cordilleran, boreal, and eastern lineages in the *Sorex palustris* complex (O'Neill et al. 2005; Mycroft et al. 2011; Hope et al. 2014). Levels of mitochondrial divergence in the range of other *Sorex* species that span several late Pleistocene glacial cycles coupled with genetic isolation support the recognition of these lineages as distinct species: *Sorex navigator* (Baird, 1858) (western water shrew), *Sorex palustris* Richardson, 1828 (American water shrew), and *Sorex albibarbis* (Cope, 1862) (eastern water shrew) (Hope et al. 2014). *Sorex navigator* and *S. palustris* were previously treated as subspecies of *S. palustris*. The eastern lineage *S. albibarbis* includes the former subspecies *Sorex palustris albibarbis* (Cope, 1862), *Sorex palustris labradorensis* Burt, 1938, *Sorex palustris gloveralleni* Jackson, 1926, and possibly *Sorex palustris punctulatus* Hooper, 1942 and *Sorex palustris turneri* Johnson, 1951, although the latter two taxa have not been genotyped (Mycroft et al. 2011; Hope et al. 2014). The three species evolved in allopatry in multiple refugial areas south of the continental ice-sheets subsequently expanding their ranges into boreal and cordilleran regions with glacial recession (Himes and Kenagy 2010; Hope et al. 2014). Either contact following lengthy isolation or repeated contact during interglacial intervals with minimal introgression would explain the current distribution of these taxa. *Sorex navigator* occupies the western cordillera and northern coastal regions from Alaska and western Canada to the southwestern United States; in western North America, *S. palustris* inhabits boreal forests from Manitoba to Alberta, the Northwest Territories, and possibly northeastern British Columbia. Their distributions are presumed to be parapatric with a contact zone in the foothills of the Rocky Mountains of Canada. Nevertheless, because subspecies maps (Banfield 1974; Hall 1981) disagree on the location of the boundary between *S. navigator* and *S. palustris* and large geographic gaps exist among individuals of known genotype (Hope et al. 2014), the scale and limits of the zone of parapatry are unknown.

We hypothesized that if phenotypic divergence among species in the *Sorex palustris* complex tracks genetic divergence, then *S. navigator* and *S. palustris* should differ in morphology. Gene and species trees generated by Hope et al. (2014) demonstrated that they are members of different clades with *S. navigator* being a sister species with *Sorex bendirii* (Merriam, 1894) (marsh shrew), a Pacific coastal water shrew (coastal mtDNA lineage), and *S. palustris* being a sister species with the eastern *S. albibarbis*. We used museum voucher specimens of known genotype associated with the O'Neill et al. (2005) and Hope et al. (2014) genetic studies to test the concordance of morphology with the three lineages *S. navigator*, *S. bendirii*, and *S. palustris*, evaluate possible morphological intergradation, and determine the patterns of morphometric differentiation. Ours is the first study of the *S. palustris* complex to apply multivariate morphometric methods and use samples of known genotype. A second objective of our study was to test if *S. navigator* and *S. palustris* are identifiable from external features and non-metric or mensural cranio-dental traits. Diagnostic morphological traits distinguishing *S. bendirii* from *S. navigator* were described by van Zyll de Jong (1983b), Verts and Carraway (1998), and Carraway (1995). In contrast, descriptions of morphological differences among *S. navigator* and *S. palustris* are limited to vague subspecies descriptions (Jackson 1928; Rand 1948; van Zyll de Jong

1983b). A set of diagnostic morphological traits is essential to identify live captures or voucher specimens of unknown genotype.

## Materials and methods

### Specimens examined

Analyses were based on 61 skulls and dentaries (9 *S. bendirii*, 38 *S. navigator*, 14 *S. palustris*) from specimens of known haplotype from mitochondrial *Cytb* gene or the nuclear gene apolipoprotein B exon (*ApoB*) described by Hope et al. (2014). The *Cytb* and *ApoB* loci are each considered to be diagnostic for species within the *Sorex palustris* complex (Hope et al. 2014). To increase our sample size of *S. palustris* for comparison with *S. navigator*, we included an additional 19 specimens from eight locations in Manitoba. Although no genetic data were associated with the Manitoba specimens, their locations are in the range extent of *S. palustris*. Moreover, genetic samples from two individuals from southeastern Manitoba (no associated museum voucher specimens) analyzed by Mycroft et al. (2011) had *Cytb* sequences of *S. palustris*. Specimens were from 38 general locations across Canada and the western United States (Fig. 1; Supplementary Table S1<sup>1</sup>). The total sample of 80 consisted of 32 females, 30 males, and 18 unknown sex.

### Qualitative characters

To evaluate possible effects of age and tooth attrition on our sample, we assigned skulls to three tooth wear categories — no abrasion: little or no wear on unicuspid, no exposure of I1 roots; moderate abrasion: unicuspid worn to <50% their length, upper incisor (I1) roots exposed up to 0.15 mm, no wear on medial tines; high abrasion: unicuspid worn >50% of their length, I1 roots exposed 0.4–0.5 mm, and I1 worn to or above medial tines.

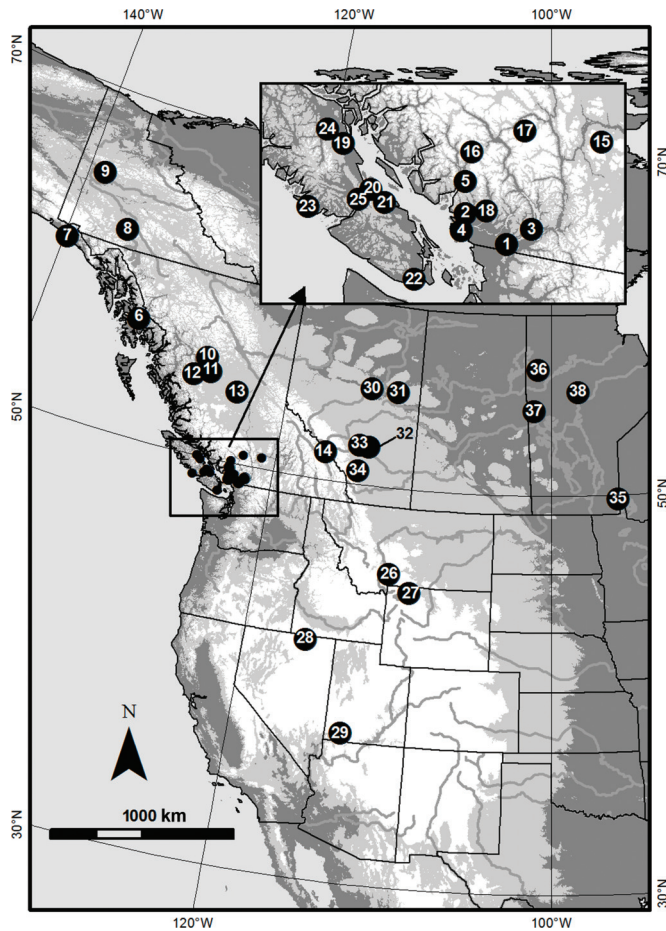
External features examined on museum study skins included the number of paired toe pads on digits 3 and 4 of the hind feet, dorsal and ventral pelage colour, the extent and size of the bristle fringe of hairs on the hind feet, degree of lateral flattening of the tail, and ventral tail colour. Colour comparisons were made under a desk lamp (Sol-Source, GretagMacbeth, New Windsor, New York, USA) with a daylight filter. We assessed five cranio-dental traits: position of infraorbital foramen relative to the mesostyle on M2 (van Zyll de Jong 1980); relative heights of the third and fourth unicuspid (U3, U4) (Junge and Hoffmann 1981); shape of medial edge of I1, size of medial tine on I1, position of medial tine relative to pigmentation on anterior face of I1 (Carraway 1990; Carraway and Verts 1994). We developed a score for medial tine size by ranking as small (1), medium-sized (2), or large (3). Mandibular traits included the following: number of denticles on i1 within the continuous pigmented area; position of mental foramen relative to protoconid and hypoconoid cusps on first lower molar (m1) (van Zyll de Jong 1980); position of posterior-most edge of i1 alveolus relative to the protoconid and hypoconoid cusps on lower premolar (p4); postmandibular foramen and canal morphology (Junge and Hoffmann 1981).

### Measurements

Four body measurements (total length, tail vertebrae length, hind-foot length, and mass) were taken from museum specimen tags. Body length was calculated from total length – tail vertebrae length. We used 15 cranio-dental (van Zyll de Jong 1980; Nagorsen and Panter 2009) and 7 dentary (Carraway 1995) measurements. The 15 cranio-dental measurements (Supplementary Figs. S1A–S1C)<sup>1</sup> were as follows — (1) greatest length of skull: greatest length from medial anterior edge of maxilla to posterior-most edge of skull; (2) width of cranium: greatest width across cranium; (3) inter-orbital width: width at narrowest point across interorbital; (4) depth of I1: width at base in lateral view; (5) length of U1: greatest length

<sup>1</sup>Supplementary table and figures are available with the article through the journal Web site at <http://nrcresearchpress.com/doi/suppl/10.1139/cjz-2017-0007>.

**Fig. 1.** General locations of specimens used in the study. Locations 1–5: *Sorex bendirii* (marsh shrew); locations 7–29: *Sorex navigator* (western water shrew); locations 30–33 and 35–38: *Sorex palustris* (American water shrew); locations 11, 12, 13, 34: hybrids with *S. navigator* nuclear *ApoB* gene and *S. palustris* mitochondrial *Cytb* gene. Locality descriptions, coordinates, and specimens examined are associated with map numbers listed in Supplementary Table S1.<sup>1</sup> White shading represents >2000 m elevation, whereas light grey shading represents >1000 m elevation. Imagery from GEO BC (<http://www2.gov.bc.ca/gov/content/data/geographic-data-services/topographic-data/topographic-map-viewer>); natural features from ESRI software (ESRI Canada, Toronto, Ontario).



of first unicuspid in lateral view; (6) angle of rostrum: angle between a line drawn along alveolus from the base of first unicuspid to fourth unicuspid and a line extending anteriorly along alveolus from third upper molar base to posterior base of fourth unicuspid; (7) basal width of I1: width across upper incisor cingulum at base in ventral view; (8) width across U1s: greatest distance across labial edges of first unicuspid; (9) width of U1: greatest width at right angles to the anterior–posterior axis of first unicuspid; (10) width of M1: greatest width at right angles to the anterior–posterior axis of first upper molar; (11) distance across U1–U5: greatest length of unicuspid row from anterior edge of first to posterior edge of fifth unicuspid; (12) distance across P4–M3: distance from anterior edge of upper premolar to posterior edge of third upper molar; (13) width between M2s: least distance between lingual edges of second upper molars; (14) width across M2s: greatest distance between labial edges of second upper molars; (15) length of M1: greatest length of first upper molar. The seven dentary measurements (Supplementary Fig. S2)<sup>1</sup> were as follows: (1) height of coronoid process: least distance from curvature in horizontal ramus to

maximum extension of coronoid process; (2) distance between i1 and p4: least distance between posterior edge of lower inciseve alveolus and cingulum of the lower premolar; (3) width of i1: width of lower incisor immediately posterior to proximal denticle; (4) length of m1: greatest length of first lower molar parallel to ventral border of mandible; (5) depth of dentary: mandible depth posterior to third lower molar; (6) length of dentary: length from proximal tip of condyloid to posterior edge of alveolus of lower incisor; (7) length of dentary toothrow: length from anterior edge of lower canine to posterior edge of third lower molar.

Skull and dentary measurements were taken with a Leica M205A stereomicroscope and associated DFC 450 camera with version 4.2.0 (build: 607) software (Leica Microsystems Switzerland, Ltd.). Digital images (4.9 Mega pixel resolution, 16 bit \*.tiff files) were four views of each specimen at fixed magnifications: dorsal skull (5.5×), ventral rostrum (9.0×), lateral rostrum (9.0×), and lateral dentary buccal view (12.0×). For all views, we used custom mounts to standardize sample positions and to ensure that measurements were taken in the same plane. We measured lengths (millimetres) and angle of the rostrum (degrees converted to radians for multivariate analyses) with tools from the Leica application suite software. We manually calibrated images using a calibration slide (0.01 mm divisions) set at the same plane as structures measured. Deviation of measurement landmarks from the plane of focus resulted in measurement errors of  $\pm 1.0$  pixel for ventral skull and  $\pm 0.5$  pixel for other images. This error is proportional to the length of each measurement:  $\pm 0.025\%$  (dorsal skull),  $\pm 0.023\%$  (lateral skull),  $\pm 0.046\%$  (ventral skull), and  $\pm 0.030\%$  (lateral dentary). Pixel size, a second source of measurement error, was  $\pm 1.0$  pixel, resulting in errors of  $\pm 0.005$  mm (dorsal skull images),  $\pm 0.0046$  mm (lateral skull images),  $\pm 0.0046$  mm (ventral skull images), and  $\pm 0.0061$  mm (lateral dentary). Total measurement error from both sources was 0.01 mm for greatest length of the skull and  $<0.01$  mm for other measurements.

### Morphometric analyses

Measurements were initially screened for outliers, normality, skewness, and kurtosis. Sample sizes of individuals of known sex of each species were too small to test for intraspecific sexual dimorphism. Therefore, we pooled the 55 (25 males, 30 females) specimens of known sex of both *S. palustris* and *S. navigator* and tested for equality of centroids with a one-way multivariate analysis of variance (MANOVA) based on the 22 cranial dentary measurements.

The focus of our study was discriminating *S. palustris* from *S. navigator*, but we included *S. bendirii* as an outgroup in an initial principal components analysis (PCA) to explore patterns of variation and distance in morpho space among the three genetic lineages. *Sorex bendirii* is a well-defined species that differs from *S. navigator* and *S. palustris* in pelage and cranio-dental traits (van Zyll de Jong 1983b; Carraway 1995; Verts and Carraway 1998). The PCA was done on a correlation matrix of the untransformed 22 cranial dentary measurements without rotation. For comparison, a second PCA was extracted from a variance–covariance matrix of log-transformed data.

Univariate and multivariate analyses comparing *S. navigator* and *S. palustris* were based on 69 specimens. Three specimens (Royal BC Museum (RBCM) 020008, RBCM 020016, RBCM 020017) from northwestern British Columbia with discordant mitochondrial and nuclear genomes (*S. palustris* mtDNA and *S. navigator* nuDNA) and a specimen from Okotoks, Alberta (Royal Ontario Museum (ROM) 109832), that had a mtDNA sequence of *S. palustris* (no previous nuDNA data available) but grouped with *S. navigator* in the PCA were excluded from these analyses. Univariate differences for 4 external measurements and 22 cranial dentary measurements were tested with one-way ANOVA with a Bonferroni correction. Box plots and one-sided tolerance limits were calculated for bivariate ratios of selected cranial dentary measurements to test for

their utility in species identification. Group differences in centroids were tested with a one-way MANOVA of 22 cranial dentary variables. We applied two-group discriminant function analyses (DFA) of untransformed cranial dentary measurements to determine variables most effective for identifying the two species and develop criteria for classifying specimens to a priori defined species groups. Separate DFA were performed on 15 cranial and 7 dentary measurements. To test if species could be identified by a reduced subset of variables, we employed forward stepwise DFA ( $p = 0.10$ ). Group membership was determined by Mahalanobis distance ( $D^2$ ) values and we used cross-validation tests with a jack-knife procedure to measure classification error. Scores of specimens were projected on the canonical variate axes for skull and dentaries to assess morphometric overlap in canonical space. The four specimens with discordant genetic or morphological identifications were treated as unknowns in the stepwise DFA and classified to species from  $D^2$  values. Tolerance limits were calculated with [The National Institute of Standards and Technology \(2012\)](#) software; all other analyses were done with SYSTAT version 12 (SYSTAT Software Inc., San Jose, California, USA).

### Genetic analyses

A specimen (ROM 109832) from Okotoks, Alberta (location 34 in [Fig. 1](#); Supplementary Table S1'), had discordant identifications from morphology and its *Cytb* haplotype in our study. Only *Cytb* data were originally available from GenBank for this specimen ([Hope et al. 2014](#)). To assess its genetic species designation, we obtained a genetic sample from the ROM and sequenced 434 bp of the nuclear *ApoB* gene. Methods for sequencing, including primers, reagents, and conditions for polymerase chain reaction and cycle sequencing, have been described in detail ([Hope et al. 2014](#)). We conducted automated sequencing of complementary strands of the *ApoB* locus using the Applied Biosystems 3110 DNA sequencer of the Department of Biology at the University of North Dakota, Grand Forks. We retrieved sequences of the *ApoB* locus and 778 bp of the *Cytb* gene from GenBank for an unambiguous specimen of *S. palustris* (RBCM 019834) and *S. navigator* (RBCM 020315) that were used in morphological analyses, the three specimens from northwestern British Columbia with mitochondrial–nuclear discordance (RBCM 20008, RBCM 20016, RBCM 20017), and a specimen of an outgroup taxon *Sorex vagrans* Baird, 1857 (vagrant shrew). We determined DNA substitution models for each locus using MrModeltest version 2.3 ([Nylander 2004](#)) and estimated genealogies using Bayesian analyses in MrBayes version 3.1 ([Ronquist and Huelsenbeck 2003](#)) as described elsewhere ([Hope et al. 2014](#)). Genealogies were visualized in FigTree version 1.2.2 ([Rambaut 2009](#)).

## Results

### Age and sex variation

Absolute age of specimens was unknown, but few specimens had worn dentition. Tooth abrasion scores were as follows: *S. bendirii*, no abrasion or moderate abrasion = 7 (78%); *S. navigator*, no abrasion or moderate abrasion = 32 (92%); *S. palustris*, no abrasion or moderate abrasion = 32 (100%). Moreover, because none of the variables used in analyses were measures of tooth height, our dental measurements were not affected by wear. The one-way MANOVA revealed no sexual dimorphism for the combined sample of males and females for *S. navigator* and *S. palustris* (Wilks'  $\lambda = 0.682$ ,  $F_{[22,32]} = 0.677$ ,  $p = 0.828$ ); therefore, we pooled sexes in subsequent statistical analyses.

### Principal components analysis of three species

Principal components 1 and 2 (PC1 and PC2, respectively) extracted from the correlation matrix accounted for 80.06% of the total variance ([Table 1](#)) among the 80 specimens. Except for angle of the rostrum, all variables were positively correlated with PC 1. This vector explained increasing skull length, dentary size, and robustness of dentition. Bipolar, PC 2 contrasted increasing length of

**Table 1.** Component correlations of 15 cranial and 7 dental measurements with the first two principal components (PC 1 and PC 2) extracted from a correlation matrix of 61 genotyped specimens of *Sorex bendirii* (marsh shrew), *Sorex navigator* (western water shrew), and *Sorex palustris* (American water shrew) and 19 specimens of *S. palustris* of unknown genotype.

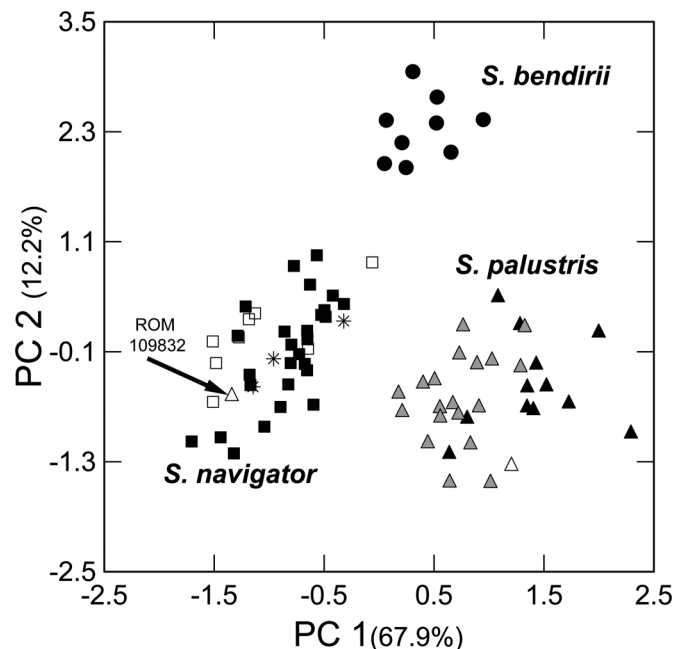
Variable	PC 1	PC 2
<b>Skull</b>		
Basal width of I1	0.897	-0.261
Depth of I1	0.936	-0.123
Length of M1	0.814	-0.408
Width of M1	0.827	-0.129
Width between M2s	0.771	0.340
Width across M2s	0.941	-0.047
Distance across P4–M3	0.923	-0.281
Width across U1s	0.967	0.022
Width of U1	0.935	-0.223
Length of U1	0.798	0.125
Distance across U1–U5	0.343	0.831
Width of cranium	0.867	0.209
Interorbital width	0.680	0.072
Greatest length of skull	0.880	0.219
Angle of rostrum	-0.126	-0.634
<b>Dentary</b>		
Height of coronoid process	0.953	0.016
Distance between i1 and p4	0.189	0.852
Width of i1	0.897	0.123
Length of m1	0.873	-0.307
Depth of dentary	0.919	-0.180
Length of dentary	0.862	0.310
Length of dentary tooththrow	0.952	0.009
Eigenvalue	14.93	2.68
Percent variance	67.86	12.20

the unicuspid tooththrow and distance between i1 and p4 with decreasing rostral angle. A bivariate ordination of factor scores on PC 1 and PC 2 ([Fig. 2](#)) revealed three nonoverlapping groups in morpho space with *S. navigator* and *S. palustris* strongly differentiated on PC 1 and *S. bendirii* individuals intermediate. PC 2 separated *S. bendirii* specimens from the other groups. The three morphological groups were congruent with the three DNA lineages. The exception was the specimen from Okotoks, Alberta (ROM 109832), that had a mtDNA sequence of *S. palustris* but clearly grouped with *S. navigator* by morphology. Three individuals from northwestern British Columbia with *S. palustris* mtDNA and *S. navigator* nuDNA all aligned within the *S. navigator* morphological group showing no evidence of intermediacy. The 19 Manitoba specimens lacking genetic data grouped with *S. palustris*; their PC 1 scores were smaller than the Alberta specimens ( $t_{[30]} = 5.22$ ,  $p < 0.000$ ). PCA based on a variance–covariance matrix with log-transformed data for the 80 specimens (not shown) produced similar results with three morphological groups, patterns of variation described by PC 1 and PC 2 similar to those extracted from the correlation matrix, and the three specimens with discordant mitochondrial and nuclear markers or morphology aligned with *S. navigator*.

### Genetic analysis

No heterozygous sites were present among the seven individuals sequenced for the *ApoB* locus. Genealogies for *Cytb* and *ApoB* loci demonstrated a discordant genetic species designation for the Okotoks, Alberta, specimen that matched the genetic designations for the three individuals from northwestern British Columbia with mitochondrial–nuclear discordance. All were *S. palustris*

**Fig. 2.** Bivariate plot of 80 specimens of *Sorex bendirii* (marsh shrew), *Sorex navigator* (western water shrew), and *Sorex palustris* (American water shrew) on the first two principal components (PC 1 and PC 2) extracted from a correlation matrix of 15 cranial and 7 dentary measurements. Black circles: *S. bendirii* lineage; black squares: *S. navigator* lineage mtDNA and nuDNA; white squares: *S. navigator* lineage mtDNA only; black triangles: *S. palustris* lineage mtDNA and nuDNA; white triangles: *S. palustris* lineage mtDNA only; grey triangles: *S. palustris*, Manitoba, no genetic data; asterisks: discordant lineage *S. palustris* mtDNA, *S. navigator* nuDNA.



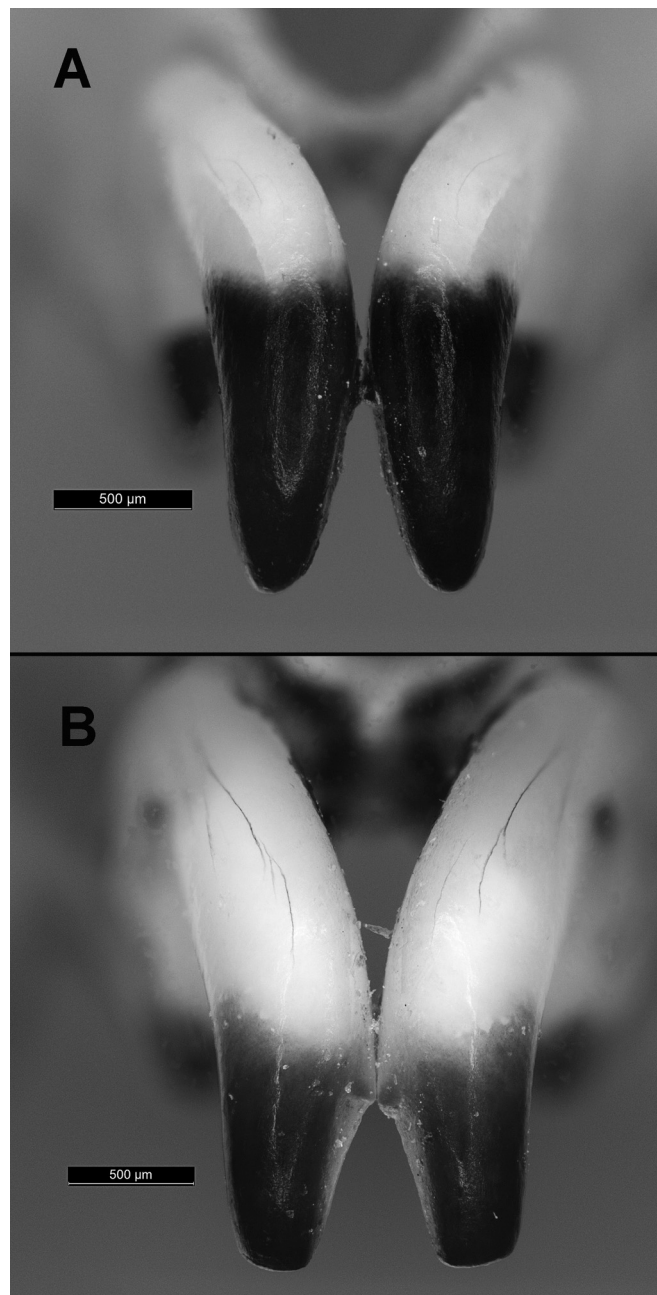
according to mtDNA, but were *S. navigator* based on nuDNA (Supplementary Fig. S3).<sup>1</sup>

#### Distinguishing *S. navigator* from *S. palustris*

We found no differences in external morphology. Both species had five paired pads on digits 3 and 4. Dorsal and ventral pelage colour and the degree of bicolouring of the tail varied among individuals, but we observed no interspecific differences. Similarly, the bristle fringe of hairs on the hind feet and degree of lateral flattening of the tail was similar in both species. In both species, ventral pelage colour ranged from silvery-grey to brown. *Sorex navigator* from Vancouver Island differed from other samples in having brown markings on the ventrum. Dorsal pelage colour of *S. navigator* and *S. palustris* ranged from brown to black with some individuals of each species showing white tips on dorsal guard hairs. Both taxa had distinctly bicoloured tails with a paler underside.

Of the five cranio-dental traits only tine size differed among species. Of the 31 *S. navigator* with unworn medial tines, size scores were 1–2 with a median score of 2. All 32 *S. palustris* had large tines that scored as 3. Pigmentation on the anterior face of I1 and its associated tine was generally darker extending higher on the tooth in *S. navigator* compared with *S. palustris* (Figs. 3A, 3B). In *S. palustris*, pigmentation on anterior I1 usually extends to the medial edge of I1 below the tines. In *S. navigator*, the medial edge of I1 is often unpigmented. For the four individuals with discordant nuclear and mitochondrial DNA, medial tine scores were 2. Pigmentation pattern and colour on their I1s was consistent with *S. navigator*. None of the four mandibular traits clearly separated the species. The posterior-most edge of i1 alveolus aligned with the metaconid in all 32 *S. palustris*; among 32 *S. navigator* that could be scored, the i1 alveolus position was more variable aligning with

**Fig. 3.** Anterior view of first upper incisors (I1) showing intensity and extent of pigmentation and size of medial tines for (A) *Sorex navigator* (western water shrew; RBCM 19823) and (B) *Sorex palustris* (American water shrew; RBCM 19841). Scale bars = 500  $\mu$ m.



either the protoconid (55%) or the metaconid (45%). Both species have three denticles on i1 with either two or three denticles enclosed in the pigmented region. The postmandibular foramen was absent among most individuals of either species, but when present, it occurred with a higher frequency in *S. navigator* (44% of 34 individuals) than in *S. palustris* (19% of 32 individuals).

Univariate statistics demonstrated differences in 4 external, 13 cranio-dental, and 6 dentary measurements with *S. palustris* larger except for tail length (Table 2). Ranges of external measurements overlapped extensively among the two species. Five cranio-dental measurements (basal width of I1, depth of I1, distance across P4–M3, width across U1s, width of U1) and one dentary measurement (height of the coronoid process) showed no overlap

**Table 2.** Body, skull, and dentary measurements of *Sorex navigator* (western water shrew) and *Sorex palustris* (American water shrew).

Variable	<i>Sorex navigator</i>					<i>Sorex palustris</i>					p
	Mean	SD	Minimum	Maximum	n	Mean	SD	Minimum	Maximum	n	
<b>Body</b>											
Total length	139.0	8.1	126	158	35	145.1	9.8	128	165	31	0.008
Body length	69.7	7.3	56	85	35	80.1	9.2	62	98	31	<0.001
Tail length	69.2	4.0	62	79	35	65.0	3.7	58	71	31	<0.001
Hind-foot length	18.8	0.9	17	20.5	35	19.1	0.9	17	21	32	0.242
Mass	10.5	3.1	6.5	21.5	26	12.6	2.5	8.7	20.0	32	0.005
<b>Skull</b>											
Basal width of I1	0.64	0.034	0.56	0.70	35	0.83	0.047	0.75	0.93	32	<0.001
Depth of I1	1.11	0.043	1.02	1.19	35	1.34	0.050	1.25	1.51	32	<0.001
Length of M1	1.46	0.055	1.35	1.55	35	1.62	0.053	1.51	1.79	32	<0.001
Width of M1	1.55	0.080	1.37	1.76	35	1.71	0.067	1.60	1.88	32	<0.001
Width between M2s	2.25	0.082	2.10	2.44	35	2.39	0.074	2.21	2.55	32	<0.001
Width across M2s	5.24	0.144	4.88	5.53	35	5.68	0.151	5.42	6.11	32	<0.001
Distance across P4–M3	4.65	0.090	4.46	4.78	35	5.13	0.172	4.89	5.62	32	<0.001
Width across U1s	1.92	0.072	1.78	2.09	35	2.29	0.087	2.10	2.46	32	<0.001
Width of U1	0.69	0.033	0.61	0.75	35	0.89	0.043	0.78	0.98	32	<0.001
Length of U1	0.94	0.039	0.86	1.03	35	1.05	0.069	0.92	1.19	32	<0.001
Distance across U1–U5	3.09	0.107	2.91	3.35	35	3.11	0.115	2.87	3.32	32	0.216
Width of cranium	9.98	0.253	9.53	10.80	35	10.54	0.250	10.01	10.97	32	0.001
Interorbital width	3.82	0.122	3.58	4.13	35	4.01	0.127	3.80	4.31	32	<0.001
Greatest length of skull	19.83	0.423	18.70	20.71	35	20.93	0.613	19.66	22.08	32	<0.001
Angle of rostrum	159.5	2.60	149.7	164.2	35	158.9	2.73	154.0	164.9	32	0.332
<b>Dentary</b>											
Height of coronoid process	4.24	0.176	3.89	4.59	35	4.98	0.149	4.68	5.25	32	<0.001
Distance between i1 and p4	0.47	0.071	0.32	0.61	35	0.47	0.056	0.35	0.59	32	0.702
Width of i1	0.80	0.042	0.72	0.89	35	0.96	0.059	0.74	1.05	32	<0.001
Length of m1	1.54	0.046	1.43	1.65	35	1.73	0.061	1.62	1.89	32	<0.001
Depth of dentary	1.30	0.067	1.14	1.46	35	1.62	0.092	1.46	1.81	32	<0.001
Length of dentary	8.49	0.247	8.06	9.03	35	9.02	0.302	8.40	9.53	32	<0.001
Length of dentary toothrow	5.59	0.136	5.36	5.88	35	6.00	0.156	5.73	6.32	32	<0.001

Note: Measurements are in millimetres except body mass, which is in grams, and angle of rostrum in degrees. The p values are based on a one-way ANOVA.

in their extreme values, but ranges of these six variables differed among species by  $\leq 0.1$  mm. Two bivariate ratios separated the species with larger ratio values for *S. palustris* — width of U1/distance across U1–U5: upper one-sided tolerance for *S. navigator* = 0.248 ( $p = 0.95$ ), lower one-sided tolerance limit for *S. palustris* = 0.251 ( $p = 0.95$ ); height of coronoid process/length of dentary: upper one-sided tolerance for *S. navigator* = 0.527 ( $p = 0.95$ ), lower one-sided tolerance limit for *S. palustris* = 0.536 ( $p = 0.95$ ).

For multivariate analyses, we treated skulls and dentaries separately to develop identification models for each. A one-way MANOVA revealed that species' centroids differed — skulls: Wilks'  $\lambda = 0.067$ ,  $F_{[15,51]} = 47.32$ ,  $p < 0.001$ ; dentaries: Wilks'  $\lambda = 0.092$ ,  $F_{[7,59]} = 83.13$ ,  $p < 0.001$ . The discriminate vector for skulls was strongly influenced by dental measurements with depth of I1 and width of U1 contributing most (Table 3). In contrast, the discriminate vector for dentaries was mostly affected by height of the coronoid process and length of the dentary; dental measurements had less influence. Jackknifed classifications derived from two-group DFAs using all variables correctly assigned all (100%) 35 *S. navigator* and 32 *S. palustris* skulls and dentaries to species. The two-group stepwise DFAs selected three cranio-dental and four dentary measurements (Table 3). The discriminant vector for skulls contrasted increasing depth of I1 and width of U1 with decreasing distance across U1–U5. The discriminant vector for dentaries mainly contrasted increasing coronoid height with decreasing length of the dentary. The discriminant function scores for skulls = 12.628(depth of I1) + 19.144(width of U1) – 4.050(distance across U1–U5) – 17.778. Discriminant function scores for dentaries = 6.155(height of coronoid process) + 9.39(length of m1) + 4.144(depth of dentary) – 2.959(length of dentary) – 23.621. The DFAs with reduced variables correctly (100%) classified all 67 specimens to species; histograms of discriminant scores showed no overlap (Figs. 4A, 4B). Group centroids for skulls

were  $-3.077$  (*S. navigator*),  $3.366$  (*S. palustris*); group centroids for dentaries were  $-2.926$  (*S. navigator*),  $3.200$  (*S. palustris*).

The four individuals with discordant genetic identifications treated as unknowns were identified from bivariate ratios and multivariate DFA as *S. navigator*. Their ratios of width of U1/distance across U1–U5 (RBCM 020008 = 0.22; RBCM 020016 = 0.23; RBCM 020017 = 0.23; ROM 109832 = 0.23) and height of coronoid process/length of dentary (RBCM 020008 = 0.50; RBCM 020016 = 0.51; RBCM 020017 = 0.50; ROM 109832 = 0.49) were within the upper tolerance value for *S. navigator* and less than the minimum tolerance value for *S. palustris*. Stepwise DFA classified all four individuals from their  $D^2$  values as *S. navigator* ( $p = 1.00$ ) congruent with their grouping in the PCA ordination. Their negative discriminant scores on the skull vector (RBCM 020008 =  $-2.715$ ; RBCM 020016 =  $-1.178$ ; RBCM 020017 =  $-2.882$ ; ROM 109832 =  $-4.731$ ) and dentary vector (RBCM 020008 =  $-2.427$ ; RBCM 020016 =  $-1.715$ ; RBCM 020017 =  $-2.943$ ; ROM 109832 =  $-3.406$ ) were within the histogram of scores for *S. navigator* scores showing no evidence for intermediacy (Figs. 4A, 4B).

## Discussion

### Morphometric differentiation

Morphometric analyses demonstrated three discrete groups in morpho space that were consistent with the coastal, cordilleran, and boreal genetic lineages in western North America defined by O'Neill et al. (2005), Mycroft et al. (2011), and Hope et al. (2014), representing *S. bendirii*, *S. navigator*, and *S. palustris*. Morphological distance between *S. navigator* and *S. palustris* was equivalent to the distances separating them from *S. bendirii*. Multivariate analyses revealed no individuals with intermediate morphologies suggesting a lack of morphological intergradation congruent with their

**Table 3.** Coefficients of canonical discriminant functions (DF) standardized by within variances for 15 skull and 7 dentary measurements of *Sorex navigator* (western water shrew) and *Sorex palustris* (American water shrew).

All variables		Stepwise	
Variable	DF	Variable	DF
<b>Skulls</b>		<b>Skulls</b>	
Basal width of I1	0.262	Basal width of I1	—
Depth of I1	0.648	Depth of I1	0.593
Length of M1	-0.192	Length of M1	—
Width of M1	-0.064	Width of M1	—
Width between M2s	0.175	Width between M2s	—
Width across M2s	-0.415	Width across M2s	—
Distance across P4–M3	-0.075	Distance across P4–M3	—
Width across U1s	0.422	Width across U1s	—
Width of U1	0.812	Width of U1	0.726
Length of U1	-0.074	Length of U1	—
Distance across U1–U5	-0.343	Distance across U1–U5	-0.449
Width of cranium	-0.367	Width of cranium	—
Interorbital width	-0.085	Interorbital width	—
Greatest length of skull	-0.035	Greatest length of skull	—
Angle of rostrum	-0.245	Angle of rostrum	—
Eigenvalue	13.89	Eigenvalue	10.68
<b>Dentaries</b>		<b>Dentaries</b>	
Height of coronoid process	0.950	Height of coronoid process	1.007
Distance between i1 and p4	-0.115	Distance between i1 and p4	—
Width of i1	0.119	Width of i1	—
Length of m1	0.445	Length of m1	0.501
Depth of dentary	0.337	Depth of dentary	0.332
Length of dentary	-0.739	Length of dentary	-0.813
Length of dentary tooththrow	-0.005	Length of dentary tooththrow	—
Eigenvalue	9.86	Eigenvalue	9.65

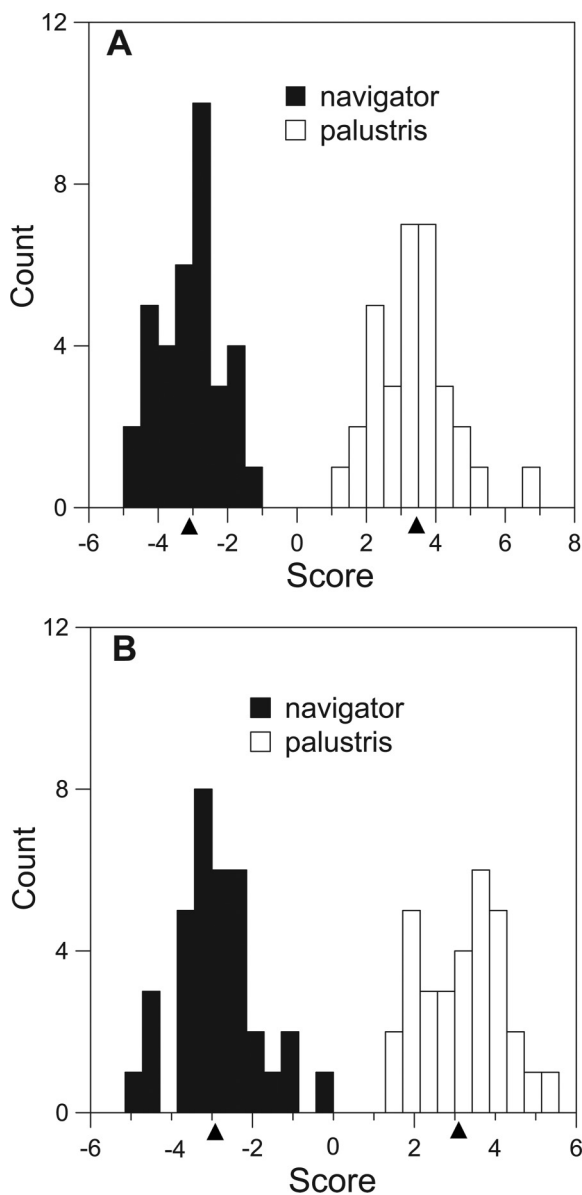
**Note:** Discriminant functions for skulls and dentaries derived from separate two-group analyses employing all variables and a stepwise methodology.

species level classification. The PCA confirmed that the British Columbian population of *S. bendirii* is larger than *S. navigator* in general, with cranial and dental size nearly approaching *S. palustris* in size. The *S. bendirii* skull differs from *S. navigator* and *S. palustris* in its more acutely angled, ventral rostrum edge, relatively longer unicuspid tooththrow length, and a longer dentary with i1 inserted more anterior to the dentary tooththrow, characteristics described by van Zyll de Jong (1983b) and Verts and Carraway (1998). According to the subspecies descriptions of Jackson (1928) and Rand (1948), *S. navigator* has a smaller skull and weaker dentition than *S. palustris*. Univariate measurements and multivariate patterns of differentiation are consistent with these early descriptions. It is evident in the PCA ordination that *S. palustris* is a scaled-up *S. navigator* with a larger skull, larger dentary, and more robust dentition, particularly U1 and I1, but subtle shape differences are also evident in the bipolar discriminant vector. *Sorex palustris* skulls have a relatively shorter unicuspid tooththrow, larger U1, and shorter rostrum compared with *S. navigator*. Dentaries of *S. palustris* differ in their higher coronoid process but relatively shorter dentary length.

An analysis of geographic variation among populations of *S. navigator* and *S. palustris* was beyond the scope and objective of this study. However, Alberta samples of *S. palustris* had larger skulls and dentaries than Manitoba samples. Specimens from Saskatchewan and western Ontario are required to test for a possible west–east cline of decreasing size in this species. The nine specimens of *S. navigator* from Vancouver Island used in our study are samples of the endemic insular subspecies *Sorex navigator brooksi* Anderson, 1934 that was described from a single specimen on the basis of pelage colour. The Vancouver Island specimens have a high prevalence of pale brown markings on their ventral fur, but they demonstrated no divergence in cranio-dental morphology from our mainland samples, consistent with their low genotypic divergence reported by Hope et al. (2014).

There are examples of genetic and morphological introgression in closely related shrew species (Brunet et al. 2002), but we found no evidence for morphological intergradation. Based on body measurements and pelage colour of specimens from Mount Assiniboine Provincial Park and Tornado Pass, Crowe (1943) suggested “a slight tendency” for intergradation among *S. navigator* and *S. palustris* in the southern Rocky Mountains of Canada. There are no specimens of known genotype from Mount Assiniboine Provincial Park or Tornado Pass. But, four individuals from nearby Yoho National Park that were genotyped from mtDNA as *S. navigator* by O’Neill et al. (2005) and Hope et al. (2014) were *S. navigator* in morphology with no evidence for intergradation with *S. palustris*. The four specimens with *S. palustris* mitochondrial and *S. navigator* nuclear lineages grouped with the *S. navigator* morphotype in medial time morphology, bivariate ratios of unicuspid teeth and dentary measures, and multivariate results from PCA and DFA morphometric analyses. Considering no morphological intergradation among these individuals, their discordant mitochondrial and nuclear genotypes likely reflects limited mitochondrial capture from *S. palustris* into *S. navigator* at some time in the past. The three “hybrid” locations in British Columbia (Twain Creek, Morice River, Cluculz Creek; locations 11, 12, 13 in Fig. 1, respectively; Supplementary Table 1<sup>1</sup>) are well within the range extent of *S. navigator* far west of the Rocky Mountains in the Interior Plateau of British Columbia, 400–550 km beyond possible contact with contemporary populations of *S. palustris* (Fig. 1). Although geographically distant from the other discordant individuals, the Okotoks specimen exhibited identical morphological and genetic designations, again suggesting mitochondrial capture. Okotoks (location 34 in Fig. 1) is east of the Rocky Mountains on the edge of the foothills (Matthews 1986), within the range extent of *S. navigator* but south of the distributional limits of *S. palustris* in Alberta and possible sympatry. Located 150 km north of Okotoks,

**Fig. 4.** Histograms of stepwise discriminant scores for (A) skulls and (B) dentaries of 35 *Sorex navigator* (western water shrew) and 32 *Sorex palustris* (American water shrew). Triangles denote group centroids. Discriminant vector for skulls based on three variables; discriminant vector for dentaries based on four variables.



the Ghostpine Creek and Red Deer River genotyped specimens used in this study are the southernmost confirmed occurrences of *S. palustris* in Alberta (Fig. 1). Given that all individuals with discordant mitochondrial and nuclear markers or morphology are located outside the potential contact zone, gene flow is likely no longer occurring in these areas and introgressed haplotypes reflect more ancient hybridization events (Toews and Brelsford 2012). However, the introgressed *S. palustris* *Cytb* haplotypes within *S. navigator* are only minimally divergent from other *S. palustris* haplotypes, suggesting that episodes of gene flow do not pre-date the last (Wisconsin) glacial phase. Additional range-wide specimen vouchers and genetic sampling, particularly from the area of parapatry, would help to characterize the extent and dynamics of both ancient and ongoing gene flow between *S. palustris* and *S. navigator*. Although our genetic results are based on putatively diagnostic loci, more specimens coupled with greater genomic

coverage would help to rule out incomplete lineage sorting as an explanation for the observed lineage designations.

The larger skull, relatively shorter and more robust dentary, and heavier dentition of *S. palustris* compared with *S. navigator* may reflect dietary differences and adaptations associated with feeding and mastication. The larger ratio of coronoid height/dentary length for the mandible of *S. palustris* suggests a stronger bite force (Vega et al. 2010). *Sorex palustris* has broader, more robust upper and lower incisors (I1, i1), which are the enlarged anterior teeth used for grasping and piercing prey. It also has larger medial tines on I1, although how differences in tine size among two species with I1s similar in medial profile would affect bite force is unclear (Carraway 1990). The limited dietary data suggest that *S. navigator* with its association with fast flowing streams feeds primarily on aquatic insect larvae (Conaway 1952), whereas *S. palustris*, which inhabits grass-sedge marsh or shrub thickets bordering ponds, creeks, and bogs (Wrigley et al. 1979), includes hard-bodied invertebrates such as carabid beetles in its prey (Beneski and Stinson 1987). Dietary studies from representative habitats across the range extent of both species are required to determine their dietary niches. We recommend a study of form that applies geometrical morphometric methodology (Rychlik et al. 2006; Vega et al. 2010) to evaluate bite force and the adaptive significance of morphometric differentiation of the two taxa.

#### Identification of *S. palustris* and *S. navigator*

Although genetically distinct, *S. navigator* and *S. palustris* are morphologically cryptic species distinguishable only by cranio-dental data. We found no external morphological traits that were diagnostic. *Sorex palustris* is larger in total length, body length, and body mass and has a shorter tail, but these measurements demonstrate substantial overlap among the two species. Moreover, linear measurements such as tail vertebrae length are subject to measuring error and body mass is affected by reproductive condition. In their subspecies descriptions, Jackson (1928) and Rand (1948) noted that the two taxa were similar in pelage except that the dorsal pelage of *S. navigator* was more grizzled with whitish-tipped hairs. However, pelage colour was not mentioned in the subspecies synopsis of van Zyll de Jong (1983b). Some individuals of both taxa have a grizzled dorsal pelage, but we found no species-specific differences in pelage colour or the degree of bi-colouring on the tail. We conclude that identification of live captures in the hand is not possible. Eight qualitative cranio-dental traits found to have taxonomic value in other shrew studies are not useful for identification because they are either invariant or their frequency of occurrence overlaps. The only nonmetric cranio-dental traits of diagnostic value are the relative size of the medial tines on I1 and the intensity and distribution of pigmentation on I1 (Figs. 3A, 3B). Pigmentation tends to be darker in *S. navigator*. Tine-size scores showed no overlap among the two taxa, but assigning a score to an individual is subjective and likely inconsistent among observers. Moreover, these traits are not discernible in individuals with heavily worn dentition. Therefore, reliable identification requires mensural data.

The only quantitative criteria given in the identification key of Jackson (1928) for discriminating *S. navigator* from *S. palustris* were condylobasal length and mastoid breadth, measurements of cranial dimensions. Neither measurement was used in our study, but we found that greatest length of the skull and width of cranium overlapped substantially. Our analyses demonstrate that *S. navigator* and *S. palustris* differ most in their cranial dentition (depth of I1, width of U1, unicuspid tooththrow length). Bivariate ratios or a discriminant function using only three cranio-dental measurements will reliably identify their skulls. Similar cranio-dental measurements, particularly the width of U1, were demonstrated by Nagorsen and Panter (2009) to be important for identifying skulls of the cryptic species *Sorex rohweri* Rausch, Feagin and Rausch, 2007 (Olympic shrew). Mandibular morphology was not described



in Jackson's (1928) subspecies synopses, but the dentaries are differentiated in relative length and height. A ratio of height of the coronoid process/dentary length or a discriminant function consisting of four dental measurements (height of the coronoid process, length of m1, depth of dentary, length of dentary) separate the species. Ruprecht (1971) found dentary length and height of the ramus effectively discriminated European species of Soricidae.

We recommend the following diagnostic traits for identification:

*Sorex navigator* — ratio of U1 width/distance across U1–U5 < 0.25; ratio of height of coronoid process/length of dentary < 0.53; discriminant score for skulls < 0.0 for  $12.628(\text{basal width of I1}) + 19.144(\text{width of U1}) - 4.050(\text{distance across U1–U5}) - 17.778$ ; discriminant score for dentaries < 0.0 for  $6.155(\text{height of coronoid process}) + 9.39(\text{length of m1}) + 4.144(\text{depth of dentary}) - 2.959(\text{length of dentary}) - 23.621$ ;

*Sorex palustris* — ratio of U1 width/distance across U1–U5 > 0.25; ratio of height of coronoid process/length of dentary > 0.53; discriminant score for skulls > 0.0 for  $12.628(\text{basal width of I1}) + 19.144(\text{width of U1}) - 4.050(\text{distance across U1–U5}) - 17.778$ ; discriminant score for dentaries > 0.0 for  $6.155(\text{height of coronoid process}) + 9.39(\text{length of m1}) + 4.144(\text{depth of dentary}) - 2.959(\text{length of dentary}) - 23.621$ .

A complete taxonomic summary of diagnostic traits for the three species of the *S. palustris* species complex awaits more genetic sampling across eastern North America. According to Hope et al. (2014), there are only three genotyped *S. albibarbis* with associated voucher specimens. Three eastern taxa originally treated as subspecies of *S. palustris* by Hall (1981), and classified as *Sorex palustris hydrobadistes* Jackson, 1926, *Sorex albibarbis punctulatus*, and *Sorex albibarbis turneri* by Hope et al. (2014) on the basis of geography, have yet to be genotyped. Moreover, because there are no individuals of known genotype from a vast region across Ontario and the Great Lakes states of the United States (Hope et al. 2014), the eastern limits of the geographic range of *S. palustris* and its extent of sympatry with *S. albibarbis* is unknown.

Unresolved issues in understanding the systematics of *S. navigator* and *S. palustris* in western North America are their historical biogeography, distributional limits, and extent of sympatry among contemporary populations in the Western Cordillera of Canada. Our morphometric criteria could be applied to address these issues. Himes and Kenagy (2010) and Hope et al. (2014) hypothesized that the two taxa expanded across their modern range from Pleistocene refugial areas located south of the continental ice in response to rapid habitat changes, but the number and location of refugial source areas where these species evolved is unclear. The genetic structure of *S. navigator* suggests derivation from a single refugium (Himes and Kenagy 2010; Hope et al. 2014). *Sorex palustris* may have evolved in multiple refugial areas in the Great Plains or eastern United States. According to the map in Himes and Kenagy (2010), there are Pleistocene fossil sites with water shrews from locations in the Rocky Mountains, southern Great Plains, and eastern regions of the United States, all identified as *S. palustris*. The identification of these water shrew fossils, however, pre-dates the recent taxonomic splitting of *S. palustris* and they should be re-examined to confirm identifications. Shrew dentaries, often well represented in fossils sites, are identifiable from various morphometric criteria (Zaitsev and Baryshnikov 2002). Intact dentaries of *S. navigator* and *S. palustris*, even those lacking dentition, can be discriminated by a simple ratio of coronoid height/dentary length. More genetic sampling from locations across the Western Cordillera and foothills in Canada is required to resolve the range limits of the two taxa and the extent of mitochondrial introgression. Nevertheless, there are existing voucher specimens lacking genotypic data from this region held in various museum collections that are a source for occurrence locations and preliminary range mapping. Our morphometric criteria provide an economical and simple alternative to DNA sequencing of biopsies taken

from study skins to confirm the identification of museum specimens.

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## Supplementary material

**Table S1.** Species of the genus *Sorex*, location data with map number referring to (Fig. 1), museum voucher specimen number, and genetic identification from mtDNA and ncDNA for the 80 specimens analyzed.

Map#	Species	State or province	Location	Coordinates (N latitude; E longitude)	Specimen	mtDNA*	nuDNA <sup>†</sup>
1	<i>S. bendirii</i>	BC	Sumas Mountain; Clayburn Creek	49.0833°, -122.2167°	RBCM 019434	bendirii	NA
1	<i>S. bendirii</i>	BC	Sumas Mountain; McKee Creek	49.0667°, -122.2833°	RBCM 019435	bendirii	NA
1	<i>S. bendirii</i>	BC	Sumas Mountain; McKee Creek	50.0606°, -123.2883°	RBCM 019436	bendirii	bendirii
1	<i>S. bendirii</i>	BC	Aldergrove; 2 km W	49.0839°, -122.5014°	RBCM 019822	NA	bendirii
2	<i>S. bendirii</i>	BC	Seymour River	49.3500°, -123.0000°	RBCM 019527	bendirii	NA
3	<i>S. bendirii</i>	BC	Hemlock Valley	49.3261°, -121.8611°	RBCM 020944	bendirii	bendirii
4	<i>S. bendirii</i>	BC	Delta; SFPR Line 80th Street	49.1350°, -123.0083°	RBCM 020945	bendirii	bendirii
4	<i>S. bendirii</i>	BC	Surrey; Hjorth Creek; Hwy 1 near 160th St	49.1911°, -122.7742°	RBCM 021087	NA	bendirii
5	<i>S. bendirii</i>	BC	Squamish; Lower Thunderbird Creek	49.7369°, -123.1103°	RBCM 020946	bendirii	bendirii
6	<i>S. navigator</i>	AK	Wrangell Island; Pat's Lake	56.3491°, -132.3383°	MSB 195374	navigator	navigator
7	<i>S. navigator</i>	AK	Yakutat Quad	59.5469°, -139.7272°	UAM 76526	navigator	navigator
8	<i>S. navigator</i>	YT	Fox Creek	61.1000°, -135.2934°	MSB 144181	navigator	navigator
9	<i>S. navigator</i>	YT	Klondike River; North Fork; Highway 5	64.0259°, -138.5789°	MSB 145600	navigator	navigator
10	<i>S. navigator</i>	BC	Bulkley River; Northern tributary Mo-37 Rip	55.2750°, -126.4617°	RBCM 020007	navigator	navigator

11	Hybrid	BC	Twain Creek; tributary ND-70 Rip	54.5433°, -125.9219°	RBCM 020008	palustris	navigator
11	<i>S. navigator</i>	BC	Twain Creek; tributary ND-69	54.5781°, -125.9542°	RBCM 020010	navigator	navigator
11	<i>S. navigator</i>	BC	Twain Creek; tributary ND-69	54.5781°, -125.9542°	RBCM 020011	navigator	navigator
12	<i>S. navigator</i>	BC	Morice River; Southern tributary Th-45	54.2072°, -127.1653°	RBCM 020012	navigator	NA
12	Hybrid	BC	Morice River; Southern tributary Th-45	54.2072°, -127.1653°	RBCM 020017	palustris	navigator
13	<i>S. navigator</i>	BC	Cluculz Creek; MT #4 Reach 615	53.7850°, -123.6931°	RBCM 020013	navigator	NA
13	Hybrid	BC	Cluculz Creek; MT #4 Reach 615	53.7850°, -123.6931°	RBCM 020016	palustris	navigator
13	<i>S. navigator</i>	BC	Cluculz Creek; Reach #618	53.7756°, -123.6939°	RBCM 020015	navigator	NA
14	<i>S. navigator</i>	BC	Yoho National Park; Yoho River Valley	51.4833°, -116.4667°	RBCM 019354	navigator	NA
14	<i>S. navigator</i>	BC	Yoho National Park; Natural Bridge	51.3833°, -116.5333°	RBCM 019388	navigator	NA
14	<i>S. navigator</i>	BC	Yoho National Park; Leanchoil	51.2167°, -116.5833°	RBCM 019400	navigator	NA
15	<i>S. navigator</i>	BC	Trojan Creek; tributary of Witches Brook	50.5522°, -120.9903°	RBCM 020251	navigator	navigator
15	<i>S. navigator</i>	BC	Axe Creek; near Logan Lake	50.5542°, -120.8675°	RBCM 020252	navigator	navigator
16	<i>S. navigator</i>	BC	Callahan Creek; tributary	50.1114°, -123.1114°	RBCM 020315	navigator	navigator
17	<i>S. navigator</i>	BC	Cayoosh Creek; Site 3	50.4986°, -122.2978°	RBCM 019956	navigator	navigator
17	<i>S. navigator</i>	BC	Cayoosh Creek; Site 3	50.4986°, -122.2978°	RBCM 019957	navigator	navigator
18	<i>S. navigator</i>	BC	Pinecone Burke Provincial Park	49.4333°, -122.6667°	RBCM 019823	navigator	NA
18	<i>S. navigator</i>	BC	Pinecone Burke Provincial Park	49.4333°, -122.6667°	RBCM 019824	navigator	NA
19	<i>S. navigator</i>	BC	Vancouver Island; Black Creek	49.8703°, -125.2569°	RBCM 019723	navigator	navigator
20	<i>S. navigator</i>	BC	Vancouver Island; Black Brook	49.3956°, -124.6344°	RBCM 019730	navigator	navigator

21	<i>S. navigator</i>	BC	Vancouver Island; Morrison Creek	49.2692°, -124.3544°	RBCM 019731	navigator	navigator
22	<i>S. navigator</i>	BC	Vancouver Island; Veitch Creek	48.4333°, -123.6022°	RBCM 019734	navigator	navigator
22	<i>S. navigator</i>	BC	Vancouver Island; Niagara Creek	48.4939°, -123.5706°	RBCM 019735	navigator	navigator
22	<i>S. navigator</i>	BC	Vancouver Island; Rithet Creek	48.5850°, -123.7231°	RBCM 019737	navigator	navigator
23	<i>S. navigator</i>	BC	Vancouver Island; Lower Lost Shoe Creek	49.0117°, -125.5894°	RBCM 019804	navigator	navigator
24	<i>S. navigator</i>	BC	Vancouver Island; Miller Creek	50.0025°, -125.5667°	RBCM 019865	navigator	navigator
25	<i>S. navigator</i>	BC	Vancouver Island; Port Alberni	49.2417°, -124.8000°	RBCM 019936	navigator	NA
26	<i>S. navigator</i>	MT	Hyalite Creek; 9 mi. S of Bozeman	45.5541°, -111.0396°	MSB 56695	navigator	navigator
26	<i>S. navigator</i>	MT	Hyalite Creek; 9 mi. S of Bozeman	45.5541°, -111.0396°	MSB 56696	navigator	navigator
27	<i>S. navigator</i>	WY	Sunlight Creek; 23 mi. NW of Cody	44.6754°, -109.5404°	MSB 123590	navigator	navigator
27	<i>S. navigator</i>	WY	Beartooth Highway	44.9334°, -109.6656°	MSB 123338	navigator	navigator
28	<i>S. navigator</i>	NV	Bull Run Mountains; Rocky Gultch	41.7773°, -115.9872°	MSB 225575	navigator	navigator
29	<i>S. navigator</i>	UT	Zion National Park; Lava Pt Ranger station	37.3834°, -113.0152°	MSB 122504	navigator	navigator
30	<i>S. palustris</i>	AB	Lake SCL20; S of Calling Lake	55.1958°, -113.6583°	RBCM 019829	palustris	palustris
30	<i>S. palustris</i>	AB	Lake SCL20; S of Calling Lake	55.1958°, -113.6583°	RBCM 019830	palustris	palustris
30	<i>S. palustris</i>	AB	Lake SCL20; S of Calling Lake	55.1958°, -113.6583°	RBCM 019831	palustris	palustris
30	<i>S. palustris</i>	AB	Lake SCL20; S of Calling Lake	55.1958°, -113.6583°	RBCM 019832	palustris	palustris
30	<i>S. palustris</i>	AB	Lake SCL20; S of Calling Lake	55.1958°, -113.6583°	RBCM 019833	palustris	palustris
30	<i>S. palustris</i>	AB	Lake SCL20; S of Calling Lake	55.1958°, -113.6583°	RBCM 019837	palustris	NA
31	<i>S. palustris</i>	AB	Lac la Biche; Lake LLB 100 Grid 4	55.1333°, -111.6611°	RBCM 019838	palustris	palustris

31	<i>S. palustris</i>	AB	Lac la Biche; Lake LLB 100 Grid 4	55.1333°, -111.6611°	RBCM 019834	palustris	palustris
31	<i>S. palustris</i>	AB	Lac la Biche; Lake LLB 100 Grid 4	55.1333°, -111.6611°	RBCM 019835	palustris	palustris
31	<i>S. palustris</i>	AB	Lac la Biche; Lake LLB 100 Grid 4	55.1333°, -111.6611°	RBCM 019836	palustris	palustris
32	<i>S. palustris</i>	AB	Ghostpine Creek; S of Pine Lake	52.0319°, -113.3889°	RBCM 019839	palustris	palustris
32	<i>S. palustris</i>	AB	Ghostpine Creek; S of Pine Lake	52.0319°, -113.3889°	RBCM 019840	palustris	palustris
33	<i>S. palustris</i>	AB	Red Deer River; 8km NW of Innisfail	52.0625°, -114.0525°	RBCM 019841	palustris	palustris
34	Hybrid	AB	Okotoks; Bob Waller's Property	50.7255°, -113.9749°	ROM 109832	palustris	navigator <sup>‡</sup>
35	<i>S. palustris</i>	ON	Ingolf	49.7936°, -95.1219°	TMM 1717	NA	NA
35	<i>S. palustris</i>	MB	Whiteshell Prov Park; McGillvary Falls	49.8095°, -95.2379°	TMM 1718	NA	NA
35	<i>S. palustris</i>	MB	Whiteshell Prov Park; McGillvary Falls	49.8095°, -95.2379°	TMM 1719	NA	NA
35	<i>S. palustris</i>	MB	Whiteshell Prov Park; Caddy Lake S end	49.7946°, -95.2038°	TMM 2949	NA	NA
36	<i>S. palustris</i>	MB	Kame Hills; Lynn Lake 68.8 k N 152 km E of	56.8500°, -101.0500°	TMM 5652	NA	NA
36	<i>S. palustris</i>	MB	Kame Hills; Lynn Lake 68.8 k N 152 km E of	56.8500°, -101.0500°	TMM 5653	NA	NA
36	<i>S. palustris</i>	MB	Kame Hills; Lynn Lake 68.8 km N 148.8 km E of	56.8500°, -101.0500°	TMM 5655	NA	NA
36	<i>S. palustris</i>	MB	Kame Hills; Lynn Lake 68.8 km N 148.8 km E of	56.8500°, -101.0500°	TMM 5656	NA	NA
37	<i>S. palustris</i>	MB	Cranberry Portage; 6.4 km S 6.4 km E of	54.5284°, -101.2770°	TMM 5659	NA	NA
37	<i>S. palustris</i>	MB	Cranberry Portage; 7.2 km S 7.2 km W of	54.5199°, -101.4873°	TMM 5661	NA	NA
37	<i>S. palustris</i>	MB	Cranberry Portage; 7.2 km S 7.2 km W of	54.5199°, -101.4873°	TMM 5662	NA	NA
37	<i>S. palustris</i>	MB	Cranberry Portage; 7.2 km S 7.2 km W of	54.5199°, -101.4873°	TMM 5664	NA	NA
37	<i>S. palustris</i>	MB	Cranberry Portage; 7.2 km S 4.8 km W of	54.5199°, -101.4505°	TMM 5667	NA	NA

37	<i>S. palustris</i>	MB	Cranberry Portage; 7.2 km S 4.8 km W of	54.5199°, -101.4505°	TMM 5668	NA	NA
38	<i>S. palustris</i>	MB	Thompson; 19.2 km S 8 km W of	55.5626°, -97.9811°	TMM 6564	NA	NA
38	<i>S. palustris</i>	MB	Thompson; 19.2 km S 8 km W of	55.5626°, -97.9811°	TMM 6565	NA	NA
38	<i>S. palustris</i>	MB	Thompson; 19.2 km S 8 km W of	55.5626°, -97.9811°	TMM 6574	NA	NA
38	<i>S. palustris</i>	MB	Thompson; 19.2 km S 8 km W of	55.5626°, -97.9811°	TMM 6576	NA	NA
38	<i>S. palustris</i>	MB	Thompson; 20.8 km S 11.2 km W of	55.5471°, -98.0284°	TMM 6566	NA	NA

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**Note:** Museum acronyms are MSB, Museum of Southwestern Biology, Albuquerque, New Mexico; RBCM, Royal BC Museum, Victoria, British Columbia; ROM, Royal Ontario Museum, Toronto, Ontario; TMM, The Manitoba Museum, Winnipeg, Manitoba; UAM, University of Alaska Museum of the North, Fairbanks, Alaska. GenBank accession numbers for specimens with genetic data are listed in Hope et al. (2014). NA indicates no genetic data available. Hybrid samples had *S. palustris* mtDNA, *S. navigator* ncDNA, *S. navigator* morphology.

\**Cytb* gene.

†Apolipoprotein B gene.

‡GenBank accession number for Apolipoprotein B gene (sequenced in this study) is KY922995.

## References

Hope, A.G., Panter, N., Cook, J.A., Talbot, S.L., and Nagorsen, D.W. 2014. Multilocus phylogeography and systematic revision of North American water shrews (genus: *Sorex*). *J. Mammal.* **95**(4): 722–738. doi:10.1644/13-MAMM-A-196.

**Fig. S1.** Skull measurements. (A) Dorsal view.



1. Greatest length of skull
2. Width of cranium
3. Interorbital width

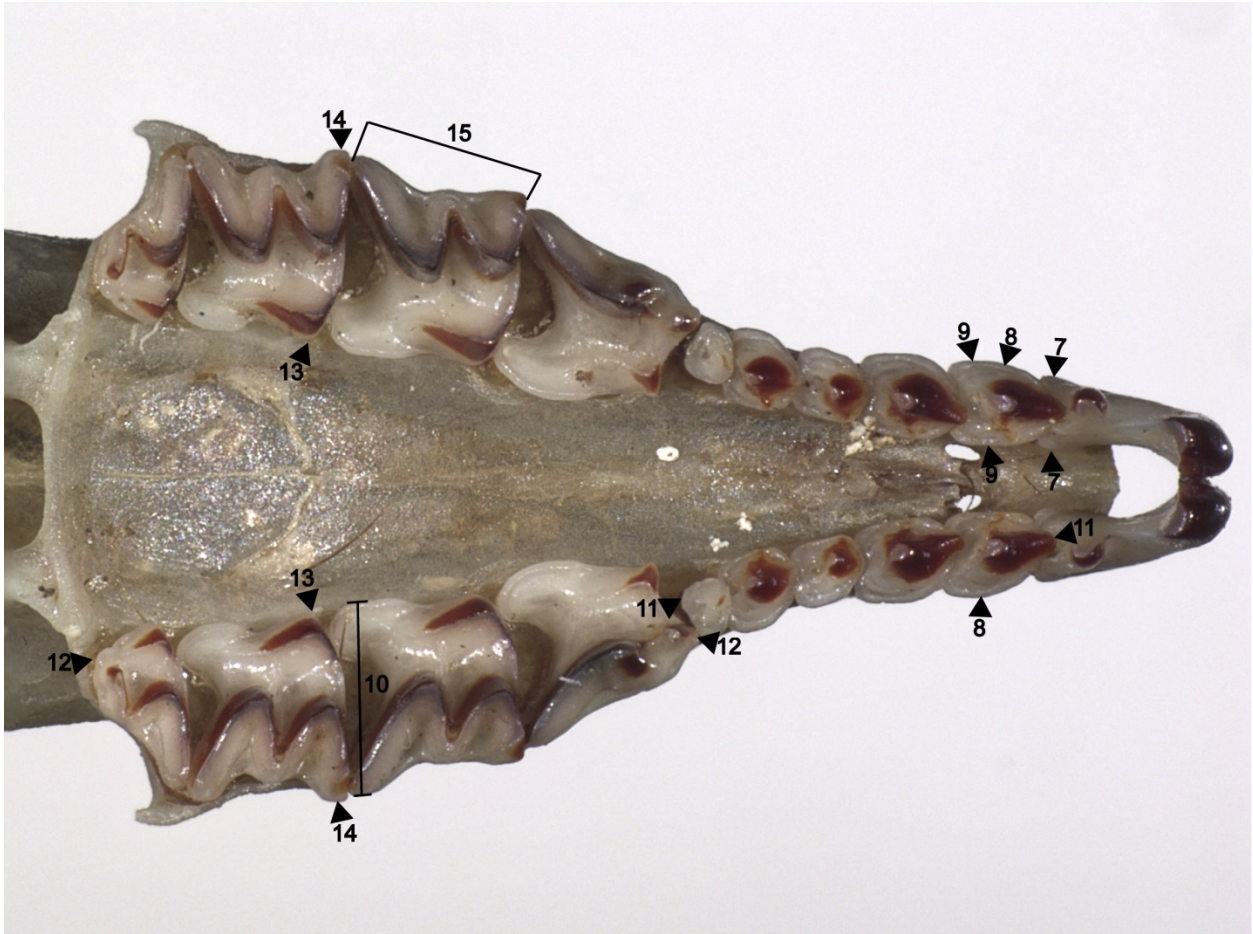


**Fig. S1.** Skull measurements. (B) Lateral view.



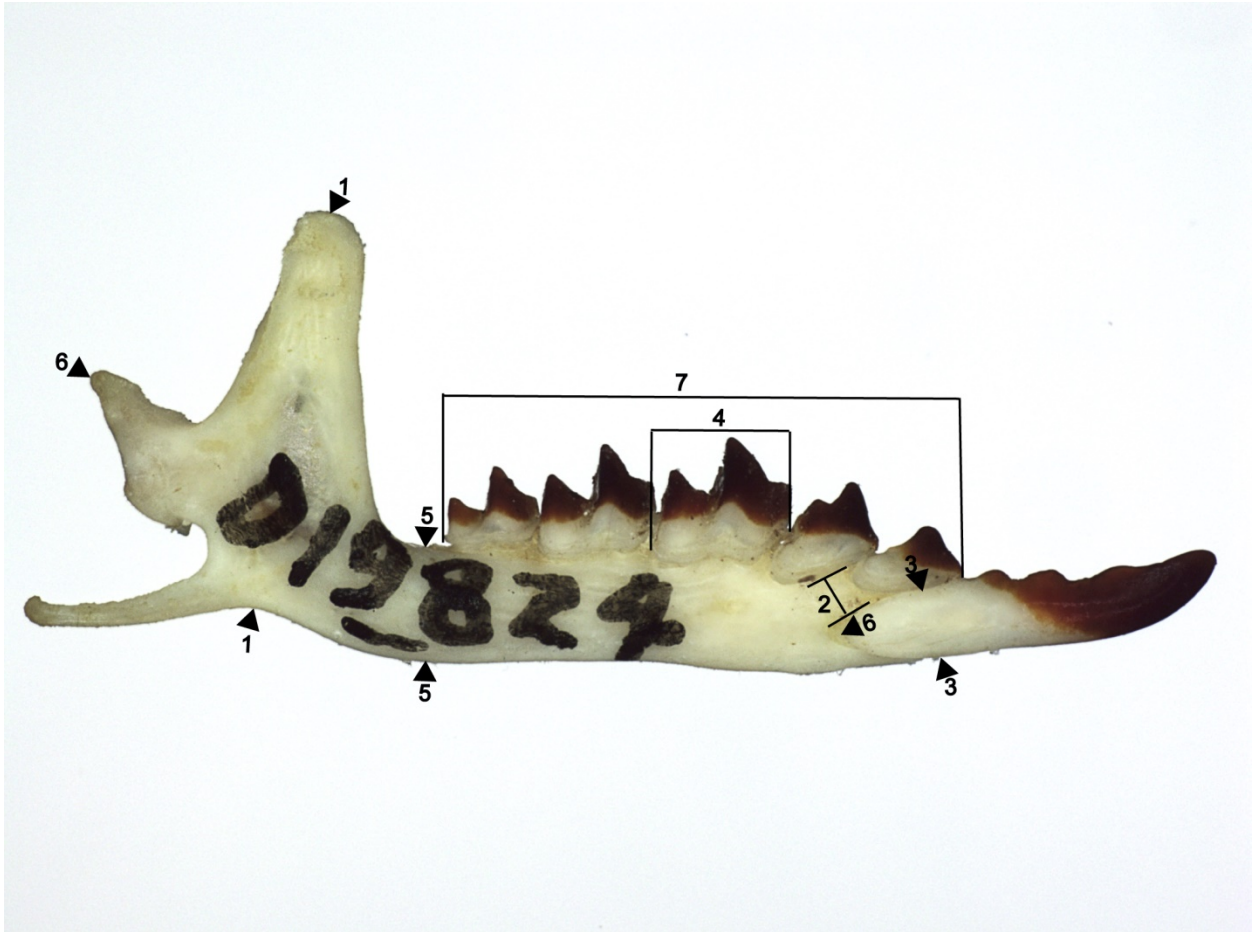
4. Depth of I1
5. Length of U1
6. Angle of rostrum

**Fig. S1.** Skull measurements. (C) Ventral view.



- 7. Basal width of I
- 8. Width across U1s
- 9. Width of U1
- 10. Width of M1
- 11. Distance across U1-U5
- 12. Distance across P4-M3
- 13. Width between M2s
- 14. Width across M2s
- 15. Length of M1

**Fig. S2.** Dentary measurements.



1. Height of coronoid process
2. Distance between i1-p4
3. Width of i1
4. Length of m1
5. Depth of dentary
6. Length of dentary
7. Length of dentary tooththrow

**Fig. S3.** Bayesian genealogies for 778 bp of the mtDNA cytochrome *b* gene and 434 bp of the apolipoprotein B gene, both presumed to be diagnostic loci. Arrows indicate putative hybrid individuals that exhibit different species designation by locus. Values at nodes indicate posterior probability support for topologies. AB, Alberta; BC, British Columbia.

