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Genetic Analysis Leads to Range Extension of the Olympic Shrew (*Sorex rohweri*) to the Eastern Slopes of the Cascade Range in Washington State

Jordan D. Ryckman[1,2] *and Kristina A. Ernest*[1,3]

[1]*Department of Biological Sciences, Central Washington University, 400 East University Way, Ellensburg, Washington 98926-7537*
[2]*Current affiliation: Conservation Northwest, 1829 10th Avenue W, Suite B, Seattle, Washington 98119*
[3]*Corresponding author, e-mail: ErnestK@cwu.edu*

***Abstract.*—The documented geographic range of the Olympic Shrew (*Sorex rohweri*) is western Washington, Oregon, and British Columbia; however, during a study on shrews in central Washington in summer 2019, we genetically verified the presence of this species on the eastern slopes of the Cascade Range. Of the 127 terrestrial shrews captured, mtDNA analysis of cytochrome *b* gene sequences identified 41 of them as *S. rohweri*. Most of these live-trapped individuals were misidentified in the field as *S. cinereus* but others were field-identified as Trowbridge's Shrew (*S. trowbridgii*), Montane Shrew (*S. monticolus*), or Vagrant Shrew (*S. vagrans*). This discovery extends the known geographic range of *S. rohweri* to the drier eastern slopes of the Cascade Range in Washington and highlights the importance of collecting genetic samples for field studies of live animals, especially for taxa that are challenging to distinguish in the field.**

Key Words.—distribution; Eulipotyphla; insectivores; mtDNA; populations; small mammals; Soricidae.

Introduction

Multiple shrew species in the genus *Sorex* often live sympatrically, occupying the same geographic range (Churchfield 1990). Shrews are notoriously difficult to identify in the field during live-trapping studies. Reliable identification to species often requires skull and dental measurements (Rausch et al. 2007; Nagorsen and Panter 2009; Woodman and Fischer 2016). This may be suitable for research on voucher specimens in mammal collections but is not feasible for live-trapping methods that aim to reduce mortality during ecological studies. One of the most accurate ways to identify shrews is through genetic analysis (Rausch et al. 2007). As part of a larger study to determine the habitat preferences and population genetic structure of six sympatric shrew species in central Washington, we sequenced mitochondrial DNA from the cytochrome *b* gene (Dubey et al. 2007; O'Neill et al. 2005; Hope et al. 2012). Here, we report the genetic identification results from these shrew populations, and the discovery of the Olympic Shrew (*Sorex rohweri*) on the east slopes of the Cascade Range in Washington state (Fig. 1).

Methods

Study site.—Our study area was located within the Okanogan-Wenatchee National Forest in central Washington on the east slopes of the Cascade Range between the southern end of Keechelus Lake and west of Easton. This area of mixed-coniferous forests contains many habitat types including wetlands, talus slopes, and old-growth forests (Washington State Department of Transportation [WSDOT] and U.S. Department of Transportation Federal Highway Administration [USDOT FHWA] 2006). For our study on shrews, we selected three sites (Fig. 2) that straddled Interstate-90 where wildlife crossing structures will be built in the future as part of a larger ecosystem connectivity and highway widening project (WSDOT and USDOT FHWA 2006). Each site encompassed secondary or mature forest through which a stream flowed from north of the highway and through a culvert to the south. Elevations ranged from 732 to 842 m (Table 1). Habitat surveys determined that these sites were dominated by Western Hemlock (*Tsuga heterophylla*), Western Red Cedar (*Thuja plicata*), and Douglas-fir (*Pseudotsuga menziesii*). The forest floor was complex, with abundant leaf litter, woody debris, and nurse logs (fallen trees that foster new vegetative growth). Understory vegetation was dominated by Vine Maple (*Acer circinatum*), Oregon Grape (*Mahonia aquifolium*), and Vanilla Leaf (*Achlys triphylla*). Skunk Cabbage (*Symplocarpus foetidus*) and Devil's Club (*Oplopanax horridus*) were commonly found within or near the streams (Ryckman 2020).

Field methods.—At each site, we placed a trapping transect and a pitfall trapping array 10–15 m away in each of three habitats, both north and south of the highway, for a total of 18 transects and 18 pitfall arrays (three sites × three habitat types × two sides of highway). Streamside habitats were adjacent to a seasonal stream; lowland habitats were relatively flat, forested areas at least 50 m from the stream channel; and upland habitats were in drier forest upslope from the stream. Each transect consisted of 20 Sherman live-traps spaced at 5-m

Figure 1. Field photographs of genetically verified Olympic Shrews (*Sorex rohweri*) captured near Easton, Washington, USA. (Left) Individual originally identified as Montane Shrew (*Sorex monticolus*). (Right) Individual originally identified as Vagrant Shrew (*Sorex vagrans*). (Photographed by Jordan Ryckman).

intervals. Each pitfall array consisted of four 19-L plastic buckets inserted into the ground, level with the surface, and connected by 30-cm-tall metal drift fencing. We also placed up to three aquatic funnel (minnow) traps partially submerged in shallow water in each streamside habitat. We fitted these 60-cm-long wire mesh traps with a cork platform to allow shrews to rest out of the water while trapped. We provided insulation and food (mealworms) in all traps to help sustain shrews overnight. We opened traps for two consecutive nights from dusk until dawn (8–12 h) during two different trapping sessions during summer 2019 (1,772 total trap-nights).

We identified live-captured individuals using a dichotomous key that we derived from multiple sources (Nagorsen 1996; Verts and Carraway 1998; http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.214.2565&rep=rep1&type=pdf) and adapted for species expected in this region. We recorded weight, standard external body measurements (body length, tail length, and hindfoot length), sex, age, and reproductive status. We evaluated body size, pelage color, dentition (observed with a hand lens), and hind feet (fringes and toepads) to identify species. To provide a genetic tissue sample of each individual, we clipped the distal 1–2 mm of the tail. We dipped the end of the tail in coagulant powder (as recommended by a veterinarian) to limit bleeding, then we released the animal at the site of capture. We directly placed each tissue sample into a 0.5-ml microcentrifuge tube filled with 95% non-denatured ethanol and immediately placed samples on ice. We quickly identified recaptured individuals (confirmed by nail polish applied to toes or a clipped tail) to species and then released them. Any shrews that died we collected as whole specimens. We identified all animals other than shrews and released them immediately. For later analysis, we kept tail tissue samples and specimens on ice for 1–2 d in the field and

Table 1. Locations of trapping pitfall arrays and number of Olympic Shrews (*Sorex rohweri*) captured in each habitat at each site in Washington state. Abbreviations are Lat. = latitude, Long. = longitude, Elev. = elevation (m) at the center of the array in each habitat type within each site (also see Fig. 2), and MP = milepost along Interstate-90.

Site	Habitat	North of I-90			South of I-90			Total # *S. rohweri*
		Lat.	Long.	Elev.	Lat.	Long.	Elev.	
Bonnie Creek	Streamside	47.314612	-121.3146	744	47.31280	-121.3164	732	11
	Lowland	47.316041	-121.3157	744	47.31444	-121.3168	740	6
	Upland	47.317373	-121.3176	762	47.31543	-121.3181	740	6
Houle Creek	Streamside	47.300615	-121.2917	741	47.29931	-121.2946	741	10
	Lowland	47.299951	-121.2906	742	47.29856	-121.2947	745	6
	Upland	47.301154	-121.2940	747	47.29847	-121.2938	754	2
MP 67.1 Creek	Streamside	47.269372	-121.2491	815	47.26702	-121.2470	778	0
	Lowland	47.269266	-121.2507	821	47.26754	-121.2433	797	0
	Upland	47.268144	-121.2533	812	47.26705	-121.2412	842	0

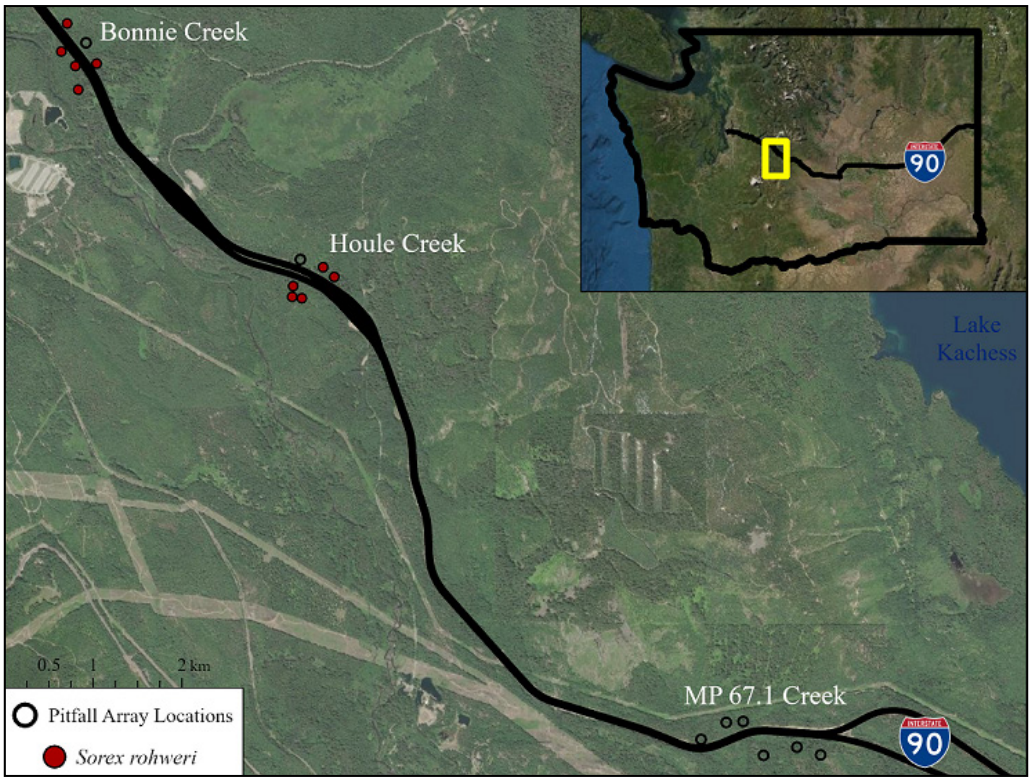

Figure 2. Locations of trapping pitfall arrays (circles) near I-90 in the central Cascades east of Snoqualmie Pass, Washington. Live-trapping took place in and around Bonnie Creek, Houle Creek, and MP 67.1 Creek. These creeks all ran through culverts under I-90. Circles filled in red indicate pitfall arrays where Olympic Shrews (*Sorex rohweri*) were captured.

then placed them in a -20° C freezer. We prepared whole specimens as museum vouchers (dried skins and skulls), and we retained liver samples for DNA extraction.

Genetic analysis.—We sent genetic samples from all individuals of terrestrial species to CD Genomics (New York, New York) for DNA extraction, amplification, and sequencing (the Aquatic Marsh Shrew, *S. bendirii*, and Western Water Shrew, *S. navigator,* were easily identifiable from the other species, so were excluded from genetic analysis). Proteinase K and zirconia beads were added to each sample and vortexed with Qiagen Tissue Lyser II. The tissue was then incubated at 55° C for no less than 3 h. Genomic DNA was extracted from the tissue lysate using the magnetic beads extraction method. The mitochondrial cytochrome *b* gene was amplified using the primers L14723 and H15915 (Nicolas et al. 2012). Cycling conditions were 96° C for 10 min, followed by 35 cycles of 95° C for 30 sec, 50° C for 30 sec, then 72° C for 10 min. Samples were then stored at 4° C. PCR products were purified with the PCR purification kit. The Sanger Sequencing method was used to sequence the mitochondrial cytochrome *b* gene (1140 bp). Two Sanger sequences were performed with both PCR primers and Bigdye 3.1 and run on an ABI 3730XI sequencer. The forward and reverse sequences from the same sample were assembled using the CodonCode Aligner, then consensus sequences were reported to us. Of the 128 samples, 127 were successfully sequenced using this method.

We conducted phylogenetic and molecular evolutionary analyses using MEGA version X (Kumar et al. 2018). Sequences were aligned using ClustalW in MEGA with a Northern Short-tailed Shrew (*Blarina brevicauda*) sequence (sample AB175134.1 from GenBank) as the outgroup. Of the 1140 base pairs in the mitochondrial cytochrome *b* gene, 1,085 base pairs were preserved in the alignment.

We compared all samples to known samples in GenBank using BLAST (Basic Local Alignment Search Tool) and set a species identification criterion as > 99% identical to the sequences of that species. One third of the samples (41) were most closely related (> 99%) to *S. rohweri*. Because this was unexpected based on previous range maps, we performed a maximum likelihood phylogenetic analysis with 100 bootstraps in MEGA to estimate the phylogenetic tree that included individuals identified as *S. rohweri* from this study plus two GenBank samples each of *S. rohweri (*GenBank samples EU088302 and EU088303.1), Trowbridge's Shrew (*S. trowbridgii*; GenBank samples FJ667520.1 and AY014956.1), Montane Shrew (*S. monticolus*; GenBank samples AB100273.1 and AB100272.1), Vagrant Shrew (*S. vagrans*; GenBank samples MK691376.1 and MK691381.1), and Masked Shrew (*S. cinereus*; GenBank samples AY014951.1 and AY014952.1). The tree was rooted by the *Blarina brevicauda* sequence (Fig. 3).

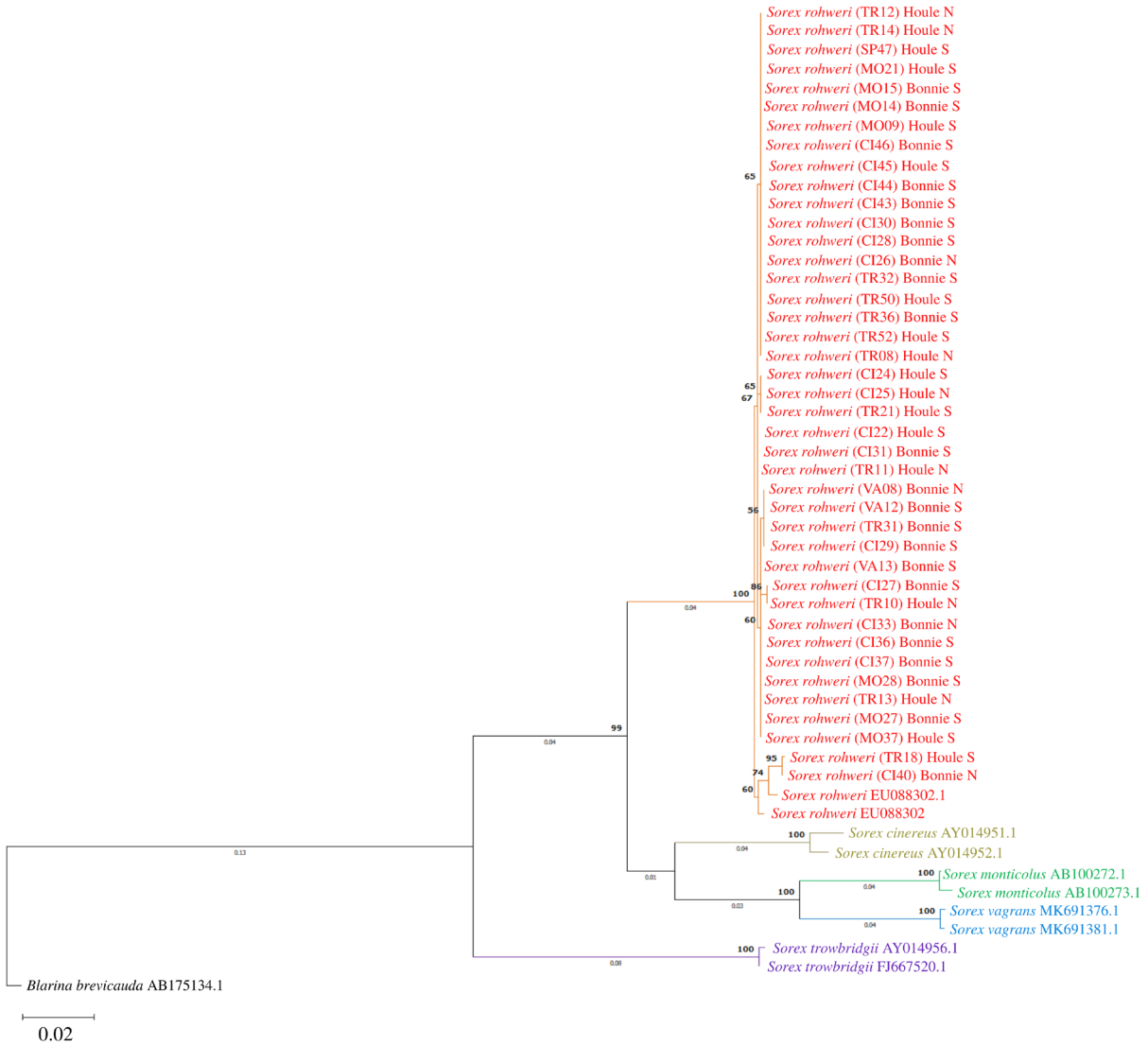

Figure 3. Maximum likelihood phylogenetic tree from 41 Olympic Shrew (*S. rohweri*) samples collected near Easton, Washington, in 2019. A Northern Short-tailed Shrew (*Blarina brevicauda*) sample from GenBank was included to root the tree, and two samples each of Trowbridge's Shrew (*Sorex trowbridgii*), Montane Shrew (*Sorex monticolus*), Olympic Shrew (*Sorex rohweri*), Vagrant Shrew (*Sorex vagrans*), and Masked Shrew (*Sorex cinereus*) from GenBank were included to verify species identifications. The percentage of trees in which the associated taxa clustered together is shown in bold text next to the branches. The tree is color-coordinated by species and drawn to scale, with branch lengths measured in number of substitutions per site (Tamura and Nei 1993; Kumar et al. 2018).

Results

The phylogenetic tree confirmed 41 *S. rohweri.* No shrews were confirmed as *S. cinereus*, and 68% of the individuals identified in the field as *S. cinereus* (n = 17) were genetically identified as *S. rohweri* (the other eight were identified as *S. monticolus* or *S. vagrans*). The remaining individuals confirmed as *S. rohweri* were originally identified in the field as *S. trowbridgii*, *S. monticolus*, *S. vagrans*, or *Sorex* sp. (Table 2). We captured most of the *S. rohweri* individuals in pitfall buckets (n = 37); only four were captured in Sherman traps. We only caught S. *rohweri* at the Bonnie Creek (n = 23) and Houle Creek (n = 18) sites (Fig. 2); none of the 14 shrews captured at the MP 67.1 Creek site, just 4.5 km east of Houle Creek, was genetically identified as *S. rohweri* (all shrews at that site were either *S. trowbridgii* or *S. vagrans*). The capture site farthest east was near Houle Creek (47.298561, -121.293831) at an elevation of about 730 m.

Discussion

Sorex rohweri was first discovered and described by Rausch et al. in 2007 through museum specimens from western Washington and British Columbia that were originally identified as *S. cinereus* or *S. vagrans*. The geographic range of the species was later extended northward into British Columbia (Nagorsen and Panter 2009) and southward into western Oregon (Woodman and

Table 2. Species identifications of 127 *Sorex* shrews captured near Easton, Washington, showing the number of individuals (n) identified to each species in the field and their confirmed genetic identifications. Numbers in bold across the diagonal represent individuals with correct field identifications.

		Genetic Identification				
Field Identification	n	*S. cinereus*	*S. trowbridgii*	*S. monticolus*	*S. vagrans*	*S. rohweri*
Masked Shrew (*S. cinereus*)	25	**0**	0	5	3	17
Trowbridge's Shrew (*S. trowbridgii*)	53	0	**32**	8	0	13
Montane Shrew (*S. monticolus*)	30	0	0	**19**	4	7
Vagrant Shrew (*S. vagrans*)	14	0	0	8	**3**	3
Unidentified (*Sorex* sp.)	5	0	0	3	1	1
Total	127	0	32	43	11	41

Fischer 2016), encompassing areas from the Pacific coast to inland sites around the crest of the Cascade Range in British Columbia, Washington, and Oregon. Most records are from the Coastal Range and western slopes of the Cascade Range in Oregon and Washington, the Olympic Peninsula of Washington, and the Fraser River Basin of southwestern British Columbia (Rausch et al. 2007; Nagorsen and Panter 2009; Woodman and Fisher 2016; Woodman 2018). All Washington records of *S. rohweri* in the University of Washington Burke Museum (207 specimens), U.S. National Museum of Natural History (USNM; 26 specimens), and iDigBio databases came from counties west of the Cascade Range crest: Clallam, Grays Harbor, Jefferson, Kitsap, Lewis, Pacific, Pierce, and Skamania (Burke Museum. 2021. Mammalogy Collection Database. Available from https://www.burkemuseum.org/collections. [Accessed 4 November 2021]; U.S. National Museum. 2021. Division of Mammals Collections. Available from https://collections.nmnh.si.edu/search/mammals/. [Accessed 4 November 2021]; iDigBio. 2021. Integrated Digital Biocollections Portal. Available from https://www.idigbio.org/portal. [Accessed 4 November 2021]). The furthest east longitude of those specimens was -121.5262 (latitude 46.9879) in Pierce County, north of Mt. Rainier, Washington.

Our captures are the first documentation of *S. rohweri* on the east slopes of the Cascade Range in Washington State. All of our sites were within Kittitas County (which extends from the crest of the Cascade Range eastward) and slightly further east (-121.293831) than all previous records (Fig. 4). The elevational limits of *S. rohweri* vary regionally but range from sea level to at least 1,585 m (recently documented in Whatcom County, Washington, 58 km east of the town of Glacier; Woodman and Fisher 2016). Our captures occurred at the midrange (732–762 m) of previously reported elevations. Due to the rain shadow effect, the eastern slopes of the Cascade Range experience increasingly warmer and drier conditions compared with the leeward western slopes. This spatially shifting climate results in a gradual change of forest habitats. Several species of small mammals that have most of their geographic distribution in western Washington extend over the Cascade crest onto the upper eastern slopes of the Cascades and eventually drop out as one continues eastward and downward in elevation; included among them are the shrews *S. bendirii* and *S. trowbridgii*. *Sorex rohweri* appears to follow this geographic pattern.

Some of our identification errors were due to the unexpected occurrence of *S. rohweri* in the study area, as it was not included in our dichotomous key. Other

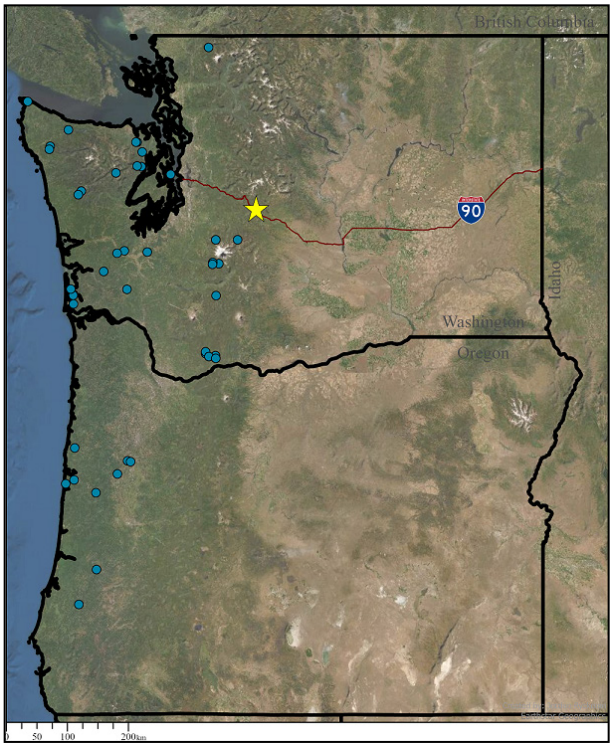

Figure 4. Location of our new Olympic Shrew (*Sorex rohweri*) records (yellow star) and locations of previously documented sites (blue circles) from database records of the University of Washington Burke Museum and the National Museum of Natural History.

errors, however, were most likely caused by the difficulty of scoring small characteristics (e.g., teeth and toepads) on live shrews. Even with a key including all possible species in the Cascade Range of Washington, several species are not reliably keyed out by morphology, especially on live animals. Individual *S. rohweri, S. vagrans,* and *S. cinereus* shrews, especially live ones, cannot be reliably distinguished due to overlapping measurements (Nagorsen and Panter 2009; Woodman and Fisher 2016). Despite an estimated 850,000-y divide (coalescence time, using cytochrome b) between *S. rohweri* and its sister group, the *S. cinereus* complex, these taxa remain morphologically similar (Hope et al. 2012). Genetic analysis proved to be crucial for the accuracy of this study, not only for *S. rohweri* but also for the other shrew species. We highly recommend its use for any field studies on live shrews.

Documentation of the longitudinal and upper elevational limits of a species is important for understanding future impacts of climate change. Future work could include more extensive sampling in this location after wildlife crossing structures are built and in other locations along the eastern slopes of the Cascade Range. We also recommend a review and genetic analysis of museum specimens from the area. Genetic expansion statistics from all samples of S. *rohweri* in Washington, Oregon, and British Columbia may help infer the source and timing of any past range extension.

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Jordan D. Ryckman is a Biologist who spent her undergraduate and graduate career studying small mammals as part of the I-90 Snoqualmie Pass East Project. She has grown a passion for wildlife and conservation and is intrigued by human activities that impact wildlife such as roads and development. After graduation, she worked on a restoration crew in central Washington, and now works with Conservation Northwest, a non-profit that works to protect, connect, and restore wildlife habitat. (Photographed by Kris Ernest).

Kris Ernest is Professor of Biological Sciences at Central Washington University, Ellensburg. She has taught Wildlife & Fisheries Ecology, Mammalogy, General Ecology, Biomes of the Pacific Northwest, and Field Techniques. Her research spans population and community ecology, with a current focus on road ecology and connectivity. Since 2008, she and her students have been investigating how American pikas (*Ochotona princeps*), shrews, and other small mammal species respond to highways and wildlife crossing structures for the I-90 Snoqualmie Pass East Project. (Photographed by Anjanette Wilson).