Western North American Naturalist 81(4), © 2021, pp. 608–614

Molecular identification of *Sceloporus* lizards in the Laramie Mountains, Wyoming

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ABSTRACT.—Accurate information on species identities and distributions is critical for informing state land use and conservation policies. However, it can often be difficult to determine species identity using morphological data alone. Using phylogenetic methods, we determined the identity of *Sceloporus* lizards occupying the Laramie Mountains of Wyoming, between known ranges for *Sceloporus tristichus* and *Sceloporus consobrinus*. The *ND1* mitochondrial gene was sequenced for 10 individuals from the Laramie Mountains and analyzed using maximum likelihood with 23 other samples of *S. tristichus* and *S. consobrinus* from throughout their ranges. The mtDNA gene tree places the Laramie Mountains populations within a clade of *Sceloporus consobrinus* that includes the Rocky Mountains in Colorado. Given the prevalence of mtDNA introgression in *Sceloporus*, we also conducted phylogenetic analyses using 4 nuclear loci (*RAG-1, R35, BDNF,* and *PNN*) for a subset of samples. Species tree analysis of the nuclear data further verified that the Laramie Mountains population belongs to *S. consobrinus.* Given the very limited data available on the range, prevalence, and ecology of *S. consobrinus* in Wyoming, as well its designation as a Species of Greatest Conservation Need in Wyoming, more research must be done to ensure protection of this population.

RESUMEN.—El contar con información precisa sobre la identidad y distribución de las especies es fundamental para el establecimiento de políticas estatales sobre el uso y preservación de la tierra. Sin embargo, a menudo, es difícil determinar la identidad de las especies a partir de datos morfológicos, únicamente. Valiéndonos del método filogenético, determinamos la identidad de las lagartijas del género *Sceloporus* que habitan en las montañas Laramie de Wyoming, dentro de los rangos de distribución conocidos de *Sceloporus tristichus* y *Sceloporus consobrinus*. Analizamos la secuencia del gen mitocondrial *ND1* de 10 individuos de las Montañas Laramie y aplicamos el método de máxima verosimilitud en otras 23 muestras de *S. tristichus* y *S. consobrinus* en todas sus áreas de distribución. El árbol genético del ADNmt coloca a las Montañas Laramie dentro de un clado de *Sceloporus consobrinus* que incluye a las Montañas Rocosas de Colorado. Dada la prevalencia de introgresión del ADNmt en los *Sceloporus*, realizamos análisis filogenéticos usando 4 loci nucleares (*RAG-1*, *R35*, *BDNF* y *PNN*), en un subconjunto de muestras. El análisis del árbol filogenético de las especies construido a partir de datos nucleares, constató que la población de las Montañas Laramie pertenece a la especie *S. consobrinus*. Dada la limitada información disponible sobre el rango, la prevalencia y la ecología de los *S. consobrinus* en Wyoming, así como su designación como especie prioritaria para la conservación, creemos necesario realizar más investigaciones que garanticen su protección.

Spiny lizards in the genus *Sceloporus* are widespread throughout North America, with a majority of the 100+ species concentrated in the arid deserts of the southwestern United States and Mexico (Sites et al. 1992). Only a few widespread species have geographic distributions extending into relatively high northern latitudes >40° (Rivera et al. 2021), including *S. graciosus* (*graciosus* group) and members of the *undulatus* group (*S. consobrinus*, *S. occidentalis*, *S. tristichus,* and *S. undulatus*; de Queiroz et al. 2017). Although the distributions and species limits of *S. graciosus* and *S. occidentalis* are clear, the geographic boundaries separating *S. consobrinus* and *S. tristichus* are uncertain across large portions of their ranges (Leaché and Reeder 2002). The distribution of these species in Wyoming is particularly interesting because it is the northernmost state containing both species (Fig. 1), and the identity of some populations, including those in the Laramie Mountains, is currently unknown.

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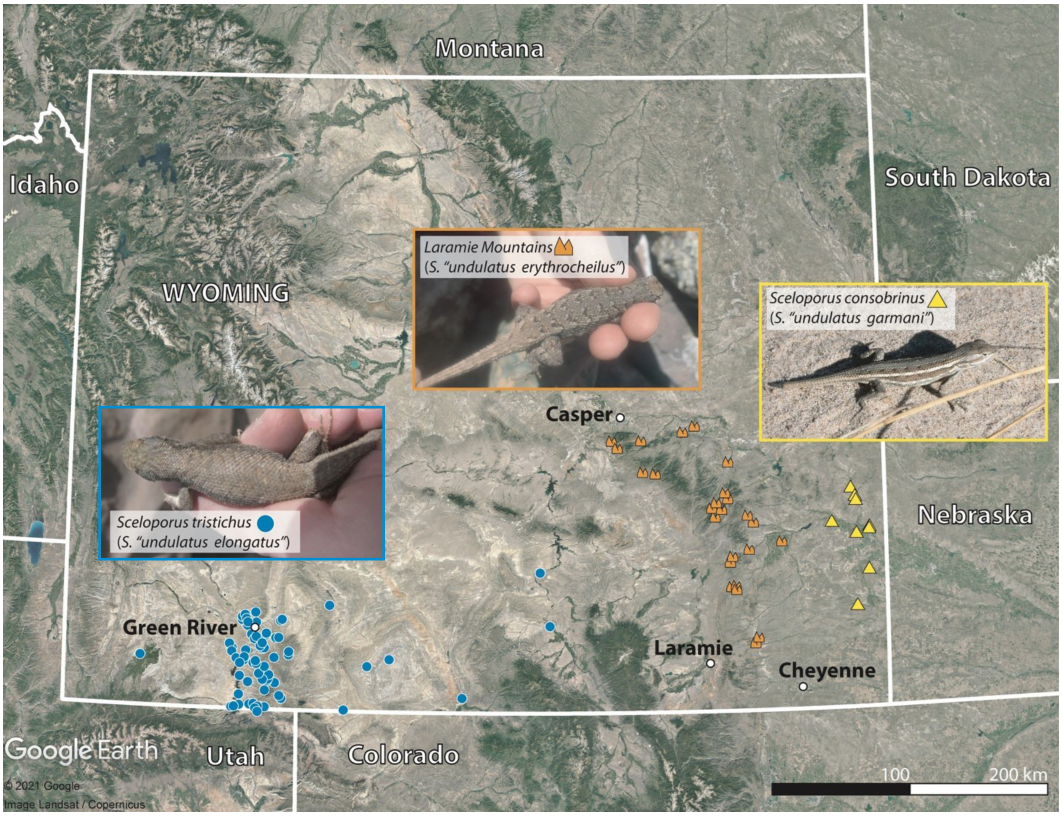

Fig. 1. Wyoming distributions of *Sceloporus tristichus* (circles, blue), *S. consobrinus* (triangles, yellow), and *S. consobrinus* populations from the Laramie Mountains (mountains, orange). Insets—Photographic examples of phenotypic diversity of Plateau Fence Lizards (*S. tristichus*) and Prairie Lizards (*S. consobrinus*) in Wyoming: *S. tristichus* (Sweetwater County, WY), *Sceloporus* sp. (Laramie Mountains, WY), and *S. consobrinus* (southeastern WY). Specimen records are from the Wyoming Game and Fish Department and the Wyoming Natural Diversity Database.

In southwestern Wyoming, *S. tristichus* is closely associated with rocky outcrops and cliffs in sagebrush shrubland and desert shrubland habitats in the Green River Basin (Knight et al. 2014). *Sceloporus consobrinus* is restricted to grassland and sandy grassland habitats located near the Nebraska border in the southeast corner of Wyoming (Fig. 1). These populations of *S. consobrinus* are vulnerable in Wyoming due to restricted ranges and habitat loss and are listed as Species of Greatest Conservation Need (WGFD 2017). These species are separated by several basins and mountain ranges including the Laramie Mountains, where another population of *Sceloporus* lizards is found (Fig. 1). Fence Lizards in the Laramie Mountains are associated with rocky outcrops in sagebrush shrubland and ponderosa pine and lodgepole pine forest habitats from the foothills (1500 m) to approximately 2450 m elevation (Knight et al. 2014). The taxonomic identity of the populations in the Laramie Mountains is poorly understood and was previously based on phenotype and ecology due to a lack of genetic data.

Prior to the taxonomic revision of the *undulatus* group based on molecular data (Leaché and Reeder 2002), geographic distributions of 3 distinct subspecies of Eastern Fence Lizards (*Sceloporus* "*undulatus*") extended into Wyoming (Baxter and Stone 1980, Smith et al. 1992). The color patterns of *Sceloporus* "*undulatus elongatus*" (now *S. tristichus*; Leaché and Reeder 2002) in southwestern Wyoming resemble other populations found throughout the Colorado Plateau to the south in coloration, habitat preference, and morphology.

TABLE 1. Samples of *Sceloporus* from Wyoming's Laramie Mountains used in the genetic analysis. Genbank accession numbers are provided for each gene.

Sample	County	Location	Latitude	Longitude	*ND1*	*BDNF*	*PNN*	*R35*	*RAG1*
2019-064	Albany	Tom Thorne Beth Williams WHMA[a]	41.782183	−105.367487	MZ287296	—	—	—	—
2019-072	Albany	Tom Thorne Beth Williams WHMA	41.781561	−105.367616	MZ287294	MZ287302	MZ287305	MZ287309	—
2019-078	Albany	Tom Thorne Beth Williams WHMA	41.781949	−105.367546	MZ287292	—	—	—	—
2019-028	Converse	Esterbrook	42.368232	−105.433697	MZ287301	—	MZ287304	MZ287307	—
2019-029	Natrona	Smith Creek	42.699187	−106.142820	MZ287300	—	—	—	—
2019-038	Natrona	Smith Creek	42.699483	−106.142959	MZ287298	—	—	—	—
2019-030	Natrona	Red Creek	42.696642	−106.393732	MZ287299	—	—	MZ287308	MZ287311
2019-039	Natrona	Smith Creek	42.699118	−106.142464	MZ287297	—	—	—	—
2019-070	Platte	South Johnson Mountain	42.182013	−105.191169	MZ287295	—	—	—	—
2019-077	Platte	South Johnson Mountain	42.183329	−105.190883	MZ287293	MZ287303	MZ287306	MZ287310	—

[a] WHMA = Wildlife Habitat Management Area.

Sceloporus "*u. garmani*" (now *S. consobrinus*; Leaché and Reeder 2002) in southeastern Wyoming resembles, in both appearance and habitat preference, populations found throughout the western Great Plains to the east and south. Lizards in the Laramie Mountains were previously classified as *S.* "*u. erythrocheilus*" and resembled populations found in rocky foothill and canyon habitat along the eastern edge (Front Range) of the Rocky Mountains in Colorado (Smith et al. 1991). Superficially, the larger body size and color pattern of *S.* "*u. erythrocheilus*" resembles *S. tristichus* (which includes the former *S.* "*u. elongatus*"); however, phylogenetic analyses place some "*erythrocheilus*" populations under *S. tristichus* and others under *S. consobrinus* (Leaché and Reeder 2002, Miles et al. 2002). Populations of "*erythrocheilus*" in the Laramie Mountains in Wyoming were placed under *S. tristichus* based on color pattern and habitat preference because no genetic samples were available (Lewis 2011). However, as geographic proximity was found to be a better predictor of phylogenetic relationships than color pattern in the *undulatus* group (Leaché and Reeder 2002, Miles et al. 2002), the *Sceloporus* populations in the Laramie Mountains could be more closely related to *S. consobrinus*. Thus, our objective was to determine the taxonomic identity of lizards in the Laramie Mountains using phylogenetic analyses.

The Wyoming Game and Fish Department (WGFD) collected 10 tissue samples using nondestructive methods (tail tips) from 5 different localities within the Laramie Mountains (Table 1). Molecular data was collected at the University of Washington. We extracted DNA using Qiagen DNA extraction kits and amplified the *ND1* mitochondrial DNA (mtDNA) gene using polymerase chain reaction (PCR) for all samples. Additionally, 4 nuclear loci (*RAG-1*, *R35*, *BDNF*, and *PNN*) were amplified and sequenced for a subset of 4 samples to verify that the mtDNA and nuclear genomes provided concordant phylogenetic relationships. Methods and primers used for PCR and sequencing of the *ND1* and nuclear genes followed Leaché and Cole (2007) and Leaché (2009), respectively.

We aligned DNA sequences using Geneious (Kearse et al. 2012). We compared mtDNA sequences for samples from the Laramie Mountains with 24 sequences available on

TABLE 2. Average pairwise sequence divergence calculated using mtDNA (*ND1* gene).

Comparison	Sequence divergence
Within Laramie Mtns.	0.083%
Laramie Mtns. vs. *S. consobrinus* (CO, Jefferson Co.)	0.15%
Laramie Mtns. vs. *S. consobrinus* (NE, Keith Co.)	5.4%
Laramie Mtns. vs. *S. tristichus* (WY, Sweetwater Co.)	8.3%

GenBank for *S. tristichus, S. consobrinus,* and *S. cowlesi* (Appendix 1). Nuclear sequences were compared with 7 samples for *S. tristichus* and *S. consobrinus* (Appendix 2).

To determine the genetic diversity among populations, we performed pairwise comparisons of sequence divergence in the program MEGA5 between the Laramie Mountains samples and *S. consobrinus* and *S. tristichus* from surrounding areas (Tamura et al. 2011). We inferred a mtDNA gene tree using maximum likelihood (ML) in program RAxML (Stamatakis 2014). We used a complex nucleotide substitution model (GTR + Γ) and rooted the tree using the Southwestern Fence Lizard (*S. cowlesi*). We performed 100 bootstrap replicates (rapid bootstrapping). Since independent loci are expected to have different genealogical histories, we analyzed the 4 nuclear loci using concatenation with ML (using program RAxML) and with a coalescent model in program BPP (Yang 2015). The ML analysis used the GTR + Γ model, and the Eastern Fence Lizard (*S. undulatus*) was used to root the tree. We performed 100 rapid bootstrap replicates. For the BPP analysis, we partitioned samples into species and treated samples from the Laramie Mountains as a separate population. We set the priors for population size to (3, 0.0029) and the tree height prior to (3, 0.047), which corresponds to approximately 0.15% sequence divergence within a species and 2.4% sequence divergence at the root of the species tree. We ran the analysis for 50,000 steps (samplefreq = 2) after discarding 10,000 steps as burn-in, and the analysis was run twice to check for convergence of parameter values on similar posterior distributions.

Average pairwise sequence divergence estimates calculated for the Laramie Mountains population using the *ND1* mtDNA gene are lowest (0.15%) in comparison to *Sceloporus consobrinus* from the Colorado Front Range (CO, Jefferson Co.) (Table 2). Sequence divergence between the Laramie Mountains and *S. tristichus* in Sweetwater County, Wyoming, is highest (8.3%); the *S. consobrinus* population in western Nebraska is 5.4% different from populations in the Laramie Mountains (Table 2).

The phylogenetic analysis of the *ND1* gene using ML places the Laramie Mountains samples within *S. consobrinus* and in a clade that includes the Colorado Front Range (bootstrap = 99%; Fig. 2). The *S. consobrinus* clade is split into 2 groups: a western group that includes samples from the western edge of the species distribution (New Mexico, Colorado, and the Laramie Mountains), and an eastern group that includes Nebraska, Kansas, Oklahoma, and Texas (Fig. 2).

The concatenated phylogenetic analysis using the nuclear genes places the Laramie Mountains within *S. consobrinus* (bootstrap = 67%; Fig. 3). Similar to the mtDNA gene tree, the Laramie Mountains population is most closely related to populations of *S. consobrinus* from Colorado (Fig. 3A). The coalescent species tree also places the Laramie Mountains population with *S. consobrinus* with a posterior probability of 0.998 (Fig. 3b).

The results of the genetic analyses indicate that the Laramie Mountains population belongs to *S. consobrinus*. Uncovering the identities and distributions of species is a challenging but critical task. Without range and identity information on species in a region, land management and conservation plans cannot be fully effective. Within Wyoming, populations with 2 distinct phenotypes belong to *S. consobrinus*; however, the external color pattern and body size of the Laramie Mountains population is more similar to *S. tristichus* than to the *S. consobrinus* population in the southeastern corner of the state. Morphology has limited utility for identifying *Sceloporus* lizards in this region, given that phylogenetic relationships do not support the monophyly of subspecies and that previously recognized subspecies were delimited on the basis of morphological characters (Leaché and Reeder 2002, Miles et al. 2002).

Due to the different rates of evolution and inheritance modes of mtDNA and nuclear DNA (Ballard and Whitlock 2004), it is critical that both DNA types are utilized in phylogenetic

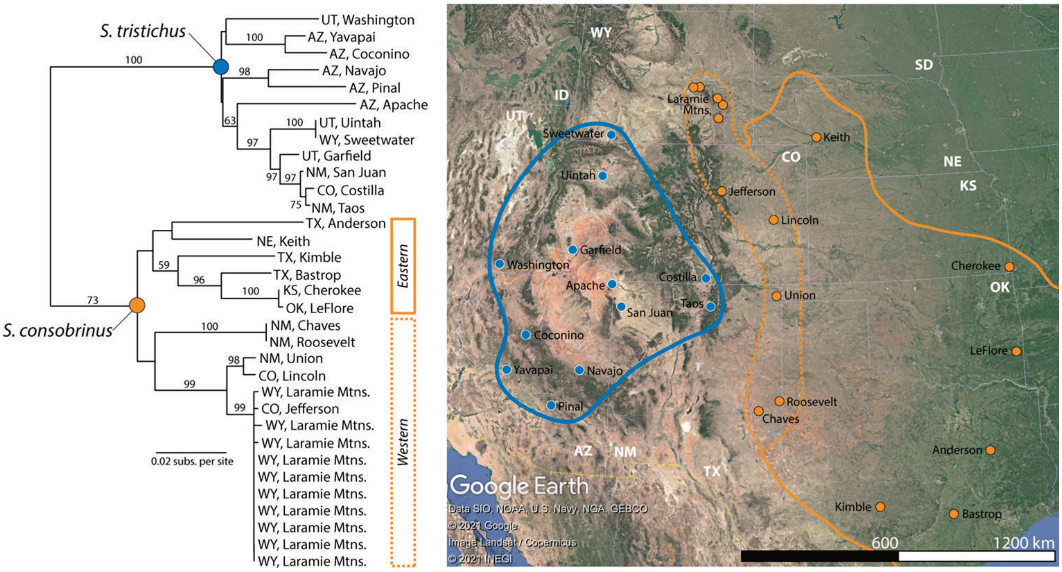

Fig. 2. Phylogenetic relationships based on mtDNA and geographic distributions of Prairie Lizards (*Sceloporus consobrinus*) and Plateau Fence Lizards (*S. tristichus*). The phylogenetic tree was estimated using maximum likelihood in program RAxML. Numbers on nodes are bootstrap values (bootstrap values ≥50% are shown).

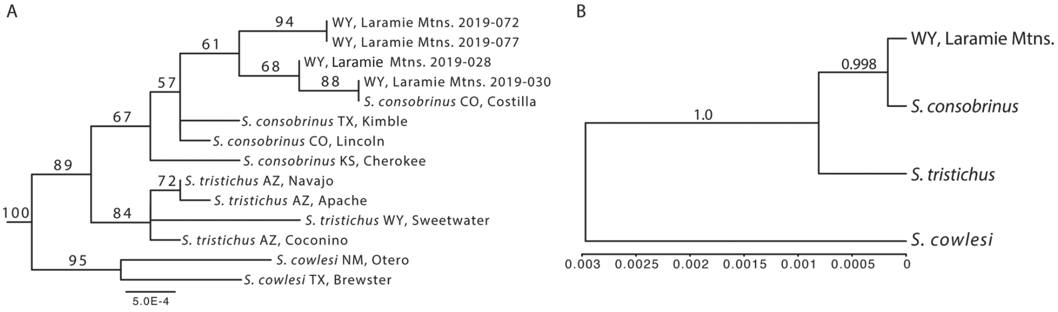

Fig. 3. Phylogenetic relationships of *Sceloporus* lizards estimated using 4 nuclear genes. ***A,*** Maximum likelihood analysis of the concatenated nuclear loci. The tree was rooted with the Eastern Fence Lizard (*S. undulatus*; not shown). Bootstrap values are shown on branches. ***B,*** Coalescent-based species tree estimated using program BPP. Posterior probability values are shown on the branches. The scale bar shows the sequence divergence.

analyses when inferring species identities. Additionally, hybridization can result in introgression of the mitochondrial genome between *Sceloporus* lizards (Leaché 2009), which further justifies the need to examine both types of data. The phylogenetic analyses presented here based on mtDNA and nuclear data place the Laramie Mountains population within *S. consobrinus*, and no evidence of hybridization with *S. tristichus* was found. Nevertheless, using both mtDNA and nuclear DNA to identify species is critical for species with known cases of mtDNA introgression. Introgression is often detected in areas where genetically distinctive populations overlap, and although no such areas of sympatry currently exist between *S. consobrinus* and *S. tristichus* in Wyoming, contemporary populations can retain genetic evidence of historical introgression events (Mallet 2005).

Phylogenetic analyses can inform conservation strategies and improve their effectiveness by identifying management units (Moritz 1994, Crandall et al. 2000). Two distinctive populations of *S. consobrinus* occur in Wyoming, and their unique distributions, habitat preferences, phenotypes, and high levels of genetic divergence warrant their consideration as separate management units. *Sceloporus consobrinus* from the Laramie Mountains belong to a clade that extends northwards from the Front Range of the Rocky Mountains in Colorado (previously classified as *S.* "*u. erythrocheilus*"). The second population of *S. consobrinus* is restricted to the southeastern corner of Wyoming and belongs to a clade that extends into the Great Plains (previously classified as *S.* "*u. garmani*"). The 2 populations of *S. consobrinus* in Wyoming are allopatric, although they may come into contact farther south in Colorado, where putative morphological intergrades have been recorded (Smith et al. 1991). Future research should focus on refining the distributional limits of populations of *S. consobrinus* from the Laramie Mountains and the Front Range versus those from the eastern plains, determining whether or not these populations come into contact, and characterizing the population genetic dynamics of any potential contact zones. The correspondence of genetic and phenotypic divergence among *S. consobrinus* populations in Wyoming suggests that these populations could represent distinct species, but a broader phylogeographic context that considers the entire distribution of the species is necessary to address this species' delimitation question.

ACKNOWLEDGMENTS

We thank K. Buxbaum and L. Reisman for collecting tissue samples that were used for genetic analysis of lizards in the Laramie Mountains. We thank members of the Leaché lab and 2 anonymous reviewers for helpful comments on the manuscript. We thank the University of Washington Biology Department for providing funding to KWM through the BEACON Field Award.

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Received 30 March 2021
Revised 7 June 2021
Accepted 11 June 2021

Appendix 1. Samples included in the phylogenetic analysis of the mtDNA data downloaded from GenBank, with location information (state, county).

Sceloporus consobrinus.—TX, Bastrop (AF440056); TX, Kimble (AF440079); CO, Jefferson (AF440034); NM, Union (AF440064); CO, Lincoln (AF440035); NE, Keith (AF440049); KS, Cherokee (AF440047); OK, LeFlore (AF440073); TX, Anderson (AF440077); NM, Chaves (AF440053); NM, Roosevelt (AF440063).

Sceloporus tristichus.—NM, San Juan (AF440065); UT, Garfield (AF440080); UT, Uintah (AF440081); UT, Washington (AF440082); WY, Sweetwater (AF440084); CO, Costilla (AF440048); AZ, Apache (AF440024); AZ, Coconino (AF440025); AZ, Pinal (AF440030); AZ, Navajo (AF440028); AZ, Yavapai (AF440032); NM, Taos (AF440058).

Sceloporus cowlesi.—AZ, Santa Cruz (AF440031).

Appendix 2. Samples of *Sceloporus consobrinus* and *S. tristichus* included in the phylogenetic analysis of nuclear DNA (*BDNF, PNN, RAG-1*, and *R35*) downloaded from GenBank, with location information (state, county).

Sceloporus tristichus.—AZ, Coconino (*BDNF* GQ494854, *PNN* GQ494839, *RAG-1* GQ494809, *R35* GQ494824); AZ, Navajo (*BDNF* GQ494853, *PNN* GQ494838, *RAG-1* GQ494808, *R35* GQ494823); AZ, Apache (*BDNF* GQ494860, *PNN* GQ494845, *RAG-1* GQ494815, *R35* GQ494830); WY, Sweetwater (*BDNF* GQ494855, *PNN* GQ494840, *RAG-1* GQ494810, *R35* GQ494825).

Sceloporus consobrinus.—KS, Cherokee (*BDNF* GQ494861, *PNN* GQ494846, *RAG-1* GQ494816, *R35* GQ494831); TX, Kimble (*BDNF* GQ494863, *PNN* GQ494848, *RAG-1* GQ494818, *R35* GQ494833); CO, Costilla (*PNN* GQ494841, *RAG-1* GQ494811, *R35* GQ494).