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GENETIC STRUCTURE NOT DETECTED IN NORTHERN IDAHO AND NORTHEAST WASHINGTON WESTERN TOAD (*ANAXYRUS BOREAS*) POPULATIONS

MICHAEL K LUCID, SHANNON EHLERS, LACY ROBINSON, AND JACK SULLIVAN

ABSTRACT—Western Toads (*Anaxyrus boreas*) are widely considered to be of conservation concern, and have high genetic variability both continentally and locally. Previous research has identified 3 major genetic clades of Western Toads in North America. We collected tissue samples from 47 larval toads representing 16 different collection sites in northern Idaho and northeastern Washington. For each sample we sequenced a 269 base-pair section of the mitochondrial cytochrome *c* oxidase I (COI) gene. We also downloaded COI sequences from GenBank, which had been used in a previous study to identify geographic clades. We used PAUP to build phylogenies in which our samples did not align geographically with each other or form distinct clades within the continental phylogeny. Our samples did, however, group in the expected Northwestern clade which was identified in a previous study. The toads examined in our study area show no evidence of cryptic diversity within the section of COI we examined and our data support the current taxonomic assignment to *Anaxyrus boreas*.

Key words: *Anaxyrus boreas*, COI, genetic structure, phylogeny, state wildlife action plan, taxonomy, Western Toad

The identification of biological lineages and taxonomic groupings is an essential step in developing conservation actions and priorities (Espindola and others 2016). Western Toads (*Anaxyrus boreas*) are widely considered to be of conservation concern and have high genetic variability, both continentally (Goebel and others 2009) and locally (Addis and others 2015). Goebel and others (2009) identified 4 major clades of Western Toads across the range of the species. This broad continental approach, however, left large portions of Western Toad range un-sampled, particularly in the Pacific Northwest. Western Toads are listed as Species of Greatest Conservation Need in Idaho (IDFG 2017) and Washington (WDFW 2015), and both states identify genetic assessment of the species as a needed conservation action to provide taxonomic clarity (IDFG 2005; WDFW 2005).

The biogeography of our study area, which included 3 mountain ranges in the Idaho Panhandle and northeastern Washington (Fig. 1), is heavily influenced by the last glacial cycle (Shafer and others 2010). During the last glacial maximum, approximately 20,000 y before present (Clark and others 2009), the Selkirk and West Cabinet Mountains were separated from the Saint Joe Mountains by the large, 300 km long, Lake Missoula (Pielou 2008). The Selkirk and West Cabinet Mountains were mostly engulfed by the Cordilleran Ice Sheet (Pielou 2008), while the Saint Joe Mountains remained ice free and part of the Pacific Northwest Refugium (Shafer and others 2010). This dynamic glacial history has influenced the distribution of many species including amphibians. For example, Coeur d'Alene Salamanders (*Plethodon idahoensis*) occur in the West Cabinet and Saint Joe Mountains, but do not occur in the Selkirk Mountains (Wilson and others 1997). Idaho Giant Salamanders (*Dicamptodon aterrimus*) occur in the Saint Joe Mountains, but not in the Selkirk or West Cabinet Mountains (Carstens and others 2005; Steele and Storfer 2006). Recent work with non-amphibian species in our study area has also revealed highly structured mammal populations (Lucid and others 2019) and cryptic species-level diversity in both terrestrial gastropods (Lucid and others 2018) and mammals (Lucid and others 2020).

The objectives of our study were to assess Western Toads for species level cryptic lineages and to compare the genetics of Western Toads in our study area to those across the range of the species. To achieve these objectives, we conducted dip-net surveys for larval amphibians as part of a regional amphibian inventory (see Lucid and others 2016 and Lucid and others In Press for full details). We used scissors to clip a portion of tail tissue from 47 larval toads representing 16 different collection sites (Fig. 1, Table 1). Samples were dried and stored at ambient room temperature prior to laboratory analysis. We extracted

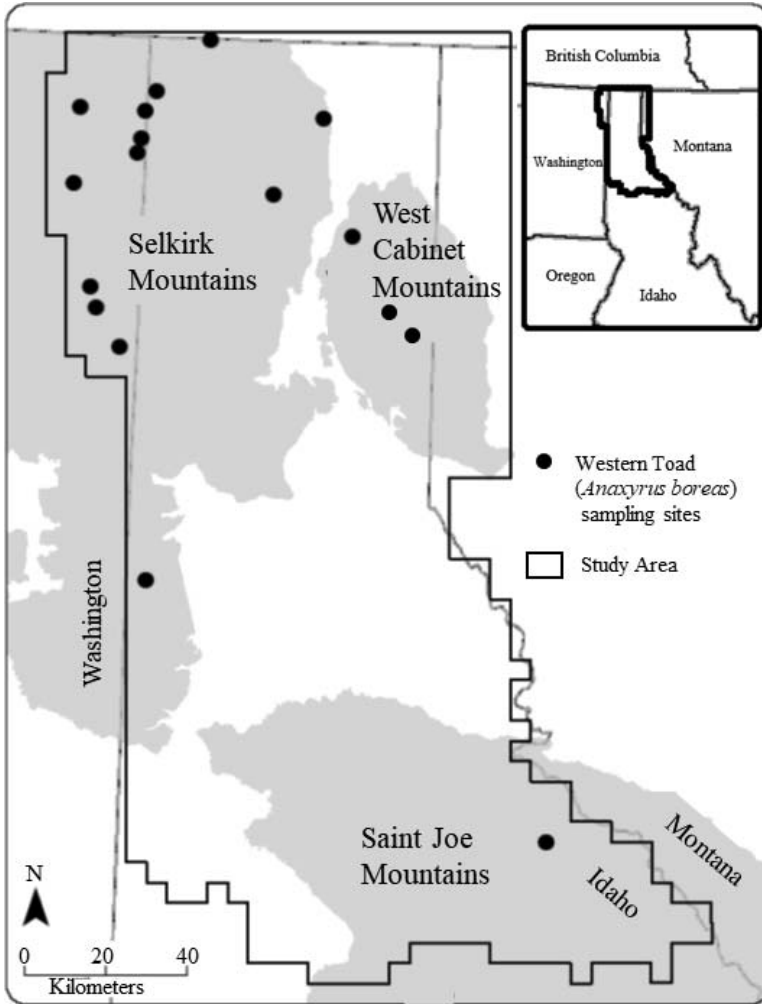





FIGURE 1. Study area with 16 locations where DNA was collected from 47 individual western toads.

TABLE 1. Western Toad sampling sites, mountain range, location, elevation (m), state, and wetland type.

Site	Range	Latitude	Longitude	Elev.	State	Type
W6	Selkirks	48.66702	-117.27137	1145	WA	Pond
W10	Selkirks	48.83778	-117.26032	813	WA	Pond
W22	Selkirks	48.30942	-117.09537	865	WA	Pond
W48A	Selkirks	48.74253	-117.06126	973	WA	Pond
W48B	Selkirks	48.77407	-117.04978	1071	WA	Pond
W50	Selkirks	48.83412	-117.04068	1632	ID	Pond
W67	Selkirks	48.87793	-117.00529	898	WA	Pond
W114	Selkirks	48.99680	-116.82925	1253	ID	Puddles
W148	Selkirks	48.65967	-116.59933	1711	ID	Pond
W171	Selkirks	48.83155	-116.43779	673	ID	Stream
W580	Selkirks	48.39373	-117.17862	1077	WA	Pond
W647A	Selkirks	47.79310	-116.97653	732	ID	Pond
W581	Selkirks	48.44117	-117.20027	1045	WA	Pond
W1097	Cabinets	48.57090	-116.32872	975	ID	Pond
W1188	Cabinets	48.37778	-116.13695	1763	ID	Pond
W1492	Saint Joe	47.23689	-115.63681	828	ID	Stream

Goebel and others (2009)
Major clades:

-  Northwest
-  East
-  Southwest

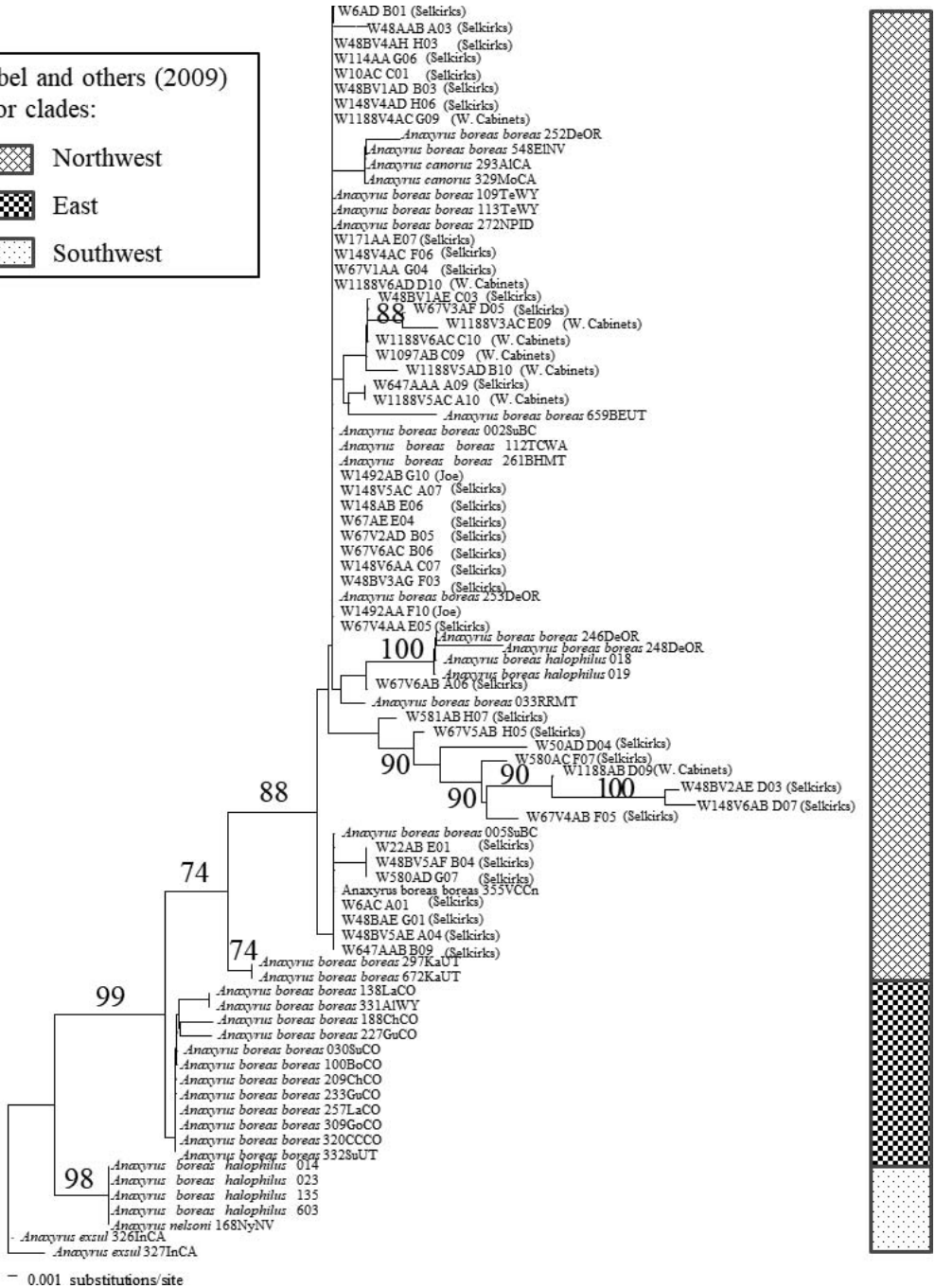


FIGURE 2. Phylogenetic reconstruction of COI mtDNA sequences generated for this study (sample name starts with W) and from Goebel and others (2009) (sample name starts with *Anaxyrus*). Goebel and others (2009) major geographic clades are shown shaded bar on right hand side of figure. Collection localities for this study are in parentheses after sample name and indicate if sample was collected in the Saint Joe (Joe), Selkirk (Selkirks), or West Cabinet (W. Cabinet) Mountain Range. Node supports show Bayesian posterior probabilities. Probabilities <50 are not included in figure. Scale bar indicates number of substitutions per site.

total DNA using a DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA) per the manufacturer's protocols, and amplified and sequenced a 269 base pair section of the mitochondrial cytochrome *c* oxidase I (COI) gene (GenBank accession numbers MT901801–MT901845) as described in Lucid and others (2018), but using primers from Goebel and others (2009). We choose to use the COI gene because of its utility in elucidating species level cryptic diversity (see Dawnay and others 2007). We accessed GenBank to obtain sequences (GenBank PopSet 146262106) of the same gene region from additional toads representing the major clades identified by Goebel and others (2009). Alignment, using Clustal Omega (Sievers and others 2011), was straightforward and no indels were required. We used PAUP* (v. 4.0 a157; Swofford 2003) to build a neighbor-joining (NJ) tree from logDet distances for use in model selection under Bayesian Information Criterion and Decision Theoretic Approach (Minin and others 2003). We then calculated distances using the HKY+I model and re-estimated the NJ tree. We then used MrBayes (v. 3.2; Ronquist and others 2012) to estimate nodal probabilities using the same model (HKY+I). We used 2 independent runs with 4 chains and 5,000,000 generations per run and sampled each run every 1000 generations. When the runs had achieved low standard deviation of split frequencies (approximately 0.015), we filtered the 1st 1,000,000 generations from each run and combined the 2 post burn-in runs.

Our samples did not align geographically with each other or form distinct clades within the phylogeny (Fig. 2). Our samples did, however, all group within the Northwest Clade identified by Goebel and others (2009). The COI sequences did not indicate genetic structure within the study area despite topographic complexity and relative large distances between sampling sites.

Individuals from the 2 West Cabinet Mountain sites are well represented across the phylogeny and grouped with samples from the Selkirk and Saint Joe Mountains. The single toad population we found in the Saint Joe Mountains, which was separated from all other samples by over 100 km, phylogenetically groups with toads from the Selkirk and Cabinet Mountains. We found no evidence of genetic structure of toad populations within our study area. It is important to keep in

mind, however, that we only used a single marker while Goebel and others (2009) used 3, and we suspect more extensive genomics would reveal at least some structure. Regardless, the toads examined in our study area show no evidence of cryptic diversity within the section of COI we examined, and our data support their current taxonomic assignment to *Anaxyrus boreas*.

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