# Phylogeography of *Pseudacris regilla*

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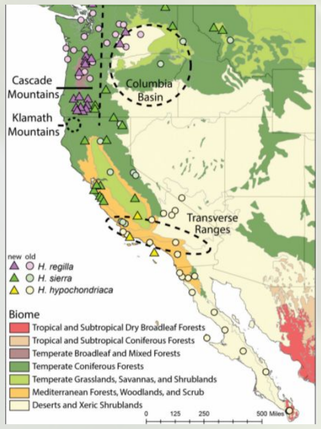
## Abstract

Current phylogeographic methods harness multilocus data to investigate the evolutionary history of closely related species and lineages, combining phylogenetics, population genetics, and biogeography to uncover evolutionary history. The phylogeographic history of the *Pseudacris regilla* complex will be revisited with more robust data and newer methods, which requires the extraction of genomic DNA from tissue samples described herein. DNA from extracted samples is quantified via Qubit, and gel electrophoresis is used to ensure that DNA in samples is of the appropriate length. High quality genomic DNA samples are necessary for further analyses which are expected to yield insight into species boundaries, historical demography, population structure, and gene flow within the complex. Resolution of *Pseudacris regilla* phylogeography can also contribute to a broader comparative framework for understanding how different groups of terrestrial vertebrates along the West coast of North America have been shaped by past climatic and geological events.

## Introduction

Previous studies of the *Pseudacris regilla* complex have been inconclusive in determining the status of populations within the complex, and determining factors and historical processes contributing to the current observed structure throughout the range. The species complex is found from southern British Columbia to Mexico along the West Coast of North America, extending as far eastward as Montana. *Pseudacris regilla* (sensu lato) is also present in Alaska, where it was introduced in the 1960s (Waters et al. 1998). Our research seeks to resolve the taxonomic confusion of the complex through phylogeographic and population genomic analyses. Recuero et al. (2006) identified three mitochondrial haplotype clades thought to have diverged around 1 million years ago. The three groups were a northwest clade from Washington and Oregon (*hyliola regilla*), a central clade (*H. sierra*), and a southern clade from southern california through the Baja California Peninsula (*H. hypochondriaca*). Jadin et al. (2021), using single-locus species delimitation methods, found support for the three species identified by Recuero et al. (2006). Our study will expand on this previous work by including nuclear loci which will help resolve species boundaries, and determine whether elevating the mitochondrial haplotype clades to species status is warranted.




The Recuero et al. (2006) study suggested that the fragmentation of the three groups was the result of vicariance. Substructuring in the population from the Baja California Peninsula could have resulted from a midpeninsular seaway. Alternatively, range expansion and contractions during the Pleistocene might have contributed to the population structure. The orogeny of the Cascade Mountains during the Pliocene was also suggested to explain the divergence between the three main clades. New data in our project will allow for further study of how geography, environmental change, and historical processes have shaped genetic diversity in the complex.

Figure 1:

Figure borrowed from Jadin et al. (2021) showing localities sampled in their study and previous studies, and the distributions of the three putative species

## Methods

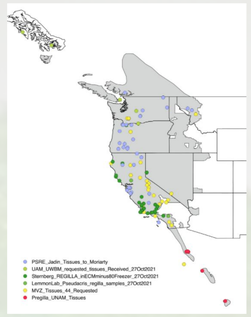



Figure 2: A map showing specimens and their localities to be used in this study, and the museum or lab collections from which they were obtained

Up to three tissue samples were chosen from localities throughout the range, with samples from each locality chosen at random. Museums were contacted to arrange for tissue samples to be borrowed. All tissue samples being used in the project were assembled into a single spreadsheet, including locality data and ID numbers. A total of 246 tissue samples were located for use in the project. DNA was extracted following the tissue DNA protocol for the omega biotek EZNA Tissue DNA Kit for 84 of the samples thus far. An RNAse treatment was used to remove RNA, and 2 elutions of 50 µL were performed for a final volume of 100 µL of genomic DNA from each sample. Following DNA extraction, a Qubit fluorometer was used to quantify the amount of DNA present in each sample to ensure adequate amounts were obtained for further analyses. Gel electrophoresis was performed to quantify the length of the DNA fragments obtained through extraction.

Figure 3: The master spreadsheet of samples being used in the study

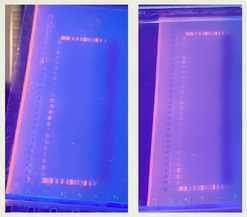
| Genus | species | prefix field number | Tissue number | Tissue Type | Collection/muse | State | County | Locality | lat | long |
|---|---|---|---|---|---|---|---|---|---|---|
| Pseudacris | regilla | UWBM# Herp: 7239 | ADL 4150 | | Burke Museum | Washington | Kittitas | Easton, Nelson S | 47.22 | -121.13 |
| Pseudacris | regilla | UWBM# Herp: 8117 | ADL 4484 | | Burke Museum | Washington | San Juan Count | N end of Henry Island, | San Juan Islands | |
| Pseudacris | regilla | UWBM# Herp: 8118 | ADL 4485 | | Burke Museum | Washington | San Juan Count | N end of Henry Island, | San Juan Islands | |
| Pseudacris | regilla | UWBM# Herp: 8096 | ADL 4513 | | Burke Museum | Oregon | Gilliam County | Fulton Canyon R | 45.64694 | -120.88028 |
| Pseudacris | regilla | UWBM# Herp: 8097 | ADL 4514 | | Burke Museum | Oregon | Gilliam County | Fulton Canyon R | 45.64694 | -120.88028 |
| Pseudacris | regilla | UWBM# Herp: 8098 | ADL 4515 | | Burke Museum | Oregon | Gilliam County | Fulton Canyon R | 45.64694 | -120.88028 |
| Pseudacris | regilla | UWBM# Herp: 8099 | ADL 4516 | | Burke Museum | Oregon | Gilliam County | Fulton Canyon R | 45.64694 | -120.88028 |
| Pseudacris | regilla | UAM:Herp:178 | | muscle | UAM | Alaska | | Ward Creek, 4-5 | 55.4 | -131.7166667 |
| Pseudacris | regilla | UAM:Herp:179 | | muscle | UAM | Alaska | | Ward Creek, 4-5 | 55.4 | -131.7166667 |
| Pseudacris | regilla | UAM:Herp:384 | | toes | UAM | Alaska | | Small pond in Si | 57.05518333 | -135.3206583 |
| Pseudacris | regilla | MVZ:Herp:137370 | | | MVZ | California | | Santa Ynez Rive | 34.6109479 | -120.1991966 |
| Pseudacris | regilla | MVZ:Herp:137371 | | | MVZ | California | | Santa Ynez Rive | 34.6109479 | -120.1991966 |
| Pseudacris | regilla | MVZ:Herp:137383 | | | MVZ | Montana | | temporary pond | 46.816956 | -113.702553 |
| Pseudacris | regilla | MVZ:Herp:137384 | | | MVZ | Montana | | temporary pond | 46.816956 | -113.702553 |
| Pseudacris | regilla | MVZ:Herp:137396 | | | MVZ | California | | pond in Meadow | 38.49078 | -119.80266 |
| Pseudacris | regilla | MVZ:Herp:137397 | | | MVZ | California | | pond in Meadow | 38.49078 | -119.80266 |
| Pseudacris | regilla | MVZ:Herp:137401 | | | MVZ | Nevada | | Beatty | 36.90861 | -116.75833 |
| Pseudacris | regilla | MVZ:Herp:137402 | | | MVZ | Nevada | | Beatty | 36.90861 | -116.75833 |
| Pseudacris | regilla | MVZ:Herp:145349 | | | MVZ | California | | Little Lake, W sic | 35.936948 | -117.90531 |
| Pseudacris | regilla | MVZ:Herp:145350 | | | MVZ | California | | Little Lake, W sic | 35.936948 | -117.90531 |
| Pseudacris | regilla | MVZ:Herp:145407 | | | MVZ | Baja California | | Isla Cedros | 28.111111 | -115.1777778 |

## Results

Frog tissue samples were successfully found and obtained from five museum collections, and organized into a catalogue of samples for this phylogeography project. The samples which were found using Herpnet and by contacting museums are evenly distributed across the range of *Pseudacris regilla* (sensu lato). Samples from the range in Mexico have not yet arrived at the Lemmon lab, however. Of the samples from which DNA has been extracted thus far, adequate quantities and appropriate lengths of genomic DNA have been obtained from all. Qubit fluorometer readings ranged from 3.1 to 37.8 ng/µL.

| Sample ID | ng/µL | Sample ID | ng/µL | Sample ID | ng/µL | Sample ID | ng/µL |
|---|---|---|---|---|---|---|---|
| 1 | 14.3 | 21 | 15 | 41 | 6.83 | 61 | 5.68 |
| 2 | 20.8 | 22 | 21.6 | 42 | 9.31 | 62 | 7.88 |
| 3 | 21.2 | 23 | 11.7 | 43 | 9.63 | 63 | 9.79 |
| 4 | 11.3 | 24 | 17.6 | 44 | 9.96 | 64 | 11.4 |
| 5 | 20.7 | 25 | 12.1 | 45 | 15.5 | 65 | 8.41 |
| 6 | 26.3 | 26 | 16.7 | 46 | 18.1 | 66 | 8.52 |
| 7 | 17.3 | 27 | 21.9 | 47 | 20.7 | 67 | 13.1 |
| 8 | 10.3 | 28 | 10.9 | 48 | 27.9 | 68 | 9.64 |
| 9 | 20.4 | 29 | 5.32 | 49 | 4.06 | 69 | 7.84 |
| 10 | 37.8 | 30 | 8.23 | 50 | 4.76 | 70 | 13.9 |
| 11 | 16.1 | 31 | 6.68 | 51 | 12 | 71 | 7.34 |
| 12 | 35 | 32 | 8.4 | 52 | 21.5 | 72 | 9.62 |
| 13 | 34.1 | 33 | 10.5 | 53 | 26.3 | 73 | 3.1 |
| 14 | 14.5 | 34 | 17.9 | 54 | 24.6 | 74 | 6.1 |
| 15 | 11.7 | 35 | 12.1 | 55 | 21.3 | 75 | 4.21 |
| 16 | 7.28 | 36 | 8.54 | 56 | 26.3 | 76 | 5.98 |
| 17 | 13.8 | 37 | 8.84 | 57 | 19.7 | 77 | 12 |
| 18 | 10.5 | 38 | 11 | 58 | 41.4 | 78 | 19.2 |
| 19 | 16.1 | 39 | 13.7 | 59 | 11.3 | 79 | 17.8 |
| 20 | 12.7 | 40 | 3.84 | 60 | 13 | 80 | 6.81 |

Figure 4: Qubit fluorometer readings for samples 1-80



Figures 5 and 6: Photos of the gels for samples 1-52

## Discussion

The successful extraction of necessary quantities of genomic DNA will allow for further resolution of the phylogeography of the *Pseudacris regilla* complex. Following completion of DNA extraction, phylogeographic methods applied to the new multilocus data will allow for comparison with past studies, and a more complete understanding of species boundaries within the complex. I hypothesize results will be in line with findings from single locus species delimitation methods employed by Jadin et al. (2021). Building on previous work, methods including Estimated Effective Migration Surfaces (EEMS) and other analyses of population structure can be used to better understand the distribution of genomic variation in the complex and patterns of gene flow between populations. EEMS allows gene flow between populations to be visualized and interpreted, as was done in the example below with the *Nerodia fasciata-clarkii* complex (Rautsaw et al. 2021). Population structure could be investigated using Discriminant analysis of principal components to identify clusters and K-means clustering to assess which model of population structure fits best. Data could also be examined for patterns of isolation-by-distance and isolation-by-environment. Additionally, admixture plots could be generated to analyze admixture proportions between putative species. Taken together, these methods will lend insight into the evolutionary history of the complex.

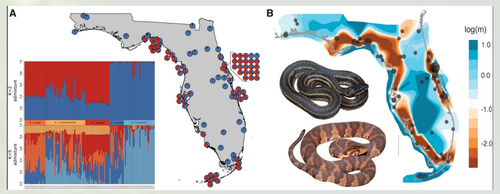



Figure 7 borrowed from Rautsaw et al. (2021): an example of admixture plots (A) and EEMS (B) used in a study of the *Nerodia fasciata-clarkii* complex. Blue colors in (B) represent areas with high migration and red with low migration.

## Literature Cited

Bonett, R. M., Boundy, J., Burbrink, F. T., Crother, B. I., de Queiroz, K., Frost, D. R., ... & Seidel, M. E. (2017). Scientific and Standard English Names of Amphibians and Reptiles North of Mexico, with Comments Regarding Confidence in Our Understanding.

Duellman, W. E., Marion, A. B., & Hedges, S. B. (2016). Phylogenetics, classification, and biogeography of the treefrogs (Amphibia: Anura: Arboranae). Zootaxa, 4104(1), 1-109.

Jadin, R. C., Orlofske, S. A., Jezkova, T., & Blair, C. (2021). Single-locus species delimitation and ecological niche modelling provide insights into the evolution, historical distribution and taxonomy of the Pacific chorus frogs. Biological Journal of the Linnean Society, 132(3), 612-633.

Rautsaw, R. M., Schramer, T. D., Acuña, R., Arick, L. N., DiMeo, M., Mercier, K. P., ... & Parkinson, C. L. (2021). Genomic adaptations to salinity resist gene flow in the evolution of Floridian watersnakes. Molecular biology and evolution, 38(3), 745-760.

Waters, D. L., Hassler, T. J., & Norman, B. R. (1998). On the establishment of the Pacific chorus frog, Pseudacris regilla (Amphibia, Anura, Hylidae), at Ketchikan, Alaska. Bulletin of the Chicago Herpetological Society, 33, 124-127.

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