Evidence for divergent patterns of local selection driving venom variation in Mojave Rattlesnakes (Crotalus scutulatus)

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(Crotalus scutulatus

venoms remains controversial (e.g.[33,35,36]). Alternatively, neurotoxic venoms may be particularly advantageous when the chance of prey escaping is high[34]. is would be expected in metabolically active ectothermic prey in warm areas. is is because Type A venoms rapidly subdue prey through neuromuscular paralysis, but do not convey the potential digestive bene ts that high SVMP activity[4] does

with abbreviations following). Tissues were stored in 95% ethanol or RNAlater and venom was collected and vacuum dried, frozen in liquid nitrogen, and/or stored at -80°C. We collected a total of 216 individuals: 114 of these had tissue and venom, 34 had only venom, and 68 had only tissue (Supplemental Table 1). Whole genom

Tracer v 1.6[84] to ensure stationarity was reached and that all ESS values for parameters from the individual and combined runs were 200. We combined the runs and generated a 50% majority rule tree.

œ ‘ … ƒ • ‡ ‹ • • ‡ – ƒ Ž Ž ‘ ’ ” ‘ – ‡ To determine if there are differences within and among venom types among populations, we performed an azocasein metalloproteinase assay on 146 samples in triplicate[46]. ese assays were performed by incubating 20 of venom with 1 mg of azocasein substrate in buffer composed of 50 mM HEPES and 100 M NaCl at a pH of 8.0 for 30 minutes at 37 C. We stopped the reaction with 250 of 0.5 M trichloroacetic acid, vortexed, and brought it to room temperature. We then centrifuged it at 2000 rpm for 10 minutes. Sample absorbance was read at 342 and reported in $_{342nm}$/min/mg of venom protein[46]. To determine the statistical significance of differences among samples at $\alpha = 0.05$, we used a Kruskal-Wallis test with venom type and lineage as factors implemented in R v. 3.1.3[55]. If there was significance globally, we used a

taxa variable specific to the forms. BIO6 was removed in favor of BIO11 because temperature of the coldest month is generally a better measure of the coldest quarter. BIO13 and BIO16 were removed in favor of BIO12, the average annual. Lastly BIO14 was removed and BIO17 was kept once again because BIO17 was more general [illegible] 14 [illegible] variable characterizing North America. MAXENT models were run with 20 replicates and average model performance was evaluated by determining Area Under the Curve (AUC) for each model. ENMs were then generated for the Sonoran, Chihuahuan, and Central Mexican Plateau lineages independently following the same methods except 10 replicates were used due to decreased sample size per lineage. [illegible] the extent of correlation between environmental variables and ecotypes at the geographic scales.

To test niche equivalency and niche similarity of Type A and Type B individual geographic distributions, we used the permutation-based method of Warren *et al*.[63] as implemented in the R package phyloclim[64]. Briefly, we ran 99 replicates to build a null distribution against which the observed values of Schoener's D and Warren's I inferred from the full data models. To test niche equivalency, the occurrence points of both groups (e.g., individuals of Type A versus Type B ecomorph) are combined and randomly partitioned into two datasets that are equal in size to Type A and Type B. ENMs are generated for each dataset (e.g., a Type A model and a Type B model) and the similarity values (D and I) are calculated. [illegible] process is repeated to build a null distribution against which the actual values of D and I inferred from each of the full data models are compared to evaluate whether the ecological niche models (Type A and Type B) are significantly

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significant difference between the two stages ($\chi^2 = 0.24$, $df = 1$, $p = 0.62$). Within the Chahai stage, there was significant differences among groups ($\chi^2 = 10.05$, $df = 2$, $p < 0.01$; Fig. 5) with Type B being lower than Type A ($p = 0.02$) but not significantly different than Type A + B ($p = 0.07$). Type A and Type A + B were also not different ($p = 0.899$). Within the Son Li stage, there was no difference among groups ($\chi^2 = 3.22$, $df = 2$, $p = 0.20$; Fig. 5). Overall, Type A values ranged from 132.77 to 733.20 [illegible] (n = 28, Average = 386.58, Median = 380.10), Type A + B from 216.87 to 382.77 [illegible] (n = 5, Average = 301.54, Median = 298.30), and Type B from 70.69 to 469.24 [illegible] (n = 27, Average = 277.231, Median =

high variation in the variables found to be signi cantly di erent between venom types and the direction of their e ects (Table 2

Previous studies examining the distribution of venom types in C

identified by Schield *et al*.[38] as Type A, Type A + B, and Type B individuals (Table 1 and Figs 1 and 3); the *C. scutulatus salvini* lineage appears fixed for Type A venom based on 13 individuals sampled. Combining venom characteristics ... *C. scutulatus* ...

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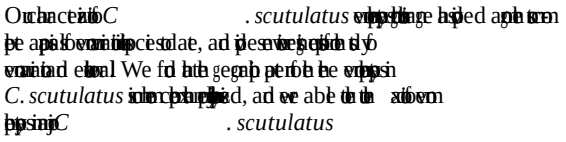

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