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Authors

Evelyn, Christopher J, PhD
Wang, Yinghui

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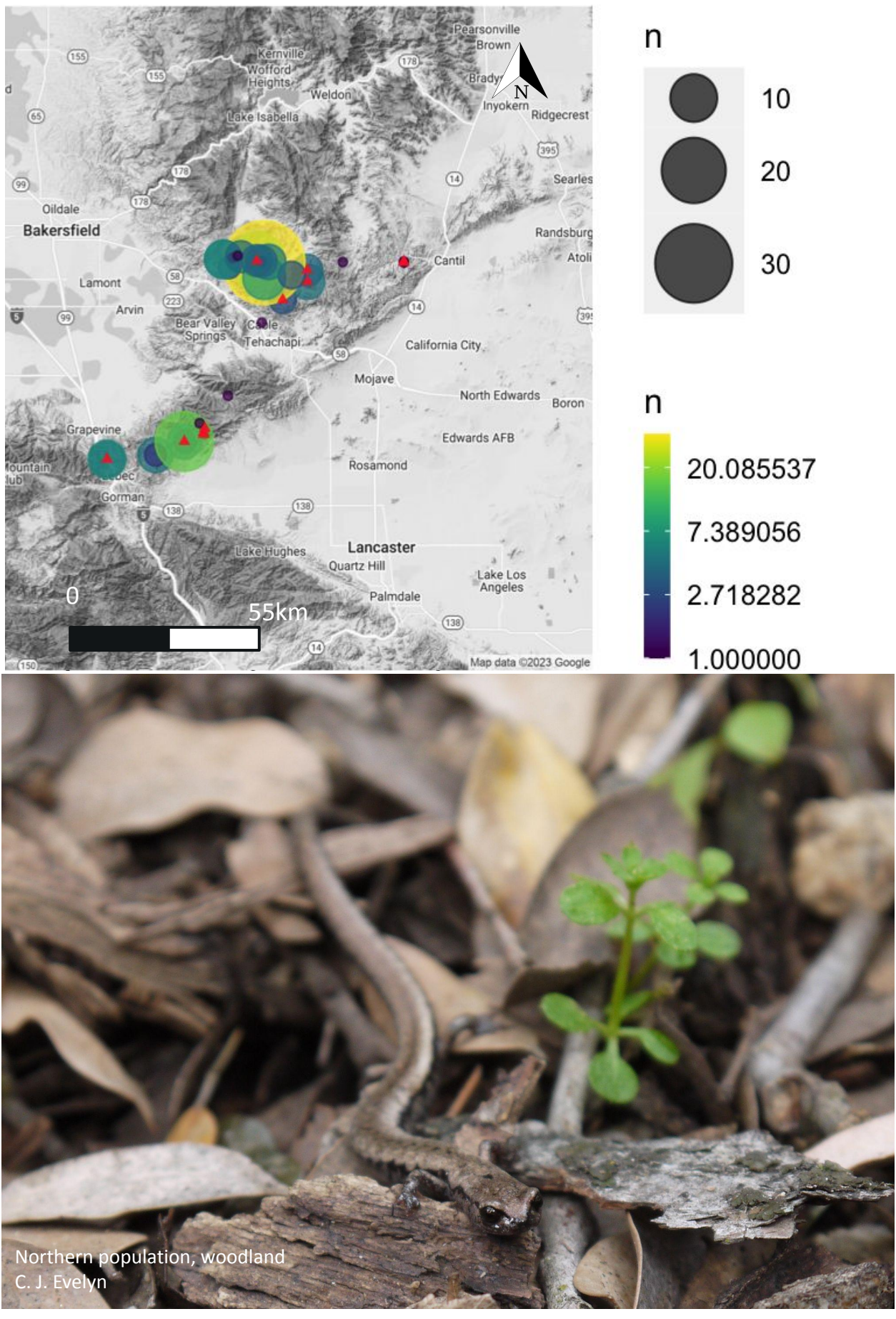
Morphological variation in a Threatened Amphibian

By: Yinghui Wang¹ and Christopher J. Evelyn¹

¹Cheadle Center for Biodiversity & Ecological Restoration University of California Santa Barbara

Abstract

Tehachapi Slender Salamander (*Batrachoseps stebbinsi*) is endemic to the Tehachapi Mountains to the Piute Mountains and is listed as a threatened species by the State of California. They are found in scattered populations from the San Andreas Fault to Walker Basin. Previous studies had found that northern and southern populations of this species have high levels of genetic, color, and size differences. It has been suggested that the two populations of *B. stebbinsi* could be separate species (Hansen and Wake 2005). We obtained morphological measurements from images of preserved specimens from both populations. No significant differences in shape or size was detected between these two populations. In future work we will increase sample size and incorporate evolution of color variation across the range of this species.



Research Goal

Test whether the northern and the southern populations of *B. stebbinsi* should be considered as separate species using morphological data focusing on the size and shape of the animals from both population.

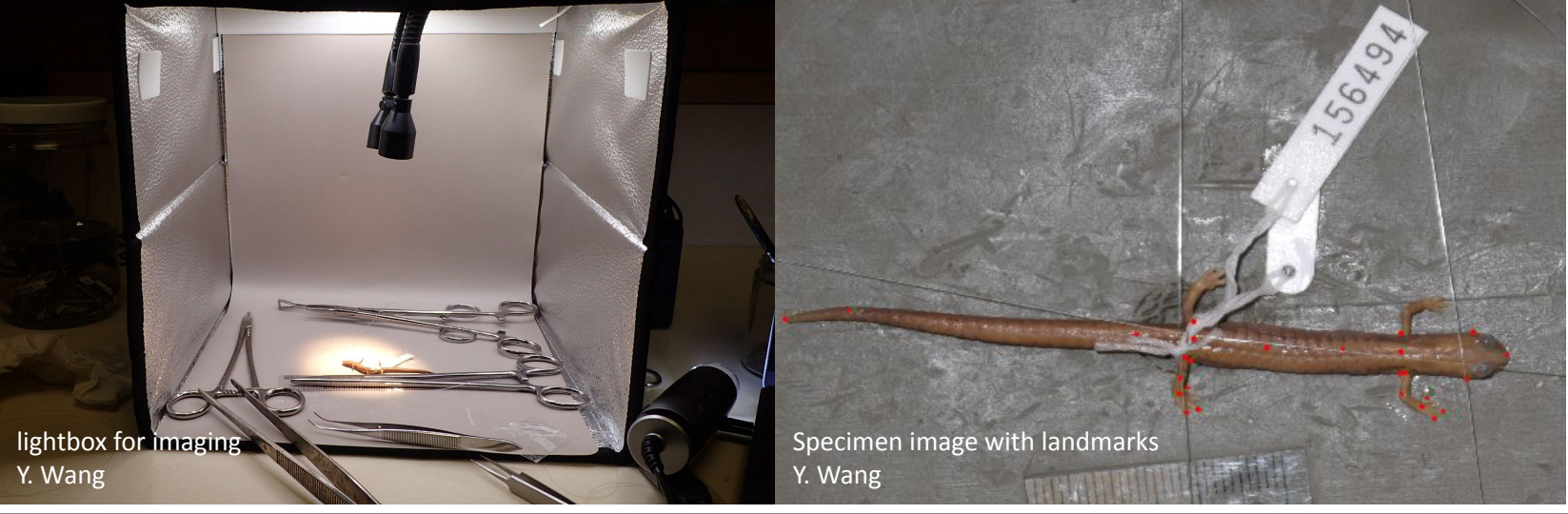
Study System

Batrachoseps stebbinsi is a fully terrestrial salamander (Amphibia) and one of >20 species of slender salamander (genus *Batrachoseps*) that occur in California. This species is found only in mountain ranges of the southern Sierra Nevada and adjacent desert canyons. Measurements were taken from preserved specimens of this species housed at Cheadle Center for Biodiversity and Ecological Restoration (UCSB), Museum of Vertebrate Zoology (UC Berkeley), and the Natural History Museum of Los Angeles County.



Methods

Measurements of 8 morphological parameters (snout-vent length, head width, front limb length, hind limb length, length of the 3rd toe (longest), forefoot width, hindfoot width, body width) taken from 32 digital images of *B. stebbinsi* specimens, 22 from northern populations and 10 from southern populations, using TpsDig2.3. For the linear measurement, landmarks were labeled on both the dorsal view picture (n = 26) and the ventral view picture (n = 14). The landmarks' coordinates, scaling data, and the geographical location of the specimens were exported to R studio. Specimens without location data and juvenile individuals (SVL < 35 mm) were excluded. The snout-vent length, chest width, and the head width between north and south population were compared using two-sample t-test. Generalized Procrustes Analysis (GPA) was performed on the landmarks coordinates to transfer and align them. Principal Component Analysis (PCA) was applied to capture the main axes of morphological variation (prcomp command in R, R Development Core Team 2008). ANOVA was used to test PC1 to PC5, using location as the fixed factor.



Results

The standard size measure for salamanders, snout-vent length, had high variance but no significant difference (North: $\mu_{SVL}=47.2\text{mm}$ South: $\mu_{SVL}=43.5\text{mm}$, $p_{SVL}=0.12$). No linear measures used in this study were found to be significantly different between northern and southern populations.

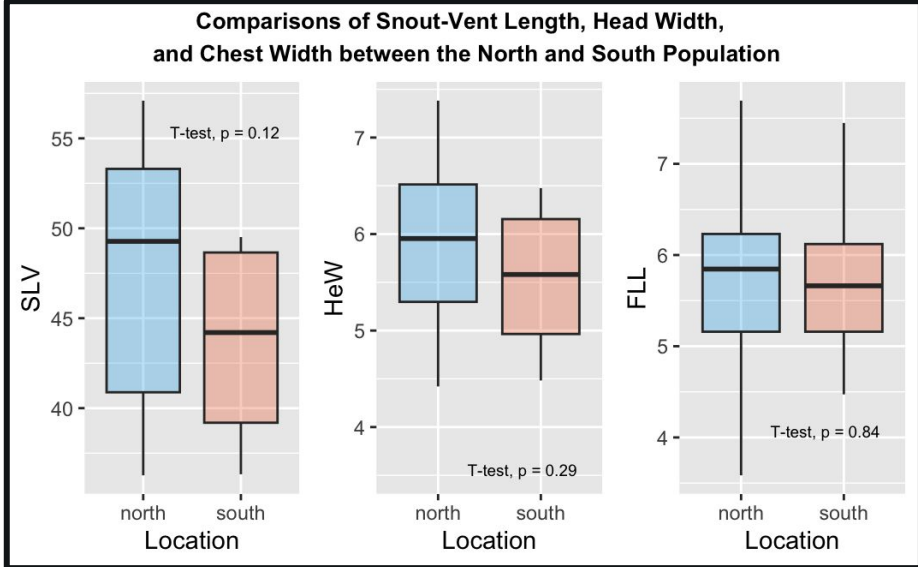


Figure 1: Boxplot of raw linear measurements.

This includes head width and limb length, traits which show interspecific divergence across genus *Batrachoseps*. In our data neither head width, nor limb length were significantly different, North: $\mu_{HeW}=5.87\text{mm}$, South: $\mu_{HeW}=5.54\text{mm}$ ($p_{HeW}=0.29$), North: $\mu_{FLL}=5.67\text{mm}$, South: $\mu_{FLL}=5.74\text{mm}$ ($p_{FLL}=0.84$).

After excluding the juvenile individuals and the specimens without location record (n = 32), the linear measurements was calculated based on the Procrustes coordinates. The 1st principal component (PC1) explained 53.44% of the variance, with head width, forefoot width, and snout-vent length as the top three contributing variables. The 2nd PC accounted for 17.22% of the variance, with hind limb length contributing the most. The 3rd PC accounted for 11.20% of the variance, contributed by the length of the longest toe. ANOVA against the population location did not show significant species effects for PC1 to PC5 ($p_{PC1}=0.40$, $p_{PC2}=0.56$, $p_{PC3}=0.14$, $p_{PC4}=0.43$, $p_{PC5}=0.52$).

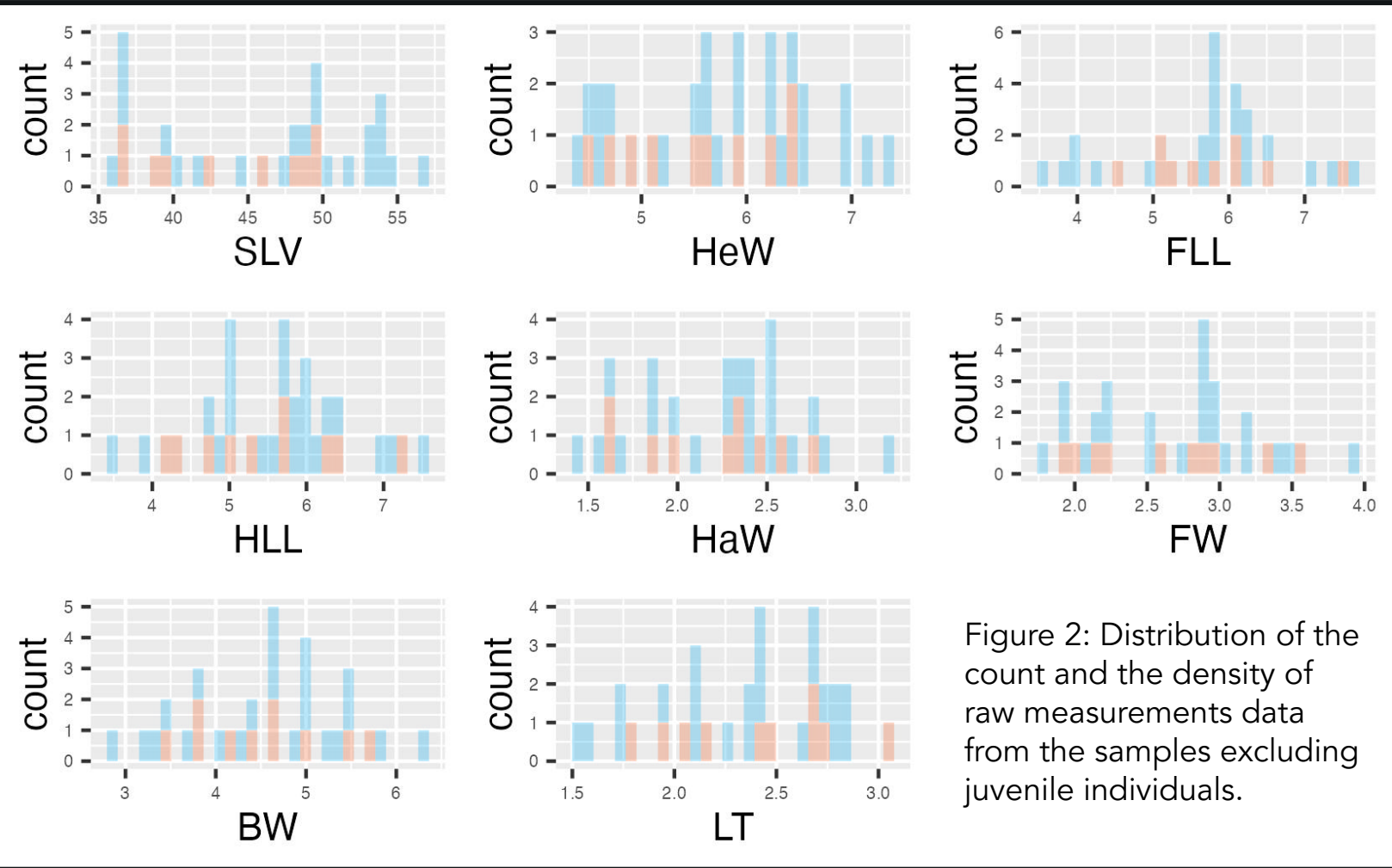


Figure 2: Distribution of the count and the density of raw measurements data from the samples excluding juvenile individuals.

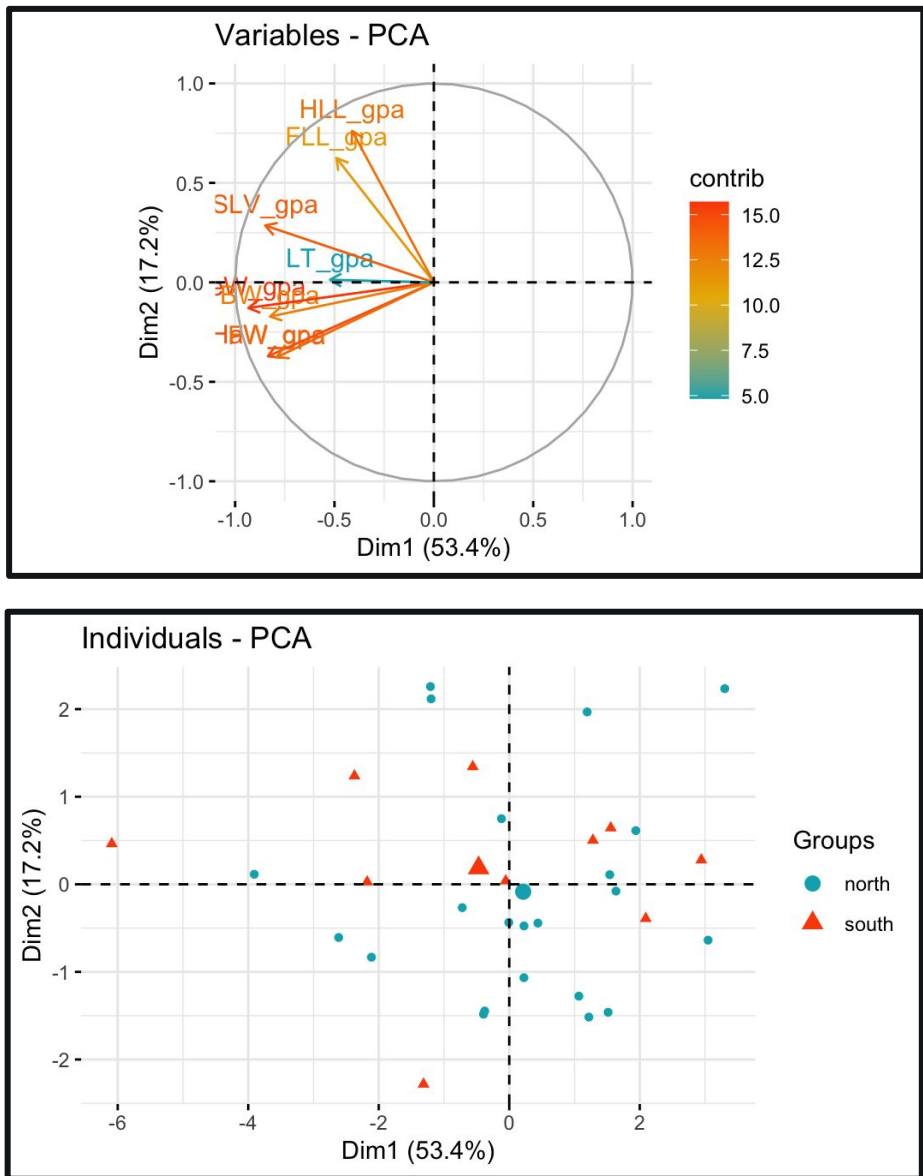


Figure 3: The correlation between each measurement and PC1 and PC2. Contribution of measurements to the projection of the data is represented by the gradient.

Figure 4: Principal components analysis showing the distribution of individual specimen from north and south population using PC1 and PC2.

Discussion

Based on our morphological measurements we do not find evidence that the northern and southern population of *B. stebbinsi* should be considered separate species. This was true for both raw size measures and for analysis of shape using a morphometrics approach (GPA). However, previous evolutionary analysis based on allozyme analysis data found that the two populations are highly divergent (Jockusch et al. 2012). This species does appear to have a disjunct range (see map) with no known records between Tehachapi Creek (northern population) and Tejon Canyon (southern population). *Batrachoseps stebbinsi* is found in both both damp oak woodland and xeric desert slopes but the relatively flat and dry Tehachapi Valley may be entirely unsuitable. The low vagility of *B. stebbinsi* means that migration across the dry valley is unlikely. This geographical barrier appears to be driving drives genetic divergence between the two populations, a pattern common in *Batrachoseps*. While our data do not show evidence of accompanying morphological divergence, ecological divergence was not considered here. One possible explanation is that the northern and southern habitat have similar environment such as similar, soil substrate, humidity, and are mainly covered by similar plants species. After the two populations formed, there is little change of the sources or the magnitude of selection pressure from the environment, so the phenotypes of the two population remain similar as genetic drift creates differences in genotypes.

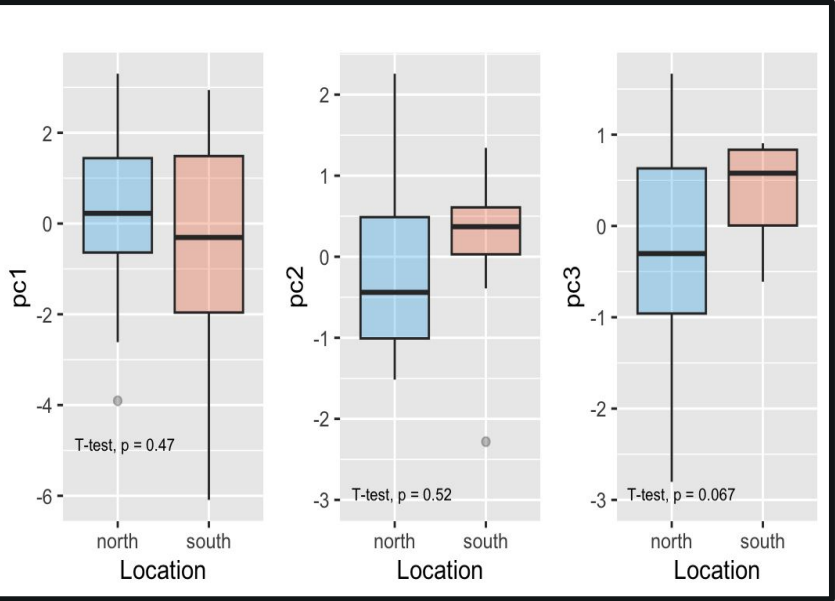


Figure 5: Boxplot of principal components 1 to 3 for Procrustes distances comparing northern and southern populations. No significant differences were detected between populations.

Table 1: Results of the principal components analysis. The top row shows the percent of the total variance explained by each principal component (PC1 to PC5). The second and third rows shows the results of the ANOVA testing for species effects. The remaining rows show loadings on each PC (PC1 to PC5).

	PC1	PC2	PC3	PC4	PC5
Proportion of Variance	53.44%	17.22%	11.20%	7.05%	4.78%
F	0.744	0.359	2.309	0.638	0.419
p-value	0.395	0.554	0.139	0.431	0.522
SVL_gpa	-0.411	0.244	-0.027	0.370	-0.071
HeW_gpa	-0.451	-0.110	0.066	0.035	-0.085
FLL_gpa	-0.237	0.533	0.193	-0.762	-0.087
HLL_gpa	-0.199	0.648	-0.359	0.331	0.291
HaW_gpa	-0.404	-0.317	-0.183	-0.004	0.262
FW_gpa	-0.382	-0.321	-0.147	-0.316	0.505
BW_gpa	-0.399	-0.146	-0.199	0.005	-0.747
LT_gpa	-0.253	0.011	0.857	0.266	0.115

Future directions

In the future, the capture process of the morphological data could be improved using a stereo camera system and 3D landmarks data. The 3D morphological data could provide more precise measurements of the limb length and foot width. Comparisons in color pattern across all known localities could be done to better understand adaptation to local conditions. We also hope to include images from live animals in the field as a way to increase sample size. A population genomic study for this species would allow us to understand morphological data in an evolutionary context and test whether observed morphological variation is the result of adaptation.

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