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# TWO SPECIES OR ONE? MORPHOLOGICAL DIVERSITY OF THE THREATENED, TEHACHAPI SLENDER SALAMANDER (CAUDATA: PLETHODONTIDAE *BATRACHOSEPS STEBBINSI*)

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## 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. This species is found in scattered populations from the San Andreas Fault to Walker Basin. Previous studies have found that northern and southern populations of this species have high levels of genetic, color, and size differences. It has been suggested that northern and southern populations of *B. stebbinsi*, divided by the Tehachapi Valley, could be separate species. We obtained morphological measurements from images of preserved specimens from northern (N = 27) and southern (N = 10) populations. No significant differences in shape or size were detected between these two populations. In future work, we will explore local adaptation, increase sample sizes, and incorporate the evolution of color variation across the range of this species.

## Introduction

Tehachapi Slender Salamander (*Batrachoseps stebbinsi*) belongs to a clade of 22-23 species (Caudata: Plethodontidae: Batrachosepini) in the genus *Batrachoseps* which are distributed along the Pacific coast of North America (Wake 2012, Jockusch et al. 2015). The dynamic tectonics of this region and low vagility of these small salamanders are considered the likely drivers of the high diversity in the genus (Jockusch et al. 2012). *Batrachoseps stebbinsi* is found only in mountain ranges of the southern Sierra Nevada and adjacent desert canyons. Their habitats are mainly damp environments in oak or mixed pine-oak forests. They are found in central-south California, with scattered populations in the Caliente Creek drainage at the juncture of the Sierra Nevada and the Tehachapi Mountains and in isolated canyons on the northern slopes of the Tehachapi Mountains from Tejon Canyon to Fort Tejon (Brame and Murray 1968). Northern and southern populations have a high level of genetic (Jockusch and Wake 2002), color, and size differentiations (Hansen and Wake 2005). These large genetic and morphological divergences suggest that the two populations of *B. stebbinsi* could be two species. They have been listed as a threatened species by the State of California and vulnerable species by International Union for Conservation of Nature (ICUN 2010). As with other amphibians, habitat loss is one of the significant threats *B. stebbinsi* currently facing. By conducting a throughout morphological analysis of all the existing specimens of *B.stebbinsi*, we can increase our understanding of the diversity within different populations of *B.stebbinsi*.

## Method

Measurements were taken from preserved specimens of *Batrachoseps stebbinsi* housed at Cheadle Center for Biodiversity and Ecological Restoration (UCSB), Museum of Vertebrate Zoology (MVZ, UC Berkeley), and the Natural History Museum of Los Angeles County (LACM) from dorsal and ventral digital images taken by cameras vertical 20-30 cm above the specimen. The images from MVZ were taken by Panasonic GF2, and the images from the Cheadle Center collection and LACM were taken by Olympus Tough TG-480. The dimensions of the MVZ collections' images are 4000 × 3000 pixels with a file size 6-7 mb, and the dimensions of the Cheadle Center and LACM collections' images are 3200 × 1800 pixels with the file size of 1-2 mb. The digital images of 40 *B.stebbinsi* specimens were collected, with 27 from northern populations, 10 from southern populations, and 3 without location information. 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) were taken 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 were excluded, and individuals with snout-vent lengths less than 35 mm were considered juvenile and excluded based on approximation from coexisting species *Batrachoseps nigriventris* (Stebbins et al. 2012). Generalized Procrustes Analysis (GPA) was performed using geomorph package in R on the landmarks coordinates to transfer and align them (Baken et al. 2021; Adams et al. 2022). 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 Principal Component 1 to Principal Component 5, using location as the fixed factor.

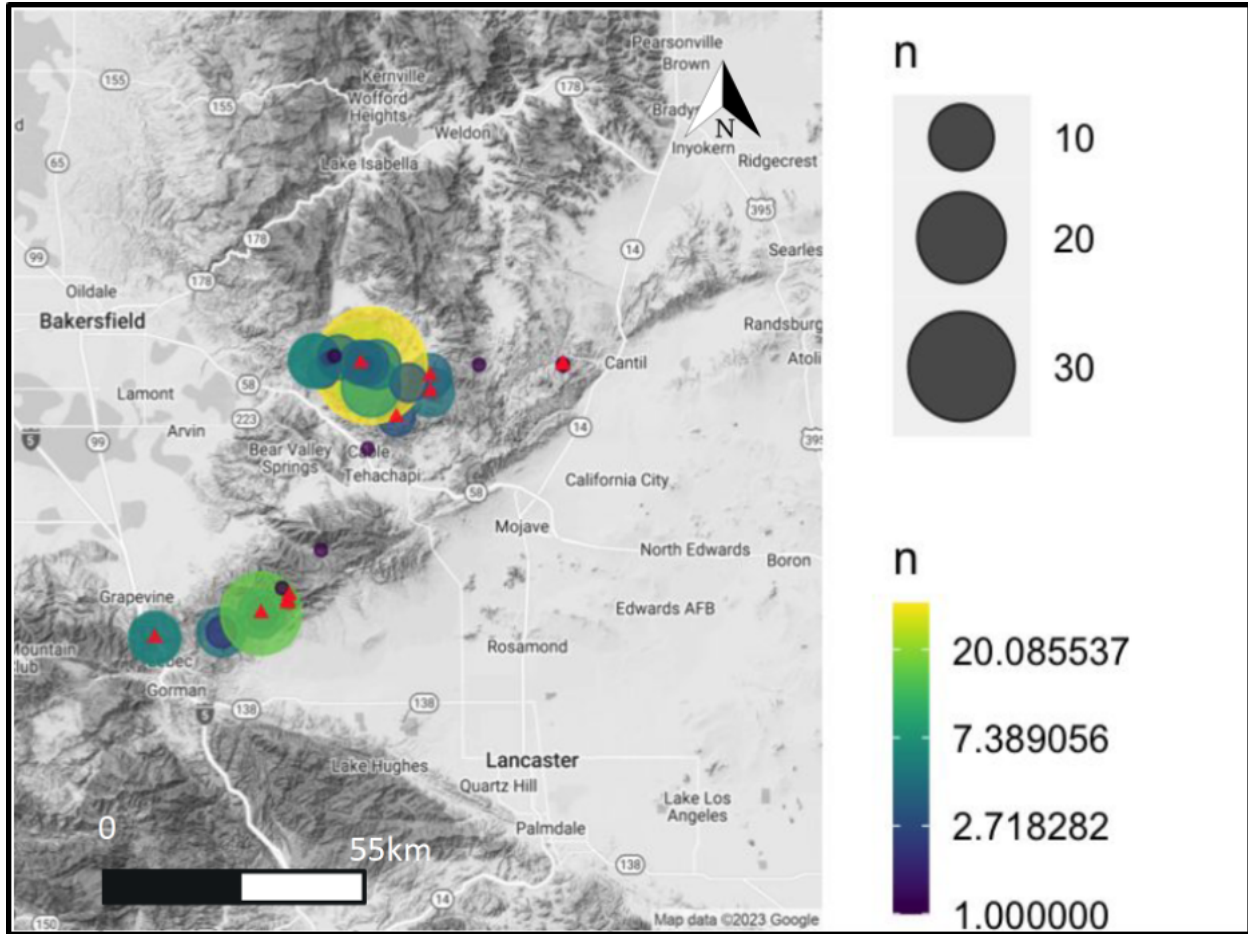


Figure 1. Known localities for *B. stebbinsi* and population. Both the color and the size of the sphere indicate the number of species discovered in the area. Triangles indicate populations that were used in morphological analyses in this paper. Note the gap in this species' known range in the vicinity of Tehachapi and Bear Valley Springs.

## Result

No linear measures used in this study were found to be significantly different between northern and southern populations. Snout-vent length had high variance but no significant difference (North:  $\mu_{\text{SLV}} = 47.2\text{mm}$  South:  $\mu_{\text{SLV}} = 43.5\text{mm}$ ,  $p_{\text{SLV}} = 0.12$ ). This includes head width and limb length, traits that show interspecific divergence across the genus *Batrachoseps*. In our data, neither head width nor limb length was significantly different, North:  $\mu_{\text{HeW}} = 5.87\text{mm}$ , South:  $\mu_{\text{HeW}} = 5.54\text{mm}$  ( $p_{\text{HeW}} = 0.29$ ), North:  $\mu_{\text{FLL}} = 5.67\text{mm}$ , South:  $\mu_{\text{FLL}} = 5.74\text{mm}$  ( $p_{\text{FLL}} = 0.84$ ).

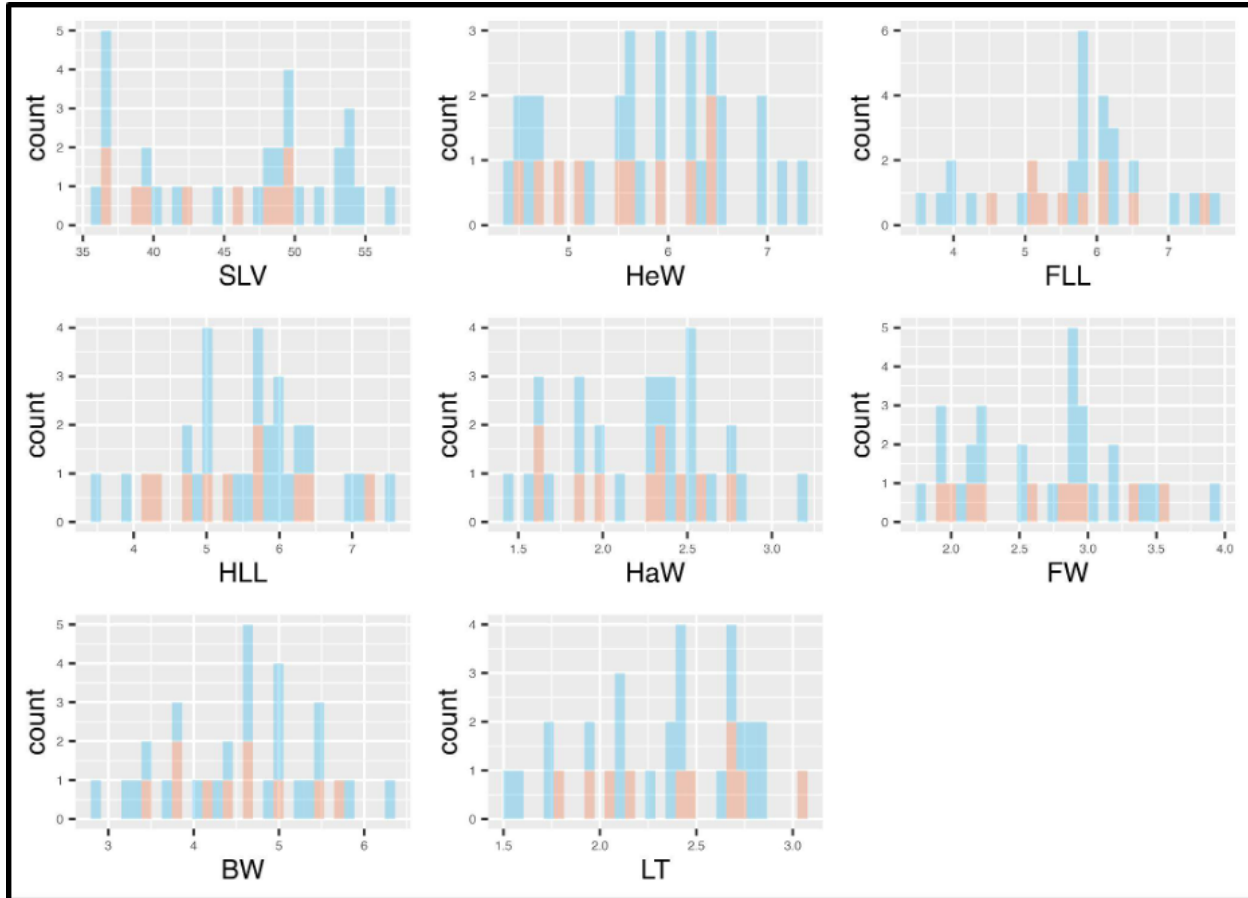


Figure 2. Distribution of the count and the density of raw measurements data from the samples ( $n = 32$ ) excluding juvenile individuals.

After excluding the juvenile individuals and the specimens without location records ( $n = 32$ ), the linear measurements were 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 ( $p_{PC1}=0.40$ ,  $p_{PC2}=0.56$ ,  $p_{PC3}=0.14$ ,  $p_{PC4}=0.43$ ,  $p_{PC5}=0.52$ ).

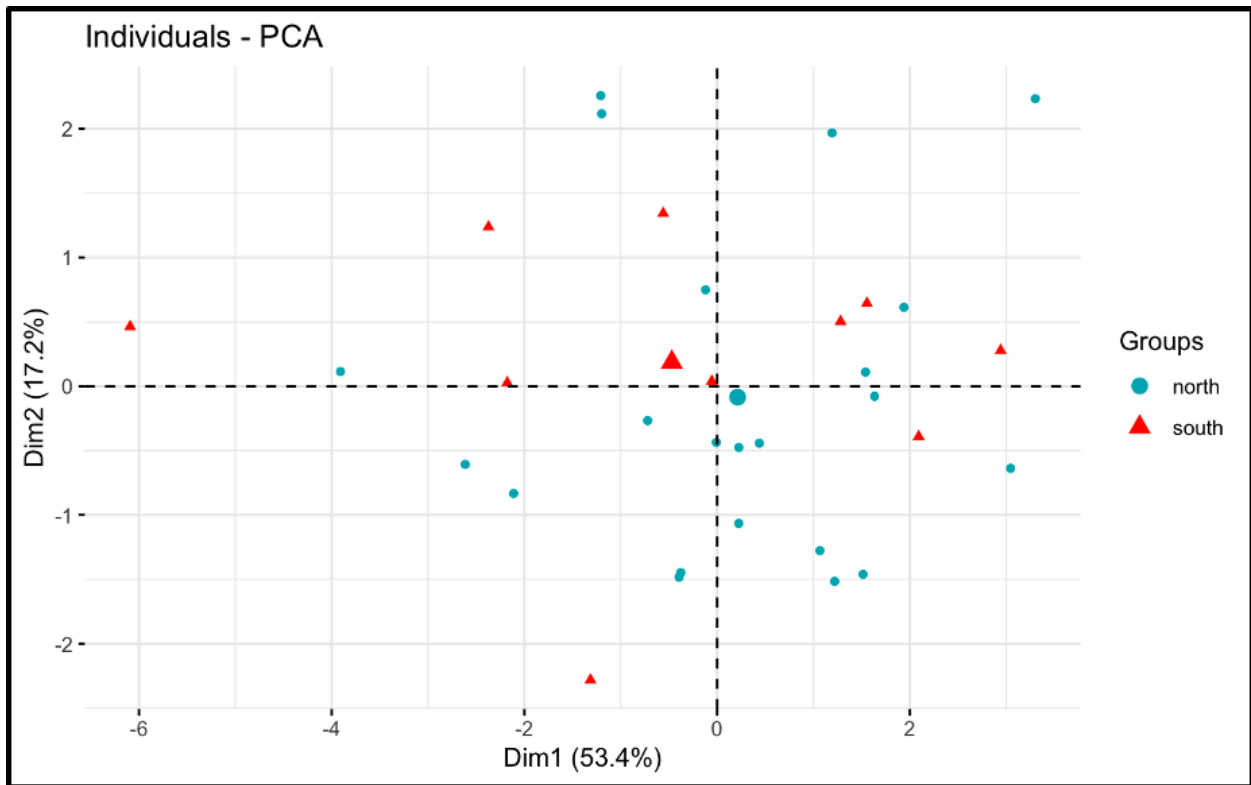


Figure 3. Principal components analysis showing the distribution of individual specimen. The red triangle represents the specimens from southern population, and the blue circle represents northern population. The x-axis is PC, and the y-axis is PC2.

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
SLV_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

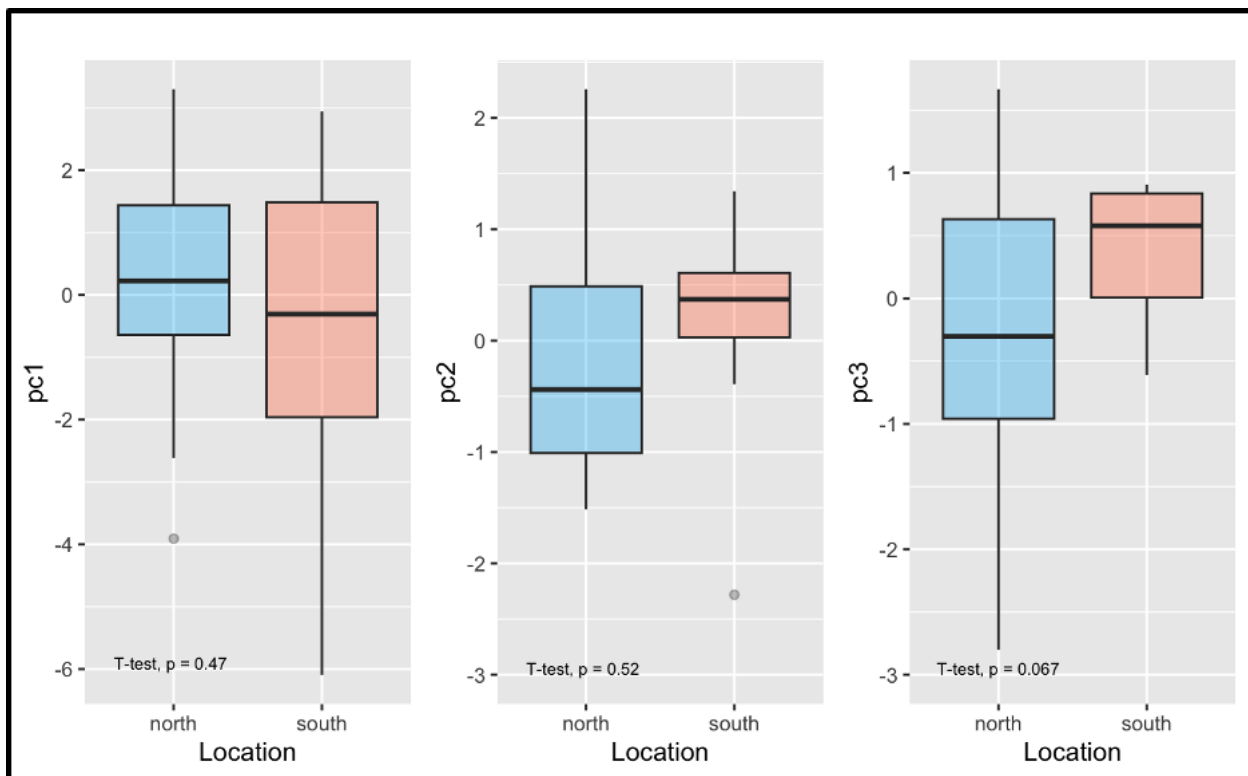


Figure 4. Boxplot comparing the morphological shape of northern and southern populations of *B. stebbinsi*. These values have been corrected for the influence of size. Scores are based on principal components 1 to 3 of Procrustes distances and explain nearly 82% of the shape variation in this species.

## Discussion

Investigating the diversity of the two major populations of *B. stebbinsi* is significant from the conservation aspect, as planned developments in the Tehachapi Mountains could disturb habitat and threaten a part of their populations. As some of its traits, such as lunglessness, sensitivity to heat and aridity, and a limited ability for long-distance dispersal, have contributed to the formation of high diversity of salamanders, those traits also make them extremely vulnerable to any changes in their habitat. It has been listed as a California State Threatened species, and it was also proposed for Federal listing in 2006 but was not listed federally (USFWS 2011). Based on our morphological measurements through images, we do not find evidence that the northern and southern populations of *B. stebbinsi* should be considered as separate species. Both raw body size measurements and analysis of shape using a morphometrics approach (GPA) did not demonstrate differences that are considered statistically significant. However, previous evolutionary analysis based on allozyme analysis data found that the two populations are highly divergent compared to other populations within one species (Jockusch et al. 2012). Furthermore, *B. stebbinsi* appears to have a disjunct range (fig.1) with no known records between Tehachapi Creek (habitat of northern population) and Tejon Canyon (habitat of southern population). The major types of habitat of the species are damp oak woodland and xeric desert slopes, while Tehachapi Valley is relatively flat and dry and therefore may be entirely unsuitable for *B. stebbinsi*. The low vagility of the species means that frequent migration across the dry valley is unlikely to happen. This geographical barrier appears to be driving genetic divergence between the two populations, which is also a pattern common in Batrachoseps, and it could further act as a condition of allopatric speciation. Shape diversity is anticipated to serve as an indication of adaptation to the local ecosystem. The influence of different characteristics of habitats, such as rocky talus or soil substrate, on morphological adaptations was not the focus of this study, and by studying those influence in the future, we can have a better understanding of what have shaped the morphological characteristics in each population. Based on the known divergence in color patterns between the two populations, comparisons of coloration combined with morphological measurements across all known localities could be done to better understand local adaptation. Images from live animals in the field will be a good way to increase the sample size as well as study the coloration.

To further explore this topic, a better understanding of the age and size of the sexual maturity of *B. stebbinsi* is still required. Additional evidence suggesting different sexual maturity sizes from the one applied in the method section would affect the result of this study, as the sexual maturity size was a criterion for including specimens in the analysis. In the study, the same sexual maturity size was assumed for the northern and the southern populations, which might be proved otherwise with further research. A difference between the two populations could change the conclusion while indicating developmental divergence between those populations.

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 can provide more precise



measurements of the limb length and foot width comparing to the 2D image data. A population genomic study for this species would allow us to understand morphological data in an evolutionary context and test whether the observed morphological variation is the result of adaptation.

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