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Resolving Questionable Records of *Pituophis ruthveni* (Louisiana Pinesnake)

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Abstract - *Pituophis ruthveni* (Louisiana Pinesnake) is considered one of the rarest snakes in North America. For that reason, *P. ruthveni* is not well represented in scientific collections, and each existing specimen is very important. Some museum records for the species are considered questionable or unverified, especially those that represent extralimital records or those from habitats not normally utilized by Louisiana Pinesnake. Clarifying these questionable Louisiana Pinesnake records will ultimately provide a better understanding of its historic and current distribution, which is necessary for listing decisions, critical-habitat designation, and overall conservation efforts for the species. To resolve this uncertainty, we performed a multivariate analysis using 13 morphological characters on 50 specimens representing 3 snake groups: (1) *P. ruthveni* ($n = 23$), (2) *P. catenifer sayi* (Bullsnake; $n = 23$), and (3) questionable or unverified snakes ($n = 4$). We included Bullsnake because they are sister to Louisiana Pinesnake genetically and also most morphologically similar. We identified all questionable records of Louisiana Pinesnake examined as Bullsnake. Blotch count, ventral-scale number, and scale-row number at mid-body were the most reliable characters for distinguishing between groups. These results have potential conservation implications for the species. The influence of these erroneous records could be substantial in future research and conservation of the species due to the relatively few known specimens of Louisiana Pinesnake. We recommend that the specimens we identified be annotated and considered erroneous records.

Introduction

Pituophis ruthveni Stull (Louisiana Pinesnake) has long been considered one of the rarest snakes in North America (Conant 1956, Rudolph et al. 2006, Stull 1940, Young and Vandeventer 1988). Prior to recent efforts to determine the status of the species, fewer than 100 records of Louisiana Pinesnake were represented in the literature or in museum collections (Rudolph et al. 2006, Thomas et al. 1976, Young and Vandeventer 1988). Currently, 246 Louisiana Pinesnake records are databased by the US Forest Service (USFS) and the US Fish and Wildlife Service (USFWS; J.B. Pierce, unpubl. data). This large, heavy-bodied snake inhabits pine savannahs of the Gulf Coastal Plain west of the Mississippi River, and is historically reported from 8 parishes in Louisiana and 12 counties in Texas (Dixon 2013, Dundee and Rossman 1989, Werler and Dixon 2000).

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Previous research concerning the conservation status of Louisiana Pinesnake has suggested that the loss of frequently burned pine savannahs, especially the *Pinus palustris* Mill. (Longleaf Pine) savannah ecosystem, is the driving factor of population declines, and this species is now widely considered one of the most imperiled snakes in the US (Reichling 1990, 1995; Rudolph et al. 2006; Young and Vandeverter 1988). Most of the Longleaf Pine ecosystem of the west Gulf Coastal Plains was lost by the 1930s due to heavy logging within the region, and it has failed to recover for a variety of reasons, including the decreased frequency of fire across the landscape (Frost 1993). It is estimated that <5% of the original extent of this open pine savannah ecosystem is extant, and much of that area is extensively altered by changes in fire regimes, silvicultural practices, and conversion to other land uses such as urban development and agriculture, which have further contributed to a reduction in suitable habitat (Frost 1993, Rudolph et al. 2006). These circumstances relegated remaining populations of Louisiana Pinesnake to isolated patches of remnant forests, primarily on public lands, within the historic range of the species (Reichling 1995; Rudolph and Burgdorf 1997; Rudolph et al. 1998, 2006).

Despite efforts to locate this species, it has only been found in 5 Louisiana parishes (Bienville, Natchitoches, Rapides, Sabine, and Vernon) and 4 Texas counties (Angelina, Jasper, Nacogdoches, and Newton) in the last 15 years (2000–2015) (Rudolph et al. 2006). Although recent research has greatly improved our understanding of Louisiana Pinesnake in regard to the natural history and current distribution of the species, the evaluation of older records is still needed to confirm its historic range. Louisiana Pinesnake specimens are not well-represented in scientific collections, and some museum records are considered questionable or unverified. Some of these records have been annotated, but uncertain records still exist. Resolving these records could have a significant impact on future research involving the conservation of this species.

Methods

To identify records that were considered questionable or unverified, we queried a database maintained by the US Forest Service's Wildlife Habitat and Silviculture Laboratory located at the Southern Research Station in Nacogdoches, TX, of all known Louisiana Pinesnake records. We identified 6 museum specimens of Louisiana Pinesnake for which the authenticity of the specimens was considered questionable or unverified (Table 1). These records included Louisiana Pinesnake specimens collected from Houston, Montgomery, and Walker counties, in Texas; and Beauregard, Calcasieu, and Jefferson Davis parishes, in Louisiana (Fig. 1). We determined that an additional specimen from Caldwell County, TX, was questionable, but it has since been resolved as a *Pituophis catenifer sayi* (Schlegel) Bullsnake (Thomas et al. 1976). We performed a morphometric analysis using 13 morphological characters on correctly identified Louisiana Pinesnake and Bullsnake specimens and on the aforementioned questionable snake specimens. We selected Bullsnake for this comparison because it is very similar morphologically and also considered sister to Louisiana Pinesnake (Rogriguez-Robles and Jesus-Escobar

2000). These characters, which are known to vary between species, included blotch count; rostral-scale length, height, and width; number of upper and lower labial scales, ventral scales, and subcaudal scales; snout-to-vent length (SVL); total body length (TBL); and scale-row number at neck, midbody, and tail (Reichling 1995, Thomas et al. 1976).

The entire morphological analysis could not be completed for 2 of the questionable Louisiana Pinesnake specimens. We could not examine the questionable

Table 1. Museum specimens of *Pituophis ruthveni* (Louisiana Pinesnake) examined in this study because the authenticity was considered questionable. MSU = McNeese State University, TCWC = Texas A&M University Biodiversity Research and Teaching Collections.

State	County or Parish	Collection date	Institution code	Catalog number	Sex	Specimen condition	Updated identification
Louisiana	Beauregard	20 Apr 1967	MSU	1339	Male	Tail only	Unknown
Louisiana	Jefferson Davis	Nov 1966	MSU	1274	Female	Good	Bullsnake
Louisiana	Calcasieu	2 Sep 1988	SML	9291	N/A	Photograph	Bullsnake*
Texas	Houston	5 May 1956	TCWC	14977	Female	Good	Bullsnake
Texas	Montgomery	Aug 1976	TCWC	81602	Female	Good	Bullsnake
Texas	Walker	24 Apr 1976	TCWC	52078	Male	Good	Bullsnake

*per Thomas et al. 1976.

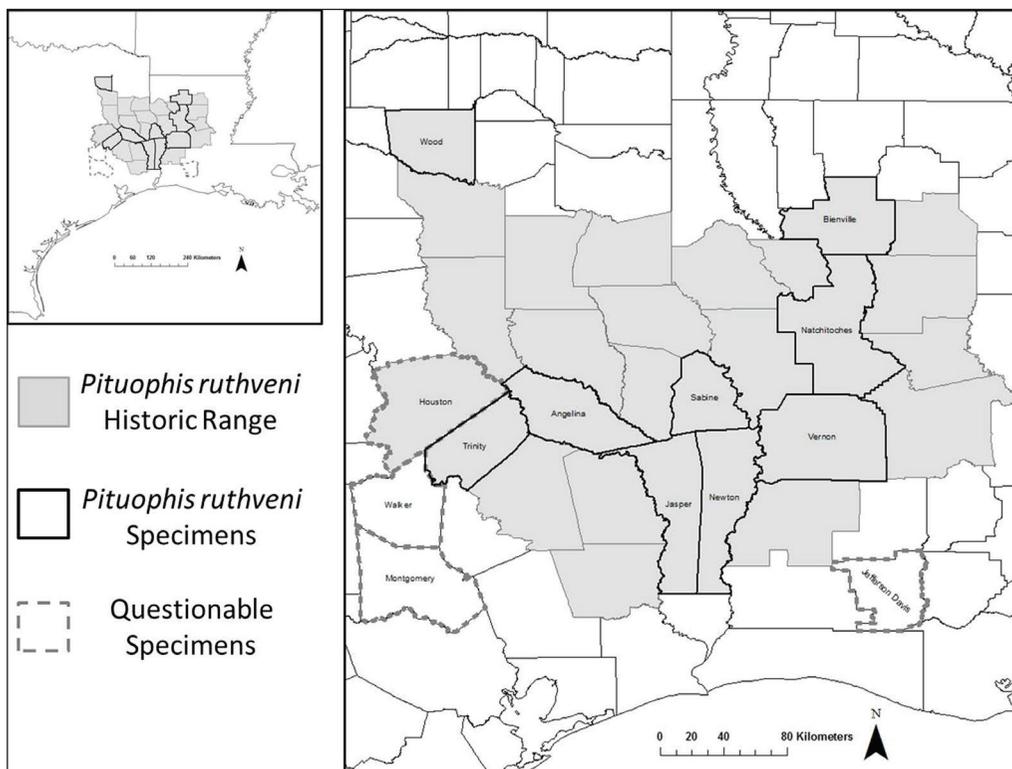


Figure 1. Map depicting specimen localities of verified and questionable *Pituophis ruthveni* (Louisiana Pinesnake).

Louisiana Pinesnake specimen from Beauregard Parish, LA, because of its poor condition (Table 1). We examined an additional questionable Louisiana Pinesnake specimen from Calcasieu Parish, LA, via photograph from the original collector, in which blotch count was the only measurement recorded (Williams and Cordes 1996). Based on blotch count only, we concluded that this specimen is most likely a Bullsnake, according to criteria described in Thomas et al. (1976; Table 1). After excluding these 2 specimens, we analyzed 50 specimens total: 4 considered questionable specimens of Louisiana Pinesnake (Table 1), 23 verified specimens of Louisiana Pinesnake, and 23 verified specimens of Bullsnake. We based our selection of verified specimens on key characteristics and distributions. We included only Bullsnakes collected from east of the Pecos River, in Texas, to avoid potential influence from western subspecies and to ensure sister relationship to Louisiana Pinesnake (Rodríguez-Robles and Jesús-Escobar 2000). We tested for differences between the Louisiana Pinesnake ($n = 23$), Bullsnake ($n = 23$), and questionable specimens ($n = 4$) groups described above using multivariate analysis of variance (MANOVA) followed by pairwise comparisons among groups using Hotelling's post-hoc tests of significance. We also used principal components analysis (PCA) to visualize the morphological characters that best distinguished between snake groups. Prior to these analyses, we used ordinary least-squares regression to conduct a residuals analysis that adjusted morphological characters for differences in the body size (i.e., snout-to-vent length) of each specimen. Pair-wise Pearson correlations among the size-adjusted characters revealed a significant correlation ($R^2 = 0.60$, $P < 0.05$) between rostral-scale height and length, so we excluded the former from our final morphometric analyses.

Results

The MANOVA revealed significant differences among groups of snakes (Wilks' Lambda = 0.95, $df_1 = 22$, $df_2 = 68$, $F = 6.93$, $P < 0.001$). Hotelling's pairwise comparisons indicated that the verified Louisiana Pinesnake specimens were significantly different from verified Bullsnake specimens ($P < 0.001$) and were also significantly different from the unverified snake specimens ($P < 0.01$). The unverified snake specimens were not significantly different from verified Bullsnake specimens ($P = 0.64$). This pattern of statistical significance between snake groups was apparent in the PCA (Table 2; Fig. 2). The first principal component axis explained 90.4% of the variation between snake groups with blotches, ventral scales, and, to a lesser extent, scales at midbody exhibiting the highest loadings (PCA loadings 0.73, 0.66, and 0.17, respectively). Along this component, verified Louisiana Pinesnake specimens were characterized by low blotch-counts, low ventral-scale counts, and low scale-counts at midbody. Verified Bullsnake specimens and unverified snake specimens exhibited high counts for each of those characters. Principal component 2 explained the remaining variation between groups; blotches, ventral scales, subcaudal scales, and tail-scale counts exhibited the highest loadings (PCA loadings: -0.49, 0.49, -0.46, and 0.51, respectively; Table 2).

Table 2. Loadings for each morphological character used in the principal components analysis (PCA) illustrating multivariate differences between the *Pituophis ruthveni* (Louisiana Pinesnake), *P. catenifer sayi* (Bullsnake), and unverified groups.

Morphological characters	Axis 1	Axis 2
Blotch count	0.733	-0.487
Rostral-scale length	-0.001	0.007
Rostral-scale width	0.048	0.054
Upper labial-scale number	0.025	0.108
Lower labial-scale number	0.010	-0.011
Scale-row number at neck	0.038	0.184
Scale-row number at midbody	0.166	0.066
Scale-row number at tail	0.016	0.509
Ventral-scale number	0.656	0.489
Subcaudal-scale number	-0.014	-0.456
Variance explained	90.4%	9.6%

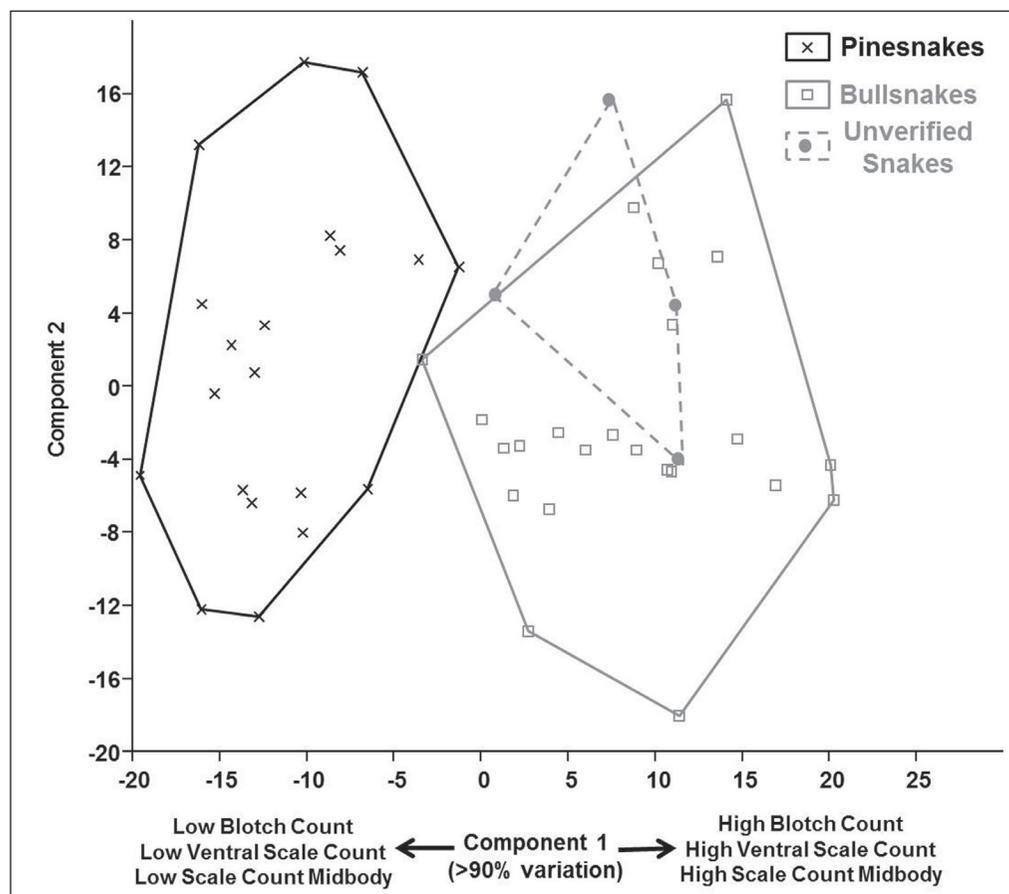


Figure 2. Results from principal components analysis (PCA) illustrating morphological multivariate differences between *Pituophis ruthveni* (Louisiana Pinesnake), *P. catenifer sayi* (Bullsnake), and unverified groups.

Discussion

Our examinations of questionable museum records indicated that the unverified specimens mentioned above are morphologically distinguishable from Louisiana Pinesnake and represent Bullsnake. Based on these findings, the specimen records from these counties and parishes (Houston, Montgomery, and Walker Counties, TX; and Jefferson Davis Parish, LA) should be considered erroneous. Our data also suggest that blotch count, ventral-scale number, and scale-row number at mid-body are reliable characters for distinguishing between groups. Similar findings concerning the taxonomy of snakes within the genus *Pituophis* exist in the literature (Conant and Collins 1998, Reichling 1995, Thomas et al. 1976, Werler and Dixon 2000, Wright and Wright 1957).

The provenance of these Bullsnake records is unknown. A large gap exists between known Bullsnake distributional records and the westernmost Louisiana Pinesnake records. It is unlikely that the Bullsnake specimens collected near the distribution of Louisiana Pinesnake represent extant or natural populations, but were probably errors made by the original collectors or represent escaped or released pets.

Clarifying these questionable Louisiana Pinesnake records is important for the conservation of the species. Erroneous records must not be used in future habitat models; the influence could be substantial due to the relatively few known Louisiana Pinesnake specimens (Wagner 2014). Knowledge of the species' correct historical distribution is vital to making informed listing decisions, critical habitat designation, and the overall conservation efforts for the species.

Natural-history collections provide researchers with a useful tool to understand the natural history and historical distributions of species (Shaffer et al 1998). However, there is still a need for researchers to identify questionable or unverified records, especially in rare species such as the Louisiana Pinesnake. There is a wealth of information residing on the shelves of natural-history museums and in their tissue collections that is underused or undervalued. Efforts to resolve questionable records of species of greatest conservation need through morphometric or genetic analyses (e.g., DNA barcoding) are necessary and important steps that will ultimately contribute to better understanding of the historic and current distributions of these species, as well as more informed decision making regarding policy, conservation, and management.

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