Project Title: Distribution, Habitat, and Species Status of the Queen Snake (*Regina septemvittata*) in Arkansas

Project Summary: The Queen Snake (*Regina septemvittata*) has the highest priority score of all SGCN reptiles in the state of Arkansas. The species is known from a small number of scattered localities in the state and little is known about its required habitat characteristics. The Arkansas population is also disjunct (>250 miles) from the rest of the species' range and the genetic uniqueness and species status of Arkansas *R. septemvittata* are unknown. We propose to: 1) conduct comprehensive and standardized surveys for *R. septemvittata* to establish the current distribution in Arkansas, 2) generate an occupancy model to determine the environmental factors and habitat characteristics that the species requires, and 3) collect DNA sequence data for *R. septemvittata* from throughout its range to assess the level of genetic divergence and species status of Arkansas populations. Our results will be beneficial to conservation efforts by identifying populations on which to focus conservation or management actions, determining what actions are likely to benefit the species, and resolving whether Arkansas populations warrant recognition as a distinct species.

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Project Budget: SWG amount requested: $55,491
Match amount provided: $30,951
Total amount of project: $86,442

PROJECT STATEMENT

Need

The Queen Snake (*Regina septemvittata*) is a medium-sized, semi-aquatic snake that ranges throughout much of eastern North America, from Michigan and southern Ontario southward to the Florida panhandle and southern Mississippi. A disjunct population of *R. septemvittata* occurs in Arkansas, principally in the Boston Mountains and Arkansas Valley ecoregions. This isolate is the only population of *R. septemvittata* west of the Mississippi River and is separated by >250 miles from the nearest eastern populations. The Mississippi River is a significant barrier to gene flow in many vertebrate species and is considered to have been the major driver of speciation in several closely related reptiles, amphibians, fishes, and mammals. For these reasons, Arkansas *R. septemvittata* may be genetically distinct from populations east of the Mississippi River and the two geographically isolated range segments may represent different species. An unpublished M.S. thesis by Weatherby (1974, Miami Univ.–Ohio) found Arkansas *R. septemvittata* were genetically unique from IL, OH, KY, and TN populations, supporting this could be the case.

Within Arkansas, *R. septemvittata* is known primarily from the Mulberry River in Franklin and Johnson counties, but scattered records exist from other systems such as Illinois Bayou, South Fork of the Little Red, Salado Creek, and Cadron Creek (Fig. 1). There is also an 1895 record for Hot Springs, Garland County. Due to the small number of localities and a paucity of recent records outside the Mulberry River, some experts have suggested that *R. septemvittata* may be nearing extirpation in Arkansas. In 2005-06, researchers from Arkansas State University conducted surveys at nine historical locations and 11 potentially new localities and found *R. septemvittata* at only two sites on the Mulberry River and two sites on Illinois Bayou. Since 2013, we have found *R. septemvittata* on the Mulberry River, Little Piney Creek, Illinois Bayou, Archey Creek, South Fork of the Little Red River, and Cadron Creek, indicating that the species still maintains populations on these rivers and may also persist in other locations.

According to the Arkansas Wildlife Action Plan, *R. septemvittata* has the highest priority score of all reptile species and is considered to be critically imperiled in Arkansas (S1 rank) with a decreasing population trend. *R. septemvittata* is a crayfish diet-specialist and historically inhabited clear, rocky streams, which have suffered from a variety of human impacts. Further distribution and abundance survey work are needed to fill data gaps and determine conservation actions. In addition, investigation of genetic and morphological characters is warranted to determine if the Arkansas population represents a distinct species.

Purpose and Objectives

The purpose of this project is to:

1) Establish the current distribution and identify required habitat characteristics of *R. septemvittata* in Arkansas.
2) Determine the species status of the isolated Arkansas population of *R. septemvittata* using genetic data.

The objectives of this project are to:

1) Conduct comprehensive and standardized surveys for *R. septemvittata* in Arkansas to establish the current distribution in the state.
2) Generate an occupancy model for *R. septemvittata* in Arkansas to determine the environmental factors and habitat characteristics that the species requires.
3) Collect DNA sequence data for *R. septemvittata* from across their distribution to assess the level of genetic divergence and species status of Arkansas populations.

Location

This project will occur primarily in the Boston Mountains and Arkansas Valley ecoregions. Counties include: Crawford, Franklin, Johnson, Pope, Conway, Faulkner, Van Buren, Cleburne, and Independence. River drainages include the Mulberry River, Little Piney Creek, Big Piney Creek, Illinois Bayou, and Cadron Creek in the Arkansas River basin and the Little Red River and Salado Creek in the White River basin (Fig. 1).

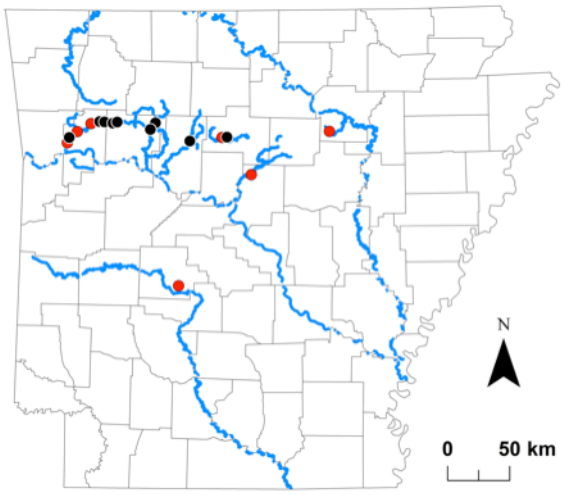

Fig. 1–Records for the queen snake (*Regina septemvittata*) in Arkansas. Red points are historic (pre-1990) and black points are recent (post-1990).

Approach

Distribution and Occupancy Modeling.—We will conduct repeated surveys at 60 sites along all rivers with records of *R. septemvittata* as well as nearby tributaries that appear to have suitable habitat. We will sample each site twice per year, for a total of four surveys per site. Each survey will consist of 4 man-hours of searching (visual, flipping rocks). All *R. septemvittata* will be sexed, measured for snout-vent length, tail length, and mass, and individually marked. We will also take a small tail clip from each individual for genetic analysis and examine snakes for clinical signs of snake fungal disease (SFD), an emerging pathogen negatively affecting snakes in the eastern U.S. We will measure habitat parameters (e.g., air and water temperature, stream width, depth, flow rate, substrate composition, conductivity, dissolved oxygen, canopy cover, crayfish abundance, etc.) during each survey and use GIS to calculate landscape characteristics (e.g., river drainage, slope, aspect, elevation, land-use, etc.) that may be important predictors of occurrence.

We will analyze presence/absence data from repeated surveys using an occupancy modeling approach in the program PRESENCE. This analysis will estimate overall proportion of sites occupied, while accounting for imperfect detection, as well as generate site-specific estimates of occupancy probability at locations where the species was not detected. Finally, we will use PRESENCE to rank a set of candidate models that include various combinations of habitat, landscape, and sampling period variables affecting occupancy and detection probability, which will allow us to determine those that are the best predictors of occurrence.

Conservation Genetics.—We will use molecular phylogenetic methods to compare DNA sequences of Arkansas *R. septemvittata* with samples from populations east of the Mississippi River in order to determine if the Arkansas population represents a distinct species. Tissue samples of *R. septemvittata* will be acquired through field collection, museum collections, and other researchers. Genomic DNA will be extracted using commercial DNA extraction kits. We will PCR and sequence the cytochrome *b* (cyt*b*) mitochondrial gene and the nuclear genes, NT3 and R35. These are commonly used markers in snake phylogeography and phylogenetic studies. We will infer phylogeographic relationships of *R. septemvittata*, calculate average sequence divergence between lineages within *R. septemvittata*, and estimate divergence times. If Arkansas and eastern U.S. populations of *R. septemvittata* are found to be well-supported, mutually exclusive groups in the phylogeny, and the level of genetic divergence is similar to or exceeds

that observed between other recognized species of snakes, then we will conclude that these two geographically isolated lineages represent different species.

Timeline.—

	2016	2017				2018		
Task	Oct-Dec	Jan-Mar	Apr-June	July-Sept	Oct-Dec	Jan-Mar	Apr-June	July-Sept
Population surveys	X		X	X	X		X	X
Molecular lab work		X	X	X	X	X	X	
Genetic analyses					X	X	X	X
Occupancy modeling								X
Prepare final report								X

Expected Results and Benefits

This project will produce the following results and benefits for *R. septemvittata*:

1) Comprehensive distribution map showing localities where *R. septemvittata* currently occurs in Arkansas. Not only will this establish the distribution in the state, but it will also allow us to assess whether the species has declined and identify extant populations on which to focus future conservation and management actions.
2) Conclusions from occupancy model identifying environmental conditions and habitat characteristics required by *R. septemvittata* in Arkansas. This knowledge will aid in diagnosing threats and setting conservation actions for the species as well as identify new localities with high potential for supporting additional populations.
3) DNA sequences for *R. septemvittata* and conclusions from genetic analyses evaluating the species status of the Arkansas population. This is important to determine so additional conservation efforts can be aimed at the Arkansas population if it is found to be a distinct population segment or species.

Budget (2 yrs: 10/01/2016 – 09/30/2018)

Expense	Party	SWG Funds	Matching Funds	Total
Salaries: Field work & Lab work	Graduate student (ULM)	$9,360	$0	$9,360
	Student worker (ULM)	$5,120	$0	$5,120
	Graduate student (UARK)	$10,740	$0	$10,740
	Student worker (UARK)	$5,522	$0	$5,522
	Shepard salary (ULM)	$0	$3,000	$3,000
	Willson salary (UARK)	$0	$4,047	$4,047
Travel: Field work	ULM	$5,220	$0	$5,220
	UARK	$3,780	$0	$3,780
Field supplies: sampling	ULM	$2,000	$0	$2,000
	UARK	$2,000	$0	$2,000
Lab supplies: extract DNA, PCR	ULM	$2,500	$0	$2,500
DNA sequencing	ULM	$2,000	$0	$2,000
Indirect costs (F&A: 10%)	ULM	$5,045	$15,638	$20,896
	UARK	$2,204	$8,266	$10,470
TOTAL		$55,491	$30,951	$86,442

QUALIFICATIONS

Donald B. Shepard obtained his Ph.D. from the University of Oklahoma in 2008, and completed postdoctoral training at Colorado State University and the University of Minnesota. He was an Assistant Professor at the University of Central Arkansas from 2013–15, and is currently an Assistant Professor at the University of Louisiana at Monroe. Don's research focuses on the evolutionary and ecological mechanisms by which species originate and diversify through time. His research employs molecular methods to determine population genetic and phylogeographic structure within species and to identify cryptic diversity. He has also worked extensively on the population ecology and conservation of the endangered eastern massasauga rattlesnake (*Sistrurus catenatus*) in Illinois. Don has authored or co-authored 40 scientific articles and is an Associate Editor for *Herpetological Conservation and Biology*.

John (J.D.) Willson joined the Department of Biological Sciences at the University of Arkansas, Fayetteville, in 2012 as an assistant professor after completing his Ph.D. at the University of Georgia and postdoctoral work at Virginia Tech. His research uses a combination of descriptive, experimental, and theoretical approaches to understand population and community dynamics of reptiles and amphibians within the context of pressing conservation issues such as habitat alteration, pollution, and invasive species. Current projects in his lab include long-term studies of semi-aquatic snake population and community dynamics, evaluations of the effects of intensive forestry on stream salamanders, and research on the ecology and management of invasive Burmese pythons in Florida. J.D. has authored or co-authored over 50 scientific articles and book chapters and recently co-authored a popular book on invasive pythons. He also serves as editor of snake natural history notes for *Herpetological Review*.

Kelly J. Irwin obtained his M.S. in Wildlife & Fisheries Science from Texas A&M University in 1997. He has worked on amphibian and reptile conservation and management projects as the Arkansas Game and Fish Commission Herpetologist for 16 years and serves on the Board of Directors of the Center for North American Herpetology.