PROJECT TITLE: Determining the taxonomic status of Least Darter, *Etheostoma microperca*, populations in Arkansas

PROJECT SUMMARY: The Least Darter occurs in tributaries to the Hudson Bay, Great Lakes, and upper Mississippi River drainages, with additional disjunct populations in the Ozarks. Previous morphological and molecular studies revealed that Ozark populations of the Least Darter are differentiated from northern populations, yet the taxonomic status of the Ozark populations remains undetermined. This project will examine morphological variation of Ozark populations in relation to northern populations with the goal of elucidating the taxonomic status of distinct Least Darter populations in Arkansas. In combination with morphological data, DNA sequences from multiple single-copy nuclear genes and one mitochondrial gene will be used with species delimitation and species tree methods to evaluate hypotheses of species distinctiveness among populations of the Least Darter. Results of this study will provide information about the taxonomic status of the Least Darter in Arkansas, which is currently considered a Species of Greatest Conservation Need.

PROJECT LEADER:

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PROJECT BUDGET:

SWG AMOUNT REQUESTED -	**$50,387**
MATCH AMOUNT (35%) -	**$27,997**
TOTAL AMOUNT -	**$78,384**

PROJECT STATEMENT:

Need: The Least Darter, *Etheostoma microperca*, occurs in tributaries to the Hudson Bay, Great Lakes, and upper Mississippi River drainages, with additional disjunct populations in the Ozarks and the Blue River in Oklahoma (Etnier and Starnes 1993). In Arkansas, *E. microperca* has a narrow distribution in the upper reaches of the Illinois River drainage in the northwestern corner of the state (Fig. 1). Robison and Buchanan (1988) listed the species as very rare in Arkansas, inhabiting only a few springs with permanent flow and gravel substrate. A survey for *E. microperca* in Arkansas by Harris and Smith (1985) revealed two new localities for the species, but failed to locate the species at historic localities in Osage and Wildcat creeks. Recent surveys by Wagner et al. (2012) suggested that *E. microperca* may be extirpated from historic localities in Wildcat Creek, Clear Creek, and Elkhorn Spring; however, new populations were discovered in Flint Creek and in a small spring tributary to Clear Creek. Given its rarity in Arkansas, and susceptibility to degradation of preferred spring habitats, *E. microperca* is listed as a species of greatest conservation need in the state (Anderson 2006; Wagner et al. 2012).

Analysis of morphological variation by Burr (1978) revealed that *E. microperca* is a highly variable species; Ozark populations exhibit substantial divergence with respect to northern populations. However, Burr (1978) found that the Blue River population in Oklahoma was more similar to northern populations than to neighboring Ozark populations. Burr (1978) noted that Ozark and Blue River populations were in the process of genetic differentiation from northern populations, but inconsistencies in morphological variation precluded elevation to the species or subspecies level. Buth et al. (1980) analyzed variation for 47 allelic products (allozymes) for *E. microperca* and found a similar pattern of genetic differentiation between Ozark and northern populations. Recently, Echelle et al. (2015) used DNA sequences from one nuclear and two mitochondrial genes to evaluate phylogenetic relationships among populations of *E. microperca*. Echelle et al. (2015) revealed that the Illinois River population was reciprocally monophyletic with respect to and all other *E. microperca* populations, diverging approx. 5.4 million years ago. In conjunction with this deep divergence, Echelle et al. (2015) suggested that the Illinois River populations in eastern Oklahoma and northwestern Arkansas and the Shoal Creek population in southwestern Missouri represented two undescribed cryptic species that required further analysis with nuclear DNA markers to determine their taxonomic status.

Resolving taxonomic uncertainty is a top priority for conservation planning and management of imperiled organisms (Allendorf and Luikart 2009). Given the morphological and genetic differences that have been uncovered between Ozark and northern populations of *E. microperca*, a formal taxonomic evaluation of Ozark and Blue River populations of the species is needed. Due to the rarity and potential decline of Illinois River populations of *E. microperca* in Arkansas (Harris and Smith 1985; Robison and Buchanan 1988; Wagner et al. 2012), determining the taxonomic distinctiveness of these populations is of utmost importance for future conservation planning.

Purpose and Objectives**:** The purpose of this study is to determine the taxonomic status of Illinois River populations of *E. microperca* in Arkansas. The objectives of this project are as follows: (1) examine morphological variation of Ozark populations in relation to each other, Blue River populations, and northern populations; (2) generate DNA sequence data from multiple single-copy nuclear genes and one mitochondrial gene and use species delimitation and species tree methods to evaluate hypotheses of species distinctiveness among populations of *E. microperca*; (3) provide an updated taxonomic revision of *E. microperca*.

Location**:** The primary focus of this study are *E. microperca* populations found in the Illinois River drainage in northwestern Arkansas (Benton and Washington counties; Fig. 1). However, comparative material will be examined throughout the range of *E. microperca*. In most cases, specimens for morphological examination will be obtained via loans from museum collections. In some cases, examination of type material may require travel to museum collections for on-site examination. When possible, tissue samples for DNA analysis of *E. microperca* will be obtained from museum collections. However, field sampling for fresh tissues and/or specimens will be conducted when they are not available from museum collections or if gifted tissues are degraded.

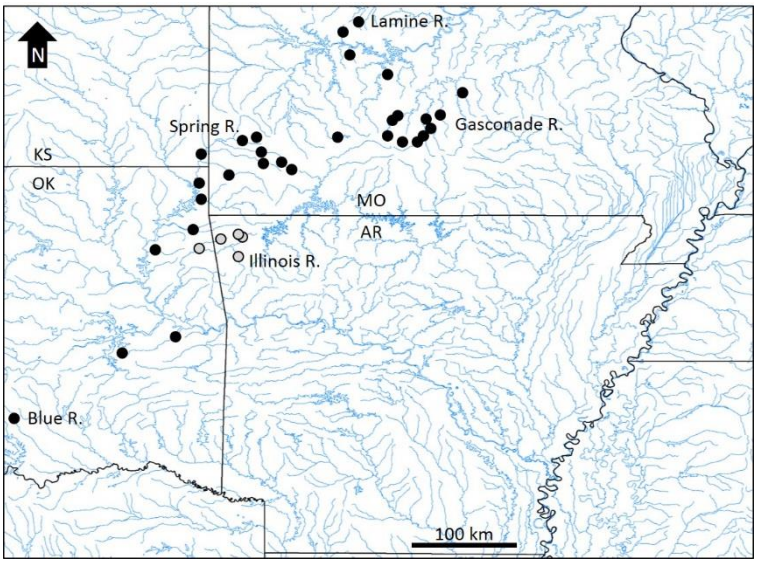

Figure 1. Map showing historic distribution of Ozark and Blue River populations of *Etheostoma microperca*; northern populations not shown for simplicity. Focal populations in the Illinois River drainage are shown with gray circles.

Approach: Morphological variation will be examined for standard meristic and mensural characteristics following Hubbs and Lagler (1958) and Page (1983). Body shape variation will be evaluated using geometric morphometric techniques. In brief, the process involves imaging the left lateral side of each specimen, digitizing homologous landmarks using tpsDIG (Rohlf 2004), and performing Procrustes superimposition and multivariate analyses of body shape using the Integrated Morphometrics Package (CoordGen, PCAgen, CVAgen; Sheets 2001).

DNA will be extracted from a subset of individuals (30-50 total), and DNA analyses will attempt to include samples from the following clades defined by Echelle et al. (2015): (1) Illinois River- Clear, Flint, Little Osage creek systems; (2) Blue River/Ozark- Blue River, Gasconade River, Lamine River, Spring River, HaHa Tonka Spring; (3) Shoal Creek; and (4) Northern Clade- upper Mississippi River and Lake Huron drainages. Up to six single-copy nuclear genes and one mitochondrial gene will be sequenced for all individuals (see Bossu and Near 2009). The multi-locus dataset will be used with newly developed species tree and species delimitation methods to test alternative hypotheses of species distinctiveness among populations of *E. microperca* (see Harrington and Near 2012). Both morphological and molecular data will be used to provide an updated taxonomic revision for *E. microperca*.

Expected Results and Benefits: Results of this study will provide an updated taxonomic revision of *E. microperca*, with special emphasis on highly diverged Illinois River populations in Arkansas. These data will serve as a foundation for conservation and management decisions for *E. microperca* in Arkansas. Results of this study will be disseminated in the form of presentations at professional meetings, annual and final written reports, and/or peer-reviewed journal publications.

Proposed start date: October 1, 2017 **Completion date: September 30, 2019**

Budget:

	Budget Justification	SWG Year 1	Match Year 1	SWG Year 2	Match Year 2	SWG Total	Match Total
PERSONNEL							
Graduate Student	$1,400/month (12 mo, yr 1; 9 mo, yr 2)	$16,800		$12,600		$29,400	
Brook Fluker (PI)	0.7 month time during 2 academic years		$4,575		$4,575		$9,149
FRINGE BENEFITS							
Brook Fluker (PI)	Faculty Fringe (34.82% of matched salary)		$1,366		$1,366		$2,732
SUBTOTAL PERSONNEL & FRINGE		**$16,800**	**$5,941**	**$12,600**	**$5,941**	**$29,400**	**$11,881**
TRAVEL							
Travel to museums	4000 miles at $0.42/mile	$840		$840		$1,680	
Lodging	12 rooms at $100/night	$600		$600		$1,200	
Travel to field sites	2500 miles at $0.42/mile	$525		$525		$1,050	
Conference Travel		$500		$500		$1,000	
TRAVEL SUBTOTAL		**$2,465**		**$2,465**		**$4,930**	
MATERIALS, SUPPLIES & SERVICES							
Specimen supplies, DNA sequencing		$3,000		$1,000		$4,000	
MATERIALS, SUPPLIES & SERVICES SUBTOTAL		**$3,000**		**$1,000**		**$4,000**	
Graduate Student Tuition	4 semesters, 8 hours each @ $257/credit hr	$4,112		$4,112		$8,224	
MODIFIED TOTAL DIRECT COSTS		**$22,265**		**$16,065**		**$38,330**	
INDIRECT COSTS (10%)		**$2,227**		**$1,607**		**$3,833**	
	Waived Indirect costs (29.73% [ASU rate is 39.73%])		**$6,619**		**$4,776**		**$11,396**
	39.73% of matched salary, fringe waived		**$2,360**		**$2,360**		**$4,720**
Totals		**$28,604**	**$14,920**	**$21,784**	**$13,077**	**$50,387**	**$27,997**

QUALIFICATIONS:

Brook L. Fluker: Received Ph.D. in Biology from the University of Alabama in 2011 with an emphasis in phylogenetics, population genetics, and conservation of freshwater fishes. Has 13 years of experience sampling, handling, and collecting tissues from freshwater fishes in North America, including experience assisting with surveys of freshwater mussels and snails and seasonal abundance surveys for several federally protected fishes. This work has resulted in six publications, 10 technical reports, and 40+ presentations at professional conferences and meetings.

Brian Wagner: Brian Wagner is the Nongame Aquatics Biologist with the Arkansas Game and Fish Commission. He has a Bachelor's Degree in Systematics and Ecology from the University of Kansas, a Master's Degree in Fisheries from Virginia Tech, is a AFS Certified Fisheries Professional, and has been involved in aquatic conservation and research with the Commission for 28 years. For the past 19 years, he has been the Commission's Nongame Aquatics Biologist. Brian coordinates the Commission's Nongame Aquatics Program and has specific oversight of nongame fish and crayfish efforts. He is a Certified Fisheries Scientist and has authored or co-authored peer-reviewed publications on sport fish, nongame fish, crayfish, reptiles, and amphibians. Brian leads the State Wildlife Grants Crayfish Taxa Team, and is also active on the Fish, Cave, and Invertebrate Taxa Teams.

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