Kentucky Creekshell (*Leaunio ortmanni*) Report

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submitted by

Office of Kentucky Nature Preserves

Frankfort, Kentucky

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Leaunio ortmanni (Clear Fork Creek, Logan Co., Ky)

To

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Introduction

The Kentucky Creekshell, *Leaunio ortmanni* (Walker 1925), is an imperiled mussel (OKNP 2019) and has been petitioned as a candidate species for federal listing. The Kentucky Creekshell is a small mussel that occurs in a wide range of stream habitats, but primarily in stable habitat patches with a mixture of pebble, gravel, and sand substrates, with constant flow. The species is endemic to the Green River drainage in central Kentucky (Haag and Cicerello 2016) and the upper headwater tributaries of the Barren River system (Green River drainage) in northern Tennessee (Dinkins et al. 2018).

A closely related species to *L. ortmanni* is the Mountain Creekshell, *Leaunio vanuxemensis* (Lea 1838). The Mountain Creekshell has a historical distribution that ranges throughout the Tennessee River basin and from the Stones River system downstream to the Eddy Creek system in the lower Cumberland River drainage. These two taxa have never been the focus of a molecular genetic study, but a few specimens were analyzed by Kuehnl (2009) during the revision of the *Villosa* complex. Kuehnl (2009) suggested that the populations *L. vanuxemensis* in the lower Cumberland River drainage (Red River system) were synonymous with the populations of *L. ortmanni* in the Green River drainage, indicating the distribution of *L. ortmanni* extends beyond the Green River drainage and into the lower Cumberland River drainage. Based on the data from Kuehnl (2009), Watters (2018) indicated this result, but did not present any new evidence to support it. Furthermore, Watters (2018) recognized the population of *L. vanuxemensis* from the Little River system of the lower Cumberland River drainage as a unique species and formally described it as the Dwarf Rainbow, *Leaunio pataecus*. If Watters (2018) is followed, the lower Cumberland River drainage from the Stones River downstream would have three recognized *Leaunio* species present, with *L. vanuxemensis* in the Stones River and Harpeth River systems, *L. ortmanni* in the Red River system, and *L. pataecus* in the Little River system, with historical records of *L. vanuxemensis* from Eddy Creek present, but not confirmed.

To properly review the status of *L. ortmanni* clarification of the *Leaunio* species present in the Green River and lower Cumberland River drainages is needed. Stream surveys of river systems in the two drainages were conducted between 2018-2022 to update occurrences and to obtain tissue samples for genetic analysis. The primary goals were to determine if the *Leaunio* ortmanni populations of the Green River were synonymous with the *L. vanuxemensis* populations of the lower Cumberland River.

Methods

Mussel surveys were conducted in the Green River and lower Cumberland River drainages in Kentucky and Tennessee. Specific streams and habitats surveyed were based on historical accounts and the likelihood of containing focal species. Surveys were conducted under workable stream conditions that provided safe and favorable conditions for successful discovery of live individuals. Surveys ranged from 1-6 person crews. Survey duration varied based on the stream size and available habitat, with the total person-hour search recorded. A catch per unit effort (CPUE) for each river system was calculated as the total abundance (focal species)/total person-hour search time. Total length measurements of all live native freshwater mussels were recorded. Occasionally, gravid females were encountered and delivered to biologist at the Kentucky Department of Fish and Wildlife Center for Mollusk Conservation in Frankfort, Kentucky. Augmentation of some populations from successful propagation efforts were made. All progenies were placed in stream systems from where the brood stock originated. Dates, location, and abundances of augmentation efforts were made. In addition, genetic tissue samples or

whole specimens were sent to the Freshwater Mollusk Conservation Center at Virginia Tech University for molecular genetic analysis.

Results

Over 200 live *Leaunio* spp. individuals were encountered from 135 stream surveys from nearly 100 sites conducted within the Green River and lower Cumberland River drainages (Table 1). A total of 78 *Leaunio ortmanni* were present at 13 sites in the Green River drainage, with all of the historical river systems having at least two live individuals present. The Rough River system was the only system with a marginal CPUE, in which 1 individual could be found in approximately 2 hours of searching. All of the other river systems in the Green River drainage required more search effort, ranging from approximately 4-50 hours of searching for the discovery of one individual. A total of 156 *L. vanuxemensis* individuals were encountered from three river systems in the lower Cumberland River drainage. The species is persistent in the upper Red River system; however, most individuals came from one stream reach, which inflates the CPUE value.

Table 1. Mussel surveys results.

Drainage	System	Sites	Sites (present)	Surveys	Effort (man-hour)	Abundance	CPUE
Green							
	Rough River	6	3	10	48	29	0.604
	Barren River	23	5	26	118	33	0.280
	Nolin River	18	3	26	104	11	0.106
	Green River mainstem	6	2	7	142	3	0.021
	Russell Creek	9	1	22	100	2	0.020
Cumberland							
	Eddy Creek	4	0	8	26	0	0.000
	Little River	12	3	16	64	19	0.297
	Red River	10	7	15	88	113	1.284
	Harpeth River	0	0	0	0	0	NA
	Stones River	5	2	5	26	24	0.923

	93	26	135	716	234

Molecular genetic analysis of the ND1 mitochondrial DNA gene and 10 nuclear DNA microsatellites was conducted by Dr. Jess Jones and Katie Ortiz at Virginia Tech University. The *ND1* mitochondrial DNA gene was sequenced from 150 individuals and obtained 13 sequences from GenBank (*L. ortmanni N*=13, *L. vanuxemensis N*=78, *C. iris N*=72) from the Green, lower Cumberland, and upper Tennessee river drainages. Two divergent mtDNA lineages were observed but were not concordant with the geography of the nominal taxa (i.e., both lineages occurred in each basin) and exhibited haplotype sharing, with minimal divergence occurring among all three taxa. Resulting in the *ND1* gene not differentiating between *Leaunio ortmanni* and *L. vanuxemensis*, with many of the *C. iris* haplotypes from the upper Tennessee River basin intermingled within the clades containing these two taxa. However, the phylogenetic analysis utilizing nuclear DNA microsatellites clearly divided *L. ortmanni* from the Green River and *L. vanuxemensis* from the Cumberland drainages into two well-diverged and distinct clades (Figure 1). An assignment test-based algorithm implemented in program STRUCTURE also supported *K*=2 across the Green and Cumberland River drainage divide.

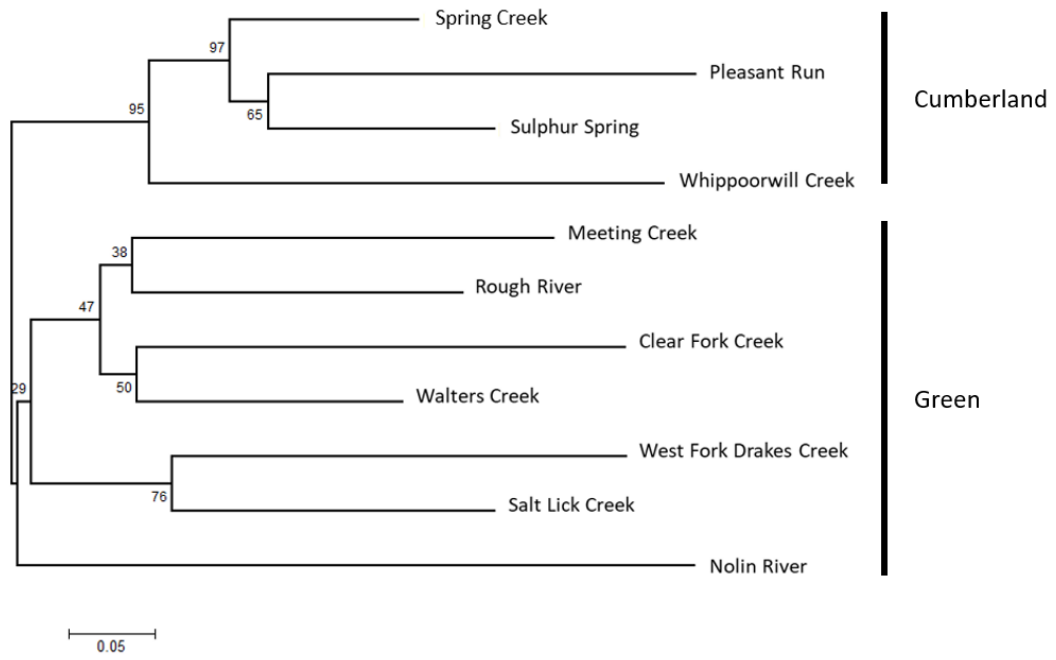

Figure 1. Neighbor-joining (NJ) tree showing relationship between investigated populations of *Leaunio ortmanni* and *Leaunio vanuxemensis*; tree was constructed from DNA microsatellite allele frequency data using Nei's standard genetic distance (D_{ST}).

Overall, genetic analyses suggested that *L. ortmanni* and *L. vanuxemensis* be recognized as separate species and retain their historical distributions until further analyses are completed. *Leaunio ortmanni* still occurs in all of the river systems in the Green River drainage; however, populations appear to have diminished greatly in spatial extent, as well as abundance in each of the river systems. The populations of *L. vanuxemensis* in the lower Cumberland River drainage also appear to have the same fate, except for a couple localized stream reaches in which dozens of specimens can be found in a short time. Augmentation of populations in both drainages could be beneficial for the persistence of the two species; however, it is highly recommended that propagation efforts ensure that progenies are returned to the localities from where the broodstock were collected until further genetic analyses are conducted.