

# **Freshwater Mussel Genetics Study**

## **In Monticello Reservoir Parr Hydroelectric Project (FERC No. 1894)**

**Fairfield and Newberry Counties, South Carolina**



*Monticello Reservoir Shoreline Habitat*

**Prepared For:**

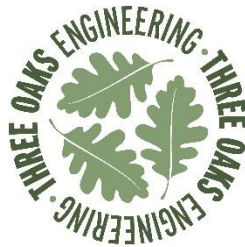
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# TABLE OF CONTENTS

1.0	INTRODUCTION .....	1
2.0	METHODOLOGY .....	1
2.1	Additional Specimen Collection .....	2
2.2	DNA Analysis .....	2
3.0	RESULTS .....	3
4.0	CONCLUSIONS.....	6
5.0	LITERATURE CITED .....	7

Appendix A: Monticello Reservoir Mussel Survey Report

Appendix B: Villosa Genetics Study Results

## 1.0 INTRODUCTION

The Parr Hydro Project (FERC No. 1894) consists of the Parr Shoals Development and the Fairfield Pumped Storage Development; both are located along the Broad River in Fairfield and Newberry Counties, South Carolina. The Parr Shoals Development forms the lower reservoir, Parr Reservoir, along the Broad River. The Fairfield Pumped Storage Development is located directly off of the Broad River and forms the 6,800-acre upper reservoir, Monticello Reservoir, with four earthen dams. The Fairfield Development has a licensed capacity of 511.2 MW and is used for peaking operations, reserve generation, and power storage and usage.

Three Oaks was retained by Kleinschmidt to perform freshwater mussel surveys in the Monticello Reservoir in Fairfield and Newberry Counties, South Carolina in the summer/fall of 2015 for the Monticello Reservoir Parr Hydroelectric Project, a SCANA Corporation (SCANA) Federal Energy Regulatory Commission (FERC) project (FERC No. 1894). The details of the survey efforts were presented in the Freshwater Mussel Survey Report for Monticello Reservoir submitted to Kleinschmidt in April 2016 (Appendix A).

Freshwater mussels were consistently found throughout the reservoir; the fauna was comprised of six species, most of which are considered common. However, a total of seven individuals identified as the Carolina Creekshell (*Villosa vaughaniana*) were found at four sites. The species is usually restricted to small or medium size streams, is rarely found in large bodies of water, and has not previously been reported from reservoirs (John Alderman and Art Bogan, personal communication). Given that it is uncommon to find this species outside of stream habitats, there was some question as to whether these individuals were simply unusual specimens of the more common Eastern Creekshell (*V. delumbis*). However, the seven individuals identified as Carolina Creekshell were done so based on conchological (shell) and soft part anatomy characteristics (ivory-white as opposed to black band on edge of marsupium). At the time, three *V. vaughaniana* and five Eastern Creekshell (*V. delumbis*) voucher specimens were preserved in 95% ethanol to allow for future genetic evaluation to be performed. Both species are listed in the South Carolina's 2015 State Wildlife Action Plan (SWAP) as conservation priority species (SCDNR 2014), with *V. vaughaniana* considered Highest Priority and *V. delumbis* considered Moderate Priority.

## 2.0 METHODOLOGY

Following agency review of these findings, SCANA and Kleinschmidt asked Three Oaks in the spring of 2017 to develop a protocol for arriving at a clearer resolution for identifying these specimens using genetic analysis. Dr. Michael Gangloff at Appalachian State University was selected to perform this task. The original specimens were preserved in 95% ethanol at the time of their collection, and remained in the Three Oaks office with no additional curation (ethanol changes) until they were delivered to Dr. Gangloff of Appalachian State University (ASU). In anticipation that some of the specimens collected in 2015 would not yield adequate DNA sequences, due to the

amount of time since collection as well as the need for a larger dataset, additional material was collected in Monticello Reservoir, as well as in a tributary to Fishing Creek. The last of the specimens collected were delivered to ASU in late June 2017, and genetic information was extracted and analyzed.

## 2.1 Additional Specimen Collection

On March 13, 2016, four specimens presumed to be *V. vaughaniana* were collected by Tim Savidge from an unnamed tributary to Fishing Creek in Chester County, South Carolina, the closest known population to the type locality (Sawney’s Creek in Kershaw County, SC) in the Santee River Basin. On June 23, 2017, Tim Savidge and Hannah Slyce with Three Oaks collected three specimens presumed to be *V. vaughaniana*, seven specimens presumed to be *V. delumbis*, and one unknown specimen of the tribe Lampsilini (of which the genus *Villosa* belongs to) adjacent to the boat ramp in Monticello Reservoir near Jenkinsville. This unknown specimen was very elongate, and the shell was weathered to a point where identification based on conchology was difficult. These mussels were preserved in 95% ethanol with a complete change of ethanol after 24 hours. The specimens were delivered to ASU on June 28, 2017.

## 2.2 DNA Analysis

Upon receipt at ASU, each specimen was assigned a unique number, which either reflected the site collection number or the date they were received at ASU (Table 1).

**Table 1. Study Specimens Analyzed**

Specimen Id #	Putative Identification	Collection Site/date
3-16-1	<i>V. vaughaniana</i>	Monticello Reservoir 2015
3-16-2	<i>V. vaughaniana</i>	Monticello Reservoir 2015
3-16-3	<i>V. delumbis</i>	Monticello Reservoir 2015
3-16-4	<i>V. delumbis</i>	Monticello Reservoir 2015
3-16-5	<i>V. delumbis</i>	Monticello Reservoir 2015
3-16-6	<i>V. vaughaniana</i>	Monticello Reservoir 2015
3-16-7	<i>V. vaughaniana</i>	UT Fishing Creek 2016
3-16-8	<i>V. vaughaniana</i>	UT Fishing Creek 2016
3-16-9	<i>V. vaughaniana</i>	UT Fishing Creek 2016
3-16-10	<i>V. vaughaniana</i>	UT Fishing Creek 2016
170623.1-1	<i>V. delumbis</i>	Monticello Reservoir 2017
170623.1-2	<i>V. vaughaniana</i>	Monticello Reservoir 2017
170623.1-3	<i>V. delumbis</i>	Monticello Reservoir 2017
170623.1-4	<i>V. vaughaniana</i>	Monticello Reservoir 2017
170623.1-5	<i>V. vaughaniana</i>	Monticello Reservoir 2017
170623.1-6	<i>V. delumbis</i>	Monticello Reservoir 2017
170623.1-7	<i>V. delumbis</i>	Monticello Reservoir 2017
170623.1-8	<i>V. delumbis</i>	Monticello Reservoir 2017
170623.1-9	<i>V. delumbis</i>	Monticello Reservoir 2017
170623.1-10	<i>V. delumbis</i>	Monticello Reservoir 2017

Specimen Id #	Putative Identification	Collection Site/date
170623.1-11	Unknown Lampsilini	Monticello Reservoir 2017

After cataloging, soft tissue clippings from each specimen were taken and DNA was extracted, then sequenced. Amplified portions of the mitochondrial COI gene were evaluated and compared to a referenced sequence of the type species Downy Rainbow (*V. villosa*). COI has been widely used to identify unknown or cryptic species in a range of taxa including freshwater mussels. The details of the methodologies and results of the genetic analysis are included in Appendix B.

### 3.0 RESULTS

Three specimens (3-16-1, 3-16-2 and 3-16-3) from 2015 and one (170623.1-3) from 2017 Monticello collections did not yield any data. The remaining 21 specimens yielded sufficient data. Analysis of the COI gene indicates that the *Villosa* specimens collected from Monticello Reservoir form two distinct clades, indicating two *Villosa* species are present in the reservoir. Sequence divergence rates within each clade were low (<1.0%) but were relatively high between the two *Villosa* taxa (8.1 to 8.4%, Table 2). Observed inter-specific divergence rates were well beyond the ~2% divergence rate seen between many freshwater mussel taxa (Perkins et al. 2017, Smith et al. 2018).

Individuals that were morphologically similar to *V. vaughaniana* from Monticello Reservoir (Fig. 1) formed a monophyletic group that was distinct from the putative *V. delumbis* clade (Fig. 2). All other specimens, including the putative *V. vaughaniana* from the UT to Fishing Creek formed a second clade that appears to represent *V. delumbis*. The Unknown Lampsilini from the reservoir (specimen 170623.1-11) appears to represent a third Lampsilini taxon and appears to be an elongate, lake-form of Eastern Lampmussel (*Lampsilis radiata*) (Fig. 2). However, sequence reads from this individual were not very robust. Further details and explanations of the results are included in Appendix B.

Table 2. Uncorrected pairwise distances among sequences obtained from *Villosa delumbis*, *V. vaughaniana* and *Lampsilis radiata* specimens collected from Monticello Reservoir (Broad River Drainage) and an unnamed tributary to Fishing Creek (Catawba River Drainage).

Sample	Taxon	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1.170623-1-2	<i>V. vaughaniana</i> - Monticello	--																
2.170623-1-4	<i>V. vaughaniana</i> - Monticello	0	--															
3.170623-1-5	<i>V. vaughaniana</i> - Monticello	0	0	--														
4.170623-1-1	<i>V. delumbis</i> - Monticello	.084	.084	.084	--													
5.170623-1-6	<i>V. delumbis</i> - Monticello	.084	.084	.084	.005	--												
6.170623-1-7	<i>V. delumbis</i> - Monticello	.084	.084	.084	.005	0	--											
7.170623-1-8	<i>V. delumbis</i> - Monticello	.084	.084	.084	.005	.005	.005	--										
8.170623-1-9	<i>V. delumbis</i> - Monticello	.081	.081	.081	.007	.002	.002	.007	--									
9.170623-1-10	<i>V. delumbis</i> - Monticello	.084	.084	.084	.005	0	0	.005	.002	--								
10. 3-16-4	<i>V. delumbis</i> - Monticello	.087	.087	.087	.007	.007	.007	.005	.010	.007	--							
11. 3-16-5	<i>V. delumbis</i> - Monticello	.087	.087	.087	.007	.007	.007	.005	.010	.007	0	--						
12. 3-16-6	<i>V. delumbis</i> - Monticello	.087	.087	.087	.007	.007	.007	.002	.010	.007	.007	.007	--					
13. 3-16-7	<i>V. vaughaniana</i> - Fishing	.084	.084	.084	.005	.005	.005	0	.007	.005	.005	.005	.002	--				
14. 3-16-8	<i>V. vaughaniana</i> - Fishing	.087	.087	.087	.007	.007	.007	.005	.010	.007	0	0	.007	.005	--			

Sample	Taxon	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
15. 3-16-9	<i>V. vaughaniana</i> - Fishing	.084	.084	.084	.005	.005	.005	0	.007	.005	.005	.005	.002	0	.005	--		
16. 3-16-10	<i>V. vaughaniana</i> - Fishing	.084	.084	.084	.005	.005	0	.005	.002	0	.007	.007	.007	.005	.007	.005	--	
17. 170623-1-11	<i>L. radiata</i> - Monticello	.094	.094	.094	.080	.080	.080	.080	.083	.080	.081	.081	.083	.080	.081	.080	.080	--



Figure 1. Specimen of *Villosa vaughaniana* collected from Monticello Reservoir on 23 June 2017.



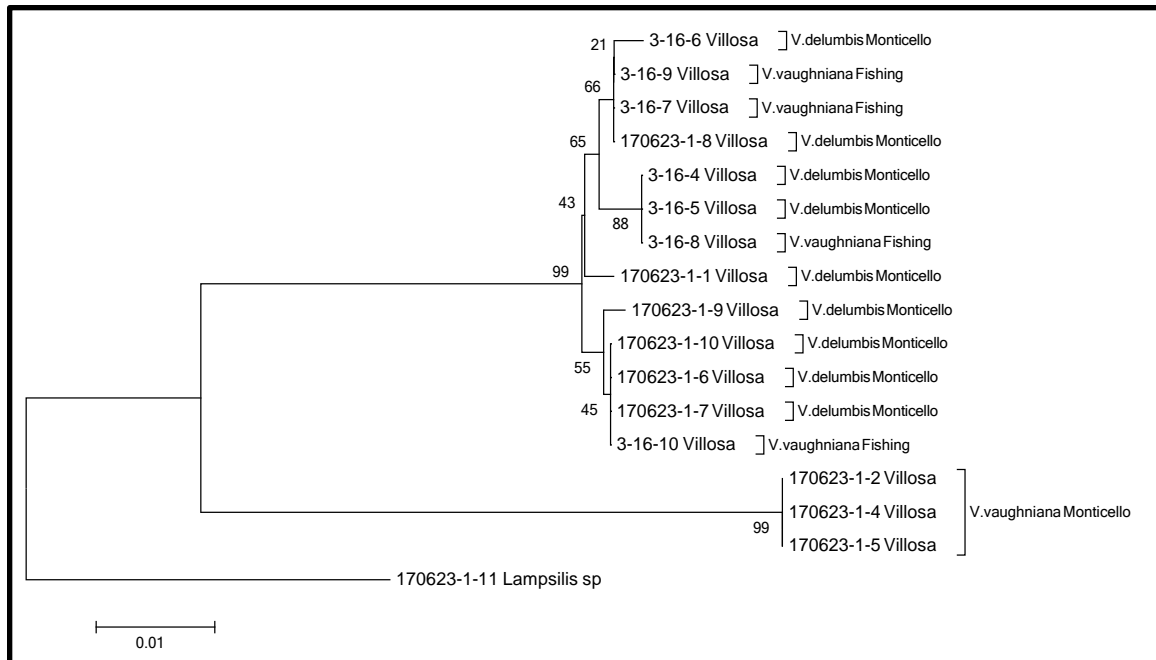


Figure 2. Neighbor-joining phylogeny showing the genetic distance between *Villosa* and *Lampsilis* taxa collected from Monticello Reservoir (Broad River Drainage) and a tributary to Fishing Creek (Catawba River Drainage). Nodal support indices are bootstrap values (10,000 replicates). Scale bar represents 1% difference among sequences.

#### 4.0 CONCLUSIONS

The results of this study support the conclusions from the survey report (Appendix A) that two *Villosa* species are present in Monticello Reservoir. The specimens from Monticello that were putatively identified as *V. vaughaniana* form a distinct clade from those identified from the reservoir as *V. delumbis*, suggesting both species are present. However, the specimens collected from a “known” *V. vaughaniana* population (UT to Fishing Creek) allied with the *V. delumbis* specimens rather than *V. vaughaniana* from the reservoir making it difficult to reach a definitive conclusion of which species are present from the small sample size. A more detailed analysis examining specimens of *V. vaughaniana* and *V. delumbis* from other populations within their respective ranges, as well as the two other *Villosa* species known to occur in South Carolina, *V. constricta* and *V. vibex*, is needed to fully understand the taxonomic relationship and distribution of the *Villosa* genus in the state. While there is some level of uncertainty, it is clear that there are two *Villosa* species in Monticello Reservoir that are morphometrically and genetically distinct from each other.

The results also indicate that a third Lampsilini taxon is present in the reservoir, as the unknown specimen appears to be *Lampsilis radiata* (Eastern Lampmussel), which is considered a “High Priority” conservation species in South Carolina (SCDNR 2014).

## 5.0 LITERATURE CITED

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Smith, C.H., Johnson, N.A., Pfeiffer, J.M., Gangloff, M.M. 2018. Molecular and morphological data reveal non-monophyly and speciation in imperiled freshwater mussels (*Anodontoidea* and *Strophitus*). *Molecular Phylogenetics and Evolution* 119:50-62.