

**Genetic Analysis of Villosa sp. Specimens from Monticello Reservoir,
South Carolina**

Final Report

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Introduction

The freshwater mussel genus *Villosa* is one of the most species-rich in North America. Williams et al. (2017) recognize 14 *Villosa* taxa, four of which (*V. constricta*, *V. delumbis*, *V. vaughaniana*) and *V. vibex* occur in Atlantic Slope drainages of South Carolina. These four species are identified in the South Carolina's 2015 State Wildlife Action Plan (SWAP) as conservation priority species (SCDNR 2014), with *V. constricta*, *V. vaughaniana* and *V. vibex* considered Highest Priority and *V. delumbis* considered Moderate Priority.

To date, there have been few published molecular analyses that include data from *Villosa* spp. although a recent thesis at Ohio State (Kuehnel 2009) examined data from nearly all of the presently-recognized *Villosa* taxa and found widespread polyphyly within the genus and also among several widespread taxa. However, the DNA sequence data from that study have yet to be released on GenBank and no peer-reviewed publications have yet resulted from this work.

The objective of this study was to use analysis of DNA sequences to determine the identity of *Villosa* species occurring in Monticello Reservoir, an impoundment adjacent to the Broad River north of Columbia, South Carolina.

Methods

Specimens were collected from Monticello Reservoir in September 2015 and July 2017. Additionally, specimens of what were presumed to be *V. vaughaniana* from an un-named tributary to Fishing Creek (Catawba River Drainage) were collected in March 2016 to provide material from near the type locality for that species (Sawney's Creek near (about 8 miles NW) Camden, South Carolina).

I used a standard bar-coding approach to identify specimens using DNA sequences. I amplified portions of the mitochondrial COI gene. COI has been widely used to identify unknown or cryptic species in a range of taxa including freshwater mussels (Perkins et al. 2017, Smith et al. 2018). Samples were

extracted and sequences were amplified using primers and PCR protocols that are widely used in genetic studies (Folmer et al. 1994, Buhay et al. 2002).

Forward DNA sequence reads were poor (sequencer could not resolve base identities) in the majority of specimens but the reverse strand read was generally much cleaner. I reverse-complemented sequence reads to obtain a forward read and aligned reads to a reference sequence from the type species *Villosa villosa*. I compared DNA sequence reads to one another to determine genetic differences within and among-taxa and examined the overall relationship of samples using a neighbor-joining analysis (1000 bootstrap replicates).

Results and Discussion

Analysis of the COI gene indicates that two *Villosa* species are present in Monticello 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 1). 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). However, Kuhnel (2009) reported similar levels of divergence between many *Villosa* spp. including several Atlantic Slope taxa.

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 formed a second clade that appears to represent *V. delumbis*. A third taxon was also apparently included in the Monticello Reservoir samples and appears to be an elongate, lake-form of *Lampsilis radiata* (Fig. 2). However, sequence reads from this individual were not very robust and so my confidence in using molecular data diagnosing this taxon is low.

From a phylogenetic perspective, these results are somewhat counter-intuitive in that they suggest that specimens identified as *Villosa vaughaniana* from the

Fishing Creek system may be more closely related to *V. delumbis*. However, my phylogenetic analyses are based on a single gene fragment and as such should be considered preliminary. Data from other drainages along with sequences from additional genes are needed to resolve species boundaries among Atlantic Slope *Villosa* taxa.

References

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Table 1. Uncorrected pairwise distances among sequences obtained from *Villosa delumbis*, *V. vaughniana* 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. vaughniana</i> - Monticello	--																
2.170623-1-4	<i>V. vaughniana</i> - Monticello	0	--															
3.170623-1-5	<i>V. vaughniana</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. vaughniana</i> - Fishing	.084	.084	.084	.005	.005	.005	0	.007	.005	.005	.005	.002	--				
14. 3-16-8	<i>V. vaughniana</i> - Fishing	.087	.087	.087	.007	.007	.007	.005	.010	.007	0	0	.007	.005	--			
15. 3-16-9	<i>V. vaughniana</i> - Fishing	.084	.084	.084	.005	.005	.005	0	.007	.005	.005	.005	.002	0	.005	--		
16. 3-16-10	<i>V. vaughniana</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	0.08	.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.