FINAL REPORT

For

Southern Hickorynut, *Obovaria jacksoniana* (Frierson, 1912), its closest congeners, and *Villosa arkansasensis*.

By

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The purpose of this agreement was to:

1. Conduct a phylogenetic analysis of *Obovaria jacksoniana* from multiple drainages, its sympatric congeners, and *V. arkansasensis*.

The following report is in the format of 2 chapters taken from Mr. Kentaro Inoue's thesis. The citation for Mr. Inoue thesis is:

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Chapter 2 :
A molecular phylogenetic analysis of *Obovaria jacksoniana* and *Villosa arkansasensis.*

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Abstract: The special concern southern hickorynut, *Obovaria jacksoniana*, occurs from the Mississippi Interior Basin to Mobile drainage. The Ouachita creekshell, *Villosa arkansasensis*, is often difficult to distinguish from *O. jacksoniana* based on conchological characters. Since both species have been ranked with conservation status, determining genetic divergences of both species are important for conservation. The goal of this study was to determine genetic divergence between *O. jacksoniana* and *V. arkansasensis*. In order to achieve this goal, we conducted molecular phylogenetic analyses. We used both mtDNA and nuclear DNA sequences. Eighty-two specimens of *O. jacksoniana* and 21 specimens of *V. arkansasensis* were collected from the Mississippi and Mobile drainages. We also used other species in genera *Obovaria* and *Villosa* to support evolutionary relationships. Our resulting phylogenetic analyses did not support monophyletic groupings of both *Obovaria* and *Villosa,* as both *O. jacksoniana* and *V. arkansasensis* occurred within the same phylogenetic clade. The results suggest that *V. arkansasensis* may be a synonymous species with *O. jacksoniana* based on genetic similarities.

Introduction

Species classification of freshwater mussels over the past 250 years has been based on morphological, anatomical, behavioral, geographical, and ecological characters. However, these methods often lead to ambiguous classification of species and can fail to recognize patterns of diversity due to convergence (Conrad 1853; Bogan & Roe 2008).

Application of molecular phylogenetics has provided the evolutionary history of freshwater mussels and, in some cases, has provided an unbiased measure of taxonomic status. In addition, molecular phylogenetic methods can be used for identification of species, as well as determining their speciation within evolutionary pathways. For example, Lydeard et al. (1996) used the 16S ribosomal RNA (rRNA) gene to compare traditional classification of North American freshwater mussels with anatomical and behavioral characters. Their analysis supported the idea that freshwater mussels can be divided into two families: Unionidae and the Margaritiferidae. Within Unionidae, Lydeard et al. (1996) reported that there are two subfamilies: the Anodontinae and Ambleminae. Traditional systematics by anatomical and behavioral characters has not shown to reveal the evolutionary pathways and genetic lineages. Another example is that of Lydeard et al. (2000) in which they conducted phylogenetic analysis to address relationships of 12 imperiled species from five closely related genera (*Fusconaia*, *Obovaria, Pleurobema, Quadrula,* and *Quincuncina*) in the Gulf Coastal drainage. Their results showed that three of the genera were polyphyletic (*Fusconaia, Obovaria*, and *Quincuncina*). Molecular phylogenetic analysis also has been used to determine the evolutionary relationships for higher-level taxonomies, such as family, subfamily, and tribe (Roe & Hoeh 2003; Graf & Cummings 2006; Walker et al. 2006). Campbell et al. (2005) provided large phylogenetic trees of a three gene dataset [cytochrome *c* oxidase gene (COI), NADH dehydrogenase (ND1), and 16S rRNA (16S)] by analyzing 107 species of 37 genera. Their results showed a clear association of the subfamilies and tribes, however, it also showed some problematic taxa, such as *Lampsilis*, *Pleurobema*, and *Fusconaia*.

Molecular phylogenetic analysis also has been used for lower-level taxonomies, such as genus and species, and to determine genetic diversity within localized areas. For example, Serb et al. (2003) studied the molecular systematics of the genus *Quadrula* using a portion of the ND1 gene. Serb et al. (2003) analyzed 66 specimens from 17 *Quadrula* species, including three closely allied species: *Tritogonia verrucosa* (Rafinesque, 1820), *Fusconaia succissa* (Lea, 1852), and *Quincuncina infucata* (Conrad, 1834). This analysis revealed non-monophyletic taxa and they suggested that the genus *Quadrula* be expanded to include three additional species. Furthermore, within the genus *Quadrula*, Serb et al. (2003) recognized three monophyletic species level taxonomic groups (*quadrula*, *metanevra*, and *pustulosa*). In other studies, the genera *Fusconaia* and *Pleurobema* (Burdick & White 2007; Campbell et al. 2008), genus *Lampsilis* (Zanatta & Murphy 2006b), and genus *Anodonta* (Mock et al. 2004; Chong et al. 2008) were phylogenetically analyzed to resolve taxonomic relationships within the genus.

It also is possible that species in localized areas may have different genetic signatures compared to other populations and that these isolated populations have a potential of introducing new evolutionary lineages (Avise 2009, for a review). Phylogeography, which is a study of spatial arrangements of genetic lineages especially within and among closely related species, has been studied in freshwater mussels as well. For example, Serb (2006) studied the genetic structure of *C. aberti* collected from 12 localities in Arkansas, Kansas, and Missouri. The results of Serb (2006) indicated that phylogenetic analysis did not support the monophyly of *C. aberti*. *Cyprogenia aberti* also was shown to be comprised with five independent lineages, including the federally endangered *Cyprogenia stegaria*. Furthermore, even in the same drainage, they found distinct sympatric lineages. Thus, it is evident that molecular phylogenetic analysis at the generic and species-level can reveal not only evolutionary processes, but also genetic relationships among species.

Meanwhile, morphological characteristics have been used traditionally to identify a species, which often leads to incorrect taxonomy and misidentification of cryptic species. Shell morphology can be affected by environmental factors, such as stream characters and habitat types (Ortmann 1920; Watters 1994). For example, *Obovaria jacksoniana* (Frierson, 1912) and *Villosa arkansasensis* (Lea, 1862) are conchologically similar, and it can be difficult to distinguish one species from the other (Valentine & Stansbery 1971; Vaughn 2003). Since both species are of conservation concern, clear taxonomic identification is key to effective conservation efforts. *Obovaria jacksoniana* occurs in southern Missouri through southern portions of the Mississippi Interior Basin and from eastern Texas through the western Alabama drainage (Oesch 1984; Howells et al. 1996). Within Arkansas, *O. jacksoniana* occurs in tributaries of the Red and Ouachita rivers and in the Poteau River (Harris 1994; Harris et al. 1997). On the other hand, *V. arkansasensis* is restricted to headwater habitats and occurs in headwaters of the Red and Ouachita rivers in Arkansas and Oklahoma and in the Poteau River (Harris 1994; Harris et al. 1997; Vaughn & Taylor 1999; Vaughn 2003). Since both species occur in the same drainages in Arkansas, it is important to examine the genetic profiles of both species to determine their phylogenetic relations and taxonomic status.

The goal of this chapter was to examine the genetic profiles of *O. jacksoniana* and *V. arkansasensis* to better manage these species. In order to achieve my goal, my first objective was to determine genetic divergence 1) between *O. jacksoniana* and *V. arkansasensis* and 2) among congener taxa using molecular phylogenetic analyses. Our second objective was to reconsider the conservation status of both species after better understanding the genetic structure of these two species. To achieve these objectives, we used molecular phylogenetics techniques to examine genetic relations between *O. jacksoniana* and *V. arkansasensis* and among *Obovaria* species from across the ranges of these taxa.

Materials and Methods

Collection sites and Specimens

Eighty-two individuals of *O. jacksoniana,* representing eight different localities, were collected from Alabama, Arkansas, and Mississippi (Figure 2.1). In Arkansas, we collected: 44 individuals from three populations in the Saline River; 17 individuals from one population in the Ouachita River; seven individuals from one population in the Little Missouri River; six individuals from three populations in the Little River; and one individual in the Cossatot River (Table 2.1). In addition, two individuals from one population in the Buttahatchee River, Mississippi and four individuals from one population in the Sipsey River, Alabama were collected (Table 2.1).

Twenty-one individual specimens of *V. arkansasensis* representing three different localities, were collected from Arkansas (Figure 2.1). Fourteen individuals from two populations in the Alum Fork of the Saline River, five individuals from one population in the Irons Fork of the Ouachita River, and one individual in the Mountain Fork of the Little River were collected (Table 2.1).

For comparison we collected other *Obovaria* species: 29 individuals of *Obovaria subrotunda* from two drainages, 17 individuals of *Obovaria olivaria* from a drainage, and 11 individuals of *Obovaria unicolor* from one drainage (Table 2.1; Figure 2.1). Additionally, published DNA sequences were used as the reference and outgroup for the phylogenetic analyses (Table 2.1).

DNA sequencing

Specimens were preserved in absolute ethanol in the field and each individual was labeled with a unique museum number. Whole genomic DNA was extracted from each specimen using standard CTAB/chloroform extraction methods followed by ethanol precipitation as described in Winnepenninckx et al. (1993). To reduce the probability of mitotype contamination from the male gonads, mantle tissues (from along with the shell margin) or foot tissues were used for DNA extraction. The quality of extracted DNA was examined by 1% agarose gel electrophoresis and the quantity was measured by a NanoDrop® ND-1000 spectrophotometer® (Thermo Fisher Scientific, Inc., Waltham, MA). Each DNA sample was diluted with molecular grade purified water to a >10 ng/µl concentration and labeled as a working DNA solution.

Polymerase chain reaction (PCR) amplification of three mitochondrial (COI, ND1, and 16S) and one nuclear (28S) DNA genes were conducted in separate 20 µL reaction in an iCycler® thermal cycler (Bio-Rad Laboratories, Inc., Hercules, CA). A 700 base pair (bp) region of the first subunit of cytochrome *c* oxidase gene (COI) was amplified using modified sequences for primers LCO1490 and HCO2198 from Folmer et al. (1994) and

Campbell et al. (2005). New COI primers were designed from complete mitochondrial DNA (mtDNA) genome of *Lampsilis ornata* (NC_005335) and were used for PCR amplification. Since only one complete mtDNA genome of North American unionid species has been analyzed from *L. ornata* (Serb & Lydeard 2003), optimized primers that are designed from the genome could be specified to the unionid species. New primers were generated using the online program Primer3 (Rozen & Skaletshky 2000) and simulated the reliability of PCR products using Amplify v3.1.4 (Engels 2005). A 820 bp region of the COI was amplified using designed primers ModCOI L and ModCOI H. Reactions of both primers were amplified for an initial denaturation cycle of 92˚C for 2 min; five cycles of 92˚C for 40 s, 40˚C for 40 s, 72˚C for 90 s; 25 cycles of 92˚C for 40 s, 50˚C for 40 s, 72˚C for 90 s; and was followed by a 10 min extension period at 72˚C (Campbell et al. 2005). A 880 bp region of the first subunit of NADH dehydrogenase gene (ND1) was amplified using Leu-uurF and a modified sequence for primer NIJ-12073 (Campbell et al. 2005). For the problematic taxa, a more reliable 3'-end primer LoGlyR that was designed from flanking the tRNA-Gly gene, was used (Serb et al. 2003). Reactions were amplified for an initial denaturation cycle of 92˚C for 2 min; five cycles of 92˚C for 40 s, 40˚C for 60 s, 72˚C for 90 s; 25 cycles of 92˚C for 40 s, 50˚C for 60 s, 72˚C for 90 s; and followed by a 10 min extension period at 72˚C (Campbell et al. 2005). A 530 bp region of 16S ribosomal RNA gene (16S) was amplified using 16sar-L-myt and 16sbr-H-myt (Lydeard et al. 1996). The reaction was amplified for an initial denaturation cycle of 92˚C for 5 min; 35 cycles of 92˚C for 40 s, 50˚C for 60 s, 68˚C for 90 s; and was followed by a 10 min extension period at 72˚C (Lydeard et al. 1996). A 750 bp of 28S ribosomal RNA gene (28S) was amplified using D23 and D6R primers (Park & Ó Foighil 2000). The reaction was set for an initial denaturation cycle of 94˚C for 4 min; 36 cycles of 94˚C for 40 s, 55˚C for 40 s, 72˚C for 105 s; and was followed by 10 min extension period at 72˚C (Park & Ó Foighil 2000). All primer sequences are shown in Table 2.2. Only a small subset of represented specimens for populations were amplified using 16S and 28S due to the slow evolved rRNA gene in bivalves (Hoeh et al. 1997).

Quality of PCR products was examined by 1% agarose electrophoresis. Each PCR product was purified either by gel-isolated (QIAquick gel extraction kit, QIAGEN) or by spin filtration columns (QIAquick PCR Purification Kit, QIAGEN). We followed the protocols provided by the manufactures for purification and the final dilution of PCR products was 16 µL.

Purified PCR products were used as template for cycle sequence reactions with DTCS Quick State kit (Beckman Coulter, Inc., Fullerton, CA). The reaction cycle was customized from the manufacture default and was the following; 35 cycles of 96˚C for 20 s, 50˚C for 30 s, and 60˚C for 4 min. The final products from cycle sequence reactions were cleaned by sodium acetate/EDTA/glycogen stop solution mixture with ethanol precipitation. Cycle sequencing products were re-suspended in 40 µL of formamide sample loading solution. Sequence reaction was visualized on a CEQ™ 8000 automated sequencer (Beckman Coulter, Inc., Fullerton, CA).

Phylogenetic Analyses

Each DNA strand was compared and aligned by eye using the alignment editor DNADynamo (Blue Tractor Software, Ltd., United Kingdom). Ambiguous sequences of both the 3’- and 5’-ends were trimmed. Paired DNA of both strands was saved as FASTA format. FASTA files were used for multiple sequence alignment in the software program

ClustalW (Larkin et al. 2007), which is included in the bioinformatics package eBioX v.1.5.1 (Bongcam-Rudloff 2008).

To determine the relationship among haplotypes and the frequencies of these haplotypes, haplotype networks were generated from the multiple sequence alignment of each gene using TCS v.1.21 (Clement et al. 2000). For haplotypes with more than one pathway, the shortest path was chosen from the most frequent haplotype. Connection limit was fixed at 30 steps of nucleotide substitutions and gaps were treated as missing data.

Multiple sequence alignments were included with published sequence data of any *Obovaria* and *Villosa* species obtained from GenBank (NCBI) (Table 2.3). Each sequence alignment was trimmed to the same length as generated sequences. To estimate phylogenetic relationships among taxa, sequences were analyzed in TreeFinder v.12.2.0 (Jobb 2008) using maximum likelihood (ML) and in BEAST v.1.4.7 (Drummond & Rambaut 2007) using Bayesian likelihood analyses. MtDNA sequence data from COI and ND1 gene portions were analyzed as separate data set (COI and ND1) and as a combined analysis (combined). Ribosomal RNA genes 16S and 28S were analyzed as separated data sets. For the ML and Bayesian analyses, substitution models for nucleotide sequence were chosen using Kakusan3 (Tanabe 2007) for each data set. ML analysis was performed with TreeFinder (Jobb et al. 2004; Jobb 2008) using the default settings and the substitution model generated from Kakusan3. Support values were generated by pseudo-bootstrapping with 1000 replicates using the expected-likelihood weights with local rearrangements of tree topology (LR-ELW) (Strimmer & Rambaut 2002). The LR-ELW edge support can be directly interpreted as confidence in the configuration of branches adjacent to a particular edge. Bayesian analysis was performed with BEAST v.1.4.7 (Drummond & Rambaut

2007) by Metropolis Coupled Markov Chain Monte Carlo (MC3). Analyses were run for 10,000,000 generations for each gene with a sample frequency of 1,000. Final trees were generated by determining a consensus with the "maximum clade creditability" using TreeAnnotator v.1.4.6 (Drummond & Rambaut 2007). Trees were drawn using FigTree v.1.1 (Drummond & Rambaut 2007).

Results

DNA Sequencing

Sequence alignment of the COI yielded a 647 bp segment for 117 individuals and contained 177 polymorphic sites, of which 143 were phylogenetically informative under maximum parsimony. Sequence alignment of ND1 yielded a 771 bp segment for 108 individuals and contained 295 polymorphic sites, of which 267 were phylogenetically informative. Sequence alignment of 16S yielded a 525 bp segment for 21 individuals and contained 88 polymorphic sites, of which 71 were phylogenetically informative. Sequence alignment of 28S yielded a 738 bp segment for 22 individuals and contained 13 polymorphic sites, of which only two were phylogenetically informative. The combined sequences of the COI and ND1 genes yielded 1418 bp for 157 individuals and contained 472 polymorphic sites, of which 410 were phylogenetically informative.

Haplotype Networks

Our haplotype analyses resulted in 40 COI haplotypes from 71 individuals of *O. jacksoniana* and *V. arkansasensis* (Table 2.4; Figure 2.2), 39 ND1 haplotypes from 69 individuals (Table 2.5; Figure 2.3), six 16S haplotypes from 11 individuals (Figure 2.4), and one 28S haplotype from 14 individuals (Figure 2.5). Some individuals of *O. jacksoniana* and *V. arkansasensis* shared the same haplotypes for COI, ND1, and 16S.

Meanwhile, *O. jacksoniana, O. olivaria,* and *V. arkansasensis* shared the same haplotypes for 28S. Based on these four haplotype analyses, there were essentially three haplotypic separations among drainages: Ouachita, Red, and Mobile River drainages (Figures 2.2 and 2.3). Both COI and ND1 showed genetically distinctive differences between Arkansas and Mobile drainage specimens (Figures 2.2 and 2.3). The shortest COI haplotype distances between the Arkansas and Mobile drainages were 19 steps of nucleotide substitutions, while ND1 was 28 steps of nucleotide substitutions. Comparatively, the shortest 16S haplotype distances between Arkansas and Mobile drainages were only eight steps, while 28S had no steps among drainages (Figures 2.4 and 2.5).

Phylogenetic Analyses

Although 28S yielded 748 bp with 13 polymorphic sites (Table 2.1), the haplotype network showed three nucleotide differences among four *Obovaria* species and *V. arkansasensis*. Because 28S contained only three phylogenetically informative variations, it was excluded from further analyses.

Phylogenetic trees for COI, ND1, 16S, and the COI+ND1 combined dataset were generated (Figures 2.6 through 2.13) with *Fusconaia ebena* being treated as an outgroup for all four analyses. None of the trees showed a monophyletic relationship for *O. jacksoniana* or *V. arkansasensis* and both *O. jacksoniana* and *V. arkansasensis* were located in same clade for all analyses. As in previous studies (Lydeard et al. 2000; Campbell et al. 2005), *O. rotulata* was not included in the *Obovaria* species clades for either of our analyses. The ML analysis on the COI gene showed that none of the *Obovaria* species, except *O. rotulata,* were monophyletic taxa. Other *Villosa* species were placed sister to the *O. olivaria* clade for the ML analysis of the COI gene, although the edge support value to support the differentiation between clades was only 38.3% (Figure 2.6). Meanwhile, Bayesian analysis of the COI showed that only *V. villosa* was sister to *O. olivaria*. Topology of clade distribution between the two analyses was dissimilar. For instance, in the ML analysis *O. jacksoniana* was sister to *O. olivaria* clade, while for the Bayesian analysis *O. jacksoniana* was sister to *O. subrotunda* and *O. unicolor* clade. Both analyses showed that the published sequence of *O. jacksoniana* was in the *O. subrotunda* clade, which might be the caused by incorrect *a priori* identification. Within the *O. jacksoniana* and *V. arkansasensis* clade for both analyses, there were distinct Mobile and Arkansas drainages clades and a slight distinction between Ouachita and Red river drainages.

Both ML and Bayesian analyses for ND1 showed a monophyletic *Obovaria* clade, which included *V. arkansasensis* (Figures 2.8 and 2.9). Likewise, both analyses for COI showed different topology of clade distributions. Additionally, the *O. jacksoniana* and *V. arkansasensis* clade showed distinctions among drainages, with high edge supports in ML analysis and posterior probability in Bayesian analysis. Even though we could only get Mountain Fork of the Little River ND1 sequences for *V. arkansasensis*, the ND1 only *V. arkansasensis* sequences were distributed with sequences of *O. jacksoniana* from the Little River. Thus, genetic structure of *O. jacksoniana* and *V. arkansasensis* showed highly similar genetic relationships arranged by drainage patterns.

Only a few individuals from each drainage were sequenced for 16S because it has a relatively slow evolutionary rate compared to other protein-coding genes (Hoeh et al. 1997) (Figure 2.4). Both ML and Bayesian analyses resulted in a monophyletic clade of *Obovaria* species, which included *V. arkansasensis* (Figures 2.10 and 2.11). Both analyses showed similar topology of clade distribution and that the clade of *O. jacksoniana* and *V. arkansasensis* was sister to the clade of *O. subrotunda* and *O. unicolor*. Furthermore, within the clade of *O. jacksoniana* and *V. arkansasensis*, there was genetic structure associated with drainage patterns.

No published sequence data were used in the COI+ND1 combined analysis (Figures 2.12 and 2.13). Both ML and Bayesian analyses showed strong divergence between the outgroup, *F. ebena,* and *Obovaria* species and *V. arkansasensis.* Although clade topology was different for each analysis, both *O. jacksoniana* or *V. arkansasensis* were monophyletic taxon and both species were located on same phylogenetic clade. Like other genes, the *O. jacksoniana* and *V. arkansasensis* clade was geographically structured, following the three drainages.

P-distance Comparisons

Genetic distances were calculated for *O. jacksoniana* and *V. arkansasensis* (Table 2.6). The mean p-distance was 1.050% (range = 0.000-5.072%) for COI, 1.103% (range = 0.000-4.929%) for ND1, and 1.200% (range = 0.000-2.519%) for 16S sequence data (Table 2.6). Genetic divergence for all genes was relatively high between the Mobile and Arkansas drainages (range = 3.246-5.072% for COI; 3.742-4.669% for ND1; and 1.934-2.519% for 16S) (Table 2.6). However, within the same drainage, the mean p-distances for all genes were < 1.0% (range = 0.000-1.356%); the mean p-distances between the Ouachita and Red River drainages for all genes were slightly greater (0.952-1.338%) than within drainages. Although the rRNA gene has relatively slow evolutionary rate compared to other protein-coding genes (Hoeh et al. 1997), p-distances of the 16S overall drainages and between the Ouachita and Red River drainages were slightly higher than COI and ND1 genes (Table 2.6).

Discussion

Phylogenetic Relationships of Obovaria jacksoniana and Villosa arkansasensis

Through all analyses for three genes, both *O. jacksoniana* or *V. arkansasensis* were not monophyletic taxa and resulted in both species being located in the same phylogenetic clade. Even though type locality specimens were not collected for either species in this study, our molecular phylogenetic results support, that, Arkansas specimens of *O. jacksoniana* and *V. arkansasensis* are synonymous species. Similar to previous studies, the published *O. rotulata* sequences were the closest to *F. ebena* (e.g., Lydeard et al. 2000; Campbell et al. 2005). Williams et al. (2008) proposed that *O. rotulata* be placed in the genus *Fusconaia* due to phylogenetic analyses in published papers, even though the taxonomical status of *F. ebena* is still under examination. If *O. rotulata* was ignored in our study, the genus *Obovaria* would be somewhat monophyletic, except for COI analyses, although the type species (*O. retusa)* of the genus *Obovaria* was not available in our study.

Phylogenetic clades of *O. jacksoniana* and *V. arkansasensis* indicated that there were three distinct phylogeographic isolations, in which there were discrete geographic clades corresponding to river drainages. High phylogenetic separations within species associated with geographic barriers or historical geographic changes have been reported in many freshwater organisms, such as fish species (Mayden 1985; Mayden 1988; Berendzen et al. 2003; Turner & Robison 2006; Berendzen et al. 2007), crayfish (Crandall & Templeton 1999; Fetzner & Crandall 2003), and mussels (King et al. 1999; Roe et al. 2001; Serb 2006; Burdick & White 2007; Elderkin et al. 2008). In our study, a species-complex clade of *O. jacksoniana* and *V. arkansasensis* was evident, and included three sub-clades representing the Mobile, Ouachita, and Red river drainages. These sub clades showed that the Mississippi River serves as a geographic barrier between the east (Mobile) and west (Ouachita and Red) sides of the Mississippi River. Even though an *O. jacksoniana* type locality specimen was not collected for our study, we assume that the high genetic divergence between the Mobile River and rivers in Arkansas is indicative of no or very low levels of current gene flow, as the drainages are separated from each other via the Mississippi River. However, considering the historic river distributions of North America, gene flow and dispersal might have occurred between drainages as the Tennessee River once connected to the Mobile River in pre-glacial period (Mayden 1988). Phylogeographic studies of fish species show the patterns of phylogenetic relationships between the Mississippi and Mobile River basins, which supports a historical connection of the Tennessee River with the Mobile Basin (Wiley & Mayden 1985; Mayden 1988; Berendzen et al. 2003). There were two possible historic connections of the Tennessee River with the Mobile River. One was the large river from the Appalachians connecting to the Mobile River via the upper Tennessee River (Mayden 1988). The second hypothetical connection was between the present lower Tennessee and Mobile rivers, via the Tombigbee River, flowing into the Gulf of Mexico (Starnes & Etnier 1986). According to the hypotheses, although the connections between the Mississippi and Mobile rivers were not simultaneous events, gene flow and dispersal might have occurred between the drainages in Arkansas (Ouachita and Red) and the Mobile River via the Mississippi and the lower Tennessee rivers.

Genetic Diversity and Genetic Distances

Population genetics studies of *Amblema plicata* (Elderkin et al., 2007) and *Elliptio* *dilatata* and *Actinonaias ligamentina* (Elderkin et al., 2008) showed that haplotype richness was significantly negatively correlated with latitude, in which southern populations had more haplotype richness than northern populations. Additionally, the number of haplotype uniqueness was strongly negatively correlated with latitude as well. In comparison to other studies (Burdick & White 2007; Elderkin et al. 2007; Elderkin et al. 2008; Zanatta & Murphy 2008), the number of haplotypes in this *O. jacksoniana* and *V. arkansasensis* study was relatively high. Although there were only slight latitudinal differences between the sites of *O. jacksoniana* and *V. arkansasensis* collections, the high haplotype uniqueness, even with our small sample size, may be explained by the fact that our sites are in relatively lower latitudes. Furthermore, haplotype frequencies distributed with drainage patterns and each drainage formed genetic structure corresponding to the results of phylogenetic analyses. This supports strong phylogeographic patterns among the Ouachita, Red, and Mobile river drainages.

According to previous studies analyzing genetic distances among species, interspecific genetic divergence has been set at 3% for invertebrate and 2% for birds and mammals on the COI (Hebert et al. 2003). For example, Lydeard and Roe (1998) obtained a p-distance of the COI gene sequences within the genus *Potamilus* ranging from 0.0 to 2.62% for intraspecific and 1.32 to 14.48% for interspecific values. Serb et al. (2003) obtained p-distances for ND1 gene sequences within the genus *Quadrula* ranging from 0.15 to 3.29% for intraspecific and 3.65 to 15.35% for interspecific. Furthermore, Serb (2006) reported extremely high intraspecific values for ND1 p-distance of *C. aberti* ranging from 0.0 to 8.9% (average 8.61%) and for COI ranging from 0.0 to 13.74% (average 13.33%).

Although Serb (2003) showed that for *C. aberti*, the average p-distances for intraspecific value ND1was greater than COI in this study, and COI and ND1 p-distances of *O. jacksoniana* and *V. arkansasensis* were not significantly different (Table 2.6). However, 16S had slightly higher genetic distances between the Ouachita and Red river drainages (mean 1.338%) compared to COI (0.952%) and ND1 (1.099%).

Taxonomic Implications

Based on our molecular phylogenetic analyses from four genes, *O. jacksoniana* and *V. arkansasensis* shared the same haplotypes and phylogenetic clade. Thus, we conclude that *O. jacksoniana* and *V. arkansasensis* in Arkansas' rivers are conspecific. However, the relatively high genetic divergences (highest 5.072% for COI) observed for all genes between Mobile and Arkansas drainages suggests significant phylogeographic structure between drainages. Previous studies of unionid COI sequences reported interspecific genetic divergences ranging from 3.65 to 16.42% (Roe & Lydeard 1998; King et al. 1999; Serb et al. 2003; Burdick & White 2007). Additionally, Hebert et al. (2003) proposed that the interspecific genetic divergence threshold for COI was >3% for invertebrates and >2% for birds and mammals. Because *O. jacksoniana* in Mobile and Arkansas drainages have high genetic divergence, at the interspecific threshold level, further study of the taxonomic status for these populations, is needed. For further genetic analyses, additional genetic markers, such as internal transcribed spacer (ITS) and microsatellite DNA, can be possible to determine genetic divergences and phylogenies of *O. jacksoniana* and *V. arkansasensis*. ITS, which is a region between nuclear ribosomal RNA genes, is useful for examining relationships among closely related species (Gonzalez et al. 1990; Kallersjo et al. 2006; Walther et al. 2006). Microsatellite DNA, which is co-dominant alleles with high allelic variation per locus, is a good indicator to examine population structure, gene flow, and kinship within and among population (Eackles & King 2002; Geist et al. 2003; Shaw et al. 2006; Zanatta & Murphy 2006a; Zanatta et al. 2007). Furthermore, analysis of *O. jacksoniana* specimens from the type locality (even though they maybe extirpated) is needed to clarify the taxonomic status of populations from Mobile River and rivers from Arkansas. Since both species are listed high as species of special concern (Williams et al. 1993), population consensus and genetic diversity studies are needed to reassess their conservation status.

Conclusions

Molecular phylogenetic analyses suggest that both *O. jacksoniana* and *V. arkansasensis* occurring in Arkansas are possibly conspecifics, due to their identical genetic structure. Although we analyzed only the maternal lineages using mitochondrial DNA genes, we observed clear phylogeographic patterns among populations. We observed high genetic distances between populations in Arkansas' rivers and the Mobile River drainages. Such high genetic divergence can be explained by low or no gene flow among drainages due to no present day connections. Furthermore, although we have not collected *O. jacksoniana* from the type locality, *O. jacksoniana* in the Mobile River drainage might be a result of allopatric speciation. Since we observed identical phylogenetic patterns in *O. jacksoniana* and *V. arkansasensis* in Arkansas' rivers, the population consensus and genetic structures of both species should be examined across their entire distributions. From the comprehensive assessments, reconsideration of conservation status of both species is needed.

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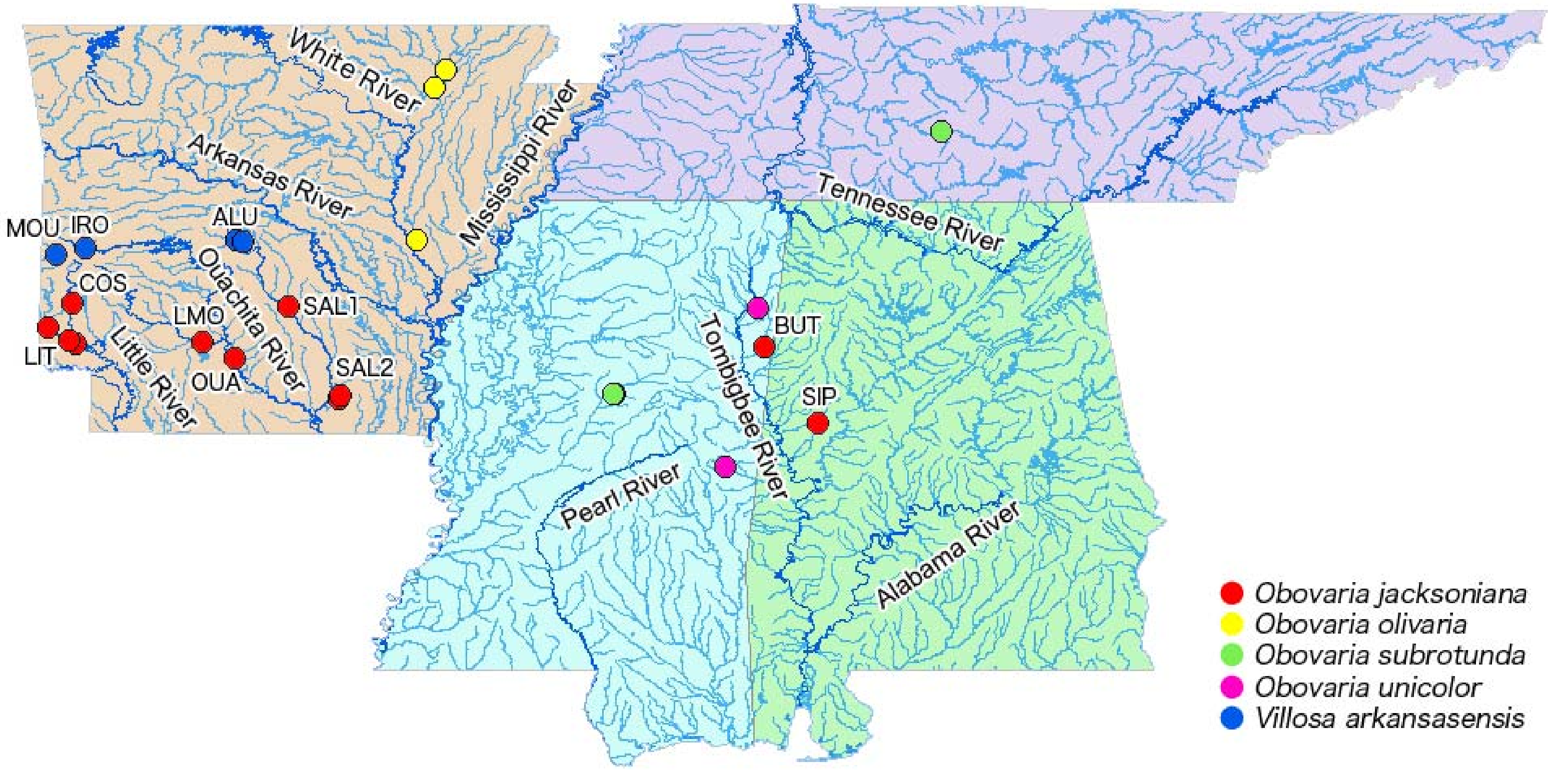

Figure 2.1. Map of collection localities used in the molecular phylogenetic analyses. Colored circles represent collected species. Specific locality information is provided in Table 2.1. The letters on *O. jacksoniana* and *V. arkansasensis* sites correspond to locality ID on Tables 2.4 and 2.5.

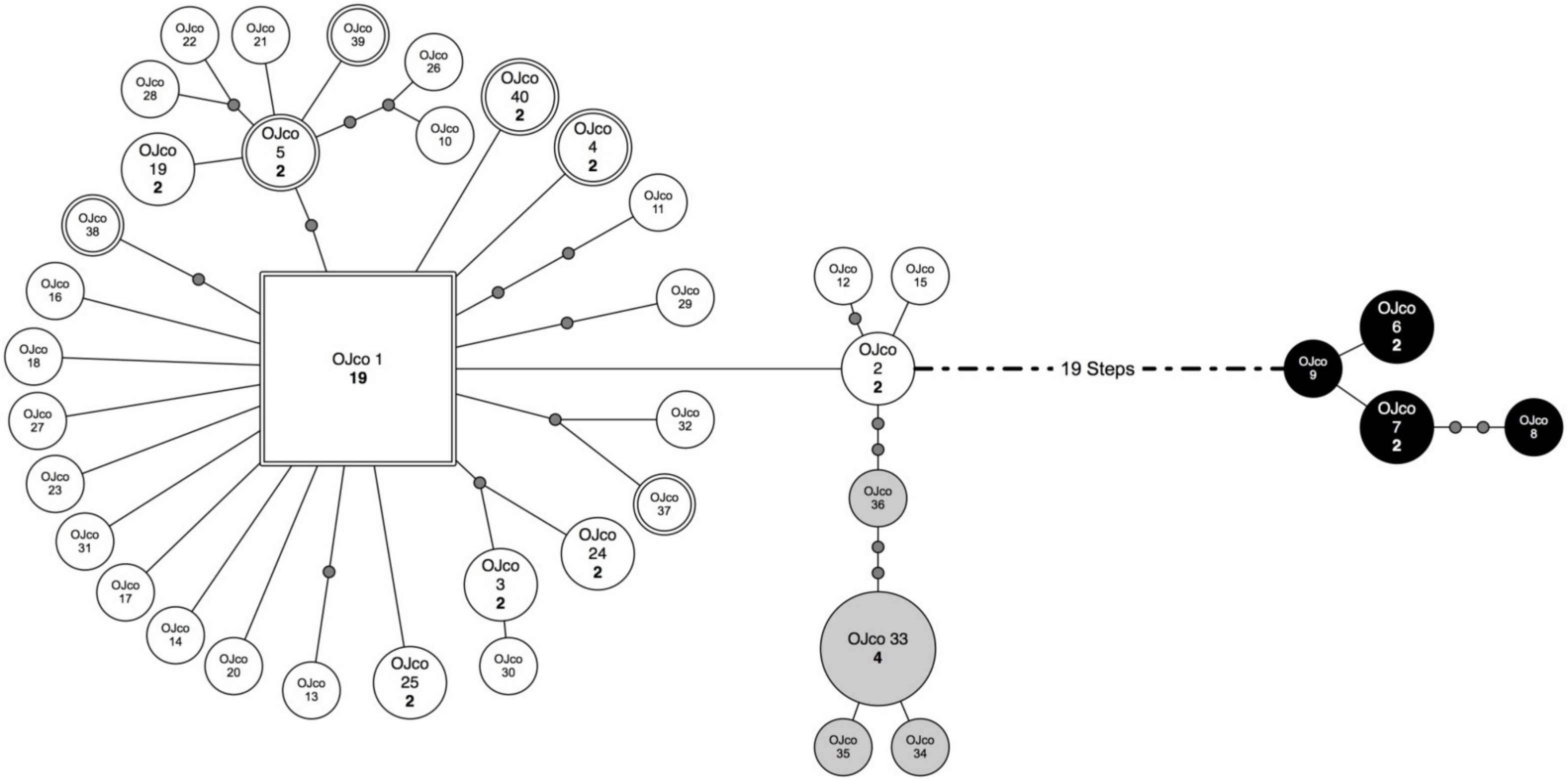

Figure 2.2. Parsimonious haplotype network of COI gene in *O. jacksoniana* and *V. arkansasensis*, where a circle represents a unique haplotype with haplotype ID and a line connecting two haplotypes represents a single nucleotide substitution. A square haplotype is based on the outgroup probability and small hatched circles represent intermediate haplotypes that were not encountered in the analysis. Size of the circle represents the frequency of the haplotype and bold numbers represent frequency. Double-lined circles contain haplotypes of both *O. jacksoniana* and *V. arkansasensis.* White circles represent the Ouachita River drainage, gray circles represent the Red River drainage, and black circles represent the Mobile River drainage.

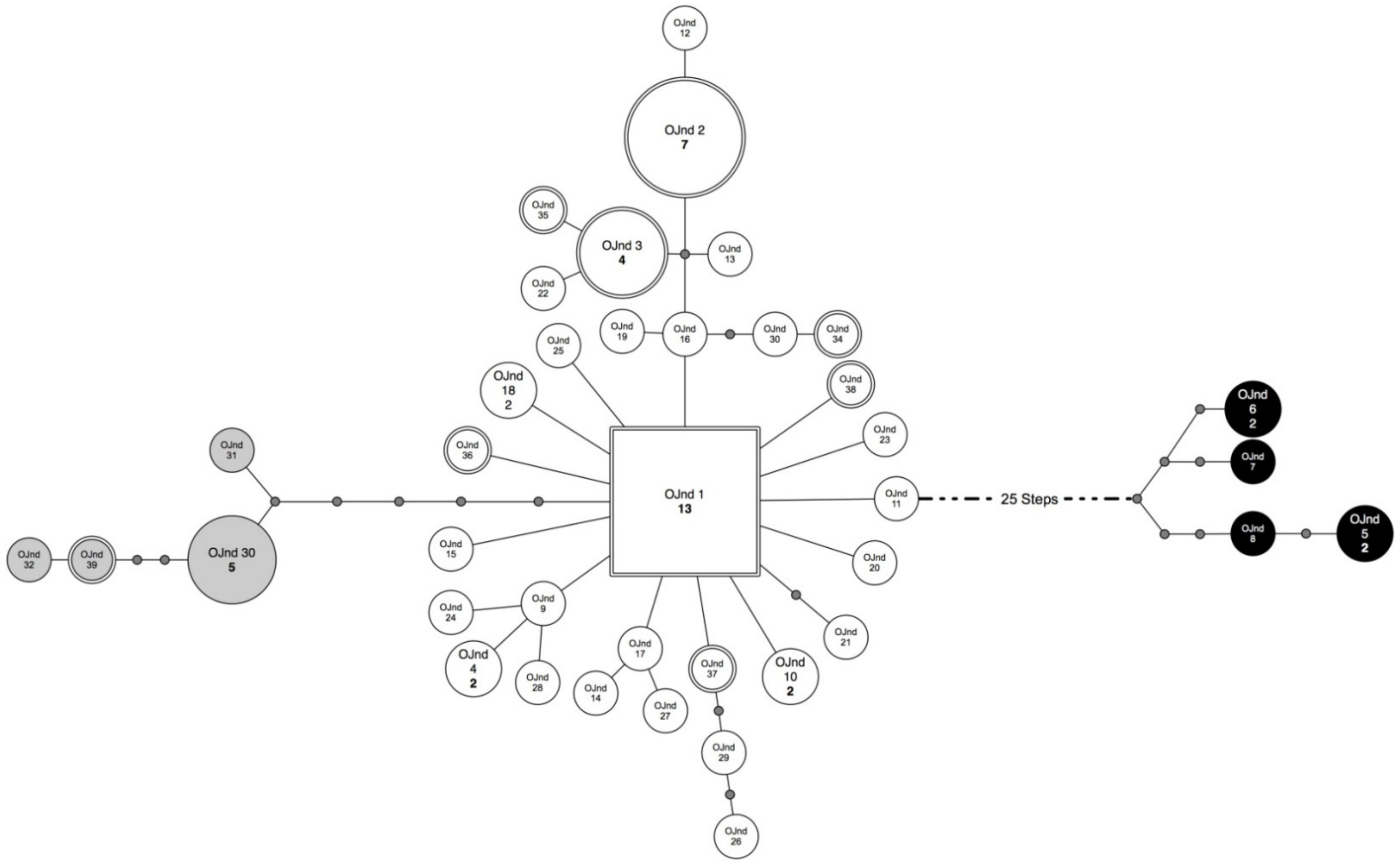

Figure 2.3. Parsimonious haplotype network of ND1 gene in *O. jacksoniana* and *V. arkansasensis*, where a circle represents a unique haplotype with haplotype ID and a line connecting two haplotypes represents a single nucleotide substitution. A square haplotype is based on the outgroup probability and small hatched circles represent intermediate haplotypes that were not encountered in the analysis. Size of the circle represents the frequency of the haplotype and bold numbers represent frequency. Double-lined circles contain haplotypes of both *O. jacksoniana* and *V. arkansasensis.* White circles represent the Ouachita River drainage, gray circles represent the Red River drainage, and black circles represent the Mobile River drainage.

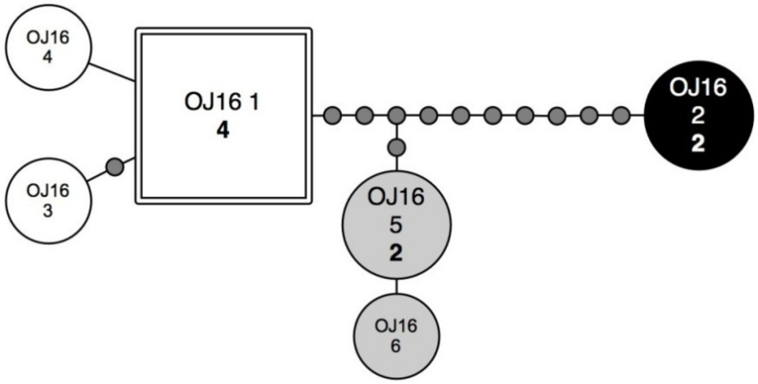

Figure 2.4. Parsimonious haplotype network of 16S gene in *O. jacksoniana* and *V. arkansasensis*, where a circle represents a unique haplotype with haplotype ID and a line connecting two haplotypes represents a single nucleotide substitution. A square haplotype is based on the outgroup probability and small hatched circles represent intermediate haplotypes that were not encountered in the analysis. Size of the circle represents the frequency of the haplotype and bold numbers represent frequency. Double-lined circles contain haplotypes of both *O. jacksoniana* and *V. arkansasensis.* White circles represent the Ouachita River drainage, gray circles represent the Red River drainage, and black circles represent the Mobile River drainage.

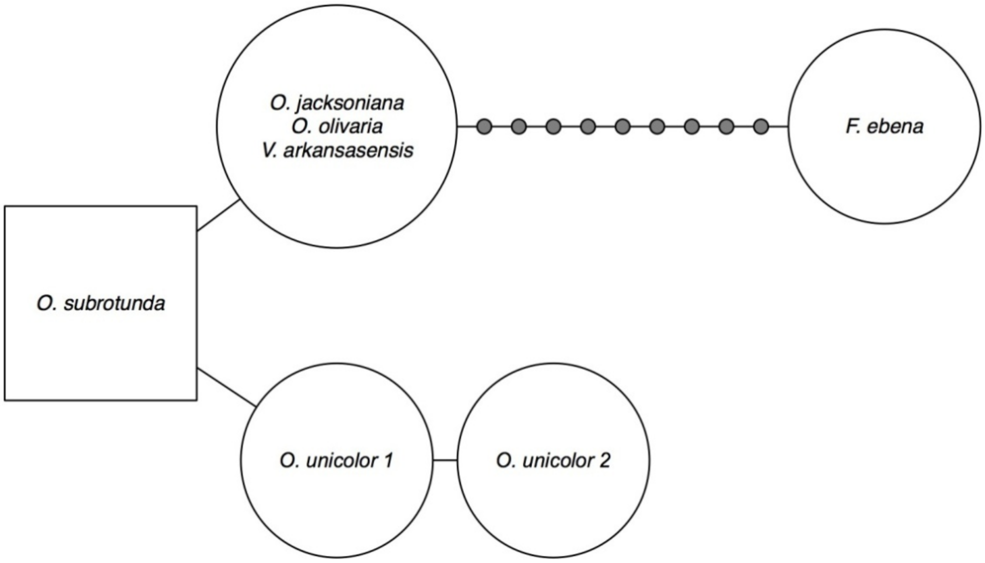

Figure 2.5. Parsimonious haplotype network of 28S gene in *Obovaria* species and *V. arkansasensis* with *F. ebena* as the outgroup, where a circle represents a unique haplotype with species and a line connecting two haplotypes represents a single nucleotide substitution. Square haplotype is based on the outgroup probability and small hatched circles represent intermediate haplotypes that were not encountered in the analysis.

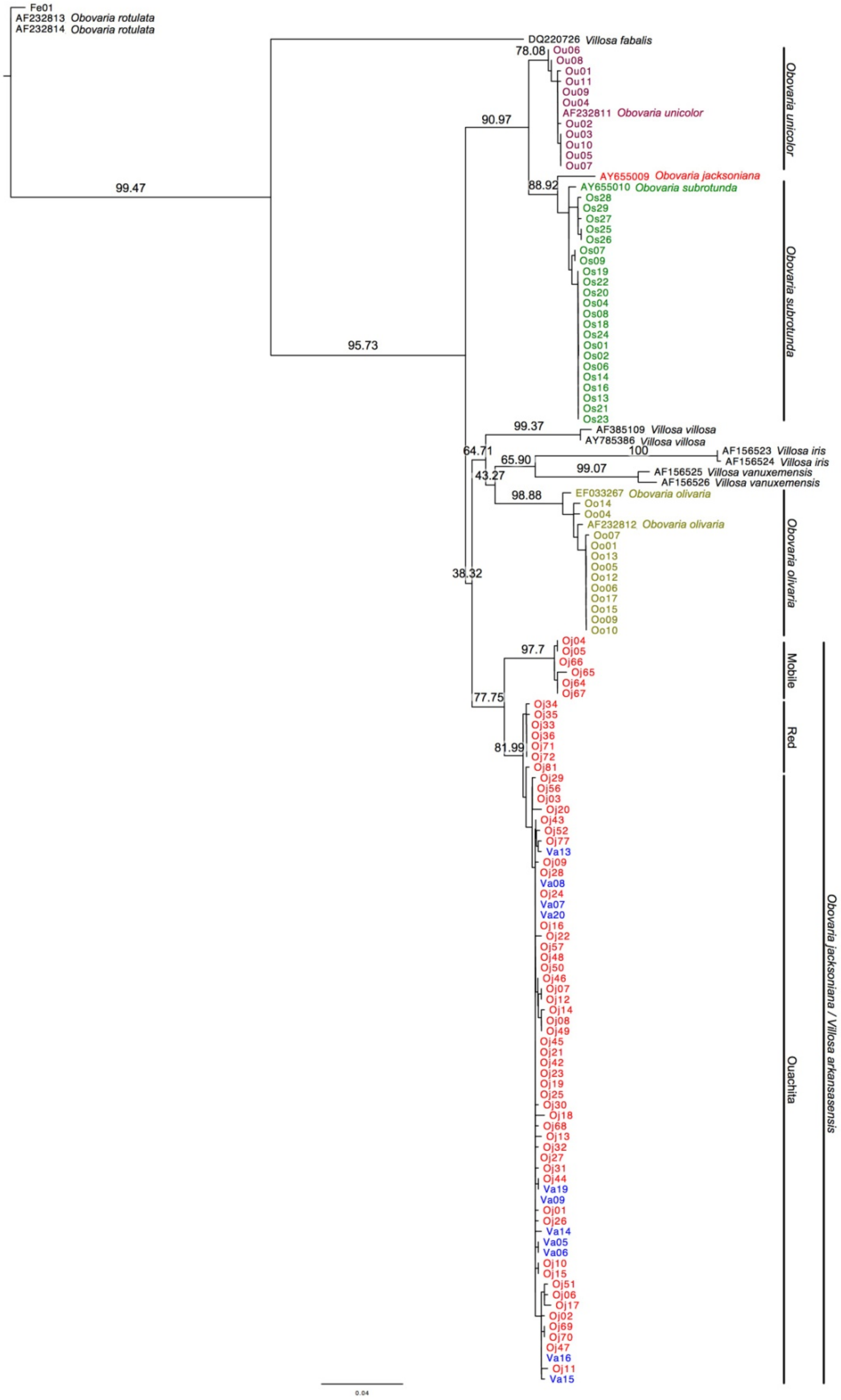

Figure 2.6. Maximum likelihood tree of COI gene using HKY+Γ model. Branch lengths are proportional to the inferred nucleotide divergence. LR-ELW edge support (1,000 replicates) showed above branches. OTU are listed by sequence ID referred to Table 2.1. Colors correspond to species on the map (Figure 2.1).

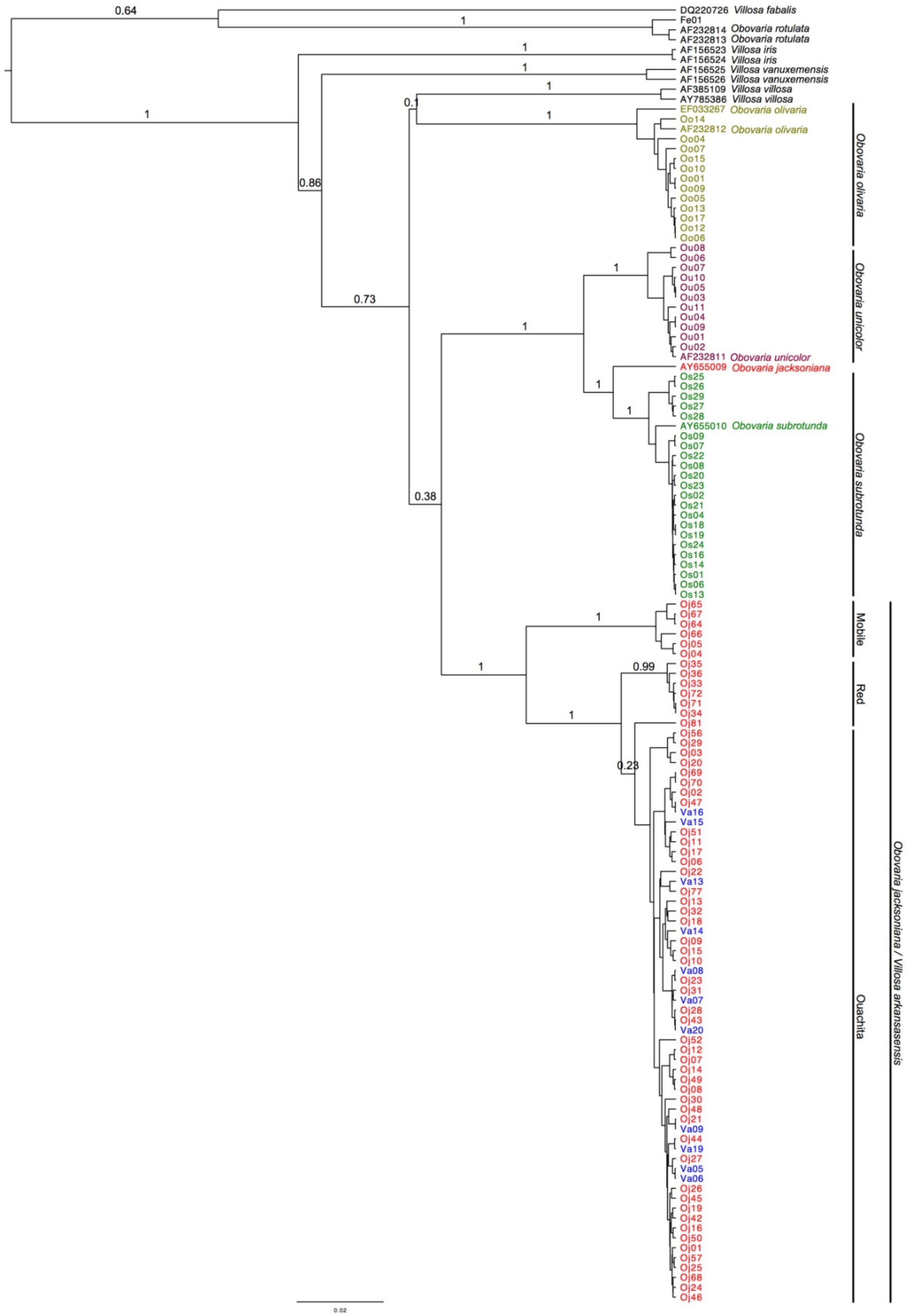

Figure 2.7. Majority consensus tree of COI gene generated from 10,000,000 Bayesian likelihood trees (burn-in = 1,000,000, mean log likelihood = -2691.747). Posterior probabilities showed above branches. OTU are listed by sequence ID referred to Table 2.1. Colors correspond to species on the map (Figure 2.1).

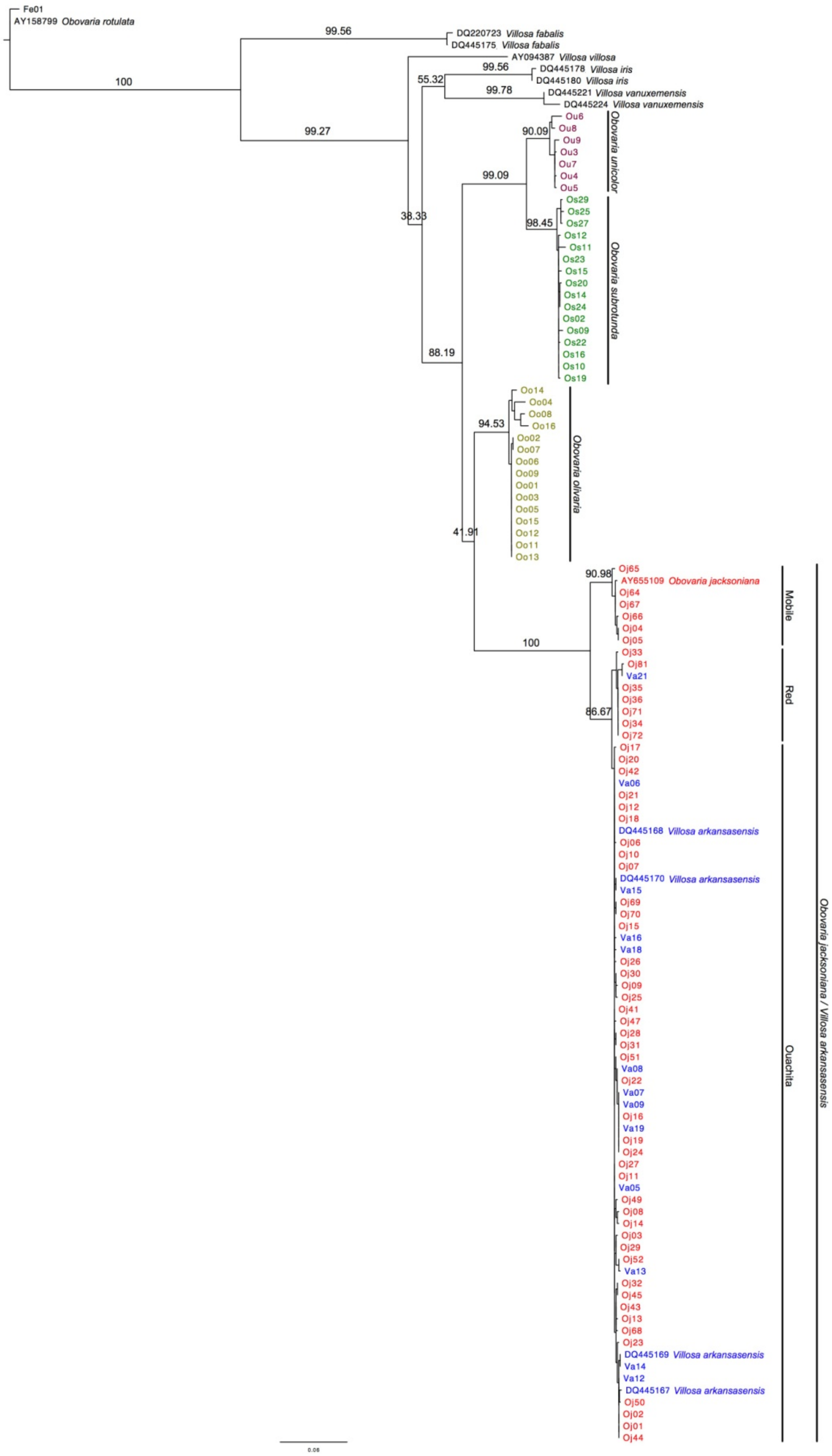

Figure 2.8. Maximum likelihood tree of ND1 gene using HKY+Γ model. Branch lengths are proportional to the inferred nucleotide divergence. LR-ELW edge support (1,000 replicates) showed above branches. OTU are listed by sequence ID referred to Table 2.1. Colors correspond to species on the map (Figure 2.1).

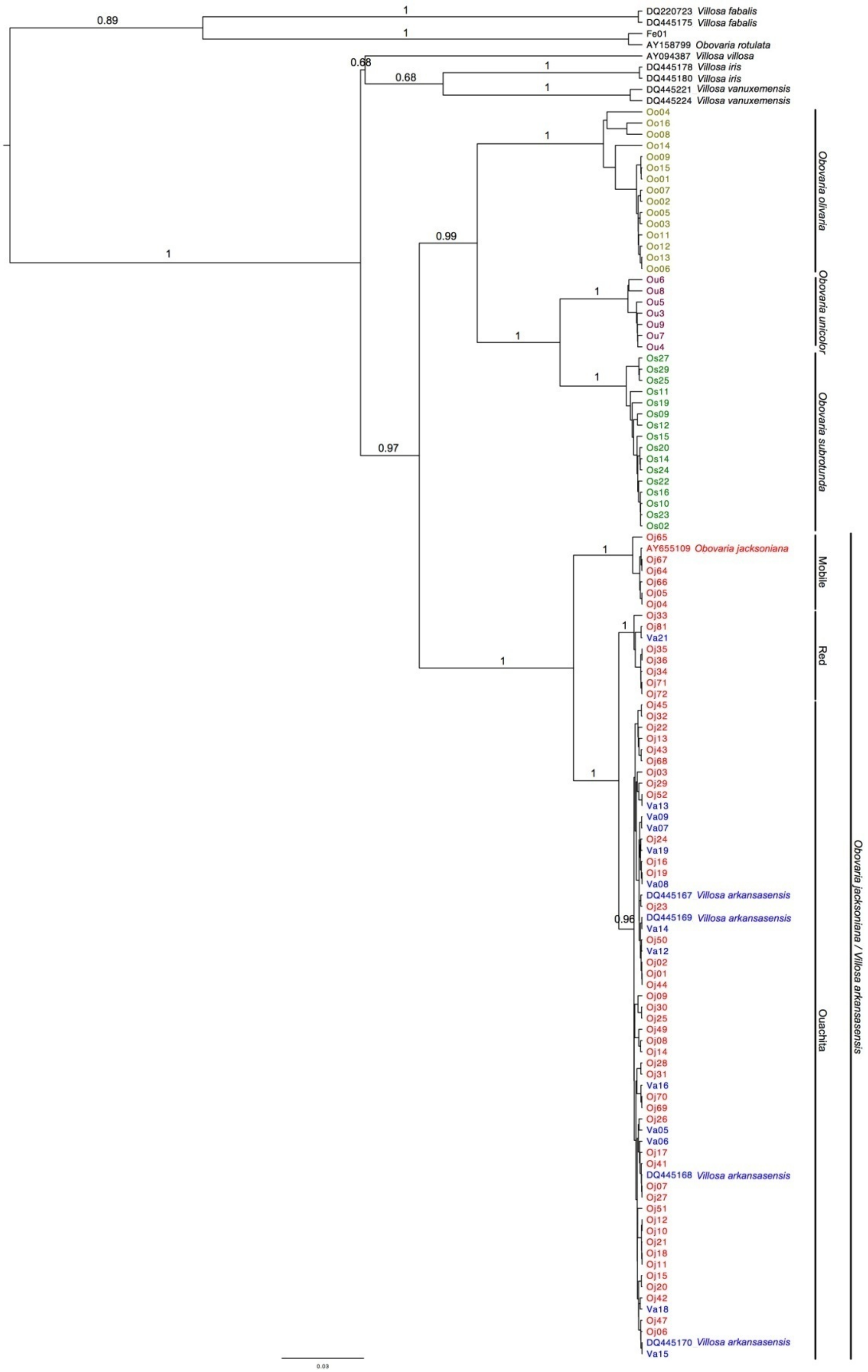

Figure 2.9. Majority consensus tree of ND1 gene generated from 10,000,000 Bayesian likelihood trees (burn-in = 1,000,000, mean log likelihood = -3859.708). Posterior probabilities showed above branches. OTU are listed by sequence ID referred to Table 2.1. Colors correspond to species on the map (Figure 2.1).

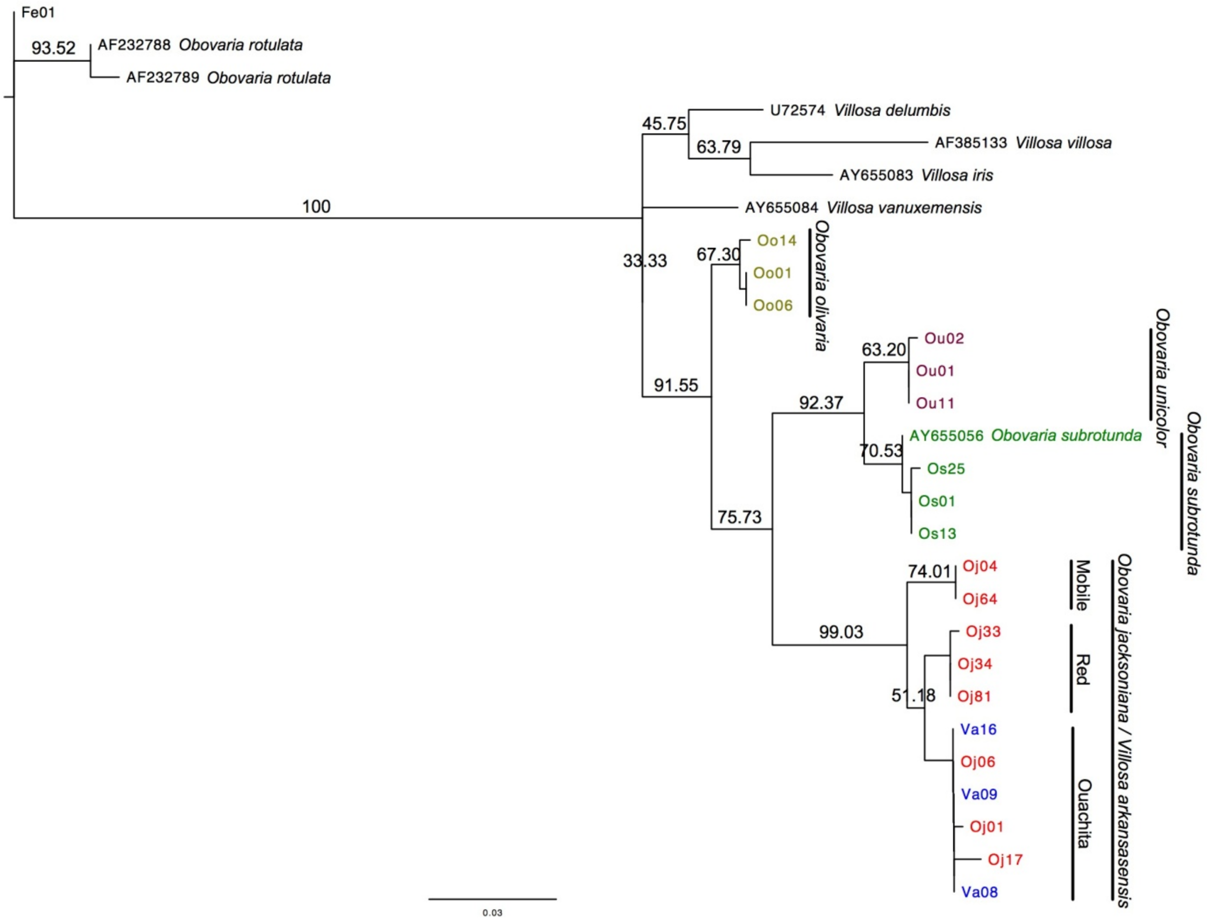

Figure 2.10. Maximum likelihood tree of 16S gene using TN+Γ model. Branch lengths are proportional to the inferred nucleotide divergence. LR-ELW edge support (1,000 replicates) showed above branches. OTU are listed by sequence ID referred to Table 2.1. Colors correspond to species on the map (Figure 2.1).

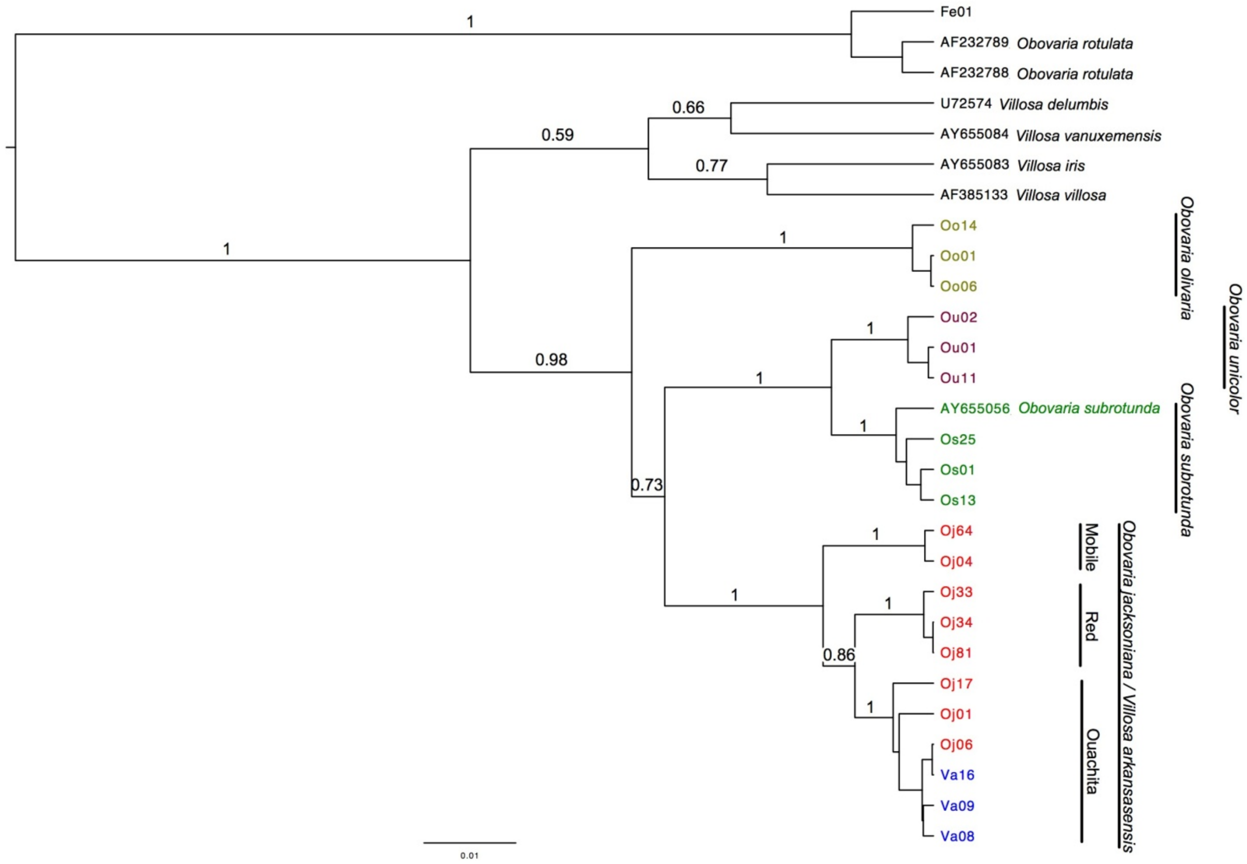

Figure 2.11. Majority consensus tree of 16S gene generated from 10,000,000 Bayesian likelihood trees (burn-in = 1,000,000, mean log likelihood = -1452.578). Posterior probabilities showed above branches. OTU are listed by sequence ID referred to Table 2.1. Colors correspond to species on the map (Figure 2.1).

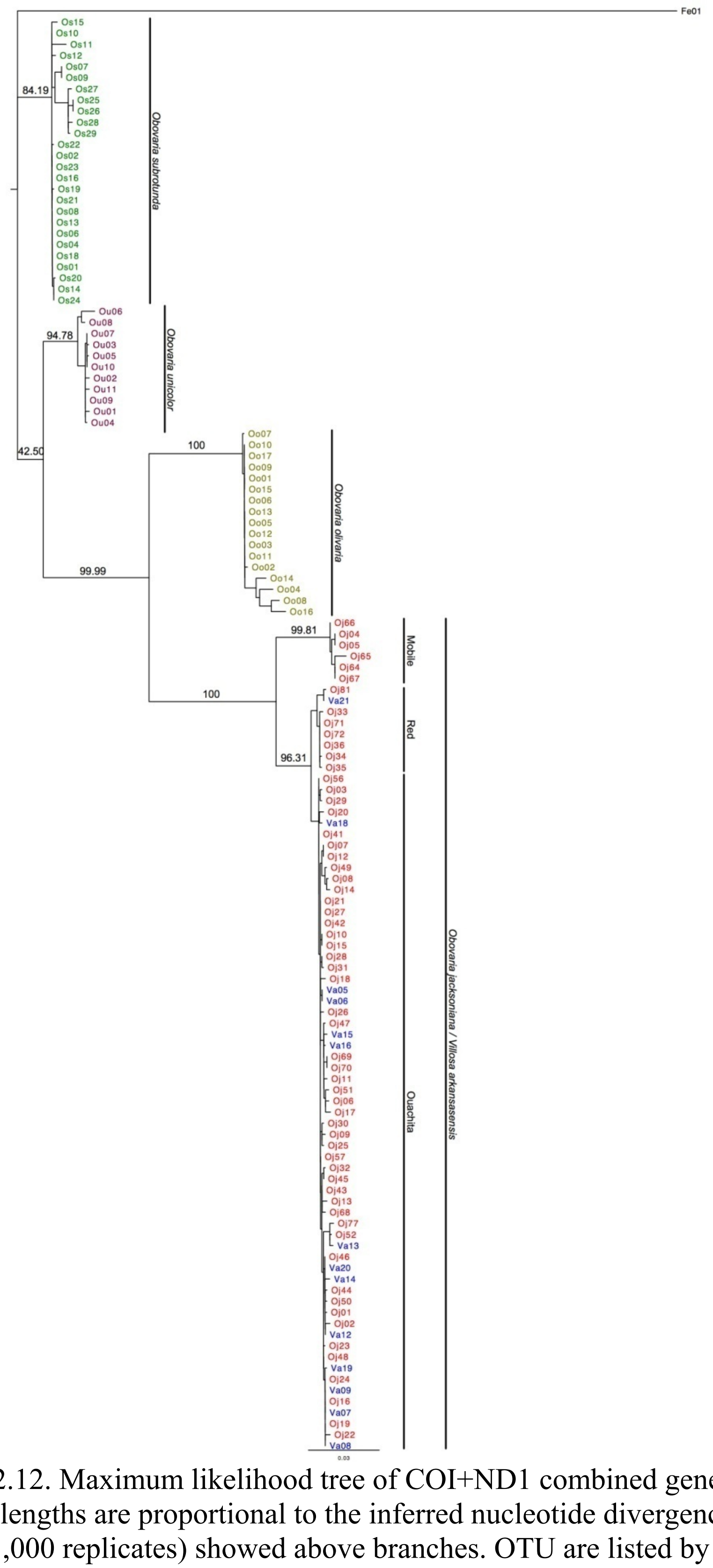

Figure 2.12. Maximum likelihood tree of COI+ND1 combined gene using J2+Γ model. Branch lengths are proportional to the inferred nucleotide divergence. LR-ELW edge support (1,000 replicates) showed above branches. OTU are listed by sequence ID referred to Table 2.1. Colors correspond to species on the map (Figure 2.1).

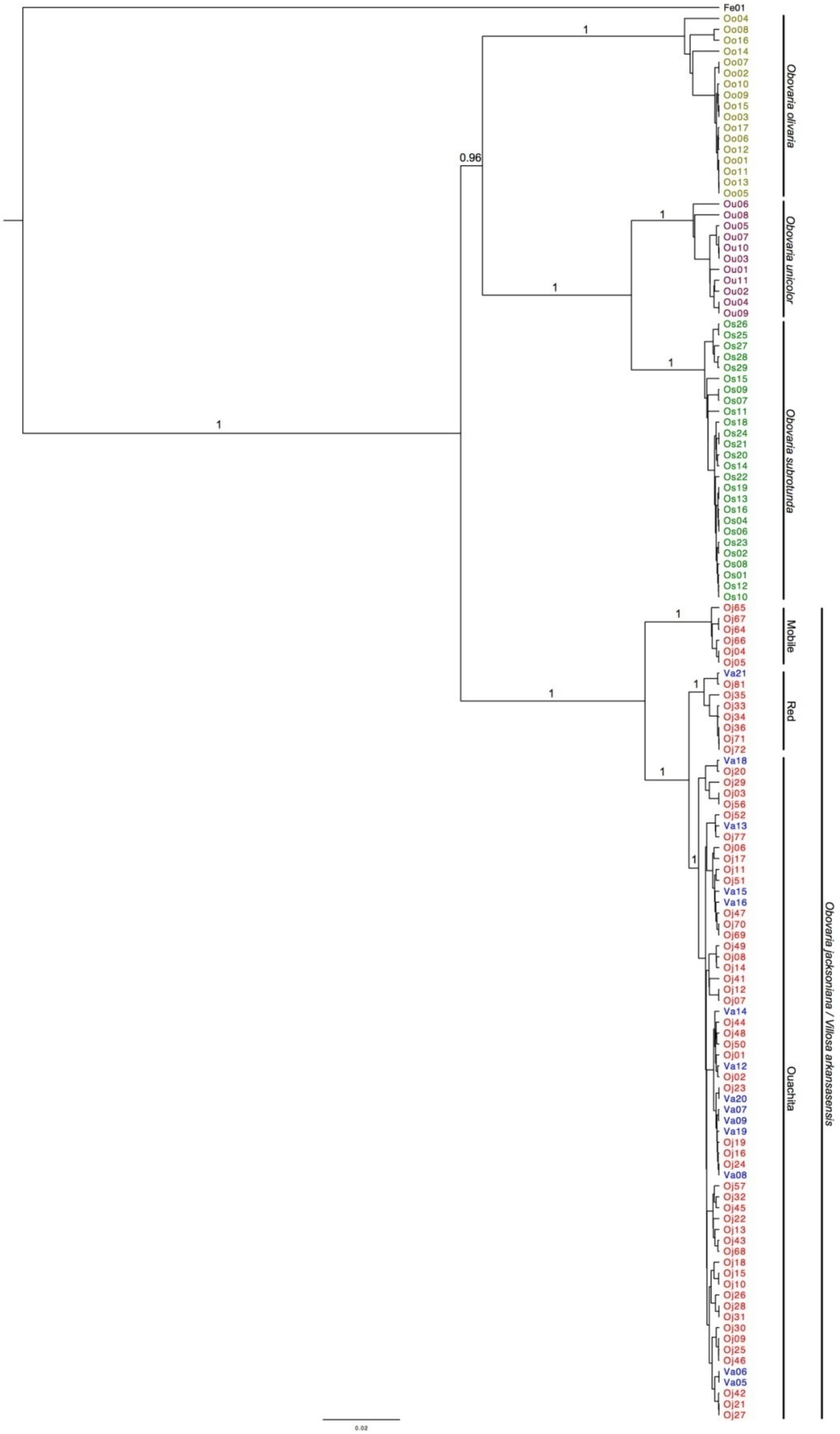

Figure 2.13. Majority consensus tree of COI+ND1 combined gene generated from 10,000,000 Bayesian likelihood trees (burn-in = 1,000,000, mean log likelihood = -5080.962). Posterior probabilities showed above branches. OTU are listed by sequence ID referred to Table 2.1. Colors correspond to species on the map (Figure 2.1).

Table 2.1. List of specimens for using on molecular phylogenetic analyses. Genes sequenced for each specimen are indicated by an “x”. Haplotype (H) ID corresponds to haplotype network (Figures 2.2, 2.3, and 2.4).

Museum #	Sequence ID	Locality	Longitude	Latitude	River Drainage	DNA Sequences						
						COI	H	ND1	H	16S	H	28S
Obovaria jacksoniana												
ASUMZ 4530	Oj 01	Saline R., Dallas/Grant Co., AR	-92.40946	34.11411	Ouachita	x	OJco 20	x	OJnd 3	x	OJ16 4	x
ASUMZ 4531	Oj 02	Saline R., Dallas/Grant Co., AR	-92.40946	34.11411	Ouachita	x	OJco 21	x	OJnd 3			
ASUMZ 4532	Oj 03	Saline R., Dallas/Grant Co., AR	-92.40946	34.11411	Ouachita	x	OJco 2	x	OJnd 19			
MMNS 8563.1	Oj 04	Buttahatchee R., Monroe Co., MS	-88.36466	33.77699	Mobile	x	OJco 6	x	OJnd 5	x	OJ16 2	x
MMNS 8563.2	Oj 05	Buttahatchee R., Monroe Co., MS	-88.36466	33.77699	Mobile	x	OJco 6	x	OJnd 5			x
ASUMZ 4533	Oj 06	Saline R., Ashley/Bradley Co., AR	-91.97575	33.32661	Ouachita	x	OJco 26	x	OJnd 25	x	OJ16 1	x
ASUMZ 4534	Oj 07	Saline R., Ashley/Bradley Co., AR	-91.97575	33.32661	Ouachita	x	OJco 24	x	OJnd 1			
ASUMZ 4535	Oj 08	Saline R., Ashley/Bradley Co., AR	-91.97575	33.32661	Ouachita	x	OJco 3	x	OJnd 26			
ASUMZ 4536	Oj 09	Saline R., Ashley/Bradley Co., AR	-91.97575	33.32661	Ouachita	x	OJco 27	x	OJnd 27			
ASUMZ 4537	Oj 10	Saline R., Ashley/Bradley Co., AR	-91.97575	33.32661	Ouachita	x	OJco 25	x	OJnd 1			
ASUMZ 4538	Oj 11	Saline R., Ashley/Bradley Co., AR	-91.97575	33.32661	Ouachita	x	OJco 28	x	OJnd 1			
ASUMZ 4539	Oj 12	Saline R., Ashley/Bradley Co., AR	-91.97575	33.32661	Ouachita	x	OJco 24	x	OJnd 1			
ASUMZ 4540	Oj 13	Saline R., Ashley/Bradley Co., AR	-91.97575	33.32661	Ouachita	x	OJco 29	x	OJnd 28			
ASUMZ 4541	Oj 14	Saline R., Ashley/Bradley Co., AR	-91.97575	33.32661	Ouachita	x	OJco 30	x	OJnd 29			
ASUMZ 4542	Oj 15	Saline R., Ashley/Bradley Co., AR	-91.97575	33.32661	Ouachita	x	OJco 25	x	OJnd 1			
ASUMZ 4543	Oj 16	Ouachita R., Ouachita Co., AR	-92.86965	33.67001	Ouachita	x	OJco 1	x	OJnd 2			x
ASUMZ 4544	Oj 17	Ouachita R., Ouachita Co., AR	-92.86965	33.67001	Ouachita	x	OJco 10	x	OJnd 11	x	OJ16 3	
ASUMZ 4545	Oj 18	Ouachita R., Ouachita Co., AR	-92.86965	33.67001	Ouachita	x	OJco 11	x	OJnd 1			
ASUMZ 4546	Oj 19	Ouachita R., Ouachita Co., AR	-92.86965	33.67001	Ouachita	x	OJco 1	x	OJnd 2			
ASUMZ 4547	Oj 20	Ouachita R., Ouachita Co., AR	-92.86965	33.67001	Ouachita	x	OJco 12	x	OJnd 1			
ASUMZ 4548	Oj 21	Ouachita R., Ouachita Co., AR	-92.86965	33.67001	Ouachita	x	OJco 1	x	OJnd 1			
ASUMZ 4549	Oj 22	Ouachita R., Ouachita Co., AR	-92.86965	33.67001	Ouachita	x	OJco 13	x	OJnd 12			
ASUMZ 4550	Oj 23	Ouachita R., Ouachita Co., AR	-92.86965	33.67001	Ouachita	x	OJco 1	x	OJnd 13			
ASUMZ 4551	Oj 24	Ouachita R., Ouachita Co., AR	-92.86965	33.67001	Ouachita	x	OJco 1	x	OJnd 2			
ASUMZ 4552	Oj 25	Ouachita R., Ouachita Co., AR	-92.86965	33.67001	Ouachita	x	OJco 1	x	OJnd 14			

(table continues)

Table 2.1 (*continued*)

Museum #	Sequence ID	Locality	Longitude	Latitude	River Drainage	DNA Sequences						
						COI	H	ND1	H	16S	H	28S
ASUMZ 4553	Oj 26	Ouachita R., Ouachita Co., AR	-92.86965	33.67001	Ouachita	x	OJco 14	x	OJnd 15			
ASUMZ 4554	Oj 27	Ouachita R., Ouachita Co., AR	-92.86965	33.67001	Ouachita	x	OJco 1	x	OJnd 1			
ASUMZ 4555	Oj 28	Ouachita R., Ouachita Co., AR	-92.86965	33.67001	Ouachita	x	OJco 1	x	OJnd 10			
ASUMZ 4556	Oj 29	Ouachita R., Ouachita Co., AR	-92.86965	33.67001	Ouachita	x	OJco 15	x	OJnd 16			
ASUMZ 4557	Oj 30	Ouachita R., Ouachita Co., AR	-92.86965	33.67001	Ouachita	x	OJco 16	x	OJnd 17			
ASUMZ 4558	Oj 31	Ouachita R., Ouachita Co., AR	-92.86965	33.67001	Ouachita	x	OJco 17	x	OJnd 10			
ASUMZ 4559	Oj 32	Ouachita R., Ouachita Co., AR	-92.86965	33.67001	Ouachita	x	OJco 18	x	OJnd 4			
ASUMZ 4560	Oj 33	Little R., Little River/Sevier Co., AR	-94.21356	33.80304	Red	x	OJco 33	x	OJnd 32	x	OJ16 6	x
ASUMZ 4561	Oj 34	Little R., Little River/Sevier Co., AR	-94.27107	33.82135	Red	x	OJco 34	x	OJnd 31	x	OJ16 5	
ASUMZ 4562	Oj 35	Little R., Little River/Sevier Co., AR	-94.27107	33.82135	Red	x	OJco 35	x	OJnd 31			
ASUMZ 4563	Oj 36	Little R., Little River/Sevier Co., AR	-94.27107	33.82135	Red	x	OJco 33	x	OJnd 31			
ASUMZ 4564	Oj 37	Saline R., Ashley/Bradley Co., AR	-91.97080	33.35090	Ouachita							
ASUMZ 4565	Oj 38	Saline R., Ashley/Bradley Co., AR	-91.97080	33.35090	Ouachita							
ASUMZ 4566	Oj 39	Saline R., Ashley/Bradley Co., AR	-91.97080	33.35090	Ouachita							
ASUMZ 4567	Oj 40	Saline R., Ashley/Bradley Co., AR	-91.97080	33.35090	Ouachita							
ASUMZ 4601	Oj 41	Little Missouri R., Nevada Co., AR	-93.13412	33.80963	Ouachita			x	OJnd 1			
ASUMZ 4602	Oj 42	Little Missouri R., Nevada Co., AR	-93.13412	33.80963	Ouachita	x	OJco 1	x	OJnd 1			
ASUMZ 4603	Oj 43	Little Missouri R., Nevada Co., AR	-93.13412	33.80963	Ouachita	x	OJco 1	x	OJnd 9			
ASUMZ 4610	Oj 44	Saline R., Dallas/Grant Co., AR	-92.40946	34.11411	Ouachita	x	OJco 4	x	OJnd 3			
ASUMZ 4611	Oj 45	Saline R., Dallas/Grant Co., AR	-92.40946	34.11411	Ouachita	x	OJco 1	x	OJnd 4			
ASUMZ 4612	Oj 46	Saline R., Dallas/Grant Co., AR	-92.40946	34.11411	Ouachita	x	OJco 1					
ASUMZ 4613	Oj 47	Saline R., Dallas/Grant Co., AR	-92.40946	34.11411	Ouachita	x	OJco 5	x	OJnd 20			
ASUMZ 4645	Oj 48	Saline R., Dallas/Grant Co., AR	-92.40946	34.11411	Ouachita	x	OJco 1					
ASUMZ 4646	Oj 49	Saline R., Dallas/Grant Co., AR	-92.40946	34.11411	Ouachita	x	OJco 3	x	OJnd 21			
ASUMZ 4647	Oj 50	Saline R., Dallas/Grant Co., AR	-92.40946	34.11411	Ouachita	x	OJco 1	x	OJnd 22			
ASUMZ 4648	Oj 51	Saline R., Dallas/Grant Co., AR	-92.40946	34.11411	Ouachita	x	OJco 22	x	OJnd 23			
ASUMZ 4649	Oj 52	Saline R., Ashley/Bradley Co., AR	-91.97080	33.35090	Ouachita	x	OJco 31	x	OJnd 30			
ASUMZ 4650	Oj 53	Saline R., Ashley/Bradley Co., AR	-91.97080	33.35090	Ouachita							

(table continues)

Table 2.1 (*continued*)

Museum #	Sequence ID	Locality	Longitude	Latitude	River Drainage	DNA Sequences						
						COI	H	ND1	H	16S	H	28S
ASUMZ 4651	Oj 54	Saline R., Ashley/Bradley Co., AR	-91.97080	33.35090	Ouachita							
ASUMZ 4652	Oj 55	Saline R., Ashley/Bradley Co., AR	-91.97080	33.35090	Ouachita							
ASUMZ 4653	Oj 56	Little Missouri R., Nevada Co., AR	-93.13412	33.80963	Ouachita	x	OJco 2					
ASUMZ 4654	Oj 57	Little Missouri R., Nevada Co., AR	-93.13412	33.80963	Ouachita	x	OJco 1					
ASUMZ 4655	Oj 58	Saline R., Ashley/Bradley Co., AR	-91.97080	33.35090	Ouachita							
ASUMZ 4656	Oj 59	Saline R., Ashley/Bradley Co., AR	-91.97080	33.35090	Ouachita							
ASUMZ 4657	Oj 60	Saline R., Ashley/Bradley Co., AR	-91.97080	33.35090	Ouachita							
ASUMZ 4658	Oj 61	Saline R., Ashley/Bradley Co., AR	-91.97080	33.35090	Ouachita							
ASUMZ 4659	Oj 62	Little Missouri R., Nevada Co., AR	-93.13412	33.80963	Ouachita							
ASUMZ 4660	Oj 63	Little Missouri R., Nevada Co., AR	-93.13412	33.80963	Ouachita							
ASUMZ 4871	Oj 64	Sipsey R., Pickens/Greene Co., AL	-86.91440	33.12170	Mobile	x	OJco 7	x	OJnd 6	x	OJ16 2	
ASUMZ 4872	Oj 65	Sipsey R., Pickens/Greene Co., AL	-86.91440	33.12170	Mobile	x	OJco 8	x	OJnd 7			
ASUMZ 4873	Oj 66	Sipsey R., Pickens/Greene Co., AL	-86.91440	33.12170	Mobile	x	OJco 9	x	OJnd 8			
ASUMZ 4874	Oj 67	Sipsey R., Pickens/Greene Co., AL	-86.91440	33.12170	Mobile	x	OJco 7	x	OJnd 6			
ASUMZ 4875	Oj 68	Saline R., Dallas/Grant Co., AR	-92.40946	34.11411	Ouachita	x	OJco 23	x	OJnd 24			
ASUMZ 4876	Oj 69	Saline R., Dallas/Grant Co., AR	-92.40946	34.11411	Ouachita	x	OJco 19	x	OJnd 18			
ASUMZ 4877	Oj 70	Saline R., Dallas/Grant Co., AR	-92.40946	34.11411	Ouachita	x	OJco 19	x	OJnd 18			
ASUMZ 4878	Oj 71	Little R., Sevier Co., AR	-94.45802	33.93519	Red	x	OJco 33	x	OJnd 31			
ASUMZ 4879	Oj 72	Little R., Sevier Co., AR	-94.36769	33.87716	Red	x	OJco 33	x	OJnd 31			
MD1	Oj 73	Saline R., Ashley/Bradley Co., AR	-91.97080	33.35090	Ouachita							
MD2	Oj 74	Saline R., Ashley/Bradley Co., AR	-91.97080	33.35090	Ouachita							
MD3	Oj 75	Saline R., Ashley/Bradley Co., AR	-91.97080	33.35090	Ouachita							
MD4	Oj 76	Saline R., Ashley/Bradley Co., AR	-91.97080	33.35090	Ouachita							
MD5	Oj 77	Saline R., Ashley/Bradley Co., AR	-91.97080	33.35090	Ouachita	x	OJco 32					
MD6	Oj 78	Saline R., Ashley/Bradley Co., AR	-91.97080	33.35090	Ouachita							
MD7	Oj 79	Saline R., Ashley/Bradley Co., AR	-91.97080	33.35090	Ouachita							
MD8	Oj 80	Saline R., Ashley/Bradley Co., AR	-91.97080	33.35090	Ouachita							
ASUMZ 4568	Oj 81	Cossatot R., Sevier Co., AR	-94.23917	34.14460	Red	x	OJco 36	x	OJnd 33	x	OJ16 5	x

(table continues)

Table 2.1 (*continued*)

Museum #	Sequence ID	Locality	Longitude	Latitude	River Drainage	DNA Sequences						
						COI	H	ND1	H	16S	H	28S
Villosa arkansasensis												
ASUMZ 4569	Va 01	Alum Fork of the Saline R., Saline Co., AR	-92.79880	34.67280	Ouachita							
ASUMZ 4570	Va 02	Alum Fork of the Saline R., Saline Co., AR	-92.79880	34.67280	Ouachita							
ASUMZ 4571	Va 03	Alum Fork of the Saline R., Saline Co., AR	-92.79880	34.67280	Ouachita							
ASUMZ 4572	Va 04	Alum Fork of the Saline R., Saline Co., AR	-92.79880	34.67280	Ouachita							
ASUMZ 4579	Va 05	Irons Fork of the Ouachita R., Polk Co., AR	-94.13941	34.61661	Ouachita	x	OJco 40	x	OJnd 1			x
ASUMZ 4580	Va 06	Irons Fork of the Ouachita R., Polk Co., AR	-94.13941	34.61661	Ouachita	x	OJco 40	x	OJnd 1			x
ASUMZ 4581	Va 07	Irons Fork of the Ouachita R., Polk Co., AR	-94.13941	34.61661	Ouachita	x	OJco 1	x	OJnd 2			
ASUMZ 4582	Va 08	Irons Fork of the Ouachita R., Polk Co., AR	-94.13941	34.61661	Ouachita	x	OJco 1	x	OJnd 2	x	OJ16 1	
ASUMZ 4583	Va 09	Irons Fork of the Ouachita R., Polk Co., AR	-94.13941	34.61661	Ouachita	x	OJco 1	x	OJnd 2	x	OJ16 1	x
Tissue clip	Va 10	Alum Fork of the Saline R., Saline Co., AR	-94.13941	34.61661	Ouachita							
Tissue clip	Va 11	Alum Fork of the Saline R., Saline Co., AR	-94.13941	34.61661	Ouachita							
ASUMZ 4661	Va 12	Alum Fork of the Saline R., Saline Co., AR	-94.13941	34.61661	Ouachita			x	OJnd 3			
ASUMZ 4662	Va 13	Alum Fork of the Saline R., Saline Co., AR	-94.13941	34.61661	Ouachita	x	OJco 37	x	OJnd 34			
Tissue clip	Va 14	Alum Fork of the Saline R., Saline Co., AR	-94.13941	34.61661	Ouachita	x	OJco 38	x	OJnd 35			x
Tissue clip	Va 15	Alum Fork of the Saline R., Saline Co., AR	-94.13941	34.61661	Ouachita	x	OJco 39	x	OJnd 36			x
ASUMZ 4663	Va 16	Alum Fork of the Saline R., Saline Co., AR	-94.13941	34.61661	Ouachita	x	OJco 5	x	OJnd 37	x	OJ16 1	x
ASUMZ 4664	Va 17	Alum Fork of the Saline R., Saline Co., AR	-94.13941	34.61661	Ouachita							
ASUMZ 4665	Va 18	Alum Fork of the Saline R., Saline Co., AR	-94.13941	34.61661	Ouachita			x	OJnd 38			x
ASUMZ 4666	Va 19	Alum Fork of the Saline R., Saline Co., AR	-94.13941	34.61661	Ouachita	x	OJco 4	x	OJnd 2			
ASUMZ 4667	Va 20	Irons Fork of the Ouachita R., Polk Co., AR	-94.13941	34.61661	Ouachita	x	OJco 1					
ASUMZ 4889	Va 21	Mountain Fork of the Little R., Polk Co., AR	-94.37800	34.56100	Red			x	OJnd 39			
Obovaria subrotunda												
MMNS 7650.1	Os 01	Big Black R., Montgomery Co., MS	-89.62614	33.37487	Big Black	x				x		x
MMNS 7650.2	Os 02	Big Black R., Montgomery Co., MS	-89.62614	33.37487	Big Black	x		x				
MMNS 7650.3	Os 03	Big Black R., Montgomery Co., MS	-89.62614	33.37487	Big Black							
MMNS 7650.4	Os 04	Big Black R., Montgomery Co., MS	-89.62614	33.37487	Big Black	x						
MMNS 7650.5	Os 05	Big Black R., Montgomery Co., MS	-89.62614	33.37487	Big Black							

(table continues)

Table 2.1. *(continued)*

Museum #	Sequence ID	Locality	Longitude	Latitude	River Drainage	DNA Sequences						
						COI	H	ND1	H	16S	H	28S
MMNS 7650.6	Os 06	Big Black R., Montgomery Co., MS	-89.62614	33.37487	Big Black	x						
MMNS 7650.7	Os 07	Big Black R., Montgomery Co., MS	-89.62614	33.37487	Big Black	x						
MMNS 7650.8	Os 08	Big Black R., Montgomery Co., MS	-89.62614	33.37487	Big Black	x						
MMNS 7650.9	Os 09	Big Black R., Montgomery Co., MS	-89.62614	33.37487	Big Black	x		x				
MMNS 7650.10	Os 10	Big Black R., Montgomery Co., MS	-89.62614	33.37487	Big Black			x				
MMNS 7650.11	Os 11	Big Black R., Montgomery Co., MS	-89.62614	33.37487	Big Black			x				
MMNS 7650.12	Os 12	Big Black R., Montgomery Co., MS	-89.62614	33.37487	Big Black			x				
MMNS 7650.13	Os 13	Big Black R., Montgomery Co., MS	-89.63897	33.37187	Big Black	x				x		
MMNS 7650.14	Os 14	Big Black R., Montgomery Co., MS	-89.63897	33.37187	Big Black	x		x				
MMNS 7650.15	Os 15	Big Black R., Montgomery Co., MS	-89.63897	33.37187	Big Black			x				
MMNS 7650.16	Os 16	Big Black R., Montgomery Co., MS	-89.63897	33.37187	Big Black	x		x				
MMNS 7650.17	Os 17	Big Black R., Montgomery Co., MS	-89.63897	33.37187	Big Black							
MMNS 7650.18	Os 18	Big Black R., Montgomery Co., MS	-89.63897	33.37187	Big Black	x						
MMNS 7650.19	Os 19	Big Black R., Montgomery Co., MS	-89.63897	33.37187	Big Black	x		x				
MMNS 7650.20	Os 20	Big Black R., Montgomery Co., MS	-89.63897	33.37187	Big Black	x		x				
MMNS 7650.21	Os 21	Big Black R., Montgomery Co., MS	-89.63897	33.37187	Big Black	x						
MMNS 7650.22	Os 22	Big Black R., Montgomery Co., MS	-89.63897	33.37187	Big Black	x		x				
MMNS 7650.23	Os 23	Big Black R., Montgomery Co., MS	-89.63897	33.37187	Big Black	x		x				
MMNS 7650.24	Os 24	Big Black R., Montgomery Co., MS	-89.63897	33.37187	Big Black	x		x				
ASUMZ 4604	Os 25	Duck R., Marshall Co., TN	-86.85385	35.61152	Tennessee	x		x		x		x
ASUMZ 4605	Os 26	Duck R., Marshall Co., TN	-86.85385	35.61152	Tennessee	x						
ASUMZ 4606	Os 27	Duck R., Marshall Co., TN	-86.85385	35.61152	Tennessee	x		x				
ASUMZ 4607	Os 28	Duck R., Marshall Co., TN	-86.85385	35.61152	Tennessee	x						
ASUMZ 4608	Os 29	Duck R., Marshall Co., TN	-86.85385	35.61152	Tennessee	x		x				
Obovaria olivaria												
20051020:01-01	Oo 01	White R., Monroe Co., AR	-91.31450	34.68120	White	x		x		x		x
20051020:01-02	Oo 02	White R., Monroe Co., AR	-91.31450	34.68120	White			x				
20051020:01-03	Oo 03	White R., Monroe Co., AR	-91.31450	34.68120	White			x				

(table continues)

Table 2.1. *(continued)*

Museum #	Sequence ID	Locality	Longitude	Latitude	River Drainage	DNA Sequences						
						COI	H	ND1	H	16S	H	28S
20051020:01-04	Oo 04	White R., Monroe Co., AR	-91.31450	34.68120	White	x		x				
20051020:01-05	Oo 05	White R., Monroe Co., AR	-91.31450	34.68120	White	x		x				
20051020:01-06	Oo 06	White R., Monroe Co., AR	-91.31450	34.68120	White	x		x		x		
20051020:01-07	Oo 07	White R., Monroe Co., AR	-91.31450	34.68120	White	x		x				
20051020:01-08	Oo 08	White R., Monroe Co., AR	-91.31450	34.68120	White			x				
20051020:01-09	Oo 09	White R., Monroe Co., AR	-91.31450	34.68120	White	x		x				
20051020:01-10	Oo 10	White R., Monroe Co., AR	-91.31450	34.68120	White	x						
20051020:01-11	Oo 11	White R., Monroe Co., AR	-91.31450	34.68120	White			x				
20051020:01-12	Oo 12	White R., Monroe Co., AR	-91.31450	34.68120	White	x		x				
20051020:01-13	Oo 13	White R., Monroe Co., AR	-91.31450	34.68120	White	x		x				
20080801BR:01-01	Oo 14	Black R., Lawrece/Randolph Co., AR	-91.16248	35.98004	White	x		x		x		x
20080801BR:01-02	Oo 15	Black R., Lawrece/Randolph Co., AR	-91.16248	35.98004	White	x		x				
20060626:09-01	Oo 16	Black R., Lawrece/Randolph Co., AR	-91.06284	36.13123	White			x				
20060626:09-02	Oo 17	Black R., Lawrece/Randolph Co., AR	-91.06284	36.13123	White	x						
Obovaria unicolor												
MMNS 7600	Ou 01	East Fork of the Tombigbee R., Itawamba Co., MS	-88.41676	34.10200	Mobile	x				x		x
ASUMZ 4890	Ou 02	Sipsey R., Pickens/Greene Co., AL	-86.91440	33.12170	Mobile	x				x		x
ASUMZ 4891	Ou 03	Sipsey R., Pickens/Greene Co., AL	-86.91440	33.12170	Mobile	x		x				
ASUMZ 4892	Ou 04	Sipsey R., Pickens/Greene Co., AL	-86.91440	33.12170	Mobile	x		x				
ASUMZ 4893	Ou 05	Sipsey R., Pickens/Greene Co., AL	-86.91440	33.12170	Mobile	x		x				
ASUMZ 4894	Ou 06	Sipsey R., Pickens/Greene Co., AL	-86.91440	33.12170	Mobile	x		x				
ASUMZ 4895	Ou 07	Sipsey R., Pickens/Greene Co., AL	-86.91440	33.12170	Mobile	x		x				
ASUMZ 4896	Ou 08	Sipsey R., Pickens/Greene Co., AL	-86.91440	33.12170	Mobile	x		x				
ASUMZ 4897	Ou 09	Sipsey R., Pickens/Greene Co., AL	-86.91440	33.12170	Mobile	x		x				
ASUMZ 4898	Ou 10	Sipsey R., Pickens/Greene Co., AL	-86.91440	33.12170	Mobile	x						
MMNS 7415	Ou 11	Sucarnoochee R., Kemper Co., MS	-88.69802	32.74914	Mobile	x				x		x
Fusconaia ebena												
ASUMZ 4899	Fe 01	White R., Monroe Co., AR	-91.31450	34.68120	White	x		x		x		x

Table 2.2. List of primer sequences. ModCOI L and H are designed from complete mtDNA genome of *Lampsilis ornata* (Serb and Lydeard, 2003). LoGlyR is alternate primer of ND1 b for problematic taxa (Serb et al., 2003).

Name	Primer sequences	Reference
LCO1490	5’- GTTCCACAAATCATAAGGATATTGG -3’	Campbell et al. (2005)
HCO2198	5’- TACACCTCAGGGTGACCAAA AAACCA -3’	Campbell et al. (2005)
ModCOI L	5’- TGTGGGGTGAATCATTCCTT -3’	Designed from mtDNA genome
ModCOI H	5’- TAAACCTCAGGATGCCCAAA -3’	Designed from mtDNA genome
Leu-uurF	5’- TGGCAGAAAAGTGCATCAGATTTAAGC -3’	Campbell et al. (2005)
HIJ-12073	5’- GCTATTAGTAGGTCGTATCG -3’	Campbell et al. (2005)
LoGlyR	5’- CCTGCTTGGAAGGCAAGTGTACT -3’	Serb et al. (2003)
16sar-L-myt	5’- CGACTGTTTAACAAAAACAT -3’	Lydeard et al. (1996)
16sbr-H-myt	5’- CCGTTCTGAACTCAGCTCATGT -3’	Lydeard et al. (1996)
28S D23	5' -GAGAGTTCAAGAGTACGTG -3'	Park and Ó Foighil (2000)
28S D6R	5' -CCAGCTATCCTGAGGGAAACTTCG- 3'	Park and Ó Foighil (2000)

Table 2.3. Published sequence data used in molecular phylogenetic analyses were obtained from GenBank (http://www.ncbi.nlm.nih.gov/).

Species	Accession #	Reference
COI		
Obovaria jacksoniana	AY655009	Campbell et al. 2005
Obovaria olivaria	AF232812	Lydeard et al. 2000
Obovaria olivaria	EF033267	Chapman et al. 2008
Obovaria rotulata	AF232813	Lydeard et al. 2000
Obovaria rotulata	AF232814	Lydeard et al. 2000
Obovaria subrotunda	AY655010	Campbell et al. 2005
Villosa fabalis	DQ220726	Zanatta & Murphy 2006
Villosa iris	AF156523	Graf & O'Foighil 2000
Villosa iris	AF156524	Graf & O'Foighil 2000
Villosa vanuxemensis	AF156525	Graf & O'Foighil 2000
Villosa vanuxemensis	AF156526	Graf & O'Foighil 2000
Villosa villosa	AF385109	Roe et al. 2001
Villosa villosa	AY785386	Hoeh et al., unpublished
ND1		
Obovaria jacksoniana	AY655109	Campbell et al. 2005
Obovaria rotulata	AY158799	Serb et al. 2003
Villosa arkansasensis	DQ445167	Buhay, unpublished
Villosa arkansasensis	DQ445168	Buhay, unpublished
Villosa arkansasensis	DQ445169	Buhay, unpublished
Villosa arkansasensis	DQ445170	Buhay, unpublished
Villosa fabalis	DQ220723	Buhay, unpublished
Villosa fabalis	DQ445175	Buhay, unpublished
Villosa iris	DQ445178	Buhay, unpublished
Villosa iris	DQ445180	Buhay, unpublished
Villosa vanuxemensis	DQ445221	Buhay, unpublished
Villosa vanuxemensis	DQ445224	Buhay, unpublished
Villosa villosa	AY094387	Buhay et al. 2002
16S		
Obovaria olivaria	AF232787	Lydeard et al. 2000
Obovaria rotulata	AF232788	Lydeard et al. 2000
Obovaria rotulata	AF232789	Lydeard et al. 2000
Obovaria subrotunda	AY655056	Campbell et al., 2005
Obovaria unicolor	AF232780	Lydeard et al. 2000
Villosa delumbis	U72574	Lydeard et al. 1996
Villosa iris	AY655083	Campbell et al., 2005
Villosa vanuxemensis	AY655084	Campbell et al., 2005
Villosa villosa	AF385133	Roe et al. 2001

Table 2.4. Relative frequencies of haplotypes for COI across all populations sampled. Locality labels follow Figure 2.1.

Species	*Obovaria jacksoniana*								*Villosa arkansasensis*	
Drainage	Mobile		Ouachita				Red		Ouachita	
Locality	BUT	SIP	LMO	OUA	SAL 1	SAL 2	LIT	COS	ALU	IRO
n =	2	4	4	17	14	12	6	1	5	6
OJco 1			0.750	0.471	0.286					0.667
OJco 2			0.250		0.071					
OJco 3					0.071	0.083				
OJco 4					0.071				0.200	
OJco 5					0.071				0.200	
OJco 6	1.000									
OJco 7		0.500								
OJco 8		0.250								
OJco 9		0.250								
OJco 10				0.059						
OJco 11				0.059						
OJco 12				0.059						
OJco 13				0.059						
OJco 14				0.059						
OJco 15				0.059						
OJco 16				0.059						
OJco 17				0.059						
OJco 18				0.059						
OJco 19					0.143					
OJco 20					0.071					
OJco 21					0.071					
OJco 22					0.071					
OJco 23					0.071					
OJco 24						0.167				
OJco 25						0.167				
OJco 26						0.083				
OJco 27						0.083				
OJco 28						0.083				
OJco 29						0.083				
OJco 30						0.083				
OJco 31						0.083				
OJco 32						0.083				
OJco 33							0.667			
OJco 34							0.167			
OJco 35							0.167			
OJco 36								1.000		
OJco 37									0.200	
OJco 38									0.200	
OJco 39									0.200	
OJco 40										0.333

Table 2.5. Relative frequencies of haplotypes for ND1 across all populations sampled. Locality labels follow Figure 2.1.

Species	*Obovaria jacksoniana*								*Villosa arkansasensis*		
Drainage	Mobile		Ouachita				Red		Ouachita		Red
Locality	BUT	SIP	LMO	OUA	SAL 1	SAL 2	LIT	COS	ALU	IRO	MOU
n =	2	4	3	17	12	11	6	1	7	5	1
OJnd 1			0.667	0.235		0.455				0.400	
OJnd 2				0.176					0.143	0.600	
OJnd 3					0.250				0.143		
OJnd 4				0.059	0.083						
OJnd 5	1.000										
OJnd 6		0.500									
OJnd 7		0.250									
OJnd 8		0.250									
OJnd 9			0.333								
OJnd 10				0.118							
OJnd 11				0.059							
OJnd 12				0.059							
OJnd 13				0.059							
OJnd 14				0.059							
OJnd 15				0.059							
OJnd 16				0.059							
OJnd 17				0.059							
OJnd 18					0.167						
OJnd 19					0.083						
OJnd 20					0.083						
OJnd 21					0.083						
OJnd 22					0.083						
OJnd 23					0.083						
OJnd 24					0.083						
OJnd 25						0.091					
OJnd 26						0.091					
OJnd 27						0.091					
OJnd 28						0.091					
OJnd 29						0.091					
OJnd 30						0.091					
OJnd 31							0.833				
OJnd 32							0.167				
OJnd 33								1.000			
OJnd 34									0.143		
OJnd 35									0.143		
OJnd 36									0.143		
OJnd 37									0.143		
OJnd 38									0.143		
OJnd 39											1.000

Table 2.6. Average genetic distance (p-distance) of COI, ND1, and 16S for *O. jacksoniana* and *V. arkansasensis* from the Mobile, Ouachita and Red rivers. The numbers of inside parenthesis show the range of p-distance.

	COI (n=71)	ND1 (n=69)	16S (n=11)
Overall	1.050% (0.000 – 5.072%)	1.103% (0.000 – 4.929%)	1.200% (0.000 – 2.519%)
Mobile	0.331% (0.000 – 0.773%)	0.363% (0.000 – 0.778%)	0.000% (0.000 – 0.000%)
Ouachita	0.400% (0.000 – 1.356%)	0.360% (0.000 – 0.908%)	0.252% (0.000 – 0.772%)
Red	0.221% (0.000 – 0.618%)	0.264% (0.000 – 0.778%)	0.132% (0.000 – 0.204%)
Mobile vs. Ouachita	4.033% (3.349 – 5.072%)	4.243% (3.742 – 4.669%)	2.131% (1.938 – 2.519%)
Mobile vs. Red	3.531% (3.246 – 4.019%)	4.350% (4.150 – 4.929%)	2.031% (1.934 – 2.130%)
Ouachita vs. Red	0.952% (0.473 – 1.696%)	1.099% (0.778 – 1.816%)	1.338% (1.015 – 1.943%)

Chapter 3 :
Traditional and geometric morphometric analyses of conchology of *Obovaria jacksoniana* and *Villosa arkansasensis*.

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Abstract: The special concern southern hickorynut, *Obovaria jacksoniana*, occurs from the Mississippi Interior Basin to the Mobile drainage. The Ouachita creekshell, *Villosa arkansasensis*, is often difficult to differentiate from *O. jacksoniana* based on conchological characters. Since both species have been ranked with high conservation status, determining genetic divergences of both species are important for conservation. The goal of this chapter was to determine morphological similarities between *O. jacksoniana* and *V. arkansasensis*. In order to achieve this goal, we conducted two morphometric analyses: traditional and geometric morphometric analyses. We analyzed conchological characteristics of 188 individuals of *O. jacksoniana* and 49 individuals of *V. arkansasensis*. In addition, we analyzed 37 *Obovaria subrotunda*, 23 *Obovaria olivaria*, and ten *Obovaria unicolor* as the outgroups in the analyses. Our resulting morphometric analyses showed that both species have distinct morphological differentiation, however, some specimens showed intermediate shapes, which could cause to hard distinguish between species. Since *O. jacksoniana* and *V. arkansasensis* occupy two different habitat types, larger stream and headwater stream, these results suggest that shell morphologies are distinct via occupying different habitat types.

Introduction

Accurate identification of biological diversity is the very first step in the conservation of an imperiled species (Rubinoff 2006). However, morphological identification of species is often difficult in the field because of shell erosion, similarities of morphological characteristics, and ecophenotypical differences by geographic variations (Ortmann 1918; Watters 1994).

Shell morphology is often the first characteristic used to indentify a species. However, studies of shell morphology between habitats have shown that shell shape can vary among populations of the same mussel species in different habitats (Utterback 1917; Ortmann 1920; Ball 1922; Clarke 1973; Watters 1994). For example, Ortmann (1918) mentioned that a large number of “species” described by Lea and Lewis are actually synonyms because shell morphologies were different in different habitat types, such as headwaters and larger rivers, leading to the same species being split into habitat defined species. Furthermore, Utterback (1917) reported that most of *Quadrula nodulata* (Rafinesque, 1820) were found to be light, rough, and in a compressed form in headwaters, while being heavier, smoother, and more inflated further downstream. The following year, Ortmann (1918) also reported differences in shell inflation correlating with river position from more detailed observations on the upper Tennessee River drainage fauna. Ortmann (1920) described this clinal morphological change as “The Law of Stream Distribution”, referring to the correlation of shell shape and river position. In the headwaters, shell shape tends to be more flat and compressed, while in the larger rivers, shell shape tends to be more convex and swollen. Ortmann (1920) noted that not all species follow this law. However, he reported that most short-term brooder species, such as those from the genera

Amblema (Rafinesque, 1820)*, Fusconaia* (Simpson, 1900)*, Pleurobema* (Rafinesque, 1819)*,* and *Quadrula* (Rafinesque, 1820), showed shell variation from compressed in headwaters to inflated in larger rivers. Even some of long-term brooder species, such as those in the genera *Dromus* (Simpson, 1900) and *Obovaria* (Rafinesque, 1819), demonstrated this law.

These variations of shell morphology are considered to function in stabilizing a mussel in the substrate (Savazzi & Yao 1992). Furthermore, Watters (1994) mentioned that shell sculptures functioned as stabilization for a mussel in the substrate. All of these variations lead to confusion in the taxonomy and systematics of freshwater mussels and has lead to inconsistencies in delimiting species boundaries.

There are several approaches to compare morphological characters among individuals such as traditional morphometric and geometric morphometric analyses. However, there are only a few studies that have analyzed the variance of shell morphology in freshwater mussels. Gangloff et al. (2006) identified a new mussel species, *Pleurobema athearni* (Gangloff, Williams, and Feminella, 2006), using the technique of traditional morphometric analysis. *Pleurobema athearni* was initially identified as *Pleurobema georgianum* (Lea, 1841) by its shell morphology. However, by using principal component analysis (PCA) and discriminant function analysis (DFA) of the ratio of shell measurements, *P. athearni* was found to be distinctive from other *Pleurobema* species. Although recent molecular analyses did not show a significant genetic difference between the two species, via the strict consensus tree from maximum parsimony analysis, the two species appeared to be relatively distinct taxa based on a Bayesian likelihood analysis tree using the same molecular data set (Campbell et al. 2005).

While traditional morphometrics has been used recently for taxonomic questions, traditional morphometrics does have several difficulties. For instance, size correction, such as a ratio converted from measurements, may yield different results when using different size correction methods. Secondly, the variables are difficult to assess as homologous points. Finally, the variables are representatives of metrics, such as maximum distances or their ratios, and results in two different shaped objects, such as oval and teardrop shapes, having the same the length and width values, but being clearly different in shape (Adams et al. 2004).

Instead of using traditional morphometrics and associated quantitative variables such as maximum distances or their ratios, geometric morphometrics emphasizes the geometry of the morphological structures and utilizes this information throughout the analysis. Geometric morphometric analysis distributes homologous points called landmarks and analyzes the similarities of landmark distributions. Each landmark is converted to a pair of coordinates for the variable and uses them in this analysis. Although the raw variables maintain their position, orientation, and scale of objects, superimposition methods eliminate variations in configuration of landmarks according to optimization criterion (e.g., Procrustes least square estimates). After superimposition, the morphology is analyzed only considering shape differences corresponding to landmark coordinates of objects. Although the geometric morphometric analysis has been only used as an exploratory technique in freshwater mussels (Christian et al. 2008), this analysis is popular in fish (Maderbacher et al. 2008), other bivalves (Roopnarine et al. 2008; Schultheiß et al. 2008), and gastropods (Hayes et al. 2007; Minton et al. 2007; Minton et al. 2008).

My previous chapter on the molecular phylogenetics of *Obovaria jacksoniana* and

Villosa arkansasensis showed that both species from rivers in Arkansas have similar genetic structures and patterns. However, *O. jacksoniana* and *V. arkansasensis* often are difficult to differentiate from each other based on conchological characters (Valentine & Stansbery 1971; Vaughn 2003). Because misidentification of both species occurs due to similar shell shapes, shell morphological variations between species possess similarities as homologous habitat type or geographical similarities.

The goal of this chapter was to examine morphological variance between *O. jacksoniana* and *V. arkansasensis* as they are difficult to conchologically distinguish, but occupy different riverine habitats. Our objectives were to determine morphological similarities analyzed by morphometric approaches among *Obovaria* species and *V. arkansasensis* and between *O. jacksoniana* and *V. arkansasensis*. To achieve our objectives, we examined shell morphological variations between species by conducting two morphometric techniques: a traditional morphometric and a geometric morphometric analyses. Although the geometric morphometric analysis has been only used for the genera *Fusconaia* and *Pleurobema* in freshwater mussel species (Christian et al. 2008) and this is an exploratory technique, we examined the feasibility of this technique for taxonomic identification and compared geometric morphometric analysis to a traditional morphometric analysis.

Materials and Methods

Collection Sites

Collection sites and individuals were the same as the molecular phylogenetic analysis chapter, except for the tissue-only specimens and the inclusion of *Obovaria olivaria* (Table 3.1; Figure 3.1). Additionally, in the analyses, we used shell midden samples collected from riverbank, museum specimens, and type specimens of *O. jacksoniana* and *V. arkansasensis*. For the midden collection, we collected 17 shells of *O. jacksoniana* from the Little Missouri River, 83 shells from the Saline River, four shells from the Little River, one shell of *V. arkansasensis* from the Irons Fork of the Ouachita River, and 25 shells from the Alum Fork of the Saline River (Table 3.1; Figure 3.1). Because several shells were missing one of the valves and some specimens were only collected as digital photographs, several individuals were only analyzed using geometric morphometric analysis.

Traditional Morphometric Analysis

Each shell was measured to the nearest 0.05 mm for maximum length, height, and width using calipers. The height/length, width/length, and width/height ratios for all specimens were calculated and normality of each ratio were confirmed using a Kolmogorov-Smirnov test using SAS® (SAS Institute 2009). Ratio data were transformed using an arcsine square root transformation to normalize the data (Sokal & Rohlf 1995). Each measurement value is shown in Table 3.1. Traditional morphometric analysis was utilized to examine morphological variation within and among species through PCA and canonical variates analysis (CVA). PCA is a technique for simplifying descriptions of variation among individuals while CVA simplifies descriptions of variation between groups. In PCA, no *a priori* assumptions are needed to group individuals. Meanwhile, an *a priori* assumption of group membership is required for CVA, as it determines the set of axes which best discriminates between groups. A PCA was performed on two data sets: 1) all species with group assigned by species (species grouping) and 2) *O. jacksoniana* and *V. arkansasensis* with groups assigned by species and drainages (drainage grouping). Both

CVA and PCA were performed on the same data sets and grouping. Additionally, DFA was utilized to determine how frequently PC scores correctly distinguished between *O. jacksoniana* and *V. arkansasensis*. Normality of PC scores was confirmed using a Hotelling's T^2 test. All statistical analyses were performed using the Palaeontological Statistics (PAST) software package (Hammer et al. 2001).

Geometric Morphometric Analysis

The left valve of each specimen was used for geometric morphometric analysis. Each valve was mounted on a sheet with radial contour lines. Photographs of external views of individual shells were taken with digital camera. Input files of the digital images were generated using tpsUtil v.1.38 (Rohlf 2003) and saved as TPS file format. The tpsDig v.2.10 software (Rohlf 2003) was used for landmark digitalization. A total of 24 landmarks, two homologous (Type I) and 22 non-homologous (Type III) landmarks, were generated from the intersection of the shell margin and the contour lines for homology and repeatability among shells (Figure 3.2).

Geometric morphometric analysis was utilized to examine morphological variation within and among species through PCA and CVA analyses. Generally, PCA suffers from the influence of size across the newly generated axes. However, in geometric morphometric analysis, all specimens are rescaled to be the same size, thus eliminating size as a factor. For CVA analysis, individuals were grouped by species and drainage.

All data analysis was performed using the PAST software package (Hammer et al. 2001) and Integrated Morphometrics Packages (IMP) Suite (Sheets 2006). The scaled landmark coordinates from tpsDig were imported into CoordGen6f (Sheets, 2006). The file was converted into Procrustes distances through least square Procrustes superimposition methods.

Initial comparisons between species were performed in TwoGroup6h (Sheets, 2006) to determine if there are significant differences in shape between species using Goodall's F statistical method. A PCA was performed with PCAGen6n (Sheets, 2006) on the data with groups assigned by species and drainage. A CVA also was performed with CVAGen6j (Sheets, 2006) with groups assigned by species and drainage.

Results

Traditional Morphometric Analysis

A total of 185 individuals of *O. jacksoniana*, 37 individuals of *V. arkansasensis*, 29 individuals of *O. subrotunda*, 23 individuals of *O. olivaria*, and ten individuals of *O. unicolor* were analyzed using traditional morphometric analysis. Each ratio of measurements was confirmed to have a normal distribution under a Kolmogorov-Smirnov test ($p<0.01$) (Table 3.2). Both PCA and CVA have relative separation of clusters among individuals of *O. jacksoniana* and *V. arkansasensis*, although *Obovaria* species were distributed in overlapping clusters (Figures 3.3 and 3.4). The PCA of all taxa yielded two distinct eigenvalues and described 99% of the total variability in shell ratios across measured taxa. The PC1 axis described 66.8% and the PC2 axis described 33.1% of the total variation (Table 3.4; Figure 3.3). The CVA of all taxa yielded two distinct axes described >99% of the total variability among taxa (Figure 3.4).

Both PCA and grouping species CVA showed relative separation between *O. jacksoniana* and *V. arkansasensis* (Figures 3.5 and 3.6). The PCA of *O. jacksoniana* and *V. arkansasensis* yielded two distinct eigenvalues and described >99% of the total variability between species. The PC1 axis described 72.6% and the PC2 axis described 27.3% of the total variation (Table 3.4; Figure 3.5). The grouping species CVA of *O. jacksoniana* and *V. arkansasensis* yielded one distinct axis and described >99% of the total variability between species (Figure 3.6). The grouping drainages CVA showed that the relative separation between drainages occupying by species, although there was no differentiation among drainages within species (Figure 3.7). By grouping drainages, the CVA yielded two distinct canonical variates and described 99% of the total variability among drainages. Based on the PCA scores, the DFA was used to describe how the PCA scores are able to separate *O. jacksoniana* from *V. arkansasensis*. The DFA revealed that PCA scores correctly distinguished *O. jacksoniana* from *V. arkansasensis* 97.3% of the time (Hotelling's T^2 $p<0.01$).

Geometric Morphometric Analysis

A total of 189 individuals of *O. jacksoniana*, 51 individuals of *V. arkansasensis*, 29 individuals of *O. subrotunda*, 23 individuals of *O. olivaria*, and 11 individuals of *O. unicolor* were analyzed using geometric morphometric analysis. The Goodall's F test showed that the all pairwise comparisons among taxa resulted in a value of $p = 0$ (Table 3.3). Although species clusters had overlapping areas, there were some separations of clusters in *O. jacksoniana, O. subrotunda,* and *V. arkansasensis* in the PCA analysis (Figure 3.8). The results of the PCA of all taxa yielded five distinct eigenvalues and described >90% of the total variability: PC1 to PC5 explained from 52.6% down to 2.0% of the variability in the PCA (Table 3.4; Figure 3.8). The CVA of all taxa yielded three distinct axes ($P<0.05$), where all means were significantly different from each other, although all taxa overlapped in CVA plots (Figure 3.9).

Both PCA and grouping species CVA of *O. jacksoniana* and *V. arkansasensis* showed relative separations between species with some overlapped area (Figures 3.10 and 3.11). The PCA of *O. jacksoniana* and *V. arkansasensis* yielded six distinct eigenvalues and described >90% of the total variability: PC1 to PC6 explained from 46.4% down to 1.3% of the variation in the PCA (Table 3.4; Figure 3.10). The grouping species CVA of *O. jacksoniana* and *V. arkansasensis* yielded one distinct axis ($p<0.01$) (Figure 3.11). Although they had overlapping areas, there was relatively clear separation along the x-axis. Based on the PCA scores between *O. jacksoniana* and *V. arkansasensis*, the DFA revealed that PCA scores correctly distinguished *O. jacksoniana* from *V. arkansasensis* 96.67% of the time (Hotelling's T^2 $p<0.01$). The grouping drainages CVA showed that the relative separation between drainages occupied by species, there was no differentiation among drainage within species (Figure 3.12). By grouping drainage, the CVA yielded four distinct canonical variates ($P<0.05$).

Discussion

Both morphological techniques showed relative correlation within taxon and distinction among taxa. Although in my previous chapter, genetic divergence between *O. jacksoniana* and *V. arkansasensis* showed very few differences, the two species showed relative distinction based on morphological characteristics. Differentiation of shell morphology can be explained by Ortmann's Law (Ortmann, 1920) in which two species occupy two different habitat types. *Villosa arkansasensis* often inhabits in the headwater streams, which have fast flow and large substrate. Shell morphology of headwater individuals tends to be flat, compressed, and rough. *Villosa arkansasensis* had a more flat and compressed shape than *O. jacksoniana*. On the other hand, *O. jacksoniana* inhabits larger streams characterized by slow flow and sand and gravel substrates. Shell morphology of larger river individuals tends to be rounded, inflated, and smooth according to Ortmann's Law. Morphological characteristics of *O. jacksoniana* are rounded and inflated in general, which suit the habitat type. However, several individuals of *O. jacksoniana* from smaller size rivers and headwaters, such as the Cossatot River and of the upper Saline River, were initially identified as "questionable *O. jacksoniana*", which showed more compressed morphologies than others. In fact, *O. jacksoniana* from the Cossatot River were clustered in between *O. jacksoniana* and *V. arkansasensis* in both traditional and geometric morphometrics analyses and both PCA and CVA; neither specimen from the upper Saline River was clustered between *O. jacksoniana* and *V. arkansasensis*.

Traditional Morphometric Analysis

Although traditional morphometric analysis only considered the ratios from three shell measurements, which provides less information than geometric morphometric analysis, it can be used for species identification at least at the generic-level. Even though molecular phylogenetic analyses showed that *O. jacksoniana* and *V. arkansasensis* possessed similar genetic structure, the morphology of the two species based on traditional morphometric analysis was able to distinguish between the two different morphotypes 97.3% of the time (Hotelling's T^2 $p<0.01$). However, there were no clear distinctions of clusters among *Obovaria* species in some the PCA and CVA analyses. This was due to the *Obovaria* species having similar conchological characters, such as a round to oval and inflated shells (Williams et al. 2008). Thus after normalization of measurements by an arcsine transformation, the values of the transformed ratio were very similar. Even though the morphological differences between *O. jacksoniana* and *O. olivaria* are clear by visual identification, the ratios of measurement were very similar because of their conchological characteristics. However, in discriminating between *O. jacksoniana* and *V. arkansasensis*, there were only few overlapping specimens in both PCA and CVA, even though there was a visual identification of a "questionable *O. jacksoniana*" from the Cossatot River and upper Saline River. Moreover, neither *O. jacksoniana* nor *V. arkansasensis* showed distinct shell morphologies among drainages in the analyses.

Geometric Morphometric Analysis

Geometric morphometric analysis considered the 2-dimentional shapes from 24 landmarks along shell margin. Although only two landmarks could be considered true homology landmarks in this study (Figure 3.2), geometric analysis showed correlations among and between taxa for both the PCA and CVA. For the analysis among taxa, the clusters of *O. jacksoniana* and *O. subrotunda* for both the PCA and CVA showed clear distinctions, unlike what was observed in the traditional morphometric analysis. This is because geometric morphometric analysis considered the shell margin and thus showed differences between the two species. However, clusters of *O. olivaria* and *O. unicolor* were mostly overlapping with *O. jacksoniana* clusters, which resulted in a similar interpretation as the traditional morphometric analysis. Mirarchi et al. (2004) mentioned that *O. unicolor* in western Mobile Basin has an almost identical distribution as *O. jacksoniana* and is often similar to or conspecific with *O. jacksoniana*. From our analyses, *O. jacksoniana* and *O. unicolor* possess similar morphological characteristics and may be difficult to distinguish between the two species. Analyses between *O. jacksoniana* and *V. arkansasensis* showed distinct cluster distributions, with a few gapped specimens. However within species, neither *O. jacksoniana* and *V. arkansasensis* had distinctions among drainages in the geometric morphometric analysis, which was similar to traditional morphometric analysis (Figure 3.12).

<u>Traditional vs. Geometric Morphometric Analysis</u>

Although there are very few publications on the morphometric analyses of mollusks (e.g., Gangloff et al. 2006; Hayes et al. 2007; Roopnarine et al. 2008) and one report on the use of geometric morphometric analysis on freshwater mussels (Christian et al. 2008), both traditional and geometric morphometric analyses showed relative distinctions of shell morphologies within and among taxa. Both PCA and CVA analyses of both morphometric methods showed clear distinction, with small overlaps, between *O. jacksoniana* and *V. arkansasensis*. For specimens in the overlap clusters, there is a chance that they could be misidentified and could be incorrectly taxonomically assigned. Although we could not find clear distinction from grouping drainage CVAs, *O. jacksoniana* specimens from upper portion of streams, such as the Cossatot and the Saline rivers, tended to be in between clusters of *O. jacksoniana* and *V. arkansasensis*.

Nevertheless, both analyses had strength and weakness. Traditional morphometric analysis on shells could only handle the ratios of three measurements, which is less information than geometric morphometric analysis. Traditional morphometric analysis could not distinguish among *Obovaria* species because of their similar conchological characteristics. On the other hand, geometric morphometric analysis could distinguish between *O. jacksoniana* and *O. subrotunda*, but this analysis on shell shape had the weakness of only having two homologous (Type I) landmarks for this analysis.

Conclusions

Although molecular phylogenetic analysis has shown that *O. jacksoniana* and *V.*

arkansasensis has similar genetic structure, both morphometric methods showed relative distinctions of shell morphologies between two species, which may be attributed to the mussels distinct habitat differences. Both traditional and geometric morphometric analysis methods have great potential use in morphological identification of freshwater mussels. Geometric morphometric analysis on internal shell morphology in future studies may show further differentiation within same genus and between species.

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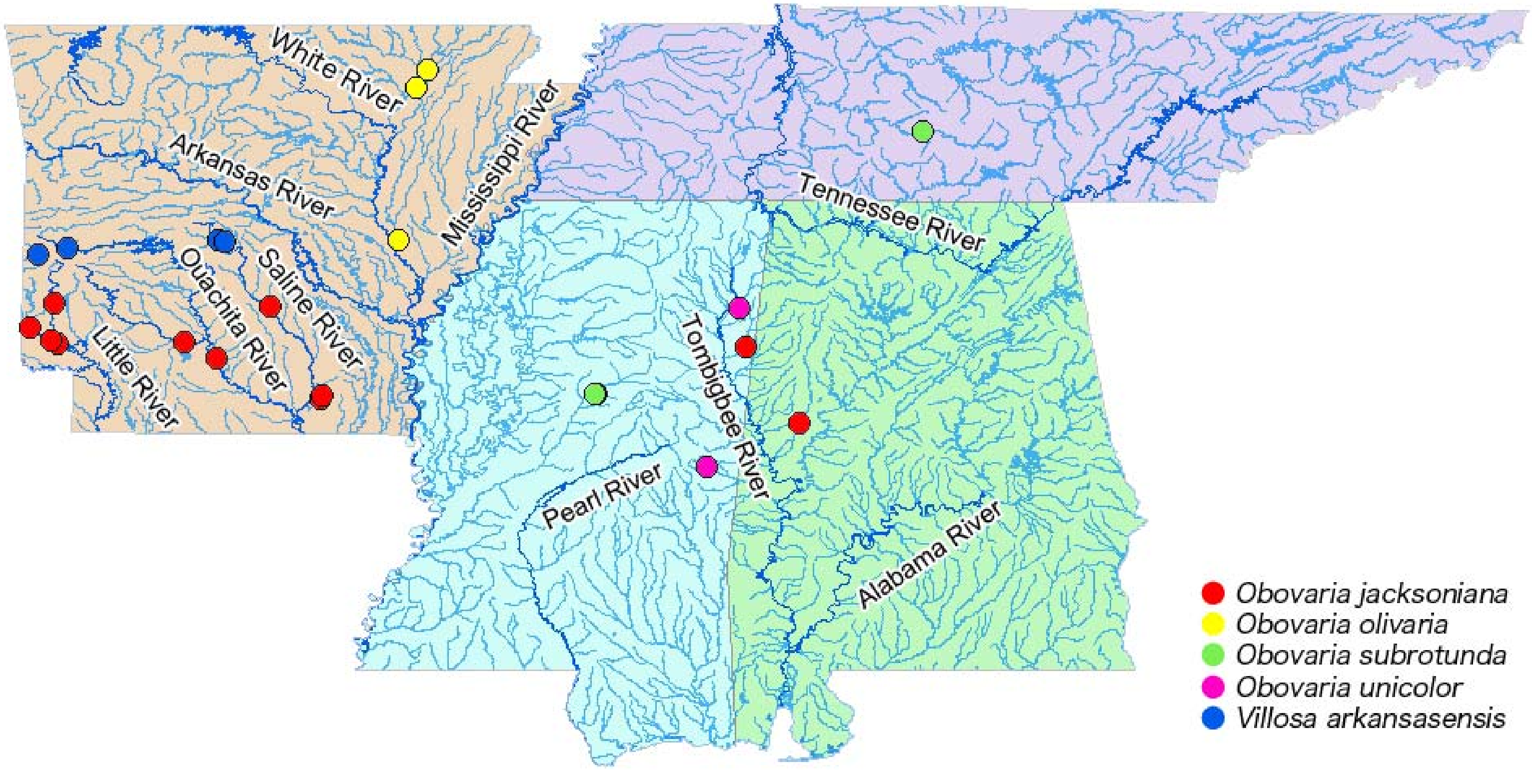

Figure 3.1. Map of collection localities used in the morphometric analyses of *Obovaria jacksoniana* (red dot), *Villosa arkansasensis* (blue dot), *O. olivaria* (yellow dot), *O. subrotunda* (green dot) and *O. unicolor* (pink dot). Specific locality information is provided in Table 3.1.

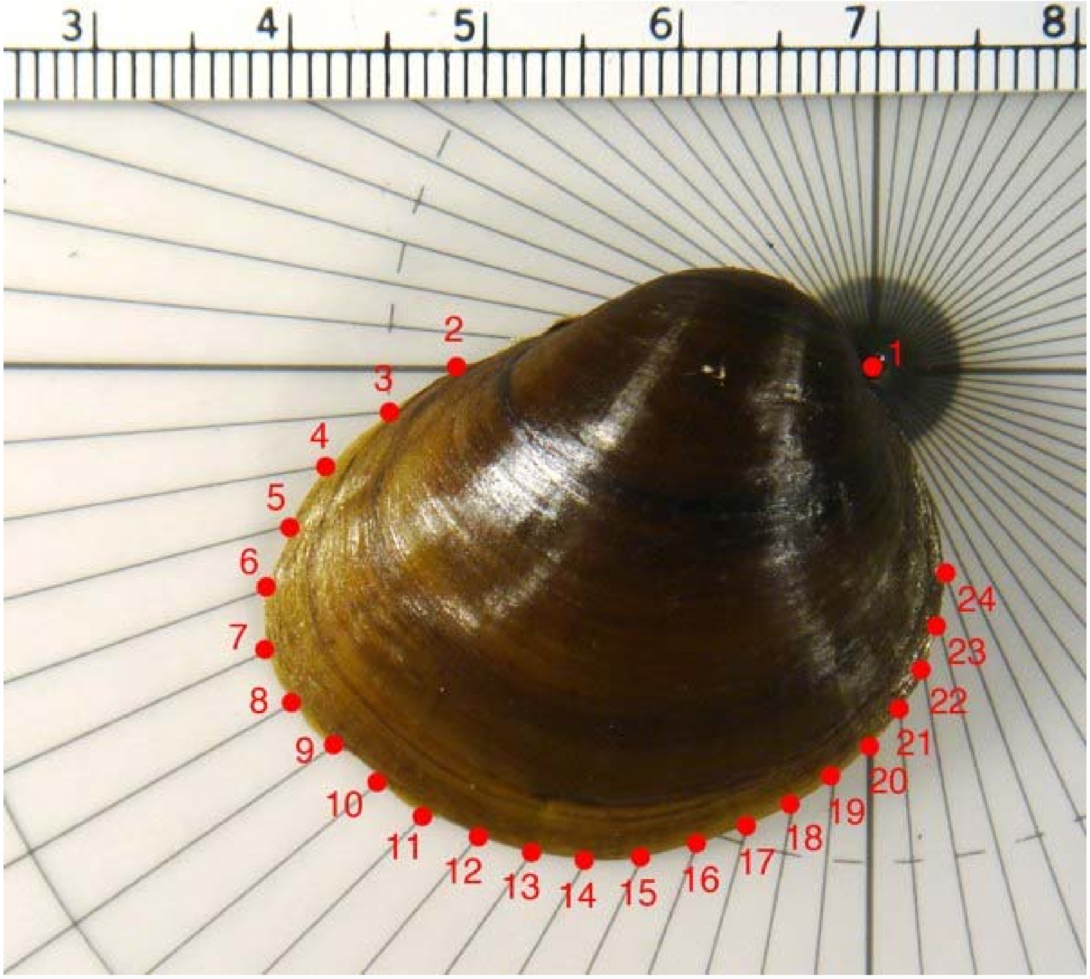

Figure 3.2. Picture of external landmarks generated for tpsDig v.2.10 (Rohlf, 2003) used in geometric morphometric analysis. The first two landmarks (1 and 2) are homologous (Type I) and the remaining 22 landmarks are non-homologous (Type III).

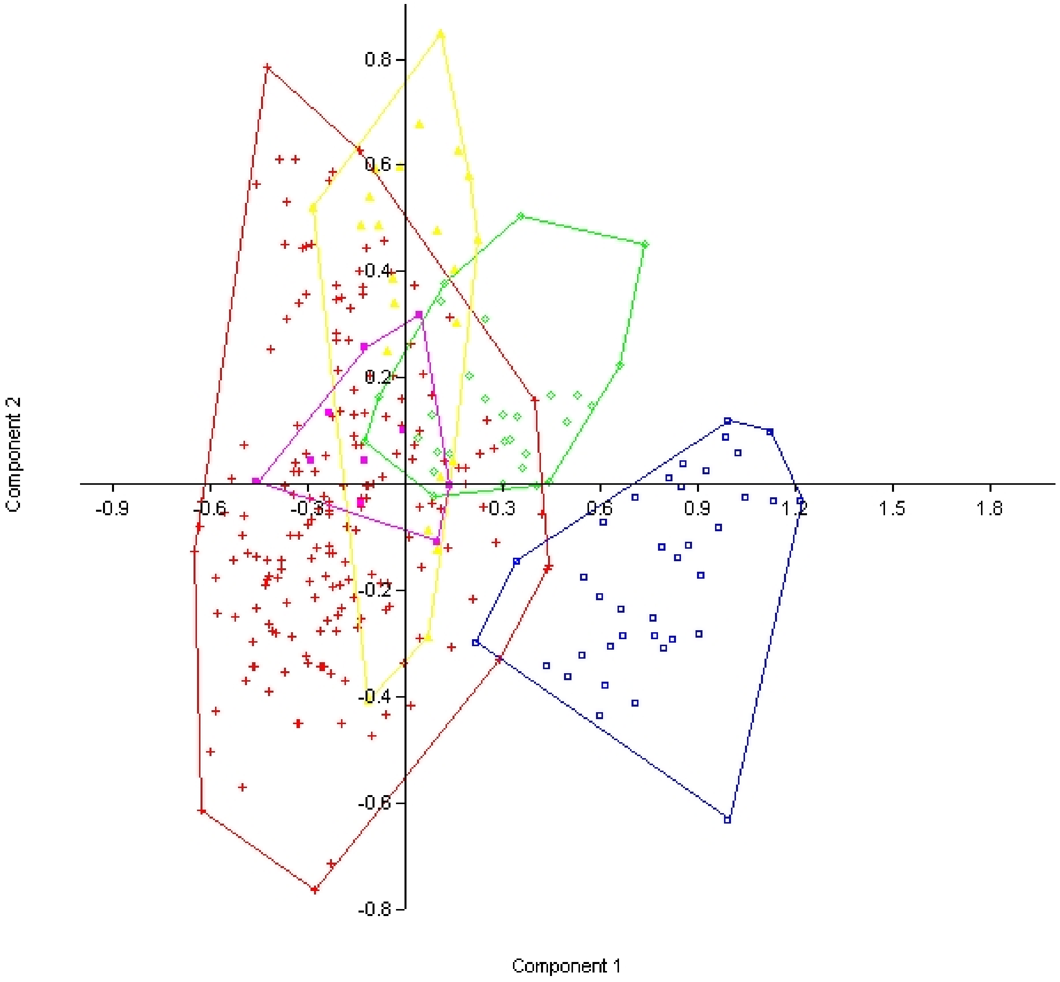

Figure 3.3. Scatter plot from principal components analysis of traditional morphometric measurement from all species (n=284). *Obovaria jacksoniana* (red cross: n=185), *V. arkansasensis* (blue open square: n=37), *O. subrotunda* (green diamond: n=29), *O. olivaria* (yellow triangle: n=23), and *O. unicolor* (magenta filled square: n=10). Lines show convex hull of each species.

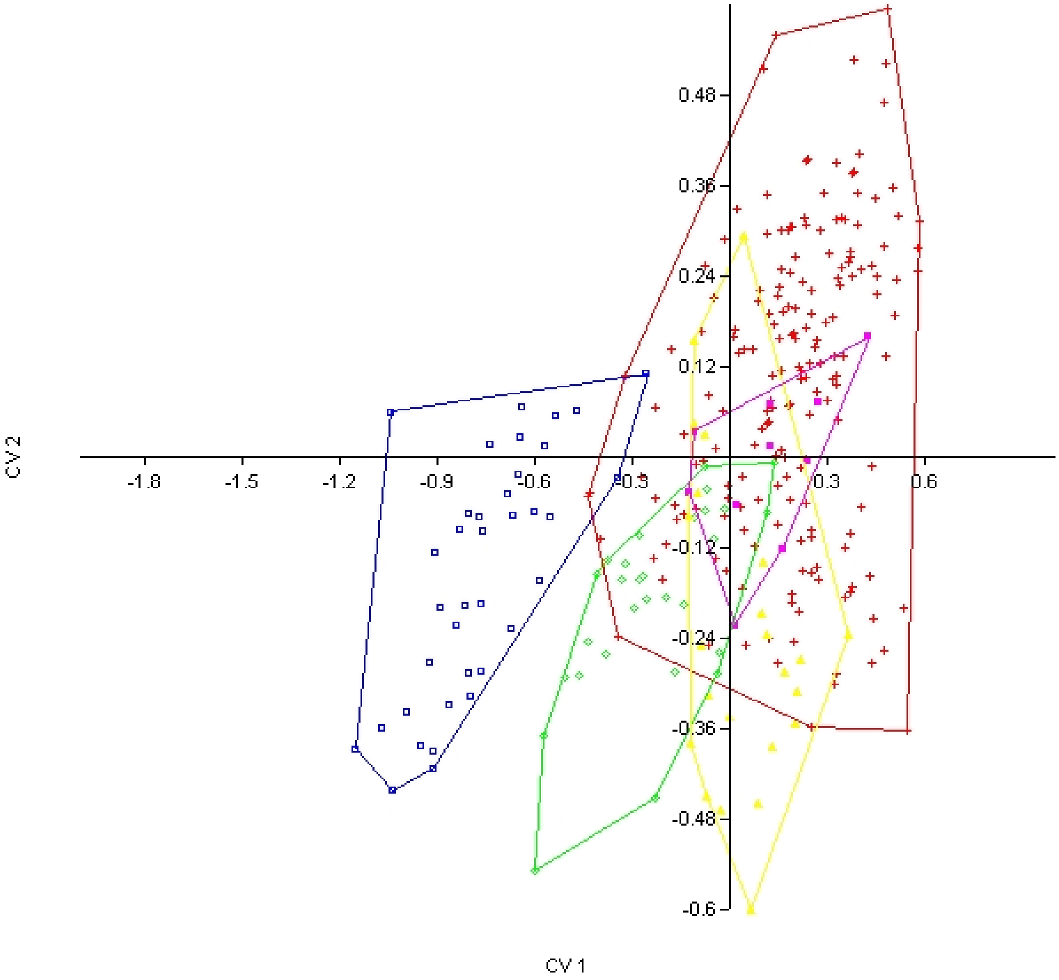

Figure 3.4. Scatter plot from canonical variates analysis of traditional morphometric measurement from all species (n=284). *Obovaria jacksoniana* (red cross: n=185), *V. arkansasensis* (blue open square: n=37), *O. subrotunda* (green diamond: n=29), *O. olivaria* (yellow triangle: n=23), and *O. unicolor* (magenta filled square: n=10). Lines show convex hull of each species.

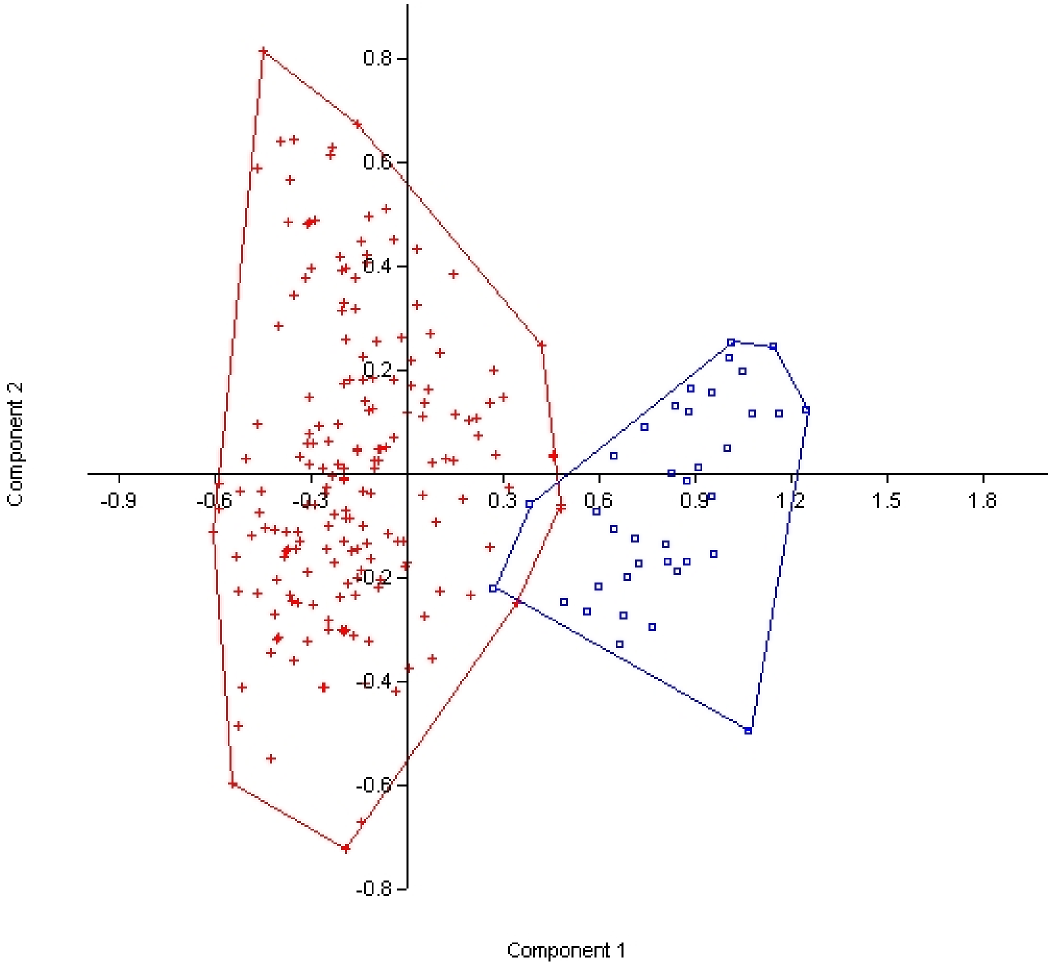

Figure 3.5. Scatter plot from principal components analysis of traditional morphometric measurement from *O. jacksoniana* and *V. arkansasensis* (n=222). *Obovaria jacksoniana* (red cross: n=185) and *V. arkansasensis* (blue open square: n=37). Lines show convex hull of each species.

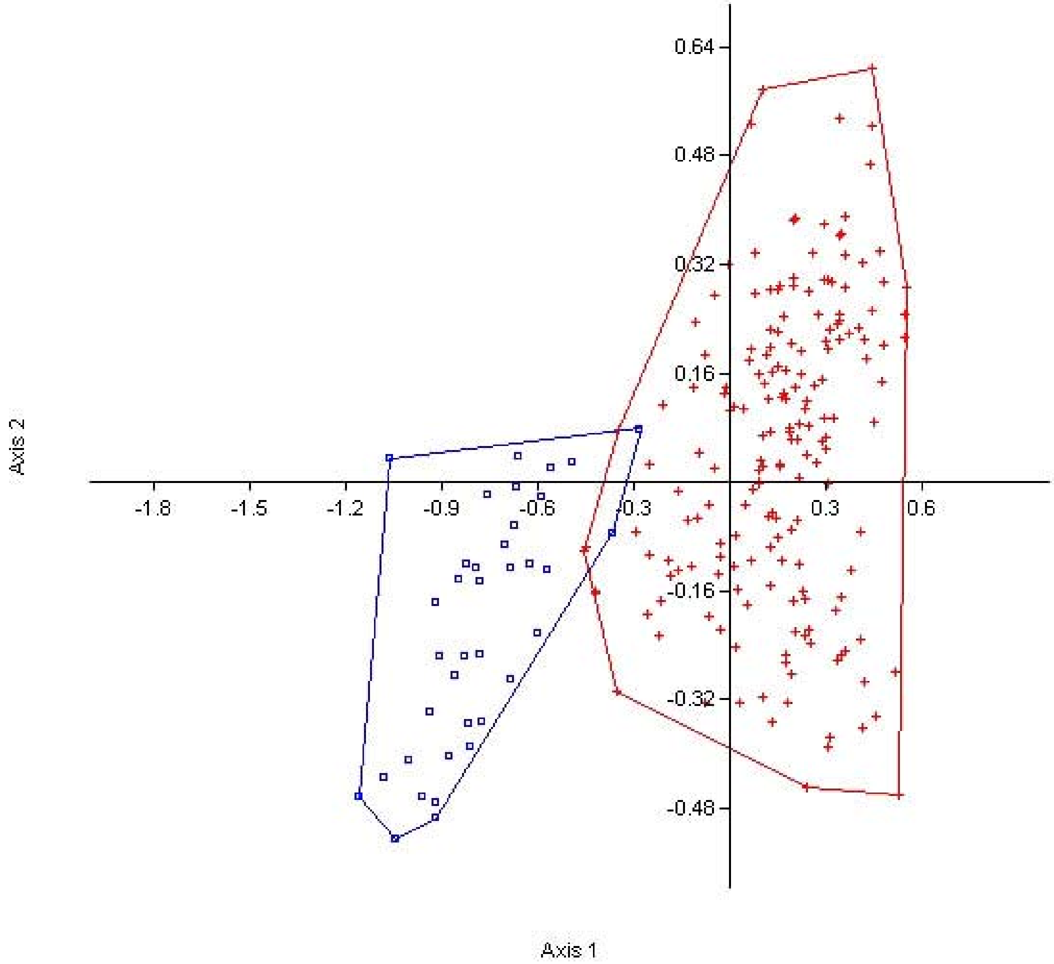

Figure 3.6. Scatter plot from canonical variates analysis of traditional morphometric measurement from *O. jacksoniana* and *V. arkansasensis* by grouping species (n=222). *Obovaria jacksoniana* (red cross: n=185) and *V. arkansasensis* (blue open square: n=37). Lines show convex hull of each species.

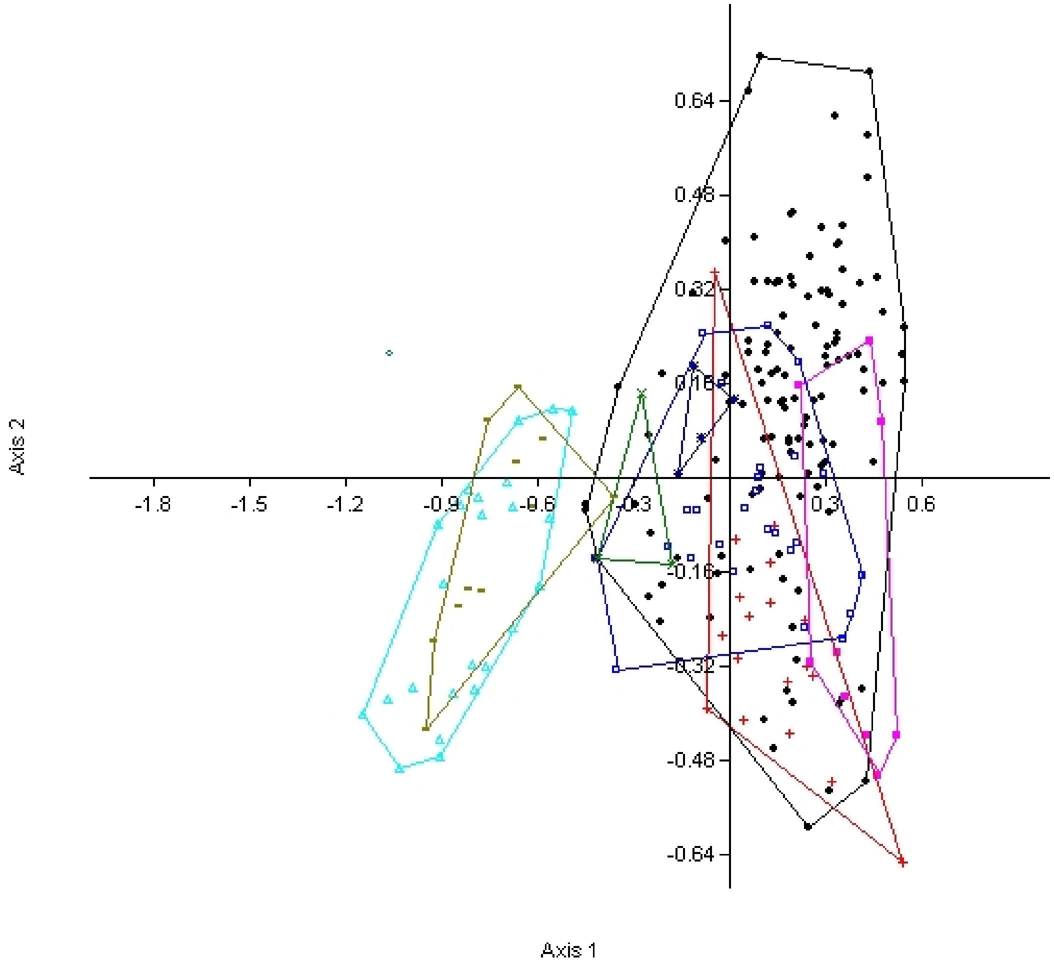

Figure 3.7. Scatter plot from canonical variates analysis of traditional morphometric measurement from *O. jacksoniana* and *V. arkansasensis* by grouping drainages (n=222). Saline River (Black dot: n=126), Ouachita River (red cross: n=18), Little Missouri River (blue open square: n=26), Little River (magenta filled square: n=9), Cossatot River (green x: n=3), Sipsey River (dark blue *: n=4), Alum Fork of the Saline River (sky blue triangle: n=24), Irons Fork of the Ouachita River (olive green -: n=11), and Mountain Fork of the Little River (moss green oval: n=1) (Table 3.1). Lines show convex hull of each species.

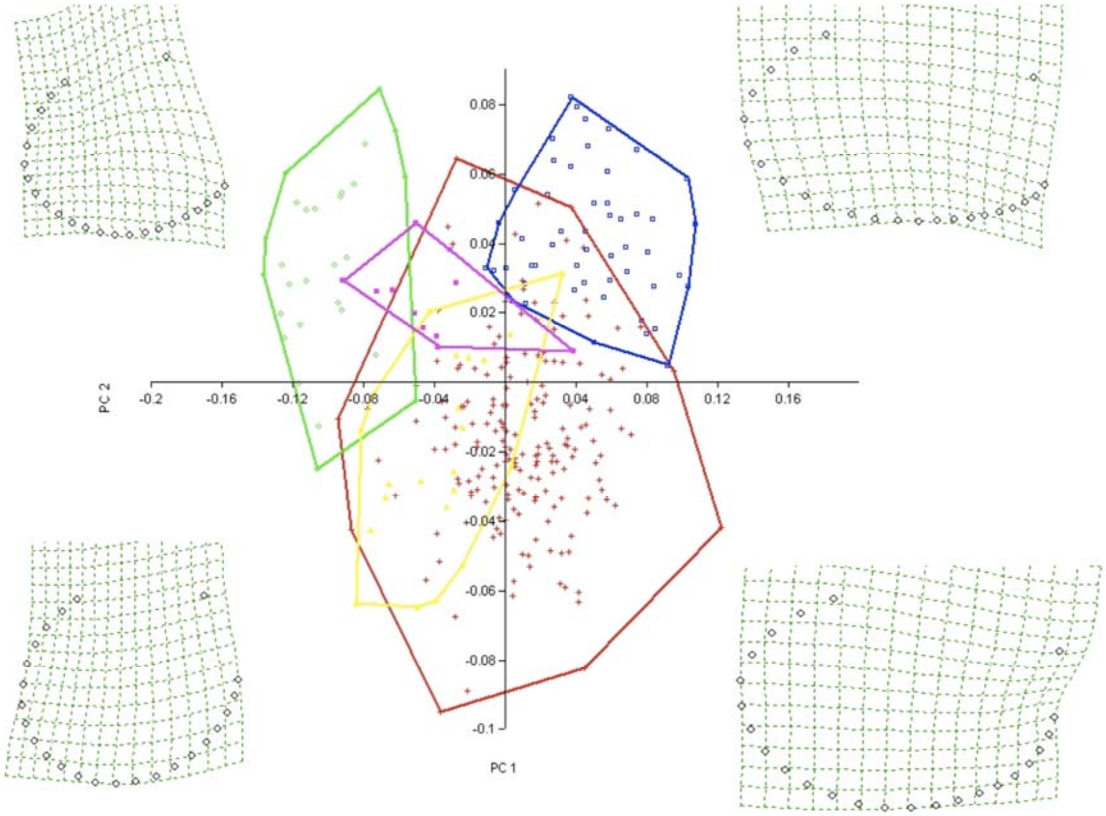

Figure 3.8. Scatter plot from principal components analysis of geometric morphometrics from all species (n=303) and changes in shape that are correlated with PCA axis 1 and 2 are shown. *Obovaria jacksoniana* (red cross: n=189), *V. arkansasensis* (blue open square: n=51), *O. subrotunda* (green diamond: n=29), *O. olivaria* (yellow triangle: n=23), and *O. unicolor* (magenta filled square: n=11). Lines show convex hull of each species. The diagrams on each corner show morphological variation on each quadrats.

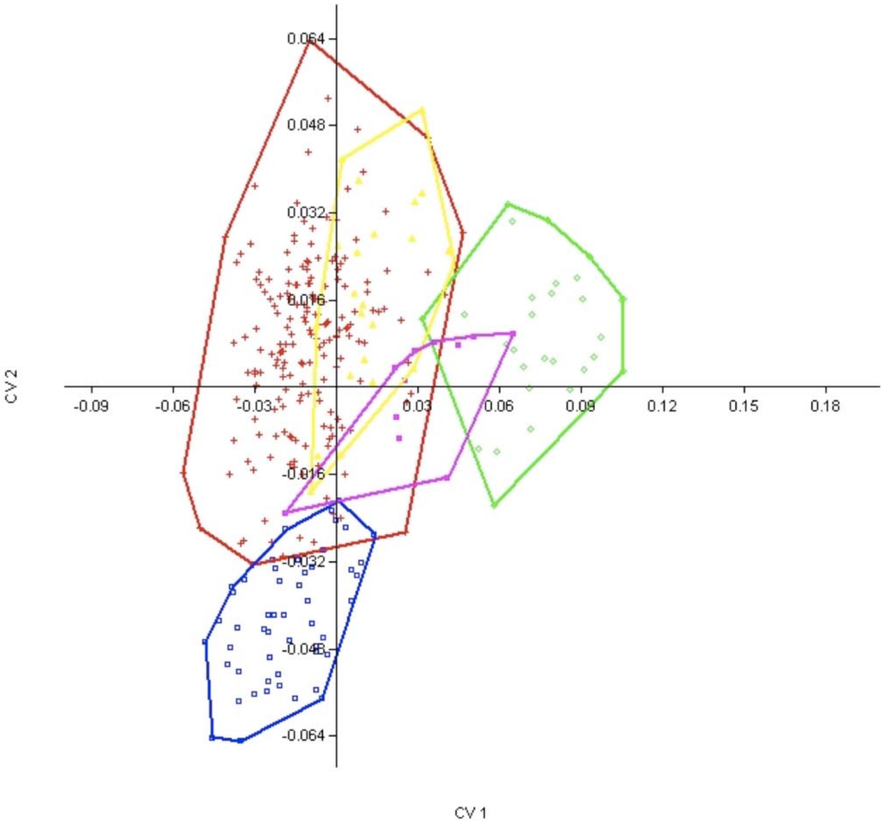

Figure 3.9. Scatter plot from canonical variates analysis of geometric morphometrics from all species (n=303) and changes in shape that are correlated with CVA axis 1 and 2 are shown. *Obovaria jacksoniana* (red cross: n=189), *V. arkansasensis* (blue open square: n=51), *O. subrotunda* (green diamond: n=29), *O. olivaria* (yellow triangle: n=23), and *O. unicolor* (magenta filled square: n=11). Lines show convex hull of each species.

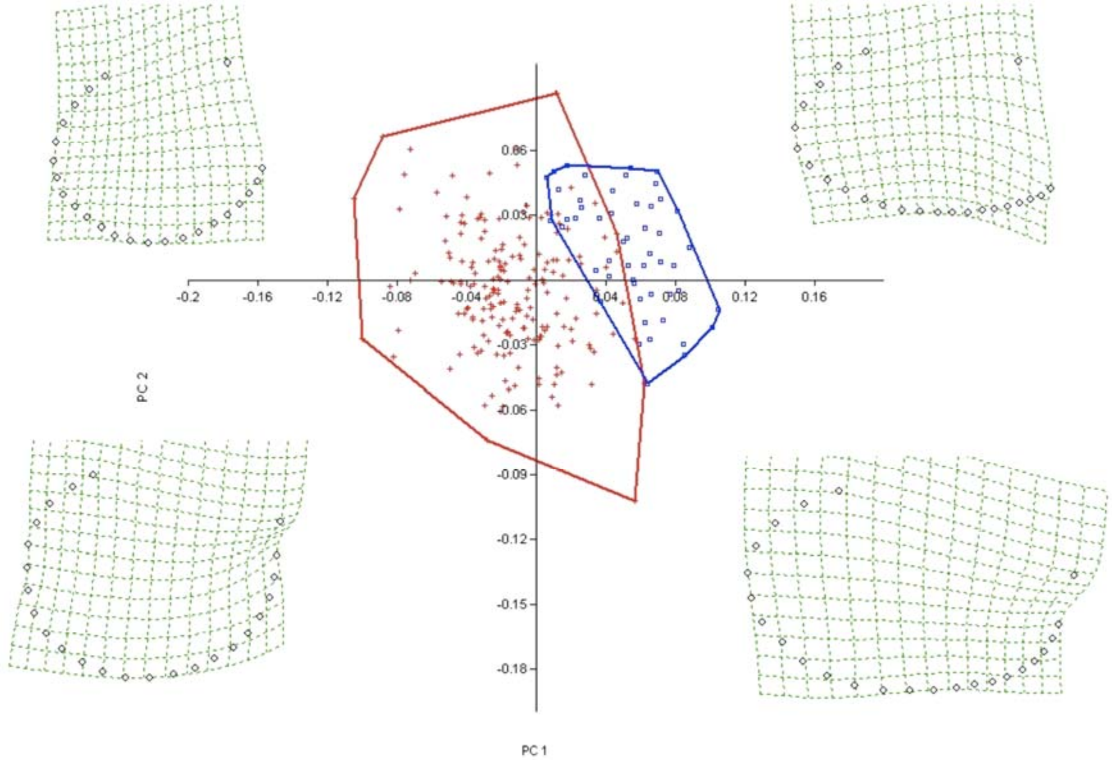

Figure 3.10. Scatter plot from principal components analysis of geometric morphometrics from *O. jacksoniana* and *V. arkansasensis* by grouping species (n=240) and changes in shape that are correlated with PCA axis 1 and 2 are shown. *Obovaria jacksoniana* (red cross: n=189) and *V. arkansasensis* (blue open square: n=51). Lines show convex hull of each species. The diagrams on each corner show morphological variation on each quadrats.

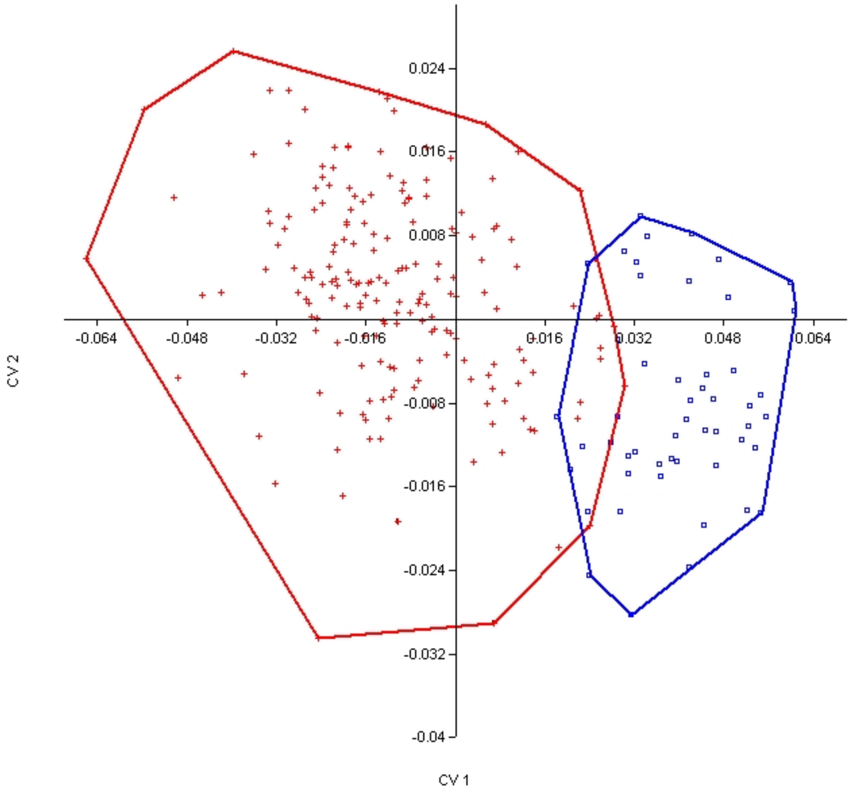

Figure 3.11. Scatter plot from canonical variates analysis of geometric morphometrics from *O. jacksoniana* and *V. arkansasensis* by grouping species (n=240) changes in shape that are correlated with CVA axis 1 and 2 are shown. *Obovaria jacksoniana* (red cross: n=189) and *V. arkansasensis* (blue open square: n=51). Lines show convex hull of each species.

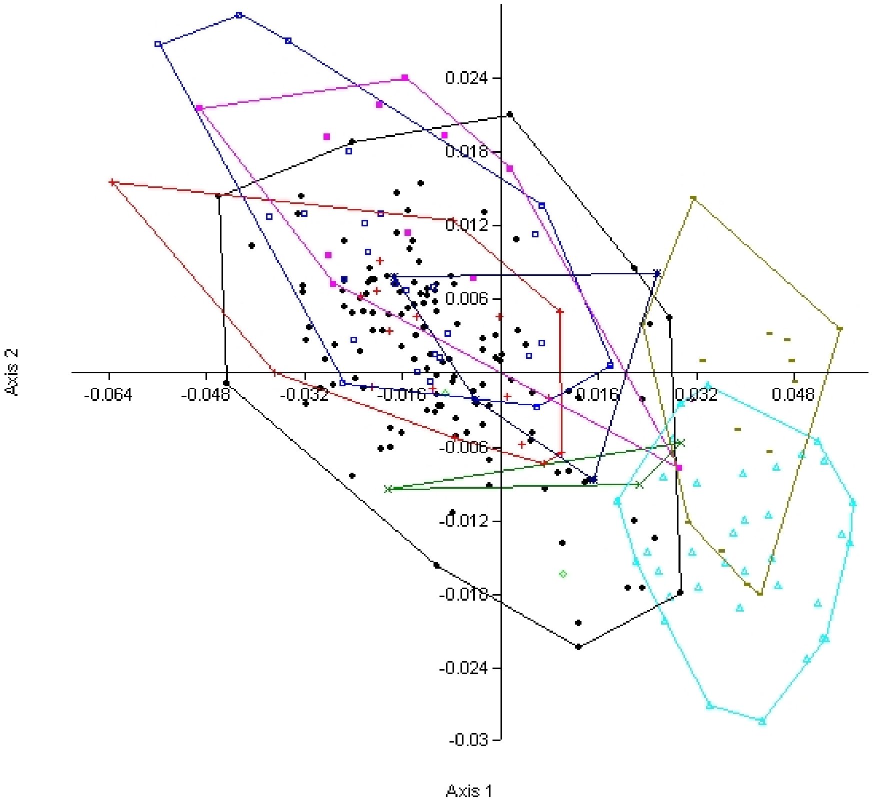

Figure 3.12. Scatter plot from canonical variates analysis of geometric morphometrics from *O. jacksoniana* and *V. arkansasensis* by grouping drainages (n=240) and changes in shape that are correlated with CVA axis 1 and 2 are shown. Saline River (Black dot: n=126), Ouachita River (red cross: n=18), Little Missouri River (blue open square: n=26), Little River (magenta filled square: n=10), Cossatot River (green x: n=3), Pearl River (red star: type specimen), Buttahatchee River (light green diamond: n=2), Sipsey River (dark blue *: n=4), Alum Fork of the Saline River (sky blue triangle: n=35), Irons Fork of the Ouachita River (olive green -: n=14), and Mountain Fork of the Little River (moss green oval: n=1) (Table 3.1). Lines show convex hull of each species.

Table 3.1. List of specimens for using on morphometric analyses.

		River	Measurement (mm)			Ratio			Transformed ratio		
Museum #	Locality	Drainage	Length	Height	Width	H/L	W/L	W/H	H/L	W/L	W/H
Obovaria jacksoniana											
ANSP 106063	Pearl R., Jackson Co., MS (Type)	Pearl									
MMNS 8563.1	Buttahatchee R., Monroe Co., MS	Mobile									
MMNS 8563.2	Buttahatchee R., Monroe Co., MS	Mobile									
ASUMZ 4530	Saline R., Dallas/Grant Co., AR	Ouachita	29.4	24.0	17.4	0.816	0.592	0.725	5.184	4.412	4.884
ASUMZ 4531	Saline R., Dallas/Grant Co., AR	Ouachita	24.0	20.6	15.0	0.858	0.625	0.728	5.316	4.534	4.895
ASUMZ 4532	Saline R., Dallas/Grant Co., AR	Ouachita	25.1	20.1	15.9	0.801	0.633	0.791	5.134	4.565	5.103
ASUMZ 4533	Saline R., Ashley/Bradley Co., AR	Ouachita	35.3	30.9	23.9	0.875	0.677	0.773	5.368	4.720	5.045
ASUMZ 4534	Saline R., Ashley/Bradley Co., AR	Ouachita	31.2	31.9	22.8	1.022	0.731	0.715	5.803	4.904	4.850
ASUMZ 4535	Saline R., Ashley/Bradley Co., AR	Ouachita	30.3	28.3	20.5	0.934	0.677	0.724	5.546	4.718	4.882
ASUMZ 4536	Saline R., Ashley/Bradley Co., AR	Ouachita	34.6	32.1	23.7	0.928	0.685	0.738	5.527	4.747	4.929
ASUMZ 4537	Saline R., Ashley/Bradley Co., AR	Ouachita	33.1	32.5	24.3	0.982	0.734	0.748	5.687	4.915	4.961
ASUMZ 4538	Saline R., Ashley/Bradley Co., AR	Ouachita	29.8	27.2	20.4	0.913	0.685	0.750	5.482	4.746	4.968
ASUMZ 4539	Saline R., Ashley/Bradley Co., AR	Ouachita	35.7	33.9	23.7	0.950	0.664	0.699	5.592	4.674	4.796
ASUMZ 4540	Saline R., Ashley/Bradley Co., AR	Ouachita	32.3	32.4	22.7	1.003	0.703	0.701	5.748	4.809	4.801
ASUMZ 4541	Saline R., Ashley/Bradley Co., AR	Ouachita	37.7	36.7	27.1	0.973	0.719	0.738	5.662	4.864	4.930
ASUMZ 4542	Saline R., Ashley/Bradley Co., AR	Ouachita	42.2	42.4	28.9	1.005	0.685	0.682	5.753	4.747	4.736
ASUMZ 4543	Ouachita R., Ouachita Co., AR	Ouachita	42.1	37.5	26.0	0.891	0.618	0.693	5.416	4.507	4.776
ASUMZ 4544	Ouachita R., Ouachita Co., AR	Ouachita	37.5	35.4	26.1	0.944	0.696	0.737	5.576	4.786	4.926
ASUMZ 4545	Ouachita R., Ouachita Co., AR	Ouachita	39.3	39.3	27.7	1.000	0.705	0.705	5.739	4.816	4.816
ASUMZ 4546	Ouachita R., Ouachita Co., AR	Ouachita	36.1	31.5	24.2	0.873	0.670	0.768	5.360	4.696	5.028
ASUMZ 4547	Ouachita R., Ouachita Co., AR	Ouachita	34.1	30.3	22.8	0.889	0.669	0.752	5.409	4.690	4.976
ASUMZ 4548	Ouachita R., Ouachita Co., AR	Ouachita	34.4	32.2	23.8	0.936	0.692	0.739	5.552	4.771	4.932
ASUMZ 4549	Ouachita R., Ouachita Co., AR	Ouachita	43.5	37.4	29.3	0.860	0.674	0.783	5.320	4.708	5.078
ASUMZ 4550	Ouachita R., Ouachita Co., AR	Ouachita	35.8	34.1	24.2	0.953	0.676	0.710	5.601	4.716	4.832
ASUMZ 4551	Ouachita R., Ouachita Co., AR	Ouachita	34.7	32.3	23.5	0.931	0.677	0.728	5.536	4.720	4.893
ASUMZ 4552	Ouachita R., Ouachita Co., AR	Ouachita	40.5	35.3	25.6	0.872	0.632	0.725	5.357	4.560	4.885
ASUMZ 4553	Ouachita R., Ouachita Co., AR	Ouachita	39.8	36.5	27.6	0.917	0.693	0.756	5.495	4.777	4.989

(table continues)

Table 3.1. *(continued)*

			Measurement (mm)				Ratio			Transformed ratio	
Museum #	Locality	River Drainage	Length	Height	Width	H/L	W/L	W/H	H/L	W/L	W/H
ASUMZ 4554	Ouachita R., Ouachita Co., AR	Ouachita	31.7	34.3	23.9	1.082	0.754	0.697	5.971	4.981	4.788
ASUMZ 4555	Ouachita R., Ouachita Co., AR	Ouachita	37.5	32.5	24.2	0.867	0.645	0.745	5.342	4.608	4.950
ASUMZ 4556	Ouachita R., Ouachita Co., AR	Ouachita	38.9	35.7	25.0	0.918	0.643	0.700	5.497	4.598	4.800
ASUMZ 4557	Ouachita R., Ouachita Co., AR	Ouachita	35.0	29.5	22.6	0.843	0.646	0.766	5.268	4.609	5.021
ASUMZ 4558	Ouachita R., Ouachita Co., AR	Ouachita	30.2	26.9	19.4	0.891	0.642	0.721	5.416	4.597	4.872
ASUMZ 4559	Ouachita R., Ouachita Co., AR	Ouachita	31.9	28.1	20.8	0.881	0.652	0.740	5.385	4.632	4.936
ASUMZ 4560	Little R., Little River/Sevier Co., AR	Red	38.0	37.0	27.4	0.974	0.721	0.741	5.663	4.871	4.937
ASUMZ 4561	Little R., Little River/Sevier Co., AR	Red	34.8	32.6	24.2	0.937	0.695	0.742	5.554	4.783	4.943
ASUMZ 4562	Little R., Little River/Sevier Co., AR	Red	32.5	33.4	24.1	1.028	0.742	0.722	5.818	4.940	4.873
ASUMZ 4563	Little R., Little River/Sevier Co., AR	Red	41.6	39.5	29.8	0.950	0.716	0.754	5.592	4.855	4.983
ASUMZ 4564	Saline R., Ashley/Bradley Co., AR	Ouachita	50.9	47.4	33.5	0.931	0.658	0.707	5.538	4.653	4.822
ASUMZ 4565	Saline R., Ashley/Bradley Co., AR	Ouachita	48.5	41.9	31.8	0.864	0.656	0.759	5.333	4.645	4.998
ASUMZ 4566	Saline R., Ashley/Bradley Co., AR	Ouachita	42.3	39.9	28.8	0.943	0.681	0.722	5.573	4.733	4.874
ASUMZ 4567	Saline R., Ashley/Bradley Co., AR	Ouachita	36.1	35.1	25.8	0.972	0.715	0.735	5.659	4.850	4.918
ASUMZ 4568	Cossatot R., Sevier Co., AR	Red	38.4	29.5	21.1	0.768	0.549	0.715	5.028	4.251	4.851
ASUMZ 4601	Little Missouri R., Nevada Co., AR	Ouachita	35.9	30.0	22.8	0.836	0.635	0.760	5.245	4.571	5.001
ASUMZ 4602	Little Missouri R., Nevada Co., AR	Ouachita	31.3	26.5	21.7	0.847	0.693	0.819	5.279	4.776	5.192
ASUMZ 4603	Little Missouri R., Nevada Co., AR	Ouachita	35.1	29.4	23.3	0.838	0.664	0.793	5.251	4.673	5.107
ASUMZ 4610	Saline R., Dallas/Grant Co., AR	Ouachita	28.8	23.5	17.4	0.816	0.604	0.740	5.183	4.458	4.936
ASUMZ 4611	Saline R., Dallas/Grant Co., AR	Ouachita	29.5	24.0	17.2	0.814	0.583	0.717	5.175	4.379	4.856
ASUMZ 4612	Saline R., Dallas/Grant Co., AR	Ouachita	31.7	21.8	21.0	0.688	0.662	0.963	4.757	4.669	5.633
ASUMZ 4613	Saline R., Dallas/Grant Co., AR	Ouachita	19.1	15.1	11.2	0.791	0.586	0.742	5.101	4.392	4.941
ASUMZ 4645	Saline R., Dallas/Grant Co., AR	Ouachita	41.0	29.5	23.3	0.720	0.568	0.790	4.866	4.323	5.099
ASUMZ 4646	Saline R., Dallas/Grant Co., AR	Ouachita	37.6	29.0	21.7	0.771	0.577	0.748	5.038	4.357	4.962
ASUMZ 4647	Saline R., Dallas/Grant Co., AR	Ouachita	31.9	23.6	19.1	0.740	0.599	0.809	4.934	4.438	5.161
ASUMZ 4648	Saline R., Dallas/Grant Co., AR	Ouachita	25.1	19.3	13.8	0.769	0.550	0.715	5.031	4.252	4.851
ASUMZ 4649	Saline R., Ashley/Bradley Co., AR	Ouachita	30.2	26.8	20.5	0.887	0.679	0.765	5.405	4.726	5.018
ASUMZ 4650	Saline R., Ashley/Bradley Co., AR	Ouachita	50.5	39.2	32.5	0.776	0.644	0.829	5.055	4.601	5.224

(table continues)

Table 3.1. (*continued*)

			Measurement (mm)			Ratio			Transformed ratio		
Museum #	Locality	River Drainage	Length	Height	Width	H/L	W/L	W/H	H/L	W/L	W/H
ASUMZ 4651	Saline R., Ashley/Bradley Co., AR	Ouachita	48.3	39.5	33.0	0.818	0.683	0.835	5.188	4.741	5.244
ASUMZ 4652	Saline R., Ashley/Bradley Co., AR	Ouachita	51.3	37.3	33.1	0.727	0.645	0.887	4.892	4.607	5.405
ASUMZ 4653	Little Missouri R., Nevada Co., AR	Ouachita	31.8	24.7	20.4	0.777	0.642	0.826	5.056	4.594	5.214
ASUMZ 4654	Little Missouri R., Nevada Co., AR	Ouachita	30.0	24.9	20.0	0.830	0.667	0.803	5.227	4.683	5.142
ASUMZ 4655	Saline R., Ashley/Bradley Co., AR	Ouachita	37.8	31.8	24.0	0.841	0.635	0.755	5.263	4.570	4.984
ASUMZ 4657	Saline R., Ashley/Bradley Co., AR	Ouachita	29.4	26.5	20.3	0.901	0.690	0.766	5.448	4.766	5.021
ASUMZ 4658	Saline R., Ashley/Bradley Co., AR	Ouachita	33.4	26.6	21.9	0.796	0.656	0.823	5.120	4.645	5.206
ASUMZ 4660	Little Missouri R., Nevada Co., AR	Ouachita	29.2	24.3	19.4	0.832	0.664	0.798	5.234	4.675	5.126
ASUMZ 4871	Sipsey R., Pickens/Greene Co., AL	Mobile	33.3	26.1	20.2	0.784	0.607	0.774	5.079	4.467	5.047
ASUMZ 4872	Sipsey R., Pickens/Greene Co., AL	Mobile	31.3	24.7	20.3	0.789	0.649	0.822	5.096	4.619	5.201
ASUMZ 4873	Sipsey R., Pickens/Greene Co., AL	Mobile	35.2	26.5	21.8	0.753	0.619	0.823	4.978	4.514	5.204
ASUMZ 4874	Sipsey R., Pickens/Greene Co., AL	Mobile	43.0	33.7	26.8	0.784	0.623	0.795	5.079	4.528	5.116
ASUMZ 4875	Saline R., Dallas/Grant Co., AR	Ouachita	27.5	22.8	16.2	0.829	0.589	0.711	5.224	4.402	4.835
ASUMZ 4876	Saline R., Dallas/Grant Co., AR	Ouachita	25.4	18.9	13.8	0.744	0.543	0.730	4.949	4.227	4.902
ASUMZ 4877	Saline R., Dallas/Grant Co., AR	Ouachita	22.6	16.8	12.3	0.743	0.544	0.732	4.946	4.231	4.909
ASUMZ 4878	Little R., Sevier Co., AR	Red	45.1	37.1	31.5	0.823	0.698	0.849	5.204	4.794	5.287
ASUMZ 4879	Little R., Sevier Co., AR	Red	36.4	32.2	27.7	0.885	0.761	0.860	5.397	5.005	5.322
MD 1	Saline R., Ashley/Bradley Co., AR	Ouachita	29.9	25.3	18.9	0.846	0.632	0.747	5.278	4.560	4.958
MD 2	Saline R., Ashley/Bradley Co., AR	Ouachita	36.0	29.7	25.0	0.825	0.694	0.842	5.211	4.780	5.264
MD 3	Saline R., Ashley/Bradley Co., AR	Ouachita	30.3	24.0	20.2	0.792	0.667	0.842	5.106	4.683	5.264
MD 4	Saline R., Ashley/Bradley Co., AR	Ouachita	37.0	31.2	28.3	0.843	0.765	0.907	5.269	5.017	5.465
MD 5	Saline R., Ashley/Bradley Co., AR	Ouachita	45.2	35.8	33.0	0.792	0.730	0.922	5.106	4.902	5.509
MD 6	Saline R., Ashley/Bradley Co., AR	Ouachita	34.2	30.2	26.7	0.883	0.781	0.884	5.392	5.069	5.395
MD 7	Saline R., Ashley/Bradley Co., AR	Ouachita	40.8	33.5	27.3	0.821	0.669	0.815	5.199	4.692	5.179
MD 8	Saline R., Ashley/Bradley Co., AR	Ouachita	37.2	29.2	28.1	0.785	0.755	0.962	5.083	4.986	5.630
ASUMZ 1889	Little Missouri R., Nevada Co., AR	Ouachita	38.4	29.5	21.1	0.768	0.549	0.715	5.028	4.251	4.851
ASUMZ 1890	Little Missouri R., Nevada Co., AR	Ouachita	45.2	33.8	28.4	0.748	0.628	0.840	4.961	4.546	5.259
ASUMZ 1891	Little Missouri R., Nevada Co., AR	Ouachita	35.8	28.0	24.2	0.782	0.676	0.864	5.074	4.716	5.334

(table continues)

Table 3.1. *(continued)*

		River	Measurement (mm)			Ratio			Transformed ratio		
Museum #	Locality	Drainage	Length	Height	Width	H/L	W/L	W/H	H/L	W/L	W/H
ASUMZ 2011	Cossatot R., Sevier Co., AR	Red	28.0	22.8	16.8	0.814	0.600	0.737	5.177	4.443	4.924
JLH 505	Caddo R., Clark Co., AR	Ouachita	47.3	34.6	30.1	0.732	0.636	0.870	4.906	4.575	5.352
Midden LM 01	Little Missouri R., Nevada Co., AR	Ouachita	40.2	32.5	24.9	0.808	0.619	0.766	5.159	4.514	5.022
Midden LM 02	Little Missouri R., Nevada Co., AR	Ouachita	35.8	31.2	25.6	0.872	0.715	0.821	5.357	4.851	5.197
Midden LM 03	Little Missouri R., Nevada Co., AR	Ouachita	35.8	29.1	25.0	0.813	0.698	0.859	5.173	4.794	5.318
Midden LM 04	Little Missouri R., Nevada Co., AR	Ouachita	31.2	27.0	21.1	0.865	0.676	0.781	5.338	4.717	5.072
Midden LM 05	Little Missouri R., Nevada Co., AR	Ouachita	29.7	25.5	19.9	0.859	0.670	0.780	5.317	4.695	5.068
Midden LM 06	Little Missouri R., Nevada Co., AR	Ouachita	33.7	29.7	23.3	0.881	0.691	0.785	5.387	4.770	5.081
Midden LM 07	Little Missouri R., Nevada Co., AR	Ouachita	33.7	28.8	21.7	0.855	0.644	0.753	5.304	4.603	4.980
Midden LM 08	Little Missouri R., Nevada Co., AR	Ouachita	32.6	26.2	20.0	0.804	0.613	0.763	5.143	4.492	5.012
Midden LM 09	Little Missouri R., Nevada Co., AR	Ouachita	33.4	31.2	24.7	0.934	0.740	0.792	5.546	4.933	5.105
Midden LM 10	Little Missouri R., Nevada Co., AR	Ouachita	35.0	29.3	22.9	0.837	0.654	0.782	5.250	4.640	5.072
Midden LM 11	Little Missouri R., Nevada Co., AR	Ouachita	32.7	31.0	23.6	0.948	0.722	0.761	5.588	4.873	5.006
Midden LM 12	Little Missouri R., Nevada Co., AR	Ouachita	31.8	30.0	23.2	0.943	0.730	0.773	5.574	4.900	5.045
Midden LM 13	Little Missouri R., Nevada Co., AR	Ouachita	29.5	24.3	18.1	0.824	0.614	0.745	5.207	4.493	4.951
Midden LM 14	Little Missouri R., Nevada Co., AR	Ouachita	27.8	24.5	19.1	0.881	0.687	0.780	5.387	4.755	5.065
Midden LM 15	Little Missouri R., Nevada Co., AR	Ouachita	28.9	23.3	17.3	0.806	0.599	0.742	5.152	4.437	4.943
Midden LM 16	Little Missouri R., Nevada Co., AR	Ouachita	27.0	22.2	15.1	0.822	0.559	0.680	5.203	4.289	4.731
Midden LM 17	Little Missouri R., Nevada Co., AR	Ouachita	30.0	27.6	20.8	0.920	0.693	0.754	5.504	4.776	4.980
Midden LS 01	Saline R., Ashley/Bradley Co., AR	Ouachita	48.3	40.4	34.2	0.836	0.708	0.847	5.247	4.827	5.279
Midden LS 02	Saline R., Ashley/Bradley Co., AR	Ouachita	50.2	41.4	36.2	0.825	0.721	0.874	5.210	4.871	5.366
Midden LS 03	Saline R., Ashley/Bradley Co., AR	Ouachita	43.3	35.5	32.4	0.820	0.748	0.913	5.195	4.962	5.482
Midden LS 04	Saline R., Ashley/Bradley Co., AR	Ouachita	51.8	37.7	32.2	0.728	0.622	0.854	4.894	4.522	5.303
Midden LS 05	Saline R., Ashley/Bradley Co., AR	Ouachita	35.8	30.9	26.8	0.863	0.749	0.867	5.331	4.964	5.344
Midden LS 06	Saline R., Ashley/Bradley Co., AR	Ouachita	44.6	35.7	29.9	0.800	0.670	0.838	5.133	4.697	5.251
Midden LS 07	Saline R., Ashley/Bradley Co., AR	Ouachita	41.5	36.0	29.7	0.867	0.716	0.825	5.344	4.853	5.211
Midden LS 08	Saline R., Ashley/Bradley Co., AR	Ouachita	47.2	39.5	32.5	0.837	0.689	0.823	5.249	4.760	5.204
Midden LS 09	Saline R., Ashley/Bradley Co., AR	Ouachita	41.5	34.3	29.8	0.827	0.718	0.869	5.216	4.861	5.348

(table continues)

Table 3.1. *(continued)*

		River	Measurement (mm)			Ratio			Transformed ratio		
Museum #	Locality	Drainage	Length	Height	Width	H/L	W/L	W/H	H/L	W/L	W/H
Midden LS 10	Saline R., Ashley/Bradley Co., AR	Ouachita	40.6	35.1	29.3	0.865	0.722	0.835	5.335	4.873	5.242
Midden LS 11	Saline R., Ashley/Bradley Co., AR	Ouachita	33.7	28.1	22.5	0.834	0.668	0.801	5.239	4.687	5.134
Midden LS 12	Saline R., Ashley/Bradley Co., AR	Ouachita	38.5	34.5	29.0	0.896	0.753	0.841	5.432	4.979	5.260
Midden LS 13	Saline R., Ashley/Bradley Co., AR	Ouachita	53.5	36.5	35.9	0.682	0.671	0.984	4.738	4.699	5.692
Midden LS 14	Saline R., Ashley/Bradley Co., AR	Ouachita	42.2	33.5	29.8	0.794	0.706	0.890	5.112	4.820	5.412
Midden LS 15	Saline R., Ashley/Bradley Co., AR	Ouachita	28.6	23.0	21.0	0.804	0.734	0.913	5.145	4.916	5.483
Midden LS 16	Saline R., Ashley/Bradley Co., AR	Ouachita	37.7	33.5	27.0	0.889	0.716	0.806	5.409	4.855	5.151
Midden LS 17	Saline R., Ashley/Bradley Co., AR	Ouachita	48.4	37.6	32.0	0.777	0.661	0.851	5.057	4.664	5.293
Midden LS 18	Saline R., Ashley/Bradley Co., AR	Ouachita	46.7	36.3	32.4	0.777	0.694	0.893	5.058	4.778	5.421
Midden LS 19	Saline R., Ashley/Bradley Co., AR	Ouachita	39.9	32.3	27.4	0.810	0.687	0.848	5.162	4.753	5.285
Midden LS 20	Saline R., Ashley/Bradley Co., AR	Ouachita	50.4	40.5	34.9	0.804	0.692	0.862	5.143	4.773	5.326
Midden LS 21	Saline R., Ashley/Bradley Co., AR	Ouachita	37.9	29.9	25.6	0.789	0.675	0.856	5.096	4.714	5.309
Midden LS 22	Saline R., Ashley/Bradley Co., AR	Ouachita	32.4	26.8	23.6	0.827	0.728	0.881	5.218	4.896	5.385
Midden LS 23	Saline R., Ashley/Bradley Co., AR	Ouachita	40.0	32.0	28.7	0.800	0.718	0.897	5.132	4.859	5.434
Midden LS 24	Saline R., Ashley/Bradley Co., AR	Ouachita	31.2	27.2	22.1	0.872	0.708	0.813	5.358	4.828	5.172
Midden LS 25	Saline R., Ashley/Bradley Co., AR	Ouachita	37.6	30.5	23.6	0.811	0.628	0.774	5.167	4.544	5.047
Midden LS 26	Saline R., Ashley/Bradley Co., AR	Ouachita	34.8	28.8	25.3	0.828	0.727	0.878	5.220	4.891	5.378
Midden LS 27	Saline R., Ashley/Bradley Co., AR	Ouachita	35.8	31.0	25.5	0.866	0.712	0.823	5.339	4.841	5.204
Midden LS 28	Saline R., Ashley/Bradley Co., AR	Ouachita	47.8	37.8	32.2	0.791	0.674	0.852	5.102	4.708	5.296
Midden LS 29	Saline R., Ashley/Bradley Co., AR	Ouachita	38.8	32.8	26.4	0.845	0.680	0.805	5.275	4.732	5.147
Midden LS 30	Saline R., Ashley/Bradley Co., AR	Ouachita	42.8	35.0	31.4	0.818	0.734	0.897	5.188	4.914	5.435
Midden LS 31	Saline R., Ashley/Bradley Co., AR	Ouachita	32.6	28.4	24.9	0.871	0.764	0.877	5.356	5.014	5.373
Midden LS 32	Saline R., Ashley/Bradley Co., AR	Ouachita	37.0	31.0	24.6	0.838	0.665	0.794	5.252	4.677	5.111
Midden LS 33	Saline R., Ashley/Bradley Co., AR	Ouachita	29.6	22.3	17.4	0.753	0.588	0.780	4.979	4.397	5.068
Midden LS 34	Saline R., Ashley/Bradley Co., AR	Ouachita	35.7	30.0	26.3	0.840	0.737	0.877	5.260	4.924	5.373
Midden LS 35	Saline R., Ashley/Bradley Co., AR	Ouachita	42.4	32.0	29.5	0.755	0.696	0.922	4.984	4.785	5.510
Midden LS 36	Saline R., Ashley/Bradley Co., AR	Ouachita	31.5	25.4	22.8	0.806	0.724	0.898	5.152	4.880	5.437
Midden LS 37	Saline R., Ashley/Bradley Co., AR	Ouachita	37.6	29.2	27.0	0.777	0.718	0.925	5.056	4.861	5.518

(table continues)

Table 3.1. *(continued)*

		River	Measurement (mm)			Ratio			Transformed ratio		
Museum #	Locality	Drainage	Length	Height	Width	H/L	W/L	W/H	H/L	W/L	W/H
Midden LS 38	Saline R., Ashley/Bradley Co., AR	Ouachita	33.8	27.8	23.2	0.822	0.686	0.835	5.203	4.752	5.241
Midden LS 39	Saline R., Ashley/Bradley Co., AR	Ouachita	41.0	32.5	30.0	0.793	0.732	0.923	5.108	4.907	5.513
Midden LS 40	Saline R., Ashley/Bradley Co., AR	Ouachita	38.2	32.2	26.6	0.843	0.696	0.826	5.268	4.787	5.215
Midden LS 41	Saline R., Ashley/Bradley Co., AR	Ouachita	40.5	31.6	28.7	0.780	0.709	0.908	5.068	4.829	5.469
Midden LS 42	Saline R., Ashley/Bradley Co., AR	Ouachita	39.3	30.3	26.8	0.771	0.682	0.884	5.037	4.737	5.396
Midden LS 43	Saline R., Ashley/Bradley Co., AR	Ouachita	44.8	34.3	30.3	0.766	0.676	0.883	5.020	4.717	5.393
Midden LS 44	Saline R., Ashley/Bradley Co., AR	Ouachita	31.6	26.7	23.5	0.845	0.744	0.880	5.274	4.947	5.383
Midden LS 45	Saline R., Ashley/Bradley Co., AR	Ouachita	46.6	36.8	31.8	0.790	0.682	0.864	5.098	4.738	5.334
Midden LS 46	Saline R., Ashley/Bradley Co., AR	Ouachita	36.4	31.4	28.5	0.863	0.783	0.908	5.329	5.076	5.467
Midden LS 47	Saline R., Ashley/Bradley Co., AR	Ouachita	44.1	35.4	31.8	0.803	0.721	0.898	5.140	4.871	5.439
Midden LS 48	Saline R., Ashley/Bradley Co., AR	Ouachita	33.7	27.6	23.1	0.819	0.685	0.837	5.192	4.749	5.249
Midden LS 49	Saline R., Ashley/Bradley Co., AR	Ouachita	36.1	30.7	25.4	0.850	0.704	0.827	5.291	4.812	5.219
Midden LS 50	Saline R., Ashley/Bradley Co., AR	Ouachita	40.5	33.4	29.5	0.825	0.728	0.883	5.210	4.896	5.393
Midden LS 51	Saline R., Ashley/Bradley Co., AR	Ouachita	45.0	35.1	29.7	0.780	0.660	0.846	5.067	4.660	5.278
Midden LS 52	Saline R., Ashley/Bradley Co., AR	Ouachita	48.2	35.9	35.2	0.745	0.730	0.981	4.951	4.902	5.683
Midden LS 53	Saline R., Ashley/Bradley Co., AR	Ouachita	30.5	26.6	23.8	0.872	0.780	0.895	5.359	5.068	5.428
Midden LS 54	Saline R., Ashley/Bradley Co., AR	Ouachita	46.8	36.1	32.0	0.771	0.684	0.886	5.039	4.743	5.402
Midden LS 55	Saline R., Ashley/Bradley Co., AR	Ouachita	47.0	39.4	33.0	0.838	0.702	0.838	5.253	4.807	5.251
Midden LS 56	Saline R., Ashley/Bradley Co., AR	Ouachita	41.2	32.5	30.3	0.789	0.735	0.932	5.096	4.920	5.541
Midden LS 57	Saline R., Ashley/Bradley Co., AR	Ouachita	37.0	32.0	25.7	0.865	0.695	0.803	5.336	4.781	5.142
Midden LS 58	Saline R., Ashley/Bradley Co., AR	Ouachita	46.8	35.3	32.5	0.754	0.694	0.921	4.982	4.780	5.506
Midden LS 59	Saline R., Ashley/Bradley Co., AR	Ouachita	42.5	32.2	28.3	0.758	0.666	0.879	4.994	4.681	5.379
Midden LS 60	Saline R., Ashley/Bradley Co., AR	Ouachita	34.5	27.7	23.5	0.803	0.681	0.848	5.141	4.734	5.285
Midden LS 61	Saline R., Ashley/Bradley Co., AR	Ouachita	44.2	34.8	28.5	0.787	0.645	0.819	5.091	4.606	5.192
Midden LS 62	Saline R., Ashley/Bradley Co., AR	Ouachita	38.5	32.0	27.7	0.831	0.719	0.866	5.231	4.866	5.338
Midden LS 63	Saline R., Ashley/Bradley Co., AR	Ouachita	35.5	27.4	24.3	0.772	0.685	0.887	5.040	4.746	5.404
Midden LS 64	Saline R., Ashley/Bradley Co., AR	Ouachita	39.0	30.7	26.8	0.787	0.687	0.873	5.090	4.755	5.361
Midden LS 65	Saline R., Ashley/Bradley Co., AR	Ouachita	43.0	36.1	30.7	0.840	0.714	0.850	5.257	4.847	5.291

(table continues)

Table 3.1. *(continued)*

			Measurement (mm)			Ratio			Transformed ratio		
Museum #	Locality	River Drainage	Length	Height	Width	H/L	W/L	W/H	H/L	W/L	W/H
Midden LS 66	Saline R., Ashley/Bradley Co., AR	Ouachita	32.1	24.0	24.3	0.748	0.757	1.013	4.960	4.991	5.775
Midden LS 67	Saline R., Ashley/Bradley Co., AR	Ouachita	36.8	29.8	24.8	0.810	0.674	0.832	5.163	4.709	5.234
Midden LS 68	Saline R., Ashley/Bradley Co., AR	Ouachita	44.3	34.1	33.5	0.770	0.756	0.982	5.033	4.989	5.688
Midden LS 69	Saline R., Ashley/Bradley Co., AR	Ouachita	36.6	30.2	24.7	0.825	0.675	0.818	5.212	4.712	5.189
Midden LS 70	Saline R., Ashley/Bradley Co., AR	Ouachita	25.4	21.3	17.5	0.839	0.689	0.822	5.254	4.761	5.201
Midden LS 71	Saline R., Ashley/Bradley Co., AR	Ouachita	26.7	22.5	18.4	0.843	0.689	0.818	5.267	4.762	5.188
Midden LS 72	Saline R., Ashley/Bradley Co., AR	Ouachita	45.8	36.7	31.0	0.801	0.677	0.845	5.136	4.719	5.273
Midden LS 73	Saline R., Ashley/Bradley Co., AR	Ouachita	48.2	41.5	33.8	0.861	0.701	0.814	5.324	4.804	5.178
Midden LS 74	Saline R., Ashley/Bradley Co., AR	Ouachita	35.8	29.0	25.5	0.810	0.712	0.879	5.164	4.841	5.381
Midden LS 75	Saline R., Ashley/Bradley Co., AR	Ouachita	40.3	33.7	26.6	0.836	0.660	0.789	5.247	4.660	5.097
Midden LS 76	Saline R., Ashley/Bradley Co., AR	Ouachita	45.4	38.8	34.0	0.855	0.749	0.876	5.304	4.965	5.371
Midden LS 77	Saline R., Ashley/Bradley Co., AR	Ouachita	35.0	29.6	23.8	0.846	0.680	0.804	5.277	4.730	5.145
Midden LS 78	Saline R., Ashley/Bradley Co., AR	Ouachita	45.7	37.8	34.8	0.827	0.761	0.921	5.218	5.006	5.506
Midden LS 79	Saline R., Ashley/Bradley Co., AR	Ouachita	36.5	30.5	26.6	0.836	0.729	0.872	5.245	4.897	5.359
Midden LS 80	Saline R., Ashley/Bradley Co., AR	Ouachita	34.7	27.1	24.1	0.781	0.695	0.889	5.070	4.780	5.411
Midden LS 81	Saline R., Ashley/Bradley Co., AR	Ouachita	50.3	37.3	33.5	0.742	0.666	0.898	4.940	4.681	5.438
Midden LS 82	Saline R., Ashley/Bradley Co., AR	Ouachita	40.1	33.7	28.1	0.840	0.701	0.834	5.260	4.802	5.239
Midden LS 83	Saline R., Ashley/Bradley Co., AR	Ouachita	36.4	31.2	26.0	0.857	0.714	0.833	5.312	4.848	5.238
Midden LR 01	Little R., Little River/Sevier Co., AR	Ouachita	30.6	31.3	23.2	1.023	0.758	0.741	5.805	4.995	4.939
Midden LR 02	Little R., Little River/Sevier Co., AR	Ouachita	41.1	34.8	31.0	0.847	0.754	0.891	5.280	4.982	5.416
Midden LR 03	Little R., Little River/Sevier Co., AR	Ouachita									
Midden LR 04	Little R., Little River/Sevier Co., AR	Ouachita	32.0	32.1	23.5	1.003	0.734	0.732	5.748	4.916	4.908
		Average =	37.2	31.1	25.4	0.840	0.681	0.814	5.254	4.730	5.171
Villosa arkansasensis											
USNM 25710	Ouachita R., Garland Co., AR (Type)	Ouachita									
ASUMZ 2924	Rock Creek, Howard Co., AR	Red	45.1	33.1	26.3	0.734	0.583	0.795	4.915	4.380	5.114
ASUMZ 4569	Alum Fork of the Saline R., Saline Co., AR	Ouachita	37.4	26.2	15.7	0.701	0.420	0.599	4.801	3.715	4.440
ASUMZ 4570	Alum Fork of the Saline R., Saline Co., AR	Ouachita	41.2	28.5	16.7	0.692	0.405	0.586	4.771	3.650	4.390

(table continues)

Table 3.1. *(continued)*

		River	Measurement (mm)			Ratio			Transformed ratio		
Museum #	Locality	Drainage	Length	Height	Width	H/L	W/L	W/H	H/L	W/L	W/H
ASUMZ 4571	Alum Fork of the Saline R., Saline Co., AR	Ouachita	27.6	20.2	11.7	0.732	0.424	0.579	4.908	3.733	4.365
ASUMZ 4572	Alum Fork of the Saline R., Saline Co., AR	Ouachita	37.1	27.6	16.6	0.744	0.447	0.601	4.948	3.835	4.448
ASUMZ 4579	Irons Fork of the Ouachita R., Polk Co., AR	Ouachita	42.1	28.0	21.2	0.665	0.504	0.757	4.678	4.069	4.992
ASUMZ 4580	Irons Fork of the Ouachita R., Polk Co., AR	Ouachita	37.7	28.5	21.2	0.756	0.562	0.744	4.988	4.301	4.948
ASUMZ 4581	Irons Fork of the Ouachita R., Polk Co., AR	Ouachita	38.2	25.2	18.5	0.660	0.484	0.734	4.659	3.991	4.915
ASUMZ 4582	Irons Fork of the Ouachita R., Polk Co., AR	Ouachita	39.9	28.2	18.7	0.707	0.469	0.663	4.823	3.926	4.671
ASUMZ 4583	Irons Fork of the Ouachita R., Polk Co., AR	Ouachita	38.7	26.7	19.4	0.690	0.501	0.727	4.765	4.060	4.890
ASUMZ 4661	Alum Fork of the Saline R., Saline Co., AR	Ouachita	43.3	29.9	22.7	0.691	0.524	0.759	4.767	4.152	4.999
ASUMZ 4662	Alum Fork of the Saline R., Saline Co., AR	Ouachita	38.2	28.4	18.2	0.743	0.476	0.641	4.946	3.958	4.592
ASUMZ 4663	Alum Fork of the Saline R., Saline Co., AR	Ouachita	37.1	27.3	17.4	0.736	0.469	0.637	4.921	3.927	4.579
ASUMZ 4664	Alum Fork of the Saline R., Saline Co., AR	Ouachita	35.4	24.1	16.9	0.681	0.477	0.701	4.733	3.962	4.804
ASUMZ 4665	Alum Fork of the Saline R., Saline Co., AR	Ouachita	37.6	26.4	18.7	0.702	0.497	0.708	4.807	4.044	4.828
ASUMZ 4666	Alum Fork of the Saline R., Saline Co., AR	Ouachita	28.0	21.0	12.5	0.750	0.446	0.595	4.968	3.831	4.425
ASUMZ 4667	Irons Fork of the Ouachita R., Polk Co., AR	Ouachita	32.2	22.8	14.9	0.708	0.463	0.654	4.827	3.901	4.637
ASUMZ 4889	Mountain Fork of the Little R., Polk Co., AR	Red	49.1	28.8	21.0	0.587	0.428	0.729	4.392	3.750	4.899
JLH 213	Ouachita River	Ouachita	42.5	30.0	19.0	0.706	0.447	0.633	4.819	3.834	4.565
JLH 214	Ouachita River	Ouachita	38.8	27.0	20.1	0.696	0.518	0.744	4.785	4.127	4.950
JLH 215	Ouachita River	Ouachita	35.3	25.2	18.0	0.714	0.510	0.714	4.847	4.095	4.848
JLH 216	Ouachita River	Ouachita									
JLH 217	Ouachita River	Ouachita	46.8	34.3	20.6	0.733	0.440	0.601	4.911	3.804	4.445
JLH 218	Ouachita River	Ouachita									
LPS105 TS-35	Irons Fork of the Ouachita R., Polk Co., AR	Ouachita	50.7	36.3	24.2	0.716	0.477	0.667	4.854	3.962	4.683
LPS50 TS-44	Alum Fork of the Saline R., Saline Co., AR	Ouachita									
LPS50 TS-45	Alum Fork of the Saline R., Saline Co., AR	Ouachita									
LPS50 TS-47	Alum Fork of the Saline R., Saline Co., AR	Ouachita									
LPS50 TS-49	Alum Fork of the Saline R., Saline Co., AR	Ouachita									
LPS50 TS-59	Alum Fork of the Saline R., Saline Co., AR	Ouachita	39.4	28.0	17.1	0.711	0.434	0.611	4.836	3.777	4.482
LPS50 TS-62	Alum Fork of the Saline R., Saline Co., AR	Ouachita	36.7	26.7	19.1	0.728	0.520	0.715	4.893	4.137	4.852

(table continues)

Table 3.1. (*continued*)

			Measurement (mm)			Ratio			Transformed ratio		
Museum #	Locality	River Drainage	Length	Height	Width	H/L	W/L	W/H	H/L	W/L	W/H
LPS50 TS-68	Alum Fork of the Saline R., Saline Co., AR	Ouachita	35.9	25.2	19.3	0.702	0.538	0.766	4.806	4.205	5.021
LPS50 TS-69	Alum Fork of the Saline R., Saline Co., AR	Ouachita	38.8	26.8	19.2	0.691	0.495	0.716	4.767	4.034	4.855
LPS50 TS-71	Alum Fork of the Saline R., Saline Co., AR	Ouachita	40.7	27.4	19.2	0.673	0.472	0.701	4.706	3.938	4.802
LPS50 TS-73	Alum Fork of the Saline R., Saline Co., AR	Ouachita	32.2	24.0	15.1	0.745	0.469	0.629	4.953	3.927	4.549
LPS50 TS-74	Alum Fork of the Saline R., Saline Co., AR	Ouachita									
LPS50 TS-75	Alum Fork of the Saline R., Saline Co., AR	Ouachita	40.4	27.3	20.3	0.676	0.502	0.744	4.715	4.065	4.947
LPS50 TS-77	Alum Fork of the Saline R., Saline Co., AR	Ouachita	40.5	28.0	18.4	0.691	0.454	0.657	4.770	3.865	4.650
LPS50 TS-78	Alum Fork of the Saline R., Saline Co., AR	Ouachita	40.0	26.7	18.1	0.668	0.453	0.678	4.686	3.857	4.723
LPS50 TS-79	Alum Fork of the Saline R., Saline Co., AR	Ouachita	33.0	24.6	16.9	0.745	0.512	0.687	4.953	4.104	4.754
LPS50 TS-82	Alum Fork of the Saline R., Saline Co., AR	Ouachita									
LPS50 TS-84	Alum Fork of the Saline R., Saline Co., AR	Ouachita	35.9	26.4	16.4	0.735	0.457	0.621	4.919	3.876	4.521
LPS50 TS-86	Alum Fork of the Saline R., Saline Co., AR	Ouachita	36.3	25.0	17.4	0.689	0.479	0.696	4.760	3.970	4.786
LPS50 TS-87	Alum Fork of the Saline R., Saline Co., AR	Ouachita									
LPS50 TS-88	Alum Fork of the Saline R., Saline Co., AR	Ouachita	35.8	26.7	17.7	0.746	0.494	0.663	4.954	4.032	4.670
LPS50 TS-89	Alum Fork of the Saline R., Saline Co., AR	Ouachita									
LPS50 TS-92	Alum Fork of the Saline R., Saline Co., AR	Ouachita									
LPS50 TS-94	Alum Fork of the Saline R., Saline Co., AR	Ouachita	37.7	25.4	17.6	0.674	0.467	0.693	4.708	3.918	4.775
LPS50 TS-98	Alum Fork of the Saline R., Saline Co., AR	Ouachita									
LPS50 TS-99	Alum Fork of the Saline R., Saline Co., AR	Ouachita									
		Average =	38.5	27.1	18.4	0.706	0.479	0.680	4.818	3.965	4.725
Obovaria subrotunda											
ASUMZ 4604	Duck R., Marshall Co., TN	Tennessee	53.0	46.5	31.5	0.877	0.594	0.677	5.375	4.422	4.721
ASUMZ 4605	Duck R., Marshall Co., TN	Tennessee	41.5	37.7	25.8	0.908	0.622	0.684	5.469	4.522	4.745
ASUMZ 4606	Duck R., Marshall Co., TN	Tennessee	31.6	27.2	15.8	0.861	0.500	0.581	5.323	4.055	4.371
ASUMZ 4607	Duck R., Marshall Co., TN	Tennessee	26.4	24.2	15.2	0.917	0.576	0.628	5.494	4.352	4.546
ASUMZ 4608	Duck R., Marshall Co., TN	Tennessee	20.6	16.7	10.5	0.811	0.510	0.629	5.166	4.094	4.548
MMNS 7650.1	Big Black R., Montgomery Co., MS	Big Black	35.1	29.6	22.2	0.843	0.632	0.750	5.269	4.561	4.968
MMNS 7650.2	Big Black R., Montgomery Co., MS	Big Black	30.3	27.3	18.9	0.901	0.624	0.692	5.447	4.530	4.773

(table continues)

Table 3.1. *(continued)*

		River	Measurement (mm)			Ratio			Transformed ratio		
Museum #	Locality	Drainage	Length	Height	Width	H/L	W/L	W/H	H/L	W/L	W/H
MMNS 7650.3	Big Black R., Montgomery Co., MS	Big Black	37.5	30.5	21.6	0.813	0.576	0.708	5.174	4.353	4.827
MMNS 7650.4	Big Black R., Montgomery Co., MS	Big Black	34.0	29.2	22.7	0.859	0.668	0.777	5.317	4.687	5.058
MMNS 7650.5	Big Black R., Montgomery Co., MS	Big Black	35.1	27.9	20.2	0.795	0.575	0.724	5.115	4.351	4.881
MMNS 7650.6	Big Black R., Montgomery Co., MS	Big Black	36.7	30.1	20.2	0.820	0.550	0.671	5.196	4.255	4.699
MMNS 7650.7	Big Black R., Montgomery Co., MS	Big Black	32.2	28.2	21.3	0.876	0.661	0.755	5.370	4.665	4.986
MMNS 7650.8	Big Black R., Montgomery Co., MS	Big Black	40.5	34.4	25.3	0.849	0.625	0.735	5.288	4.533	4.920
MMNS 7650.9	Big Black R., Montgomery Co., MS	Big Black	41.1	33.8	25.5	0.822	0.620	0.754	5.203	4.518	4.983
MMNS 7650.10	Big Black R., Montgomery Co., MS	Big Black	39.5	33.8	23.8	0.856	0.603	0.704	5.308	4.452	4.814
MMNS 7650.11	Big Black R., Montgomery Co., MS	Big Black	41.8	35.1	24.7	0.840	0.591	0.704	5.258	4.409	4.812
MMNS 7650.12	Big Black R., Montgomery Co., MS	Big Black	35.0	28.9	21.4	0.826	0.611	0.740	5.214	4.485	4.936
MMNS 7650.13	Big Black R., Montgomery Co., MS	Big Black	36.1	28.9	20.3	0.801	0.562	0.702	5.133	4.301	4.808
MMNS 7650.14	Big Black R., Montgomery Co., MS	Big Black	36.5	29.3	19.7	0.803	0.540	0.672	5.140	4.213	4.703
MMNS 7650.15	Big Black R., Montgomery Co., MS	Big Black	41.8	33.9	22.3	0.811	0.533	0.658	5.167	4.189	4.652
MMNS 7650.16	Big Black R., Montgomery Co., MS	Big Black	31.8	25.8	19.7	0.811	0.619	0.764	5.168	4.514	5.013
MMNS 7650.17	Big Black R., Montgomery Co., MS	Big Black	26.5	20.7	14.5	0.781	0.547	0.700	5.071	4.242	4.801
MMNS 7650.18	Big Black R., Montgomery Co., MS	Big Black	26.7	20.9	14.8	0.783	0.554	0.708	5.076	4.270	4.827
MMNS 7650.19	Big Black R., Montgomery Co., MS	Big Black	35.8	29.4	20.4	0.821	0.570	0.694	5.199	4.329	4.778
MMNS 7650.20	Big Black R., Montgomery Co., MS	Big Black	28.2	22.6	14.8	0.801	0.525	0.655	5.136	4.154	4.642
MMNS 7650.21	Big Black R., Montgomery Co., MS	Big Black	36.3	28.9	20.5	0.796	0.565	0.709	5.119	4.310	4.831
MMNS 7650.22	Big Black R., Montgomery Co., MS	Big Black	35.1	29.0	20.3	0.826	0.578	0.700	5.215	4.362	4.799
MMNS 7650.23	Big Black R., Montgomery Co., MS	Big Black	30.5	24.8	17.5	0.813	0.574	0.706	5.174	4.344	4.819
MMNS 7650.24	Big Black R., Montgomery Co., MS	Big Black	33.6	27.9	20.8	0.830	0.619	0.746	5.228	4.513	4.953
		Average =	34.9	29.1	20.4	0.833	0.584	0.701	5.235	4.379	4.801
Obovaria olivaria											
ASUMZ 395	White R., White Co., AR	White	76.7	57.6	47.1	0.751	0.614	0.818	4.971	4.494	5.188
ASUMZ 396	White R., White Co., AR	White	70.0	62.0	42.9	0.886	0.613	0.692	5.400	4.490	4.772
ASUMZ 398	White R., White Co., AR	White	84.8	63.0	55.1	0.743	0.650	0.875	4.945	4.624	5.366
ASUMZ 399	White R., White Co., AR	White	85.7	67.4	52.6	0.786	0.614	0.780	5.088	4.493	5.068

(table continues)

Table 3.1. (*continued*)

			Measurement (mm)			Ratio			Transformed ratio		
Museum #	Locality	River Drainage	Length	Height	Width	H/L	W/L	W/H	H/L	W/L	W/H
ASUMZ 400	White R., White Co., AR	White	69.3	55.3	43.0	0.798	0.620	0.778	5.125	4.518	5.059
ASUMZ 576	Black R., Clay Co., AR	White	66.1	54.3	40.2	0.821	0.608	0.740	5.200	4.473	4.936
ASUMZ 577	Black R., Clay Co., AR	White	70.6	66.2	44.4	0.938	0.629	0.671	5.557	4.548	4.698
ASUMZ 578	Black R., Clay Co., AR	White	74.0	72.3	50.0	0.977	0.676	0.692	5.673	4.715	4.770
ASUMZ 579	Black R., Clay Co., AR	White	71.1	73.9	45.0	1.039	0.633	0.609	5.851	4.563	4.476
ASUMZ 580	Black R., Clay Co., AR	White	70.4	63.0	46.3	0.895	0.658	0.735	5.428	4.652	4.918
ASUMZ 1179	White R., Jackson Co., AR	White	59.4	58.9	42.5	0.992	0.715	0.722	5.715	4.852	4.873
ASUMZ 1180	White R., Jackson Co., AR	White	71.8	66.7	47.2	0.929	0.657	0.708	5.531	4.651	4.826
ASUMZ 1181	White R., Jackson Co., AR	White	75.0	68.7	49.1	0.916	0.655	0.715	5.492	4.641	4.850
ASUMZ 1182	White R., Jackson Co., AR	White	81.1	79.7	53.2	0.983	0.656	0.668	5.689	4.646	4.686
ASUMZ 1183	White R., Jackson Co., AR	White	87.8	71.8	54.0	0.818	0.615	0.752	5.188	4.498	4.975
ASUMZ 1184	White R., Jackson Co., AR	White	89.4	82.2	53.8	0.919	0.602	0.655	5.502	4.449	4.640
ASUMZ 1185	White R., Jackson Co., AR	White	83.3	82.5	56.1	0.990	0.673	0.680	5.711	4.707	4.730
ASUMZ 1186	White R., Jackson Co., AR	White	96.7	88.2	59.5	0.912	0.615	0.675	5.480	4.499	4.711
ASUMZ 1641	Black R., Clay Co., AR	White	47.2	47.1	30.4	0.998	0.644	0.645	5.733	4.603	4.608
ASUMZ 1642	Black R., Clay Co., AR	White	57.4	54.8	35.0	0.955	0.610	0.639	5.607	4.479	4.584
ASUMZ 1644	Black R., Clay Co., AR	White	75.9	73.7	46.9	0.971	0.618	0.636	5.655	4.509	4.575
ASUMZ 1645	Black R., Clay Co., AR	White	76.4	73.8	52.0	0.966	0.681	0.705	5.640	4.732	4.815
ASUMZ 1646	Black R., Clay Co., AR	White	84.6	81.2	56.6	0.960	0.669	0.697	5.622	4.692	4.789
		Average =	75.0	68.0	48.0	0.911	0.640	0.708	5.470	4.588	4.822
Obovaria unicolor											
MMNS 7415	Sucarnoochee R., Kemper Co., MS	Mobile									
MMNS 7600	East Fork of the Tombigbee R., Itawamba Co., MS	Mobile	64.3	53.5	42.9	0.832	0.667	0.802	5.234	4.685	5.138
ASUMZ 4890	Sipsey R., Pickens/Greene Co., AL	Mobile	30.0	25.5	20.0	0.850	0.667	0.784	5.290	4.683	5.081
ASUMZ 4891	Sipsey R., Pickens/Greene Co., AL	Mobile	32.0	28.3	22.2	0.884	0.694	0.784	5.396	4.778	5.081
ASUMZ 4892	Sipsey R., Pickens/Greene Co., AL	Mobile	26.4	23.8	16.8	0.902	0.636	0.706	5.448	4.575	4.819
ASUMZ 4893	Sipsey R., Pickens/Greene Co., AL	Mobile	45.8	39.7	32.2	0.867	0.703	0.811	5.342	4.810	5.167
ASUMZ 4894	Sipsey R., Pickens/Greene Co., AL	Mobile	44.0	35.7	26.8	0.811	0.609	0.751	5.168	4.476	4.971

(table continues)

Table 3.1. *(continued)*

Museum #	Locality	River Drainage	Measurement (mm) Length	Height	Width	Ratio H/L	W/L	W/H	Transformed ratio H/L	W/L	W/H
ASUMZ 4895	Sipsey R., Pickens/Greene Co., AL	Mobile	50.0	39.5	30.7	0.790	0.614	0.777	5.099	4.494	5.058
ASUMZ 4896	Sipsey R., Pickens/Greene Co., AL	Mobile	41.0	34.9	26.3	0.851	0.641	0.754	5.294	4.594	4.980
ASUMZ 4897	Sipsey R., Pickens/Greene Co., AL	Mobile	36.7	32.1	27.2	0.875	0.741	0.847	5.366	4.939	5.282
ASUMZ 4898	Sipsey R., Pickens/Greene Co., AL	Mobile	22.9	20.7	15.4	0.904	0.672	0.744	5.456	4.704	4.948
		Average =	39.3	33.4	26.1	0.857	0.665	0.776	5.309	4.674	5.052

Table 3.2. Summary statistics for the length, height, and width pairwise ratios of each measurement (n=284). P-values were based on Kolmogorov-Smirnov test and significant at the level of 0.05.

	Height/Length	Width/Length	Width/Height
Mean	0.828	0.641	0.775
Median	0.824	0.664	0.765
Mode	0.768	0.676	0.715
SD	0.082	0.084	0.086
SE	0.005	0.005	0.005
p-value	<0.01	<0.01	<0.01

Table 3.3. Pairwise Goodall's F (Procrustes) analyses for geometric morphometrics of *O. jacksoniana, V. arkansasensis, O. subrotunda, O. olivaria,* and *O. unicolor*. D = minimized partial Procrustes distance between means.

	Villosa arkansasensis (n=51)	*Obovaria subrotunda* (n=29)	*Obovaria olivaria* (n=23)	*Obovaria unicolor* (n=11)
Obovaria jacksoniana (n=189)	F=78.53, P=0; df=44, 10472; D=0.0722	F=138.78, P=0; df=44, 9504; D=0.1211	F=18.69, P=0; df=44, 9240; D=0.0493	F=16.90, P=0; df=44, 8712; D=0.0659
Villosa arkansasensis (n=51)		F=171.99, P=0; df=44, 3432; D=0.1511	F=68.26, P=0; df=44, 3168; D=0.1036	F=33.67, P=0; df=44, 2640; D=0.0953
Obovaria subrotunda (n=29)			F=42.44, P=0; df=44, 2200; D=0.0884	F=13.21, P=0; df=44, 1672; D=0.0609
Obovaria olivaria (n=23)				F=8.48, P=0; df=44, 1408; D=0.0512

Table 3.4. Percent of variance explained for each PC axis for each morphometric analysis by overall species (*O. jacksoniana, O. olivaria, O. subrotunda, O. unicolor, V. arkansasensis*) and between *O. jacksoniana* and *V. arkansasensis*.

		Overall species	Between species
Traditional Morphometrics			
	PC1	66.8%	72.6%
	PC2	33.1%	27.3%
Geometric Morphometrics			
	PC1	52.6%	46.4%
	PC2	25.0%	24.0%
	PC3	9.7%	12.7%
	PC4	3.9%	5.6%
	PC5	2.0%	2.8%
	PC6	-	1.2%