

Final Report

Intraspecific phylogenetic relationships in the freshwater bivalve genus *Alasmidonta* (Bivalvia: Unionidae).

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16. Abstract The genus <i>Alasmidonta</i> currently contains 12 species with 3 species presumed extinct. Six species of <i>Alasmidonta</i> occur in North Carolina, including the presumed extinct <i>Alasmidonta robusta</i> . Tissue samples from all taxa of living <i>Alasmidonta</i> species were included in a test of the monophyly of the genus and the relationships of the species using mitochondrial DNA sequences from cytochrome oxidase c subunit 1 (COI) and NADH dehydrogenase subunit (ND1). <i>Alasmidonta</i> (<i>Prolasmidonta</i>) <i>heterodon</i> and <i>Alasmidonta</i> (<i>Pressodonta</i>) <i>viridis</i> are significantly different from the rest of <i>Alasmidonta</i> and the two subgenera are elevated to generic level. <i>Alasmidonta varicosa</i> is split into two separate species as is <i>A. viridis</i> . <i>Alasmidonta raveneliana</i> is represented by two separate conservation units corresponding to the French Broad and Little Tennessee River drainages. Further work is needed to understand the variation in <i>A. marginata</i> between the upper Mississippi River basin and the Ohio River drainage populations.			
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Executive Summary:

The genus *Alasmidonta* currently contains 12 species with 3 species presumed extinct. Six species of *Alasmidonta* occur in North Carolina, including the presumed extinct *Alasmidonta robusta*. Tissue samples from all taxa of living *Alasmidonta* species were included in a test of the monophyly of the genus and the relationships of the species using mitochondrial DNA sequences from cytochrome oxidase c subunit 1 (COI) and NADH dehydrogenase subunit (ND1). *Alasmidonta* (*Prolasmidonta*) *heterodon* and *Alasmidonta* (*Pressodonta*) *viridis* are significantly different from the rest of *Alasmidonta* and the two subgenera are elevated to generic level. *Alasmidonta varicosa* is split into two separate species as is *A. viridis*. *Alasmidonta raveneliana* is represented by two separate conservation units corresponding to the French Broad and Little Tennessee River drainages. Further work is needed to understand the variation in *A. marginata* between the upper Mississippi River basin and the Ohio River drainage populations.

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Introduction

The 12 species and their synonyms currently included in the genus *Alasmidonta* Say, 1818 were originally described in the genera *Unio* and *Margaritana* and those described more recently in *Alasmidonta* (Simpson 1914; Clarke, 1981; Turgeon et al., 1998). Early malacologists thought the shell morphology of these taxa to be intermediate between what was referred to as the genus *Unio* (with well developed teeth on the hinge plate) and *Anodonta* (without any teeth on the hinge plate). Simpson (1914) recognized the genus as containing 4 subgenera and 12 species. He included *Unio collina* Conrad, 1837 in *Alasmidonta* which has subsequently been placed in *Pleurobema*. Frierson (1927) divided the genus among five subgenera and included seven species. Haas (1969) in his monograph of the order Unioniformes divided the genus *Alasmidonta* into five subgenera and recognized six species. The most recent monographic treatment of the genus was prepared by Clarke (1981) where he recognized 4 subgenera, including 1 new subgenus and 11 species (Table 1). Six species of freshwater mussels in the genus *Alasmidonta* are known to have inhabited North Carolina creeks and rivers (Bogan, 2002). One species, *Alasmidonta robusta* Clarke, 1981, was considered extinct until North Carolina Department of Transportation (NCDOT) biologists recently identified a possible specimen in Poison Fork, Montgomery County (J. Alderman, personal communication). Another species, *A. varicosa*, also inhabits North Carolina's rivers draining to the Atlantic Ocean. Determining the taxonomic validity of these two species in North Carolina is essential to efforts to assess the need for federal listing and expanded conservation efforts of *A. robusta*. Similarly, taxonomic confusion surrounds two morphologically similar species: *A. undulata* and *A. triangulata*. Historically, specimens assigned to *A. undulata* were identified as *A. triangulata*.

Biologists often find morphologically similar "shell forms" of recognized species that may actually be distinctly different endemic species. Occasionally, they may rediscover a species thought extinct. Therefore, a taxonomy based solely on morphological characteristics may not reveal phylogenetic relationships among these constantly evolving groups. In contrast, molecular data provide a useful heritable genetic basis with the number of characteristics limited only by genome size for resolving taxonomic issues (Hillis et al., 1996) and may more accurately reflect true phylogenetic relationships (Avice, 1994). Several high-level phylogenetic studies based on genetic data have been conducted on the Unionidae that have included only a few representatives of *Alasmidonta* (Davis & Fuller, 1981; Graf and O'Foighil, 2000; Lydeard et al., 1996, 2000;); however, no study has included all extant members of the genus and no intraspecific phylogeny for the component species has been proposed.

This study focused on identifying valid species in the genus *Alasmidonta* and characterizing phylogenetic relationships of *Alasmidonta* within NC using mitochondrial DNA sequences from cytochrome oxidase c subunit 1 (COI) and NADH dehydrogenase subunit 1 (ND1). By combining sequences from both the COI and ND1 genes, we present a phylogenetic hypothesis of relationships within the species of the *Alasmidonta*. We test the monophyly of *Alasmidonta* by including representatives of 10 other anodontine genera, and representative species of *Amblema*, *Quadrula* and *Margaritifera* as outgroups.

Materials and methods

Samples and DNA extraction

Eight species of *Alasmidonta* and representatives of other Anodontine genera were included in this analysis (Tables 2, 4.). Mantle tissue was collected from each specimen and total cellular DNA extracted using the DNeasy Tissue Kit (Qiagen) following the manufacturer's recommendation. Integrity of the DNA was assessed visually on 1-2% agarose gels. PCR primers (Folmer et al., 1994; Serb et al., 2003) and conditions are shown in Table 3. Sequencing of DNA was performed using the same primers used for PCR following the recommended protocol with the ABI BigDye v. 3.1 sequencing kit (Applied Biosystems). Sequences were purified for sequencing using the Qiagen DyeEx Spin Kit (Qiagen) and visualized using either an ABI 377 or ABI 3130 XL automated sequencer (Applied Biosystems). Outgroup sequences were obtained from GenBank (National Center for Biotechnology Information, National Institutes of Health; <http://www.ncbi.nlm.nih.gov>) or generated *de novo*.

Sequence data was compiled using Sequencher™4.1. Alignments were compiled and manipulated using Clustal_X (Thompson et al., 1997) and assembled into a PAUP*4 matrix for phylogenetic analysis. Collapse v. 1.2 was used to define unique haplotypes. Phylogenetic analysis was conducted using PAUP*4.0 b10 (Swofford, 2002) by combining ND1 and COI data into a single continuous data matrix.

Distribution of *Alasmidonta* species:

Eight species of *Alasmidonta* were examined in these analyses and represented three of the four subgenera recognized by Clarke (1981) (Table 1). *Alasmidonta* (*Alasmidens*) *mccordi* Ahearn, 1964, *Alasmidonta* (*Alasmidonta*) *wrightiana* (Walker, 1901) and *Alasmidonta* (*Decurambis*) *robusta* Clarke, 1981 were not included, as no known recent live specimens have been collected and are presumed extinct. Another extinct, undescribed *Alasmidonta* species is known from the Etowah River in Georgia (Williams et al., in press).

***Alasmidonta arcula*, Altamaha Arcmussel:**

A species endemic to the Altamaha River basin, Georgia, including the Ocmulgee River, Little Ocmulgee River and Ohoopsee River drainages (Clarke, 1981). However, more recently this species was identified in the Ogeechee River of North Georgia (Williams et al., 2004; Plate 1).

***Alasmidonta atropurpurea*, Cumberland Elktoe:**

Restricted to the upper middle portion of the Cumberland River drainage including: North and South Fork Cumberland River Tennessee; Lynn Camp, Marsh and Rock creeks, Kentucky and from the Clear Fork River, Whiteoak Creek and the Collins River in Tennessee (Clarke, 1981; Call and Parmalee, 1981; Plate 1).

***Alasmidonta heterodon*, Dwarf Wedgemussel:**

The distribution of the Dwarf Wedgemussel is listed as occurring from the Neuse River basin, North Carolina, in the south, north to the Petitcodiac River Basin, New Brunswick, Canada (Clarke, 1981). Distribution of *A. heterodon* is sporadic in the river basins between these two river basins. This species occurs in the Neuse and Tar-Pamlico River basins in North Carolina (Clarke, 1981; Bogan, 2002; Plate 1).

Alasmidonta marginata, Elktoe:

Alasmidonta marginata is reported from: eastern portions of the Great Lakes drainage from the Ottawa River to Lake Michigan; the Atlantic Coast section of New York and Pennsylvania in the Susquehanna and Hudson River drainages; throughout the Mississippi Basin from Wisconsin and Minnesota south to Arkansas, and from headwaters of the Ohio River drainage in New York west to eastern Kansas. It occurs in the Cumberland River drainage downstream of Cumberland Falls, Kentucky and Tennessee; from throughout the Tennessee River drainage in Alabama, Tennessee and Virginia (Williams et al., in press; Plate 1). This species has not yet been found in western North Carolina (Bogan, 2002).

Alasmidonta raveneliana, Appalachian Elktoe:

The Appalachian Elktoe is restricted to the French Broad and Little Tennessee River drainages, tributaries of the Tennessee River in western North Carolina. This species is found in the Little Tennessee River drainage and the Nolichucky River [French Broad River drainage] and the French Broad River. The species is presently comprised of six more or less disjunct populations: upper Nolichucky river drainage (includes Cane, Toe, North Toe, and South Toe rivers), upper French Broad River drainage (includes Little and Mills rivers), upper Pigeon and West Fork Pigeon rivers, Little Tennessee River, Tuckasegee River, and Cheoah River. (Clarke, 1981, T. Savidge, Pers. Comm. March 2002; Plate 1).

Alasmidonta robusta, Carolina Elktoe:

This species was described from Long Creek, a tributary of the Catawba River, Mecklenburg County, North Carolina and is listed as extinct (Clarke, 1981; Turgeon et al., 1998; Plate 1).

Alasmidonta triangulata, Southern Elktoe

Alasmidonta triangulata is an Apalachicola Basin endemic, known from the Apalachicola, Chattahoochee, Chipola and Flint River drainages in Alabama, Georgia and Florida (Williams et al., in press; Plate 2).

Alasmidonta undulata, Triangle Floater:

The range of *Alasmidonta undulata* extends from the Bosquet River of the lower St. Lawrence River Basin south to the Ogeechee River Basin, Georgia (Clarke, 1981). This species is found in the Chowan, Tar-Pamlico, Roanoke, Neuse, Cape Fear, and Pee Dee River basins in North Carolina (Clarke, 1981; Bogan, 2002; Plate 2)

Alasmidonta varicosa, Brook Floater:

The broadly distributed *Alasmidonta varicosa* is found from the Lower St. Lawrence River Basin south in streams and rivers draining into the Atlantic Ocean to the Savannah River Basin in South Carolina and Georgia (Clarke, 1981). It is found in Roanoke, Neuse, Cape Fear, Yadkin-Pee Dee and Catawba River basins in North Carolina (Clarke, 1981; Bogan, 2002; Plate 2).

Alasmidonta viridis, Slippershell Mussel:

Alasmidonta viridis occurs in the Great Lakes Basin from the Ottawa River west to Lake Michigan; in the Mississippi Basin it occurs from southern Wisconsin south to Arkansas and from the Ohio River drainage in Ohio west to Missouri and Arkansas; is found throughout the Cumberland River drainage, Kentucky and Tennessee and is widespread in the Tennessee River

drainage in Alabama, North Carolina, Tennessee and Virginia (Williams et al., in press) This species is known from the Little Tennessee, Mills and French Broad Rivers in North Carolina (Clarke, 1981; Bogan 2002; Plate 2).

Results

Combined CO1 and ND1

Figure 1 is the result of analysis of the combined data sets of CO1 and ND1 mitochondrial DNA gene sequences. Relationships of genera, subgenera and species will be discussed by species and the genus *Alasmidonta* will be discussed separately.

Alasmidonta arcula:

This species has been considered an Altamaha River basin endemic in Georgia (e.g. Johnson, 1970). The samples of this species form a clade sister to *A. undulata* (Figure 1). It is interesting that specimens identified as *A. triangulata* from the Ogeechee River are included in the clade with *A. arcula*. The specimens identified as *A. triangulata* from the Ogeechee River represent a northern population of *A. arcula*. *Alasmidonta arcula* based on this information is no longer an Altamaha River endemic. Historically some specimens from the Neuse River basin have been identified as *A. triangulata* but these analyses have placed specimens from the Neuse River with specimens of *A. undulata*.

Alasmidonta atropurpurea:

A single tissue sample of *A. atropurpurea* was available from the University of Alabama collections. In our analyses this specimen came out sister to *Strophitus undulatus* (Figure 1). This appears to be either a sequencing error or more likely, a mis-identification of the specimen. The shells of *A. atropurpurea* have been historically lumped under *A. marginata* and only separated by Clarke (1981). The relationships of this species must await more specimens.

Alasmidonta heterodon change to *Prolasmidonta heterodon*:

Microsatellite primers were developed for *Alasmidonta heterodon* (Shaw et al., 2006). However, when these primers were applied to other taxa in *Alasmidonta*, they did not work which raised questions (T. King, Pers. Com. 2007). Based on analysis of the gene sequences, *Alasmidonta heterodon* is placed in a clade separate from the type species of *Alasmidonta*, *Alasmidonta undulata* (Figure 1). This placement would argue for the elevation of the subgenus *Prolasmidonta* Ortmann, 1914 with the type species *Alasmidonta heterodon*. Clarke (1981) had placed *Prolasmidonta* as a junior synonym of the subgenus *Pressodonta* Simpson, 1900 with the type species *Unio calceolus* Lea [= *Alasmidonta viridis*] by original designation. Since *Alasmidonta heterodon* and *A. viridis* are found in separate clades (Figure 1), *Pressodonta* can not be used for *A. heterodon*. The elevation of the subgenus *Prolasmidonta* for *Alasmidonta heterodon* was proposed and used by Fuller (1977). He argued that *A. heterodon* should be in a separate genus based on its discontinuous range, presence of lateral teeth and sexual dimorphism of the shell. Our genetic data support the re-elevation of *Prolasmidonta* to generic status.

Alasmidonta marginata:

This species is the type species of the subgenus (*Decurambis*). Two synonyms, *A. atropurpurea* and *A. raveneliana*, were split out of the synonymy of *Alasmidonta marginata* by Clarke (1981). Based on results presented here *A. raveneliana* is a valid separate species. The relationship of *A. atropurpurea* is still unknown as the single sample available appears to have been from a mis-identified specimen. There does appear to be some structure or population divergence between samples from the upper Mississippi River basin (in the lower clade) vs. those from the Ohio

River drainage in the upper clade. This separation will require a larger series of samples from throughout *A. marginata*'s range. The status of *Alasmidonta marginata susquehannae* Ortmann, 1919 from the Susquehanna River in Pennsylvania is unknown at this time.

***Alasmidonta raveneliana*:**

This species was resurrected from the synonymy of *A. marginata* by Clarke (1981) based on shell characters such as the lack of sculpture on the posterior slope. The type locality for this species is the Swannanoa River, Buncombe County, North Carolina in the French Broad River drainage (Clarke, 1981). The samples included in our analyses are from the North Toe River a tributary of the Nolichucky River, in the French Broad River drainage and the Little Tennessee River drainage in North Carolina. The samples of *A. raveneliana* analyzed here divide into two clades along river drainage lines, the three samples from the North Toe River, and the larger series of samples from the Little Tennessee River drainage in North Carolina. These two populations are distinct and represent two separate and distinct conservation units. Further work is required to clarify the level of divergence of these two populations. Little Tennessee River and French Broad River drainage populations should not be mixed to avoid altering the current population distinctions. *Alasmidonta raveneliana* is sister to *Alasmidonta marginata*, type species of the subgenus (*Decurambis*).

***Alasmidonta robusta*:**

This species was described by Clarke (1981) based on a single lot of five dry shell specimens from Long Creek (near Charlotte), Mecklenburg County, North Carolina. This was in the nineteenth century collection of Charles M. Wheatley now housed at the Academy of Natural Sciences of Philadelphia. The species was listed as extinct when described. *Alasmidonta robusta* was not reported in surveys in the Catawba and Pee Dee river drainages by Keferl and Shelly (1988) and noted as extinct by Adams et al. (1990). It has been subsequently listed as presumed extinct by Williams et al. (1993) and Turgeon et al. (1998). Recently J.M. Alderman (Pers. Comm. 2005) and Tim Savidge (Pers. Comm. 2006) have reported finding shells of *Alasmidonta* in tributaries to the Uwharrie River drainage that are consistent in shell form with *Alasmidonta robusta*. Several attempts to find live specimens with the same shell characteristics as *A. robusta* have not been successful. However, specimens identified as *A. varicosa* have been found in these small streams. Specimens identified as *A. varicosa* from the Uwharrie River drainage form a separate clade from the rest of the *A. varicosa* specimens sequenced (Figure 1). Specimens from the Savannah, Santee, Catawba, Cape Fear River basins all cluster together as *A. varicosa*. This unidentified *Alasmidonta* species may be identified subsequently as *A. robusta*. However, until there are live specimens consistent with the *A. robusta* shell form found either near the original type locality or elsewhere in the Catawba River system, this unidentified species will be listed as *Alasmidonta* sp.

***Alasmidonta triangulata*:**

There has been some confusion surrounding the identification of this species. Some of the large inflated specimens found in North Carolina have been identified as *A. triangulata*. Lea (1858) reported specimens of this species from the Chattahoochee River and Potato Creek, both in Georgia and Sawney's Creek, South Carolina. In the monographic treatment of *Alasmidonta*, Clarke (1981) examined the variation of shell characters used to distinguish species in this genus. He considered *Margaritana triangulata* to represent the southern end of the clinal

variation of *Alasmidonta undulata* and included *M. triangulata* as a junior synonym of *A. undulata*. Turgeon et al. (1998) recognized *A. triangulata* as a valid species. *Alasmidonta triangulata* was recognized as a valid species by Brim Box and Williams (2000). They noted that Clench and Turner (1956) had designated a lectotype and restricted the type locality to the [Chattahoochee River] Columbus [Muscogee County], Georgia. Clarke (1981) invalidly again designated the same specimen as a lectotype. Williams et al. (2004) reported four historic museum records of this species from Ogeechee River, Georgia and reported collecting live specimens from a single site. Live specimens from the Ogeechee River were identified on shell shape as *A. triangulata*. These specimens would test the hypothesis of Clarke (1981) that the shell forms found in North and South Carolina identified as *A. triangulata* were actually *A. undulata*. Analyses presented in Figure 1 do not place *A. triangulata* from the Ogeechee River with *A. undulata* but with *A. arcuata*. It is clear that the shell form identified in the Ogeechee River basin as *A. triangulata* is not a separate species and represents a northern population of *A. arcuata*, a presumed Altamaha River basin endemic. The shell shapes identified from North Carolina that have been identified as *A. triangulata* all are placed in the clade with *Alasmidonta undulata*.

***Alasmidonta undulata*:**

Alasmidonta undulata is the sister species to *A. arcuata* (Figure 1) and this placement confirms Clarke's (1981) placement of *A. arcuata* together with *A. undulata*, type species of the genus and nominate subgenus. One specimen of *A. varicosa* is placed in the clade with *Alasmidonta undulata* and represents a mis-identification. Specimens of *A. undulata* used in these analyses are from the Neuse and Tar River drainages in North Carolina and the James River basin, Virginia. At this time specimens from the Northeastern United States were not available.

***Alasmidonta varicosa*:**

Specimens from the Savannah, upper Catawba, Cape Fear River and Potomac River basins all cluster together as *A. varicosa*. Shells of these specimens are all consistent with shells of this species from the northern end of the species range. Specimens identified as *A. varicosa* from the Uwharrie River drainage form a separate clade from the rest of the sequenced specimens of *A. varicosa* (Figure 1). This unidentified *Alasmidonta* species may be identified subsequently as *A. robusta*. However, until there are live specimens consistent with the *A. robusta* shell form found either near the original type locality or elsewhere in the Catawba River system, this unidentified species will be listed as *Alasmidonta* sp.

***Alasmidonta viridis* change to *Pressodonta viridis*, New Combination.**

Clarke (1981) recognized the *Alasmidonta* subgenus *Pressodonta* Simpson, 1900 with the type species *Unio calceolus* Lea [= *Alasmidonta viridis*] by original designation. He included both *A. viridis* and *A. heterodon* in this subgenus. However, these analyses have clearly separated these two species and *A. heterodon* has been placed in *Prolasmidonta* leaving *A. viridis* in *Pressodonta* (Figure 1). Based on the distance and separation shown in Figure 1, this subgenus should be elevated to generic level, with the new combination *Pressodonta viridis*. There are two separate clades of *A. viridis* present in Figure 1, the larger series of samples including specimens from the Big South Fork Cumberland River, Little Tennessee River and the Mills River. The other two samples form a distinct clade separate from *A. viridis*. These two samples are from Missouri and may represent an undescribed species.

Alasmidonta

Our analyses do not support the current concept that the genus *Alasmidonta* is a monophyletic unit with four subgenera (Figure 1). Species assigned historically to the genus *Alasmidonta* were split by Clarke (1981) into four subgenera. Based on these analyses, two of these subgenera [(*Alasmidonta*) and (*Decurambis*)] can be recognized with changes and two subgenera are elevated to generic rank: *Pressodonta* and *Prolasmidonta*. Explanations for their elevation were discussed above, under *A. viridis* and *A. heterodon* respectively. *Alasmidonta* (*Decurambis*) included five species in Clarke's (1981) classification while here we include two species (Table 5). The placement of *A. robusta* is still problematic but most likely will be aligned with *A. varicosa*. *Alasmidonta varicosa* is placed in the clade of *Alasmidonta* (*Alasmidonta*) along with *A. undulata* and *A. arcua*, not in *A. (Decurambis)* *fide* Clarke (1981). The placement of *A. atropurpurea* is uncertain at this time awaiting more specimens. The correct placement of *Alasmidonta wrightiana* and *Alasmidonta mccordi* are unknown and due to their being presumed extinct, are likely to remain unclear until a large set of morphological characters can be applied to the shells of this genus. Using overall shell shape it may be suggested that *A. wrightiana*, *A. mccordi* and *A. triangulata* would be placed in *Alasmidonta* (*Alasmidonta*).

The two subgenera or clades recognized here in *Alasmidonta* split along major physiographic boundaries. *Alasmidonta* (*Alasmidonta*) species are restricted to the Atlantic slope and eastern Gulf Coast drainages while *Alasmidonta* (*Decurambis*) species are restricted to the Interior Basin west of the Appalachian Mountains.

Discussion

Alasmidonta as monographed by Clarke (1981) is not a monophyletic group based on these analyses (Figure 1). Recognition of *Pressodonta viridis* and *Prolasmidonta heterodon* as separate clades within the Anodontinae explains the lack of results experienced by King (T.L. King, Pers. Comm. 2007; Shaw et al., 2006) when applying his microsatellites techniques for *Prolasmidonta heterodon* to other taxa in *Alasmidonta*. *Prolasmidonta* and *Alasmidonta* are distantly related (Figure 1). The analyses presented here have a monophyletic *Alasmidonta* clade minus *Pressodonta* and *Prolasmidonta* (Figure 1). *Alasmidonta* is composed of two main clades, recognized as two separate subgenera (*Decurambis*) and (*Alasmidonta*). It is interesting to note that the species in the (*Alasmidonta*) clade are all Atlantic Coast taxa while the (*Decurambis*) species are all Interior Basin taxa. These two clades have diverged at some time in the past. The extinct taxa, *A. wrightiana* from the Ochlockonee River basin and *A. mccordi* from the Mobile basin represent the Gulf Coast drainages. Based solely on shell shape, these two species would most likely be placed in the subgenus (*Alasmidonta*). Based on shell shape *Alasmidonta triangulata* from the Apalachicola River basin, although not included in these analyses, should fall in the subgenus (*Alasmidonta*). Frierson (1927) described the Alasmidontinae as a subfamily separate from the Anodontinae. Starobogatov (1970) recognized Alasmidontinae as a subfamily, but Haas (1969) did not. Clarke (1981, 1985) in his monographic treatment of the group, recognized the tribe Alasmidontini within the subfamily Anodontinae. The concept of a clade containing the genera *Alasmidonta*, *Lasmigona*, *Arcidens*, *Arkansia*, *Pegias*, *Strophitus* and *Simpsonaias* as defined by Frierson (1927) and modified by Clarke (1981, 1985) is not supported by these analyses (Figure 1). The Anodontinae are recognized as a major clade in the Unionidae but the tribe Alasmidontini as used by Clarke (1981, 1985) is shown here to be polyphyletic.

Implementation and Technology Transfer Plan:

The object of this project was to clarify problems of species identifications related to species of *Alasmidonta* reported from North Carolina. Five of the six species of *Alasmidonta* from North Carolina were examined. No live specimens of the presumed extinct, *A. robusta* were identified. The correct identification of *A. undulata* is confirmed and the previous identification of specimens as *A. triangulata* from North Carolina is dismissed. The specimens initially identified as *A. varicosa* from the Uwharrie River drainage are not *A. varicosa* but represent an undescribed species. *Alasmidonta heterodon* is moved to the genus *Prolasmidonta* and *Alasmidonta viridis* is moved to the genus *Pressodonta*. The implications for NCDOT aquatic biologists are the confirmation of *A. undulata* and *A. varicosa* as good species and the discovery of an undescribed species that looks like *A. varicosa* in the Uwharrie River drainage. This new species should be treated as a special conservation priority that is probably endangered. *Alasmidonta raveneliana* is a valid species separate from *A. marginata*. *Alasmidonta raveneliana* is comprised of two separate conservation units.

The results of this work are important to NCDOT and their aquatic biologists in planning conservation and mitigation plans. Several areas are identified where further field and laboratory work are required for a more complete understanding of the distribution and relationships of *Alasmidonta* species occurring in North Carolina.

The taxonomic implications presented in this report represent the results of our current research. The elevation of *Prolasmidonta* and *Pressodonta* to generic status is recommended. We will add additional specimens of several taxa including *A. heterodon* and revise our analyses. The elevation of these two genera will be submitted to a peer-reviewed journal for publication. The results will not become formal until these results are published in a peer-reviewed journal.

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Table 1. Comparison of the classifications of the genus *Alasmidonta* presented by Haas (1969) and Clarke (1981).

Haas (1969) classification			Clarke (1981) classification		
Genus	Subgenus	Species	Genus	Subgenus	Species
<i>Alasmidonta</i>	(<i>Alasmidonta</i>)	<i>undulata</i> (Say, 1817)	<i>Alasmidonta</i>	(<i>Alasmidonta</i>)	<i>undulata</i> (Say, 1817)
		<i>triangulata</i> (Lea, 1858)			<i>arcula</i> (Lea, 1838)
	(<i>Prolasmidonta</i>)	<i>heterodon</i> (Lea, 1830)			<i>wrightiana</i> (Walker, 1901)
	(<i>Bullella</i>)	<i>arcula</i> (Lea, 1836)		(<i>Alasmidens</i>)	<i>mccordi</i> Athern, 1964
	(<i>Pressodonta</i>)	<i>calceolus</i> (Lea, 1830) ¹		(<i>Decurambis</i>)	<i>marginata</i> Say, 1818
	(<i>Decurambis</i>)	<i>marginata</i> Say, 1818			<i>atropurpurea</i> (Rafinesque, 1819)
					<i>raveneliana</i> (Lea, 1834)
					<i>robusta</i> Clarke, 1981
					<i>varicosa</i> (Lamarck, 1819)
				(<i>Pressodonta</i>)	<i>viridis</i> (Rafinesque, 1820)
					<i>heterodon</i> (Lea, 1830)

¹ *Alasmidonta* (*Pressodonta*) *calceolus* (Lea, 1830) changed to *Alasmidonta* (*Pressodonta*) *viridis* (Rafinesque, 1820) based on priority of names (Clarke, 1981).

Table 2. Number of sequenced individuals used in these analyses.

Museum catalog number	Genus	Species	GenBank Number	COI	ND!
NCSM 30080	<i>Alasmidonta</i>	<i>arcula</i>		9	8
NCSM 28236	<i>Alasmidonta</i>	<i>marginata</i>		3	0
NCSM 28547	<i>Alasmidonta</i>	<i>marginata</i>		2	2
NCSM 29158	<i>Alasmidonta</i>	<i>marginata</i>		0	2
NCSM 29517	<i>Alasmidonta</i>	<i>marginata</i>		0	1
NCSM 29922	<i>Alasmidonta</i>	<i>marginata</i>		1	1
NCSM 27215	<i>Alasmidonta</i>	<i>raveneliana</i>		0	2
NCSM 27755	<i>Alasmidonta</i>	<i>raveneliana</i>		1	1
NCSM 29606	<i>Alasmidonta</i>	<i>raveneliana</i>		3	5
NCSM 29746	<i>Alasmidonta</i>	<i>raveneliana</i>		3	7
NCSM 30861	<i>Alasmidonta</i>	<i>raveneliana</i>		2	0
NCSM 35105	<i>Alasmidonta</i>	<i>raveneliana</i>		3	0
NCSM 40284	<i>Alasmidonta</i>	<i>raveneliana</i>		1	1
NCSM 40285	<i>Alasmidonta</i>	<i>raveneliana</i>		1	0
NCSM 29995	<i>Alasmidonta</i>	<i>triangulata</i>		3	3
NCSM 28312	<i>Alasmidonta</i>	<i>undulata</i>		1	1
NCSM 28550	<i>Alasmidonta</i>	<i>undulata</i>		1	1
NCSM 30035	<i>Alasmidonta</i>	<i>undulata</i>		0	1
NCSM 30272	<i>Alasmidonta</i>	<i>undulata</i>		1	1
NCSM 45082	<i>Alasmidonta</i>	<i>undulata</i>		2	0
NCSM 27445	<i>Alasmidonta</i>	<i>varicosa</i>		0	2
NCSM 28110	<i>Alasmidonta</i>	<i>varicosa</i>		1	1
NCSM 29140	<i>Alasmidonta</i>	<i>varicosa</i>		5	5
NCSM 29409	<i>Alasmidonta</i>	<i>varicosa</i>		0	5
NCSM 29435	<i>Alasmidonta</i>	<i>varicosa</i>		1	1
NCSM 29520	<i>Alasmidonta</i>	<i>varicosa</i>		1	1
NCSM 29536	<i>Alasmidonta</i>	<i>varicosa</i>		1	1
NCSM 29537	<i>Alasmidonta</i>	<i>varicosa</i>		1	1
NCSM 29538	<i>Alasmidonta</i>	<i>varicosa</i>		1	1
NCSM 30074	<i>Alasmidonta</i>	<i>varicosa</i>		1	1
NCSM 28947	<i>Alasmidonta</i>	<i>viridis</i>		3	0
NCSM 29179	<i>Alasmidonta</i>	<i>viridis</i>		0	1
NCSM 29215	<i>Alasmidonta</i>	<i>viridis</i>		2	3
NCSM 29216	<i>Alasmidonta</i>	<i>viridis</i>		1	1
NCSM 29406	<i>Alasmidonta</i>	<i>viridis</i>		12	5
NCSM 29485	<i>Alasmidonta</i>	<i>viridis</i>		3	9
NCSM 29661	<i>Alasmidonta</i>	<i>viridis</i>		2	3
NCSM 27752	<i>Amblema</i>	<i>plicata</i>		2	0
NCSM 27986	<i>Anodonta</i>	<i>cygnea</i>		2	0
NCSM 30219	<i>Anodonta</i>	<i>implicata</i>		3	0
	<i>Anodonta</i>	<i>oregonensis</i>	AY655087	0	1
NCSM 30224	<i>Anodontoides</i>	<i>radiatus</i>		1	1
AUM924	<i>Anodontoides</i>	<i>radiatus</i>		0	1
NCSM 27750	<i>Arcidens</i>	<i>confragosus</i>		1	0
	<i>Cristaria</i>	<i>plicata</i>	DQ156178	0	1
NCSM 27247	<i>Lasmigona</i>	<i>complanata</i>			2
NCSM 27234	<i>Lasmigona</i>	<i>compressa</i>		1	1

Museum catalog number	Genus	Species	GenBank Number	COI	ND!
NCSM 26954	<i>Lasmigona</i>	<i>costata</i>		1	1
NCSM 30857	<i>Lasmigona</i>	<i>decorata</i>		1	1
NCSM 27486	<i>Lasmigona</i>	<i>holstonia</i>		1	1
NCSM 27754	<i>Lasmigona</i>	<i>holstonia</i>		1	1
UAUC 27280	<i>Pegias</i>	<i>fabula</i>		0	1
NCSM 27936	<i>Pseudanodonta</i>	<i>complanata</i>		1	0
NCSM 29797	<i>Pyganodon</i>	<i>grandis</i>		1	0
	<i>Quadrula</i>	<i>quadrula</i>	AY158772	0	1
NCSM 30607	<i>Simpsonaias</i>	<i>ambigua</i>		1	0
NCSM 29439	<i>Strophitus</i>	<i>undulatus</i>		1	0
NCSM 30249	<i>Utterbackia</i>	<i>imbecillis</i>		1	0

AUM – Auburn University, Auburn, Alabama

UAUC – University of Alabama, Tuscaloosa, Alabama

NCSM – North Carolina State Museum of Natural Sciences, Raleigh, North Carolina

Table 3. Primers used in the analysis

	Name	Sequence	Amplification
	Leu-urF	5'-TGGCAGAAAGTGCATCAGATTAAGC-3'	30cycles: 94°C, 1min; 52°C, 1min; 72°C, 2min
ND1	LoGlyR	5'-CCTGCTTGGAAGGCAAGTGTACT-3'	
COI	COI-H	5'-TAAACTTCAGGGTGACCAAAAAATCA-3'	35cycles: 94°C, 30s; 54°C, 30s; 72°C, 1min30s
	COI-L	5'-GGTCAACAAATCATAAAGATATTGG-3'	

Table 4. List of locality data for all specimens used in these analyses

catalog number	genus	species	subspecies	Basin	state	county	locality	lat NS start	lat start	long EW start	long start	collector	date	day	year
26954	Lasmigona	costata	n/a	Atchafalaya Basin	Arkansas	Saline	Saline River upstream of Benton, [point estimated at the end of Hutchinson Road, 1.78 air miles NW center Benton], [Benton Quad.]	N	34.581699	W	92.610031	Davidson, Gosse	June	n/k	2002
27215	Alasmidonta	raveneliana	n/a	Mississippi Basin	North Carolina	Mitchell-Yancey	North Toe River at NC 197, [0.64 air miles SSW center of Red Hill], [Bakersville Quad.]	N	36.013172	W	82.229141	TW Savidge	September	9	2002
27234	Lasmigona	compressa	n/a	Mississippi Basin	Pennsylvania	Erie	Le Boeuf Creek, from mouth [0.5 air miles ENE center Indian Head] 500 yards upstream near Indian Head, [Waterford Quad.]	N	41.902302	W	79.985901	AE Bogan, D Locy	September	22	2002
27247	Lasmigona	complanata	n/a	Mississippi Basin	Pennsylvania	Crawford	Conneaut Lake, Conneaut outlet above sewage treatment plant, [point estimated 2.4 air miles SE center town of Conneaut Lake], [Conneaut Lake Quad.]	N	41.5994	W	80.299301	AE Bogan, D Locy	September	20	2002
27445	Alasmidonta	varicosa	n/a	Santee Basin	North Carolina	Caldwell	Johns River, first bridge on SR 1356 (Old Johns River Road) upstream of intersection of Highway 90, [1.09 air miles NW Collettesville], [Collettesville Quad.]	N	35.933857	W	81.690392	JA Fridell, MA Cantrell, TW Savidge, et al.	August	26	1999
27486	Lasmigona	holstonia	etowahensis	Mobile Bay Basin	Georgia	Floyd	Little Cedar Creek at GA 100 [Freezer Locker Road], [0.45 air miles SSW center Cave Spring], [Cedartown West Quad.]	N	34.1021	W	85.341682	PD Johnson	December	n/k	2002
27750	Arcidens	confragosus	n/a	Mississippi Basin	Arkansas	Poinsett	Ditch 10 at AR Hwy 14 crossing, ca. 5.0 road miles E of Harrisburg, [2.96 air miles SSW center McCormick], [McCormick Quad.]	N	35.566666	W	90.616943	JL Harris, W Bailey	November	20	2002
27752	Amblema	plicata	n/a	Atchafalaya Basin	Arkansas	Hempstead-Little River	Little River at gravel island ca. 1.0 river km downstream of Old Allen Ferry boat ramp and ca. 3.0 river km upstream of confluence with Red River, [point estimated 2.58 air miles W center Fulton], [Fulton Quad.]	N	33.615932	W	93.858162	JL Harris, J Seagraves	August	18	2002
27754	Lasmigona	holstonia	n/a	Mississippi Basin	Virginia	Tazewell	Indian Creek at SR 627, about 2 mi NE [town of] Mouth of Laurel, 250m above railroad trestle, [Amonate Quad.]	N	37.148594	W	81.702934	BT Watson, et al.	March	10	2003
27755	Alasmidonta	raveneliana	n/a	Mississippi Basin	North Carolina	Swain	Little Tennessee River, USGS gage station, [0.7 air miles NNW] Needmore, [Wesser Quad.]	N	35.336388	W	83.526665	JA Fridell, TW Savidge, SJ Fraley	March	12	2003
27936	Pseudanodonta	complanata	n/a	Ukraine Black Sea Minor Coastal (misc. creek tribs)	Zhytomyr	n/a	River Zherv, Luzhny?, point estimated directly south of Zherv.	N	50.243732	E	28.662472	A Kornushin	n/k	n/k	2001
27986	Anodonta	cygnea	n/a	Ukraine Black Sea Minor Coastal	Zhytomyr	n/a	Grushkivtsi, pond in the basin of River Grylopyat.	N	0	W	0	A Kornushin	May	n/k	2001
28110	Alasmidonta	varicosa	n/a	Cape Fear Basin	North Carolina	Randolph	Brush Creek at NC 22/42, ca. 3.5 mi. NW of Bennett,	N	35.601624	W	79.582993	AE Bogan, C Eades, ME Raley, et al.	July	17	2003

catalog number	genus	species	subspecies	Basin	state	county	locality	lat NS start	lat start	long EW start	long start	collector	date	day	year
							[Bennett Quad.]								
28236	<i>Alasmidonta</i>	<i>marginata</i>	<i>marginata</i>	Mississippi Basin	Wisconsin	Barron	Red Cedar River under Highway 8 bridge and 50 yd upstream, [3.13 air miles ESE center of Barron], [Barron Quad.]	N	45.393673	W	91.785942	T Balding	August	4	2003
28312	<i>Alasmidonta</i>	<i>undulata</i>	n/a	Pamlico Sound Basin	North Carolina	Warren	Fishing Creek at Hamme's Mill, at end of Hamme Mill Road, off Baltimore Road, [ca. 2.35 air miles] South of Warrenton, [Afton Quad]	N	36.369415	W	78.153603	AE Bogan, R Butler, M Hughes, et al.	September	4	2003
28547	<i>Alasmidonta</i>	<i>marginata</i>	<i>marginata</i>	Mississippi Basin	Tennessee	Polk	Hiwassee River, above Powerhouse, Appalachia cut-off, [1.58 air miles Hiwassee], [McFarland Quad.]	N	35.184299	W	84.437897	SA Ahlstedt	August	20	2003
28550	<i>Alasmidonta</i>	<i>undulata</i>	n/a	Pamlico Sound Basin	North Carolina	Johnston	Swift Creek, just above Lee Road (SR 1562), [10.04 air miles WNW from center of Selma], [Powhatan Quad.]	N	35.552399	W	78.4608	TW Savidge, T Dickinson, K Lynch, et al.	August	22	2003
28947	<i>Alasmidonta</i>	<i>viridis</i>	n/a	Mississippi Basin	North Carolina	Henderson	Mills River at crossing of NC 280/191, [0.48 air miles NW center of Oak Hill Court], [Skyland Quad.]	N	35.3913	W	82.568298	TW Savidge, J Burelson, T Dickinson	May	22	2002
29140	<i>Alasmidonta</i>	<i>varicosa</i>	n/a	Savannah Basin	Georgia-South Carolina	Rabun-Oconee	Chattooga River at crossing of US 76/[SR 2], [6.92 air miles SE center of Clayton], [Rainy Mountain Quad].	N	34.814098	W	83.306503	JM Alderman	November	1	2003
29158	<i>Alasmidonta</i>	<i>marginata</i>	<i>marginata</i>	Mississippi Basin	Tennessee	Polk	Hiwassee River, Appalachia cut-off, [0.89 river] miles above TN Highway 68 bridge, [0.59 air miles NNW Apalachia], [Farner Quad.]	N	35.175701	W	84.327301	SA Ahlstedt	October	29	2003
29179	<i>Alasmidonta</i>	<i>viridis</i>	n/a	Mississippi Basin	Tennessee	Scott	Big South Fork Cumberland River at Rough Shoals Branch, [1.99 air miles NW center of Black Oak], [Barthell SW Quad.]	N	36.507599	W	84.633598	SA Ahlstedt, S Bakolitz	August	22	2000
29215	<i>Alasmidonta</i>	<i>viridis</i>	n/a	Mississippi Basin	North Carolina	Henderson	Mills River at crossing of NC 280/191, [0.48 air miles NW center of Oak Hill Court], [Skyland Quad.]	N	35.3913	W	82.568298	TW Savidge, J Burelson, S Snider	May	22	2002
29216	<i>Alasmidonta</i>	<i>viridis</i>	n/a	Mississippi Basin	North Carolina	Henderson	Mills River approx. 50 yards upstream of crossing of NC 280 and 191 bridge, [0.5 air miles NNW center of Oak Hill Court], [Skyland Quad.]	N	35.391602	W	82.568604	TW Savidge, JA Fridell	February	25	2000
29406	<i>Alasmidonta</i>	<i>viridis</i>	n/a	Mississippi Basin	North Carolina	Henderson	Mills River, Hooper Lane [SR 1353] crossing and upstream, [2.43 air miles SW center Brickton], [Skyland Quad.]	N	35.3871	W	82.545998	JW Mays, SJ Fraley	July	23	2003
29409	<i>Alasmidonta</i>	<i>varicosa</i>	n/a	Santee Basin	North Carolina	Caldwell	Johns River at Old Johns River Road [SR 1356], [1.0 air miles NW center Collettsville], [Collettsville Quad.]	N	35.932999	W	81.690102	SJ Fraley, JW Mays, S Bryan	September	12	2003
29435	<i>Alasmidonta</i>	<i>varicosa</i>	n/a	Pee Dee Basin	North Carolina	Randolph	Little River at SR 1135, Howard Auman Road, 8.04 [air] miles [SSW] of Asheboro, 50 m. below and 200m above the bridge, [Seagrove Quad.]	N	35.573719	W	79.841469	AE Bogan, JM Smith, ME Raley, et al.	January	16	2004

catalog number	genus	species	subspecies	Basin	state	county	locality	lat NS start	lat start	long EW start	long start	collector	date	day	year
29439	<i>Strophitus</i>	<i>undulatus</i>	n/a	Pee Dee Basin	North Carolina	Randolph	Little River at SR 1135, Howard Auman Road, 8.04 [air] miles [SSW] of Asheboro, 50 m. below and 200m above the bridge, [Seagrove Quad.]	N	35.573719	W	79.841469	AE Bogan, JM Smith, ME Raley, et al.	January	16	2004
29485	<i>Alasmidonta</i>	<i>viridis</i>	n/a	Mississippi Basin	North Carolina	Henderson	Mills River at Hooper Lane [SR 1353], [ca. 1.1 air miles ESE center of town of Mills River], [Skyland Quad.]	N	35.3871	W	82.545998	CB Eades, P Hubert, L Humphries, et al.	March	4	2003
29517	<i>Alasmidonta</i>	<i>marginata</i>	<i>marginata</i>	Mississippi Basin	West Virginia	Pocahontas	Greenbrier River, below Thorny Creek, [3.0 air miles NE center Marlinton], Marlinton Quad.	N	38.244446	W	80.046112	JL Clayton	August	7	2003
29520	<i>Alasmidonta</i>	<i>varicosa</i>	n/a	Chesapeake Bay Basin	West Virginia	Mineral	Patterson Creek at junction of CR 28-10 [Plum Run Road] and CR 28-3 (Patterson Creek), [3.19 air miles NNE Fort Ashby], [Petterson Creek Quad.]	N	39.544998	W	78.739723	JL Clayton	July	1	2003
29536	<i>Alasmidonta</i>	<i>varicosa</i>	n/a	Cape Fear Basin	North Carolina	Randolph	Little River at crossing of SR 1135 (Howard Auman Road), [4.48 air miles NW center of Seagrove], [Seagrove Quad.]	N	35.573799	W	79.841202	CB Eades, P Hubert, E Schubert	March	29	2004
29537	<i>Alasmidonta</i>	<i>varicosa</i>	n/a	Cape Fear Basin	North Carolina	Randolph	Little River at NC 134, [0.6 air miles south of Aumans Crossroads], [Seagrove Quad.]	N	35.551998	W	79.839302	CB Eades, P Hubert, E Schubert	March	29	2004
29538	<i>Alasmidonta</i>	<i>varicosa</i>	n/a	Pee Dee Basin	North Carolina	Montgomery	Barnes Creek at [Ophir Road], [4.6 air miles WNW center Lovejoy], [Lovejoy Quad.]	N	35.439999	W	79.999443	CB Eades, P Hubert, E Schubert	March	29	2004
29606	<i>Alasmidonta</i>	<i>raveneliana</i>	n/a	Mississippi Basin	North Carolina	Macon	Little Tennessee River, at Coggins Bend, approx. 0.25 RM downstream from mouth of Rose Creek, [2.65 air miles SE center of Stiles], [Alarka Quad.]	N	35.2593	W	83.445702	SJ Fraley, MA Cantrell, CB Eades, et al.	April	7	2004
29661	<i>Alasmidonta</i>	<i>viridis</i>	n/a	Mississippi Basin	North Carolina	Macon	Little Tennessee River at Needmore Swinging bridge, [ca. 0.280 km SSW Needmore], [Wesser Quad.]	N	35.324299	W	83.523399	SJ Fraley, JW Simmons, T Dickinson, et al.	April	22	2004
29746	<i>Alasmidonta</i>	<i>raveneliana</i>	n/a	Mississippi Basin	North Carolina	Macon	Little Tennessee River at Needmore Swinging bridge, [ca. 0.280 km SSW Needmore], [Wesser Quad.]	N	35.324299	W	83.523399	SJ Fraley, JW Simmons, T Dickinson, et al.	April	22	2004
29797	<i>Pyganodon</i>	<i>grandis</i>	n/a	Apalachicola Basin	Florida	Liberty	Apalachicola River on left bank at RM 33, E shore, across from and downstream of dike field, [3.13 air miles E center Honeyville], [Upper Piney Reach], [Wewahitchka Quad.]	N	30.056267	W	85.134804	SJ Walsh, et al.	June	16	2004
29922	<i>Alasmidonta</i>	<i>marginata</i>	<i>marginata</i>	Mississippi Basin	Tennessee	Hancock	Clinch River (RM 172.2), at Swan Island, [1.07 air miles ENE Lawson Mill], [Swan Island Quad.]	N	36.476112	W	83.290558	SA Ahlstedt	July	20	2004
29995	<i>Alasmidonta</i>	<i>triangulata</i>	n/a	Ogeechee Basin	Georgia	Bulloch	Ogeechee River on Highway US 301, [8.91 air miles] NNE of Statesboro, [Dover Quad.]	N	32.564602	W	81.715103	JD Williams, et al.	August	7	2004
30035	<i>Alasmidonta</i>	<i>undulata</i>	n/a	Pamlico Sound Basin	North Carolina	Wake	Neuse River between US 401 and Buffalo Road [SR 2715], [5.55 air miles NNW center of Knightdale], [Raleigh East	N	35.854698	W	78.529297	TW Savidge, M Wood, S Luginbuhl	May	9	2003

catalog number	genus	species	subspecies	Basin	state	county	locality	lat NS start	lat start	long EW start	long start	collector	date	day	year
							Quad.]								
30074	<i>Alasmidonta</i>	<i>varicosa</i>	n/a	Cape Fear Basin	North Carolina	Orange	New Hope Creek at Turkey Farm Road [SR 1370] crossing, [8.26 air miles W Durham], downstream, [Chapel Hill Quad.]	N	35.992188	W	79.045563	M Wood, T Dickinson, S Medlin	September	15	2004
30080	<i>Alasmidonta</i>	<i>arcula</i>	n/a	Altamaha Basin	Georgia	Jeff Davis	Ocmulgee River app. 0.55 air miles NE of Haddock Landing, [8.44 air miles WNW Roper], [Snipesville Quad.]	N	31.834	W	82.79258	GR Dinkins, JE Dinkins, JE Daniel, et al.	June	16	2004
30219	<i>Anodonta</i>	<i>implicata</i>	n/a	Albemarle Sound Basin	North Carolina	Northampton	Roanoke River above US 258/NC 561, [7.76 air miles NW center of Norfleet], [Scotland Neck Quad.]	N	36.232361	W	77.377853	TW Savidge, T Dickinson	November	11	2004
30224	<i>Anodontoides</i>	<i>radiatus</i>	n/a	Mobile Bay Basin	Mississippi	Winston	Mill Creek at end of Forest Service Road 986, 4.0 km. SW [center] Betheden, 11.0 km. NE [center] Louisville, [Betheden Quad.]	N	33.195278	W	88.969444	WR Haag, ML Warren Jr	November	18	2004
30249	<i>Utterbackia</i>	<i>imbecillis</i>	n/a	Ogeechee Basin	Georgia	Evans	[Sands Pond], front pond, Evans County Public Fishing Area, [2.8 air miles WSW Groveland], [Daisy Quad.]	N	32.130833	W	81.791389	KE Sukkestad	November	4	2004
30272	<i>Alasmidonta</i>	<i>undulata</i>	n/a	Pamlico Sound Basin	North Carolina	Johnston	Moccasin Creek at Buck Road [SR 2105], [2.68 air miles SSW center Middlesex], [Middlesex Quad.]	N	35.754501	W	78.220901	AJ Rodgers, et al.	July	28	2004
30334	<i>Anodonta</i>	<i>heardi</i>	n/a	Apalachicola Basin	Florida	Gulf	Chipola River, FL Hwy 22, [CR 22/Lakegrove Road], [1.76 air miles NE] of Wewahitchka, [Dead Lakes Quad.]	N	30.127655	W	85.176384	RS Butler	August	6	1988
30607	<i>Simpsonaias</i>	<i>ambigua</i>	n/a	Mississippi Basin	Pennsylvania	Greene	Dunkard Creek, Brave, located approximately 400 feet below Dam, toward RDB, [Wadestown Quad.]	N	39.725533	W	80.258797	DD Locy	May	11	2005
30742	<i>Alasmidonta</i>	<i>marginata</i>	<i>marginata</i>	Mississippi Basin	Missouri	Jasper	Spring River, [point estimated 0.72 air miles N center] Carthage, (Neosho/Arkansas system), [Carthage Quad.]	N	37.187046	W	94.309494	n/k	July	19	2003
30743	<i>Alasmidonta</i>	<i>marginata</i>	<i>marginata</i>	Mississippi Basin	Missouri	Jasper	Spring River, [point estimated 0.72 air miles N center] Carthage, (Neosho/Arkansas system), [Carthage Quad.]	N	37.187046	W	94.309494	n/k	July	19	2003
30746	<i>Alasmidonta</i>	<i>viridis</i>	n/a	Mississippi Basin	Missouri	Taney	Swan Creek, [Origin unknown.]	N	0	W	0	n/k	August	6	2004
30749	<i>Alasmidonta</i>	<i>viridis</i>	n/a	Mississippi Basin	Missouri	Taney	Swan Creek, [Origin unknown.]	N	0	W	0	n/k	August	6	2004
30857	<i>Lasmigona</i>	<i>decorata</i>	n/a	Pee Dee Basin	North Carolina	Union	Duck Creek, tributary to Goose Creek, roughly [1 km] 2 km upstream of US 601 bridge, [4.05 air miles SW center Midland], [Midland Quad.]	N	35.18399	W	80.548607	JA Fridell, 12 others	September	30	2005
30857	<i>Lasmigona</i>	<i>decorata</i>	n/a	Pee Dee Basin	North Carolina	Union	Duck Creek, tributary to Goose Creek, roughly [1 km] 2 km upstream of US 601 bridge, [4.05 air miles SW center Midland], [Midland Quad.]	N	35.18399	W	80.548607	JA Fridell, 12 others	September	30	2005

catalog number	genus	species	subspecies	Basin	state	county	locality	lat NS start	lat start	long EW start	long start	collector	date	day	year
30861	<i>Alasmidonta</i>	<i>raveneliana</i>	n/a	Mississippi Basin	North Carolina	Macon	Little Tennessee River between mouth of Watauga Creek and Lake Emory Dam, [point estimated 2.32 air miles SW center Brendletown], [Corbin Knob Quad.]	N	35.22052	W	83.37162	SJ Fraley, MA Cantrell, J Simmons, et al.	April	23	2004
35105	<i>Alasmidonta</i>	<i>raveneliana</i>	n/a	Mississippi Basin	North Carolina	Swain	Little Tennessee River, ~200m upstream from swinging bridge, off of Needmore Road (SR 1113), [3.8 air miles E center Wesser], [Wesser Quad.]	N	35.32584	W	83.325844	JW Simmons, DL Bell, SJ Fraley, et al.	June	23	1994
40284	<i>Alasmidonta</i>	<i>raveneliana</i>	n/a	Mississippi Basin	North Carolina	Mitchell-Yancey	North Toe River, [along SR 1187], [0.13 air miles SW center Toecane], [Bakersville Quad.]	N	36.007469	W	82.193222	SJ Fraley, JW Simmons, JA Hartsell, et al.	October	4	2005
40285	<i>Alasmidonta</i>	<i>raveneliana</i>	n/a	Mississippi Basin	North Carolina	Swain	Little Tennessee River @ 50 m upstream from Needmore swinging bridge, Needmore Road [SR 1113], [0.24 air miles S center Needmore], [Wesser Quad.]	N	35.32325	W	83.522697	JW Simmons, SJ Fraley, JA Hartsell	October	12	2005
45082	<i>Alasmidonta</i>	<i>undulata</i>	n/a	Chesapeake Bay Basin	Virginia	Richmond	James River [0.61 river miles] below Powhite Parkway (VA 76), approximately 2.4 [air] miles W [center] of Richmond, [Richmond Quad.]	N	37.53529	W	77.486099	TW Savidge, T Dickinson, S Garriock	July	10	2006

Table 5. Comparison of the classifications of the genus *Alasmidonta* provided by Haas (1969) and Clarke (1981) compared with the results of this research.

Haas (1969) classification			Clarke (1981) classification			New proposed classification		
Genus	Subgenus	Species	Genus	Subgenus	Species	Genus	Subgenus	Species
<i>Alasmidonta</i>	(<i>Alasmidonta</i>)	<i>undulata</i> (Say, 1817)	<i>Alasmidonta</i>	(<i>Alasmidonta</i>)	<i>undulata</i> (Say, 1817)	<i>Alasmidonta</i>	(<i>Alasmidonta</i>)	<i>undulata</i> (Say, 1817) [type species of genus]
		<i>triangulata</i> (Lea, 1858)			<i>arcula</i> (Lea, 1838)			<i>arcula</i> (Lea, 1838)
	(<i>Prolasmidonta</i>)	<i>heterodon</i> (Lea, 1830)			<i>wrightiana</i> (Walker, 1901)			<i>mccordi</i> Athearn, 1964 ²
	(<i>Bullella</i>)	<i>arcula</i> (Lea, 1836)		(<i>Alasmidens</i>)	<i>mccordi</i> Athearn, 1964			<i>robusta</i> Clarke, 1981 ²
	(<i>Pressodonta</i>)	<i>calceolus</i> (Lea, 1830) ¹		(<i>Decurambis</i>)	<i>marginata</i> Say, 1818			<i>triangulata</i> (Lea, 1858)
	(<i>Decurambis</i>)	<i>marginata</i> Say, 1818			<i>atropurpurea</i> (Rafinesque, 1819)			<i>varicosa</i> (Lamarck, 1819)
					<i>raveneliana</i> (Lea, 1834)			<i>wrightiana</i> (Walker, 1901) ²
					<i>robusta</i> Clarke, 1981			Undescribed species
					<i>varicosa</i> (Lamarck, 1819)		(<i>Decurambis</i>)	<i>marginata</i> Say, 1818 [type species of subgenus]
				(<i>Pressodonta</i>)	<i>viridis</i> (Rafinesque, 1820)			<i>atropurpurea</i> (Rafinesque, 1819) ³
					<i>heterodon</i> (Lea, 1830)			<i>raveneliana</i> (Lea, 1834)
						<i>Pressodonta</i>		<i>viridis</i> (Rafinesque, 1820)
						<i>Prolasmidonta</i>		<i>heterodon</i> (Lea, 1830)

¹ *Alasmidonta* (*Pressodonta*) *calceolus* (Lea, 1830) changed to *Alasmidonta* (*Pressodonta*) *viridis* (Rafinesque, 1820) based on priority of names (Clarke, 1981).² Placement of this presumed extinct taxon in a subgenus is provisional.³ Specimen used was misidentified and taxon is tentatively placed here pending new samples.

Figure 1. Tree based on analysis of COI and ND1 mitochondrial DNA sequences.

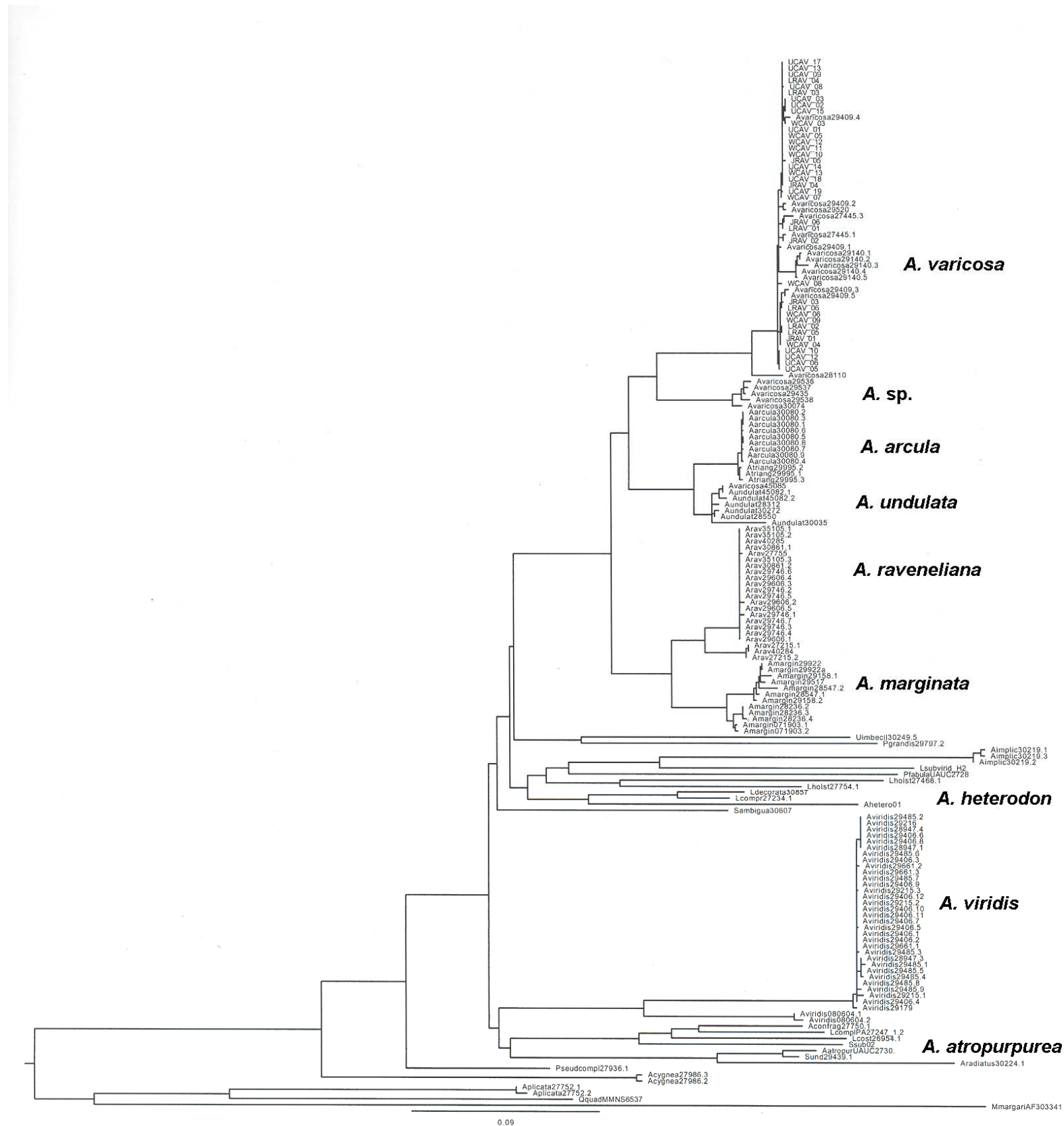


Plate 1. Figures of the *Alasmidonta* species used in these analyses.



Alasmidonta arcula



Alasmidonta atropurpurea



Prolasmidonta heterodon
(+ *Alasmidonta heterodon*)



Alasmidonta marginata



Alasmidonta raveneliana



Alasmidonta robusta

Plate 2. Figures of the *Alasmidonta* species used in these analyses.



Alasmidonta triangulata



Alasmidonta undulata



Alasmidonta varicosa



Pressodonta viridis
(+ *Alasmidonta viridis*)