THE PHLYOGENETICS AND BIOGEOGRAPHY OF THE FRESHWATER PEARLY MUSSEL GENUS ELLIPTIO (BIVALVIA:UNIONIDAE)

A Thesis
by
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Abstract

THE PHLYOGENETICS AND BIOGEOGRAPHY OF THE FRESHWATER PEARLY MUSSEL GENUS ELLIPTIO (BIVALVIA:UNIONIDAE)

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The taxonomy of North American freshwater pearly mussels (Unionidae) has been problematic since the earliest species descriptions. Based upon morphology alone, taxonomists have long disputed what constitutes a species and there is still a debate as to how to classify all the potential morphotypes of a given taxon. *Elliptio* is thought to be the most speciose genus of Unionids. Early taxonomists described hundreds of taxa and despite once being synonymized to 13 species, there currently are upwards of 38 recognized species. With the advent of molecular techniques, there have been many attempts to resolve this troubled nomenclature and to better understand the evolutionary relationships of both *Elliptio* and Unionid taxa as a whole. Although many higher order discrepancies have been resolved, *Elliptio* still remains unresolved. A total of 79 new sequences representing 13 species were generated for the mitochondrial gene regions of COI and ND1. Three of these species (including federally listed *Elliptio chipolaensis*) had no previous genetic data available and one (*E. congaraea*) had no previous topotypic material available. In combination with previously available data from both published and unpublished work a total of 311 sequences representing 27 currently recognized species of 25 river basins were obtained for this study. Both Bayesian inference and maximum likelihood analyses indicate polyphyly on a genus and species level. *Elliptio sensu stricto* group has been determined as the type, *Elliptio crassidens* and 18 closely related species. Haplotype reconstructions of currently recognized species of the *Elliptio sensu stricto* failed to uphold the current nomenclature in this group. Haplotypes were shared between numerous species and basins across vast distances. A closer investigation of fish host use in this group is recommended to better understand this trend. Although a conclusion cannot be reached as to how nomenclature can be improved based upon this study, it reveals new avenues of investigation in order to do so. In conjunction with a better life history understanding, the use of more recent molecular techniques, namely RADseq, may help elucidate the evolutionary relationships of this group.

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Dedication

I would like to dedicate this work to my husband,

Justin Fischer,

and my daughter,

Veronica “Nica” Fagundo Fischer.

Thank you for all your love, support, patience, smiles, hugs, and inspiration.

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Foreword

This research will be submitted to the peer-reviewed journal, *Journal of Biogeography*. It has been formatted to fit the requirements for that journal.

INTRODUCTION

The taxonomy of the freshwater pearly mussels and the genus *Elliptio* have both been long disputed. Early North American taxonomists described well over 1000 species of unionids and many descriptions were based only a few specimens or relied primarily upon shell morphology in the days before soft parts were commonly vouchered by collectors. Many of these taxa are now regarded as synonyms (Simpson, 1914; Ortmann, 1921). In 1970, Johnson while acknowledging the wide array of morphologies observed in *Elliptio*, only recognized 13 species along the Southern Atlantic Slope (Johnson, 1970). However, Johnson's conservative taxonomy was not well-received by field biologists and, as a result, *Elliptio* is thought to be the most diverse and widespread freshwater pearly mussel genus in North America. As with most freshwater mussel genera, *Elliptio* species richness is greatest (36 to 38 species are currently recognized) in streams draining the Southern Atlantic Slope of North America (Table 1, Turgeon *et al.*, 1998; Williams *et al.*, 2014).

Arnold Ortmann was among the more conservative unionid taxonomists of the early 20th century and was keenly interested in how environmental conditions influenced the morphology of freshwater mussel shells (i.e., ecophenotypic plasticity). Ortmann demonstrated that numerous widely-accepted species of the Pleurobemini tribe were ecophenotypes of more widely-distributed species (Ortmann, 1920). Ortmann hypothesized that a gradual shift in shell morphology occurred along stream continuums. According to 'Ortmann's Rule', freshwater bivalve populations in upstream reaches were generally more elongated and laterally-compressed compared to downstream populations. Ortmann speculated that this was due to predictable changes in environmental factors within stream networks (Ortmann, 1920). For example, the concentrations of dissolved ions that mollusks use to construct their shells typically increase as one moves from the headwaters to the lower mainstem reaches of large rivers.

Elliptio was first recognized as a subgenus in 1819 by Rafinesque who approximated that there were 12 species (Rafinesque, 1819). However, due to the language barriers and taxonomic competition at the time (see Frierson, 1927), colleagues largely ignored this nomenclature for nearly a century until Ortmann elevated *Elliptio* to the genus level and included 8 recognized species (Ortmann, 1912).

Early genetic studies using allozymes were the first to show that lanceolate Elliptios were distinct from the *E. complanata* group and *Elliptio* spp. that are conchologically more similar (more ovate) to the type species, *E. crassidens* (Davis *et al.*, 1981). Attempts to further resolve the lanceolate taxonomy were never published (see Davis, 1984). One clear implication of these early molecular studies was the realization that many *Elliptio* species are very closely related, despite their seemingly divergent morphologies. Davis *et al.* (1981) and Davis (1984) also hypothesized that *Elliptio* evolved relatively recently and speculated that the genus included 16-18 species.

Subsequent to Davis (1984), numerous synonyms were recognized based largely on morphological data (Williams *et al.*, 1993; Turgeon *et al.*, 1998). Although targeted sequencing of mitochondrial and nuclear genes have been widely used in subsequent studies of freshwater mussel evolution (Lydeard *et al.*, 1996, 2000; Roe &

Lydeard, 1998; King *et al.*, 1999; Bogan & Hoeh, 2000; Buhay *et al.*, 2002; Serb *et al.*, 2003), most of these studies included only a single *Elliptio* taxon, *E. dilatata*. Campbell *et al.* (2005) was the first genetic study since Davis (1984) to include more than one *Elliptio* taxon in phylogenetic analyses. These analyses revealed that *Elliptio* is likely a polyphyletic taxon and called attention to the need to revisit *Elliptio* phylogenetic relationships yet again (Campbell *et al.*, 2005).

Studies that have sequenced mitochondrial genes from large numbers of individuals from across multiple taxa have suggest that many *Elliptio* species are indeed closely related and call to question the currently accepted taxonomy. Sommer (2007) tested the hypothesis that *E. waccamawensis* is a distinct taxon endemic to Lake Waccamaw and the Waccamaw River in southeastern North Carolina (Lea, 1863) and compared sequences from that system to congeners from the Pee Dee drainage. Her results found that *E. waccamawensis* shared haplotypes with a range of *Elliptio* species from adjacent drainages including *E. congaraea* and that most taxa examined did not form well-supported monophyletic groups (Sommer, 2007). A study by Campbell and Lydeard (2012) included data from a relatively small number of individuals but across a somewhat broader taxonomic scale (9 species) and showed that *Elliptio* was likely polyphyletic. Because *E. dilatata* appeared distantly related to *E. crassidens* and other *Elliptio* taxa, Campbell and Lydeard (2012) assigned *E. dilatata* to the monotypic subgenus, *Eurynia*. Most recently, research by Perkins (2014) revealed that two additional species do not belong in the genus *Elliptio*. The

Tar River spinymussel *E. steinstansana* and the Altamaha spinymussel *E. spinosa* belong in two distinct monophyletic clades distinct from *Elliptio sensu stricto* (Perkins, 2014).

Based on these results, species currently classified as *Elliptio* comprise at least five paraphyletic clades within the unionid tribe Pleurobemini (*Elliptio sensu stricto*-*E. crassidens, E. complanata*, Lanceolate *Elliptio* taxa, two distinct groups of spiny mussels and *Eurynaia dilatata*. However, it is likely that other important divisions occur within *Elliptio* that need to be elucidated. Additionally, there is limited support for basal nodes linking these genera to one another and to other closely related taxa (*Pleurobema*, *Fusconaia*) in all published phylogenies (e.g. Campbell *et al*., 2005; Campbell & Lydeard, 2012; Perkins, 2014).

Here, I examine genetic differences within large sample sizes of *Elliptio* taxa from several rivers across the southern Atlantic Slope to get a better idea of molecular diversity within and among taxa in this widespread group of freshwater mussels. This study represents the most comprehensive examination of *Elliptio* to date in terms of taxonomic inclusion and geographic breadth. Furthermore, this study represents the first to test currently recognized species boundaries within *Elliptio sensu stricto* using multiple mitochondrial DNA markers in an effort to resolve the taxonomic ambiguities found within the group. A more parsimonious understanding of the relationships within and among putative species groups will also improve conservation and management of rare species and allow agencies to more effectively direct precious resources.

MATERIALS AND METHODS

Collection and sequences

Taxonomy follows Turgeon *et al.* (1998), Williams *et al.* (2008) and Williams *et al.* (2014) but I also recognized several species recognized by state agencies (e.g., *E. buckleyi*, *E. mediocris*, and *E. nasutilus*) although several are synonymized by other taxonomic authorities (See Turgeon *et al.*, 1998; Williams *et al.*, 2014).

For sensitive taxa (e.g., federally-listed *E. chipolaensis*), tissue cells were collected via non-lethal buccal swabs (Isohelix SK-1 swabs, Boca Scientific Inc., Boca Raton, FL). Non-listed specimens were collected by hand and vouchered soon afterwards. Adductor tissue was clipped and placed in 95% EtOH and the animals were immediately vouchered in the Appalachian State University Zoological Collections in Boone, North Carolina (Table 1). MOBIO UltraClean Tissue & Cells DNA Isolation Kit (MOBIO Laboratories, Carlsbad, CA) kits were used to isolate DNA following the recommended protocol with the optional Proteinase K step. DNA extraction for pre-2014 collections followed the same protocol with the exception of a modified Proteinase K step: 20 ul of Proteinase K was added to each museum tissue sample, vortexed on max speed for 15 minutes, and then incubated at 60°C for 1 hour.

DNA concentration and purity was evaluated using a NanoDrop 2000 nano-spectrophotometer (Thermo Scientific, Waltham, MA). Polymerase chain reactions (PCR) used 10 µl of GoTaq® Green Master Mix 2X (Promega Corporation, Madison, WI), 50 ng of template DNA, 0.04 µL (10µM) of both upstream and downstream primers and nuclease-free water for a final volume of 20 μL per reaction. Folmer universal primers (forward 5'- GGTCAACAAATCATAAAGATATTGG -3'; reverse 5' – TAAACTTCAGGGTGACCAAAAAATCA-3') were used to amplify a fragment of the mitochondrial cytochrome oxidase subunit I (COI) gene. Primers adapted from Serb *et al.* 2003 (forward 5' - TGGCAGAAAAGTGCATCAGATTAAAC -3'; reverse 5' - GATTTTCAAGCTATTGCTAT -3') were used to amplify a fragment of the mitochondrial NADH subunit I (ND1) gene. Thermocycler conditions were as follows for COI amplification: 95°C for 2:00, followed by 34 cycles of 95°C for 0:40, 50°C for 1:00, 72°C for 1:30, followed by 72°C for 7:00 and then held at 10°C ∞. For amplification of ND1, PCR conditions were as follows: 95°C for 2:00, followed by 34 cycles of 95°C for 0:40, 48°C for 1:00, 72°C for 1:30, followed by 72°C for 7:00 and held at 10°C ∞. All PCR reactions were performed on an Eppendorf Mastercycler. PCR product was visually inspected on 1% agarose gel stained with ethidium bromide and successful reactions were sent off site for sequencing by Retrogen, Inc. (San Diego, CA).

Taxonomic coverage

One hundred and thirty-seven sequences were also used from previous unpublished Gangloff Lab projects that were obtained prior to the start of my thesis work. (Table 2). Spiny mussel (e.g., *E. spinosa* and *E. steinstansana*) sequences were obtained from Perkins (2014). Ninety-five GenBank *Elliptio* species sequences were used to supplement and add robustness to the dataset (Table 3). *Elliptio* sequences were only used if geographic information was available. One hundred GenBank sequences from more distantly-related taxa from tribe Pleurobemini were used as outgroups and all trees were rooted with *Strophitus subvexus* (Table 3). Outgroup sequences were only used if a given individual, with a unique identifier, had both COI and ND1 gene fragments available.

Sequence analysis

Sequences were compiled, aligned, edited and concatenated in Geneious R7 (Biomatters Ltd., Aukland, New Zealand). Sequences were aligned using the Muscle algorithm and visually inspected for stop codons, numts, and male mitotypes (Curole & Kocher, 2002; Hoeh *et al.*, 2002; Buhay, 2009). COI sequences were trimmed to 438 base pairs (bps) and ND1 sequences were trimmed to 567 bps and concatenated for a total concatenated alignment length of 1005 bps. Genetic distances were estimated using maximum composite likelihood using MEGA version 6 (Tamura *et al.*, 2013). The number of haplotypes and conserved regions were determined using DNAsP (Librado & Rozas, 2009).

Phylogenetic analysis

jModelTest version 2 was utilized to calculate the best-fit nucleotide substitution model within a 95% confidence interval, HKY+I+G (Darriba *et al.*, 2012). To observe the implied evolutionary relationships of my dataset, a maximum likelihood tree was reconstructed using 1000 iterations in MEGA version 6 (Tamura *et al.*, 2013). To test the hypothesis that *Elliptio* forms a monophyletic clade, a Bayesian inference analysis with Metropolis-coupled Markov Chain Monte Carlo (MCMC) was implemented using MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003). 1,000,000 iterations were performed with sampling every 1000 generations. The first 100,000 iterations were discarded as burn-in and the remaining were combined to a 50% majority consensus tree.

***Elliptio sensu stricto* phylogeography**

Using pairwise distances and phylogenetic reconstructions the *Elliptio sensu stricto* group was determined. I define the *Elliptio sensu stricto* group as the taxa that are closely related to *Elliptio crassidens*, the type species of the genus *Elliptio*. Taxa that were separated by a pairwise distance substantially greater than an intra-specific level and did not group with the *Elliptio sensu stricto* were considered separate. The intra-specific and inter-specific distances greatly overlapped, taxa were grouped according to previous work (lanceolate *Elliptios, E. (Eurynia) dilatata, E. spinosa, and E. steinstansana*), in order to better determine members of *Elliptio sensu stricto*. Taxa that were not part of the *Elliptio sensu stricto* group were excluded from this part of the analysis.

Haplotype networks were utilized to further examine the genetic relationships of the *Elliptio sensu stricto* group (Hart & Sunday, 2007). TCS haplotype networks were constructed using PopART (Clement *et al.*, 2002; Leigh & Bryant, 2015). Haplotypes were examined by defining sequences based upon their current nomenclature, river basin, and geographic region (defined as Northern, Mid-Atlantic, Southern, Gulf, Mississippi and Suwannee). Haplotypes that were shared between basins were further examined by plotting the coordinates of the individuals that shared the haplotypes.

RESULTS

A total of 79 new sequences were generated for 65 individuals and 13 species. Fifty-seven sequences of 12 species were generated for ND1, and 22 sequences of 7 species were generated for COI, with a total of 15 sequences for 5 species available for concatenation (Table 4). This study represents the first presented genetic data for three species (*E. ahenea*, *E. chipolaensis*, and *E. roanokensis*), ND1 data for one species (*E. mediocris*), and topotypic material for one species (*E. congaraea*).

The COI dataset contained 122 sequences of 21 currently recognized *Elliptio* taxa; the ND1 dataset contained 184 sequences of 26 taxa; and the concatenated dataset contained 106 sequences of 18 taxa. Among the three datasets, COI was the most conserved (108 informative sites, C=0.753), and ND1 the least conserved (206 informative sites, C=0.637). The concatenated dataset, although containing the highest number of taxonomically-informative sites (280) was more conservative than ND1 (C=0.721).

COI intra-specific pairwise distances ranged from 0 (*E. producta*) and 0.016 (*E. congaraea*) and inter-specific pairwise distances ranged from 0.004 (*E. pullata:E. waccamawensis, E. pullata:E. mcmichaeli, E. waccamawensis:E. mcmichaeli*) and 0.084 (*E. nasutilus:E. steinstansana*) (Tables 5 and 6). ND1 intra-specific pairwise distances ranged from 0.0005 (*E. steinstansana*) and 0.06 (*E. fisheriana*) and inter-specific pairwise distances ranged from 0.004 (*E. hopetonensis:E. roanokensis*) and 0.143 (*E. spinosa:E. steinstansana*) (Table 5 and 6). Intra-specific pairwise distances for the concatenated dataset ranged from 0.001 (*E. steinstansana*) and 0.017 (*E. congaraea*) and inter-specific pairwise distances ranged from 0.008 (*E. crassidens:E. mcmichaeli, E. crassidens:E. pullata*) and 0.122 *(E. spinosa:E. steinstansana*) (Tables 5 and 7).

When treating the taxa as groups following previous work (lanceolate Elliptios, *E. (Eurynia) dilatata, E. spinosa, and E. steinstansana*), the COI marker intra-group distances ranged from 0.001 (*E. spinosa*) and 0.026 (lanceolate Elliptios) and inter-group pairwise distances ranged from 0.044 (*Elliptio sensu stricto*:lanceolate Elliptios) and 0.082 (*E. spinosa:E. steinstansana*) (Tables 8 and 9). ND1 intra-group pairwise distances ranged from 0.0005 (*E. steinstansana*) and 0.058 (lanceolate Elliptios) and inter-group pairwise distances ranged from 0.076 (*Elliptio sensu stricto*:lanceolate Elliptios) and 0.143 *(E. spinosa:E. steinstansana*) (Tables 8 and 10). Concatenated dataset intra-group pairwise distances ranged from 0.001 (*E. steinstansana*) and 0.064 (lanceolate Elliptios) and inter-group pairwise distances ranged from 0.059 (*Elliptio sensu stricto*:lanceolate Elliptios) and 0.122 (*E. spinosa:E. steinstansana*) (Tables 8 and 11).

All maximum likelihood and Bayesian inference phylogenies had strong support for a polyphyletic *Elliptio* (Figs. 1-6). *Elliptio (Eurynaia) dilatata* consistently grouped outside the *Elliptio sensu stricto*s. *Elliptio steinstansana* and *E. spinosa* each formed their distinct monophyletic clades, respectively. The lanceolate Elliptios (*E. angustata, E. fisheriana, E. nasutilus, E. product, E. shepardiana*) consistently claded outside the *Elliptio sensu stricto* but did not always comprise a monophyletic group. Additionally, in the ND1 trees indicate that *E. ahene*a is sister to *E.* ja*yensis* and *E.* o*ccults*, rather than the predicted lanceolate group and *E. chipolaensis* does not strongly group with the *Elliptio sensu stricto*s (Figs. 2 and 5). Aside from the aforementioned outliers, the remaining species are either polyphyletic, lack nodal support, or a combination of the two (Figs. 1-6).

Following the discovery of the placement of *E. chipolaensis*, additional analysis was performed regarding pairwise distances to better determine its inclusion to the *Elliptio sensu stricto* group for downstream analysis. When treated as its own separate group, the pairwise distances of *E. chipolaensis* to other respective groups ranged from 0.102 – 0.139 (Table 10). Due to the high amount of divergence from the *Elliptio sensu stricto* group, it was not included in the haplotype analysis.

Haplotype analyses of the COI *Elliptio sensu stricto* dataset included 85 individuals representing 14 species, 14 river basins, and all geographic regions (Figs. 7-9). There were 54 haplotypes represented, 9 of which were shared among multiple individuals. Of these 9 shared haplotypes, 5 were shared by the same species occurring in the same basin, two were shared by different species occurring in the same basin, and two were shared by different species occurring in different basins (Figs. 7-10). The 2 haplotypes shared by the different species in the same basin were both comprised of *E. jayensis* and *E. occulta* in the Suwannee Basin, Florida. One of the haplotypes shared by different species in different basins was comprised of one *E. congaraea* from the Neuse Basin, North Carolina and *E. complanata* from the Apalachicola basin, Florida. The other was comprised of one *E. pullata* from the

Mobile basin, Alabama, one *E. arctata* from the Choctawhatchee Basin, Alabama, and one *E. mcmichaeli* and two *E. pullata* from the Apalachicola Drainage, Florida.

Haplotype analyses of the ND1 *Elliptio sensu stricto* dataset included 146 individuals representing 16 species, 25 river basins, and all geographic regions (Figs. 11-13). There were 100 haplotypes represented, 21 of which were shared among multiple individuals. Of these 21 shared haplotypes, 6 were shared by the same species occurring in the same basin, 4 were shared by the same species occurring in different basins, 4 were shared by different species occurring in the same basin and 7 were shared by different species occurring in different basins (Figs. 11-14). Of the 6 haplotypes shared by the same basin, two were *E. occulta* of the Suwannee basin, Florida; one was *E. roanokensis* of the Pee Dee Basin, North Carolina; one was *E. pullata* of the Apalachicola basin, Florida; one was *E. complanata* of the Catawba Basin, North Carolina; one was of *E. icterina* of the Pee Dee. Of the haplotypes shared by the same species in different basins one was shared by *E. complanata* of the Penobscot Basin, Maine and the York Basin, Virginia; one was shared by *E. complanata* of the Cape Fear and Pee Dee basins; one was shared by *E. complanata* of the Pee Dee Basin and of the York Basin; and one was shared by *E. complanata* of the St. Croix Basin, Maine and of the James Basin, Virginia. Of the haplotypes shared by multiple species occurring in the same basin 3 were shared by *E. jayensis* and *E. occulta* in the Suwannee Basin and one was shared by *E. waccawensis* and *E. icterina* in the Pee Dee Basin. Of the 7 haplotypes shared by multiple individuals and basins, one was shared by *E. pullata* and *E. icterina* from the Apalachicola Basin, Florida and *E. pullata* from the Ecofina Basin, Florida; one was shared by *E. pullata* of the

Lumber basin, North Carolina and *E. complanata* from the Penobscot Basin, Maine; one was shared by *E. pullata* of the Lumber Basin, North Carolina and *E. complanata* from the James Basin, *E. complanata* from the York Basin and one *E. complanata* from the Penobscot Basin; one was shared by *E. complanata* of the Apalachicola Basin, *E. icterina* of the Neuse Basin, *E. complanata* of the Tar Basin and *E. complanata* of the James Basin; one was shared by *E. icterina* of the Cape Fear Basin and *E. complanata* of the Tar Basin; one was shared by *E. congaraea* of the Pee Dee Basin and *E. complanata* form the Cape Fear and Roanoke Basins; and one was shared by *E. complanata* of the Neuse Basin and *E. icterina* and *E. complanata* from the York Basin.

Haplotype analyses of concatenated dataset included 74 individuals representing 11 species from 13 river basins and all geographic regions (Figs. 15-17). There were 58 haplotypes represented, 7 of which were shared among multiple individuals. Of these 7 shared haplotypes, 5 were shared by the same species occurring in the same basins, respectively, and two were shared by different individuals from the same basin (Figs. 15-17). Of the 5 haplotypes shared by the same species in the same basin, 3 haplotypes were shared by *E. occulta* in the Suwannee Basin and two haplotypes were shared by *E. complanata* in the Catawba Basin. The two haplotypes shared by different species in the same basin were *E. jayensis* and *E. occulta* in the Suwannee Basin.

DISCUSSION

Overall, these data support the previous findings of polyphyly of *Elliptio*. Many taxa currently recognized under the genus *Elliptio* represent separate lineages apart from *Elliptio sensu stricto*. Moreover, the current nomenclature of *Elliptio sensu stricto* is unsupported. There is lack of genetic structure and mitochondrial haplotype sharing is extensive across a large geographic range and between many putative species. It is evident from these data that the current taxonomy of this group needs to be revised to more effectively manage these taxa.

New species and localities

This study is the first to examine genetic data from the federally-threatened species *E. chipolaensis* (USFWS 1998) as well as data from several candidate or state listed species (e.g., *E. ahenea*, *E. roanokensis;* Bogan, 2002; USFWS 2011).

The relationship of *E. chipolaensis* to the rest of the currently recognized *Elliptio* taxa is of particular interest. The placement of *E. chipolaensis* within *Elliptio* has not previously been seen as a taxonomic issue and there are surprisingly no taxonomic synomyms (Williams *et al.,* 2008; Williams *et al.*, 2014). My data suggest that it is not closely related to any of the other five *Elliptio* lineages.

Although thought to be considered part of the lanceolate *Elliptio* clade based upon morphology (Williams *et al.*, 2014), my data suggest that *E. ahenea* is more closely related to the *Elliptio sensu stricto* group. In fact, *E. ahenea* appears to be closely related to *E. jayensis* of the Suwannee basin, a sympatric species which has numerous synonyms (Johnson, 1972; Williams *et al.*, 2014). *Elliptio ahenea* was historically recognized by Frierson (1927), synonymized with *E. jayensis* by Johnson (1972) but recognized by Williams *et al.* (1993) and subsequent authors (Turgeon *et al.*, 1998, Williams *et al.* 2014). Although I had only one specimen of *E. ahenea* in my alignment and did not have material from the type locality (Black Creek, St. John's River Basin, Florida), my results support the conservative taxonomy of Johnson (1972).

Elliptio roanokensis grouped closely with syntopic *E. complanata*, *E. icterina* and *E. hopetonensis* from the Altamaha river basin (Figs. 11-13). Although *E. roanokensis* does not share a haplotype with any of the individuals, it does not display the traditional genetic variation used to barcode species (pairwise distance ranges from 0.02 and 0.07). However, within the *Elliptio sensu stricto* group, this does not necessarily mean that *E. roanokensis* is not a valid taxon. To draw any substantial conclusions on the taxonomic standing of this taxon, both topotypic material and additional markers are needed.

The topotypic material for *E. congaraea* revealed interesting relationships between the newly presented sequences and those used in previous studies. Topotypic specimens did not cluster or clade closely with putative *E. congaraea* from other localities (Figs. 7, 11 and 15). When revisiting the pairwise distances between the type locality specimens and specimens from other localities, genetic distances approach that of intra-specific rather than inter-specific (ND1 dataset range (0.018 – 0.022); COI dataset range (0.006 - 0.02); concatenated dataset range (0.016 – 0.021)). Future studies should take this into consideration when selecting material to include in the analysis as certain localities may result in varying results.

Elliptio Phylogenetics

Previous studies have widely reported polyphyly in the genus *Elliptio* (Campbell *et al.*, 2005; Sommer, 2007; Campbell & Lydeard, 2012; Abernethy *et al.*, 2013; Perkins, 2014). Results of my study were largely congruent with published work but show that the taxonomic issues within *Elliptio* have deep, if largely unresolved, roots. My study clearly shows that *Elliptio dilatata* and both spinymussel lineages identified by Perkins (2014) clade separately from the *Elliptio sensu stricto* group and both lineages displayed greater inter-specific distances compared to members of *Elliptio sensu stricto*, similar to previous findings (Campbell *et al*., 2005; Campbell & Lydeard, 2012; Perkins, 2014). Members of the lanceolate group represent a clade, albeit possibly not monophyletic, separate from the *Elliptio sensu stricto* group, similar to previous work and in support of earlier hypotheses (Davis *et al.*, 1981; Davis, 1984; Sommer, 2007). Moreover, *E. chipolaensis* may belong to a unique lineage as it did not clade with any sampled *Elliptio* or Pleurobemini groups. However, more than one specimen and more markers will be needed to confirm this finding.

Elliptio sensu stricto Phylogeography

Unlike previous studies, my results clearly show that many currently-recognized *Elliptio* species do not comprise monophyletic groups. There were numerous polytomys within *Elliptio sensu stricto*. Haplotype networks utilizing current nomenclature fail to find any structure with regards to any currently recognized species (Figs. 7, 11 and 15). Interestingly, halplotype networks show that geographic range better explained much of the clustering of haplotypes and putative taxa. The best structure is visible when haplotypes were sampled from across a broad geographic range demonstrating that mtDNA haplotypes are shared across both morphologically dissimilar species (*E. complanata* and *E. icterina*) as well as across basins (Figs. 9, 12 and 17).

One of the most interesting findings is that mtDNA haplotypes are shared across broad geographic regions. Haplotype sharing appears to be greatest further downstream and presumably closer to the sites of former linkages between adjoining coastal stream drainages (Figs. 10, 14). It is possible that reliance on catadromous or anadromous host fishes may explain this genetic structure. Although the host fish for most *Elliptio* species are unknown, they are generally thought of as generalists (Williams *et al.*, 2014). Further study of potential catadromous and anadromous fish hosts may reveal suitable hosts that are more likely to move laterally between coastal estuaries and rivers. Additionally, a more geographically robust dataset covering the area between Virginia and Maine may reveal this haplotype along the Northeastern Atlantic Slope between the locations represented in this study.

Congruence of datasets

Although the three datasets differed in gene conservation, sample size, and robustness, each portrayed similar relationships in the resulting phylogenetic trees. Additionally, the three datasets yielded similar results with respect to the pairwise distance groupings. The most distinguishable differences in the dataset were seen in the haplotype analyses. Although the COI dataset contained more species and individuals, it consistently underestimated haplotype diversity compared to the concatenated and ND1 datasets. The concatenated dataset contained the fewest shared haplotypes. However, this is likely due to the sampling limitations of this dataset (Table 15). The ND1 dataset displayed the greatest haplotype diversity. This may either be due to a greater number of individuals and species available, or that it is the least conserved marker used in this study. It may also be that ND1 is a more quickly evolving gene. Given the apparently recent radiation of *Elliptio* and the repeated result of polyphyly in this group (Campbell *et al*., 2005; Sommer, 2007; Campbell & Lydeard, 2012; Perkins, 2014), ND1 may represent a much less conservative marker (especially compared to the more widely used bar-coding gene COI) that may be of more use in ascertaining biogeographic trends in these taxa.

Taxonomic implications

This study confirms earlier hypotheses that the lanceolate *Elliptios* are distinct from *Elliptio sensu stricto*s (Davis *et al.*, 1981; Davis, 1984). As well as being genetically distinct from the *Elliptio sensu stricto* group, members of this group are morphologically distinct as well. Members of the *Elliptio sensu stricto* group exhibit a more ovate shell morphology whereas lanceolate taxa are distinguished by their long slender shells. Lanceolate taxa frequently co-occur with *Elliptio sensu stricto* taxa and show no signs of hybridization. It is believed that this group is on its own evolutionary trajectory and may warrant recognition as a distinct genus. However, more rigorous study should be conducted as this group also appears to be paraphyletic (Figs 1-6).

This study supports the findings of Campbell and Lydeard (2012). With the addition of new *Elliptio* taxa, *E.* (*Eurynia*) *dilatata* remains separate from the *Elliptio sensu stricto* lineage. This makes sense, considering this is the only *Elliptio* species that is restricted to interior river drainages. There is substantial evidence of range restrictions and molecular evidence that this species is on its own evolutionary trajectory. Therefore, in consideration of future taxonomic revisions of *Elliptio* and other Pleurobemini taxa, it is recommended that this species be recognized as the sole member of the genus *Eurynia*, rather than of the subgenus *Eurynia*.

Analysis of datasets with a large number of taxa from *Elliptio sensu stricto* and lanceolate *Elliptio* groups continued to support Perkins (2014) hypothesis that both *E. spinosa* and *E. steinstansana* comprise unique lineages that are distinct from the *Elliptio sensu stricto* lineage. Perkins (2014) recommended that the genus *Canthyria* be resurrected for *E. spinosa* and that a new genus (*Parvaspina*) be created comprising *E. steinstansana* and *Pleurobema collina*.

Based upon one marker of one individual, it appears that *E. chipolaensis* is distinct from the *Elliptio sensu stricto* group. Although more data are needed to further analyze this relationship, it would not be surprising if this taxon is, indeed, separate from the *Elliptio sensu stricto* group. Most species of *Elliptio* are wide-ranging, occur in multiple river systems and are common and generally abundant. *Elliptio chipolaensis* is a federally-threatened species endemic to the Apalachicola-

Chipola-Flint Basin. Moreover, unlike most (save for *E. crassidens*) members of *Elliptio sensu stricto*, *E. chipolaensis* releases juveniles via conglutinates (Preister, 2008). Other *Elliptio* taxa that do not clade with *Elliptio sensu stricto* (e.g., *E. (Eurynia) dilatata*. *E. spinosa*, and *E. steinstansana*) also package glochidia in conglutinates and provide further support for the conclusion that *E. chipolaensis* may not belong in *Elliptio* (Perkins, 2014; Williams *et al.*, 2014).

At this stage of understanding the genetic relationships among the members of *Elliptio sensu stricto*, it is inadvisable to recommend taxonomic revisions or recognize additional species. Although *Elliptio* likely contains many synonyms, further analysis will be needed to identify names that have taxonomic priority. This study demonstrates that morphological differences are not particularly useful in identifying these animals. Geographic region and river basin provided the only apparent levels of organization observed for all of the mtDNA datasets. It is therefore recommended that biologists and managers consider implementing conservation efforts that are appropriate for sub-populations but that efforts to manage these taxa across their biological ranges are likely to lead to frustration.

To better decipher the relationships within taxa in the *Elliptio sensu stricto* group, further studies should include alternative means of evaluating genetic relationships. For example, RADseq has successfully been used to resolve relationships between post-glacial taxa (Emerson *et al.*, 2010) and may be of use with this group. Suggested revisions will not be made here, however, it is strongly recommended to cease species descriptions based upon morphological characteristics.

CONCLUSIONS

Freshwater mussels within the nominal genus *Elliptio* exhibit pronounced polyphyly at both the genus and species level. Results of this study are in line with previous research that identified several unique lineages within *Elliptio* (Campbell & Lydeard, 2012; Perkins, 2014). Additionally, by including multiple previously un-sequenced taxa in my analyses, I provided more support for the hypothesis that *Eurynia dilatata*, both spiny mussel clades and the lanceolate clade are all distinct from the *Elliptio sensu stricto* group (Davis *et al.*, 1981). However, further analyses will likely be needed to better understand relationships within the lanceolate *Elliptio* and *Elliptio sensu stricto* groups. Finally, I found that *E. chipolaensis* may comprise a unique lineage that does not appear to be closely related to other *Elliptio* lineages.

Moreover, the current taxonomy of much of the *Elliptio sensu stricto* group was not well-supported by my molecular data. There was a lack of structure at the population level and evidence for widespread hybridization among species. The only pronounced structuring in the data was observed across broad geographic (i.e., drainage or regional) scales. I hypothesize that a more complete understanding of host fish use within this group may help explain this phenomenon. However, the patterns observed may also reflect past biogeographic processes including orographic uplift, changes in sea level and drainage capture events (April *et al.*, 2013).

Future work should focus on implementing new molecular approaches that may better interpret the genetic relationships and possibly lead to a finer scale resolution in which a better nomenclature can be determined. While this study does not bring resolution to this issue, it does identify a means of broad organization according to geographic region rather than taxonomy. It is on this basis that it is recommended to consider conservation measures on a basin or region wide basis, rather than species level.

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Table 1. Currently recognized *Elliptio* species. Numbers indicate representation in this study and are used as identifiers in consequent tables.

	Species	Common Name	Authority	Type locality	Type basin
1	*E.ahenea*	Southern Lance	Lea 1845	Black Creek, FL	St. Johns River basin
2	*E.angustata*	Carolina Lance	Lea 1831	Cooper River, SC	Santee-Cooper
3	*E.arca*	Alabama Spike	Conrad 1834	Alabama River, AL	Mobile
4	*E.arctata*	Delicate Spike	Conrad 1834	Alabama River, AL	Mobile
5	*E.buckleyi*	Florida Shiny Spike	Lea 1843	Lake George and Lake Monroe, FL	
6	*E.chipolaensis*	Chipola Slabshell	Walker 1905	Chipola River, FL	Chipola
	E.cistellaeformis	Box Spike	Lea 1863	Neuse River, NC	Neuse
7	*E.complanata*	Eastern Elliptio	Lightfoot 1786	Potomac River, Washington DC	Potomac
8	*E.congaraea*	Carolina Slabshell	Lea 1831	Congaree River, SC	Saluda
9	*E.crassidens*	Elephantear	Lamarck 1819	Ohio River,OH	Ohio
	E.cylindracea	Sad Elliptio	Frierson 1927	Savannah River, GA	Savannah
	E.dariensis	Georgia Elephantear	Lea 1842	Altamaha River, GA	Altamaha
10	*E.dilatata*	Spike	Rafinesque 1820	Kentucky River, KY	Mississippi
	E.downiei	Satilla Elephantear	Lea 1858	Buck Lake, GA	Satilla
	E.errans	Oval Elliptio	Lea 1856	Savannah River, GA	Savannah
11	*E.fisheriana*	Northern Lance	Lea 1838	Chester River, MY	Chesapeake Bay
12	*E.folliculata*	Pod Lance	Lea 1838	Savannah River, SC	Savannah
	E.fraterna	Brother Spike	Lea 1852	Chattahoochee River, GA	Chattahoochee
	E.fumata	Gulf Slabshell	Lea 1857	Chattahoochee River, GA	Chattahoochee
	E.hepatica	Brown Elliptio	Lea 1859	Salkehatchie River, SC	Salkehatchie
13	*E.hopetonensis*	Altamaha Slabshell	Lea 1838	Altamaha River, GA	Altamaha
14	*E.icterina*	Variable Spike	Conrad 1834	Savannah River, GA	Savannah
15	*E.jayensis*	Flat Spike	Lea 1838	Florida	
	E.judithae	Plicate Spike	Clarke 1986	Neuse River, NC	Neuse
	E.lanceolata	Yellow Lance	Lea 1828	Tar River, NC	Tar
	E.lugubris	Sad Elliptio	Lea 1834	Hopeton, NC	Altamaha
	E.marsupiobesa	Cape Fear Spike	Fuller 1972	Caper Fear River, NC	Cape Fear
16	*E.mcmichaeli*	Fluted Elephantear	Clench & Turner 1956	Choctawhatchee River, FL	Choctawhatchee
17	*E.mediocris*	N/A	Lea 1863	Neuse River, NC	Neuse
	E.monroensis	St. John's Elephantear	Lea 1843	Lake Monroe, FL	Cape Fear
18	*E.nasutilus*	N/A	Lea 1863	Livingston's Creek, NC	Cape Fear

Table 1. Continued

	Species	Common Name	Authority	Type locality	Type basin
	E.nigella	Winged Spike	Lea 1952	Chattahoochee River, GA	Chattahoochee
19	*E.occulta*	Hidden Spike	Lea 1843	Black Creek, FL	St. Johns River basin
20	*E.producta*	Atlantic Spike	Conrad 1836	Savannah River, GA	Savannah
21	*E.pullata*	Gulf Spike	Lea 1856	creeks near Columbus, GA	Chattahoochee
22	*E.purpurella*	Inflated Spike	Lea 1857	Flint River, GA	Flint
	E.raveneli	Carolina Spike	Conrad 1834	Wateree Canal, SC	Santee-Cooper
23	*E.roanokensis*	Roanoke Slabshell	Lea 1838	Roanoke River, NC	Roanoke
24	*E.shepardiana*	Altamaha Lance	Lea 1834	Altamaha River, GA	Altamaha
25	*E.spinosa*	Altamaha Spinymussel	Lea 1836	Altamaha River, GA	Altamaha
26	*E.steinstansana*	Tar River Spinymussel	Johnson & Clark 1983	Tar River, NC	Tar
27	*E.waccamawensis*	Waccamaw Spike	Lea 1863	Lake Waccamaw	Waccamaw

Table 2. Sequences obtained from previous Gangloff lab projects. Geographic information is provided. I.D. indicates individual identifier. GPS coordinates and additional metadata are available for non-listed species upon request.

Marker	Genus	Species	State	Waterbody	Basin	I.D.
COI	*Elliptio*	*dilatata*	NC	South Fork of the New River	New	TF16
COI	*Elliptio*	*dilatata*	NC	South Fork of the New River	New	TF18
ND1	*Elliptio*	*fisheriana*	NC	Chowan River	Chowan	EF160426-1trb2
COI	*Elliptio*	*jayensis*	FL	Santa Fe River	Suwannee	SFR34
ND1	*Elliptio*	*jayensis*	FL	Santa Fe River	Suwannee	SFR34
COI	*Elliptio*	*jayensis*	FL	Santa Fe River	Suwannee	SFR35
ND1	*Elliptio*	*jayensis*	FL	Santa Fe River	Suwannee	SFR35
COI	*Elliptio*	*jayensis*	FL	Santa Fe River	Suwannee	SFR37
ND1	*Elliptio*	*jayensis*	FL	Santa Fe River	Suwannee	SFR37
COI	*Elliptio*	*jayensis*	FL	Santa Fe River	Suwannee	SFR41
ND1	*Elliptio*	*jayensis*	FL	Santa Fe River	Suwannee	SFR41
COI	*Elliptio*	*jayensis*	FL	Santa Fe River	Suwannee	SFR44
ND1	*Elliptio*	*jayensis*	FL	Santa Fe River	Suwannee	SFR44
COI	*Elliptio*	*jayensis*	FL	Santa Fe River	Suwannee	SFR45
ND1	*Elliptio*	*jayensis*	FL	Santa Fe River	Suwannee	SFR45
COI	*Elliptio*	*jayensis*	FL	Santa Fe River	Suwannee	SFR46
ND1	*Elliptio*	*jayensis*	FL	Santa Fe River	Suwannee	SFR46
COI	*Elliptio*	*jayensis*	FL	Santa Fe River	Suwannee	SFR47
ND1	*Elliptio*	*jayensis*	FL	Santa Fe River	Suwannee	SFR47
COI	*Elliptio*	*occulta*	FL	Santa Fe River	Suwannee	SFR1
ND1	*Elliptio*	*occulta*	FL	Santa Fe River	Suwannee	SFR1
COI	*Elliptio*	*occulta*	FL	Santa Fe River	Suwannee	SFR11
ND1	*Elliptio*	*occulta*	FL	Santa Fe River	Suwannee	SFR11
COI	*Elliptio*	*occulta*	FL	Santa Fe River	Suwannee	SFR12
ND1	*Elliptio*	*occulta*	FL	Santa Fe River	Suwannee	SFR12
COI	*Elliptio*	*occulta*	FL	Santa Fe River	Suwannee	SFR15
ND1	*Elliptio*	*occulta*	FL	Santa Fe River	Suwannee	SFR15
COI	*Elliptio*	*occulta*	FL	Santa Fe River	Suwannee	SFR16
ND1	*Elliptio*	*occulta*	FL	Santa Fe River	Suwannee	SFR16
COI	*Elliptio*	*occulta*	FL	Santa Fe River	Suwannee	SFR17
ND1	*Elliptio*	*occulta*	FL	Santa Fe River	Suwannee	SFR17
COI	*Elliptio*	*occulta*	FL	Santa Fe River	Suwannee	SFR18
ND1	*Elliptio*	*occulta*	FL	Santa Fe River	Suwannee	SFR18
COI	*Elliptio*	*occulta*	FL	Santa Fe River	Suwannee	SFR19

Table 2. Continued

Marker	Genus	Species	State	Waterbody	Basin	I.D.
ND1	*Elliptio*	*occulta*	FL	Santa Fe River	Suwannee	SFR19
COI	*Elliptio*	*occulta*	FL	Santa Fe River	Suwannee	SFR20
ND1	*Elliptio*	*occulta*	FL	Santa Fe River	Suwannee	SFR20
COI	*Elliptio*	*occulta*	FL	Santa Fe River	Suwannee	SFR21
ND1	*Elliptio*	*occulta*	FL	Santa Fe River	Suwannee	SFR21
COI	*Elliptio*	*occulta*	FL	Santa Fe River	Suwannee	SFR22
ND1	*Elliptio*	*occulta*	FL	Santa Fe River	Suwannee	SFR22
COI	*Elliptio*	*occulta*	FL	Santa Fe River	Suwannee	SFR23
ND1	*Elliptio*	*occulta*	FL	Santa Fe River	Suwannee	SFR23
COI	*Elliptio*	*occulta*	FL	Santa Fe River	Suwannee	SFR25
ND1	*Elliptio*	*occulta*	FL	Santa Fe River	Suwannee	SFR25
COI	*Elliptio*	*occulta*	FL	Santa Fe River	Suwannee	SFR26
ND1	*Elliptio*	*occulta*	FL	Santa Fe River	Suwannee	SFR26
COI	*Elliptio*	*occulta*	FL	Santa Fe River	Suwannee	SFR27
ND1	*Elliptio*	*occulta*	FL	Santa Fe River	Suwannee	SFR27
COI	*Elliptio*	*occulta*	FL	Santa Fe River	Suwannee	SFR28
ND1	*Elliptio*	*occulta*	FL	Santa Fe River	Suwannee	SFR28
COI	*Elliptio*	*occulta*	FL	Santa Fe River	Suwannee	SFR3
ND1	*Elliptio*	*occulta*	FL	Santa Fe River	Suwannee	SFR3
COI	*Elliptio*	*occulta*	FL	Santa Fe River	Suwannee	SFR30
ND1	*Elliptio*	*occulta*	FL	Santa Fe River	Suwannee	SFR30
COI	*Elliptio*	*occulta*	FL	Santa Fe River	Suwannee	SFR33
ND1	*Elliptio*	*occulta*	FL	Santa Fe River	Suwannee	SFR33
COI	*Elliptio*	*occulta*	FL	Santa Fe River	Suwannee	SFR4
ND1	*Elliptio*	*occulta*	FL	Santa Fe River	Suwannee	SFR4
COI	*Elliptio*	*occulta*	FL	Santa Fe River	Suwannee	SFR40
ND1	*Elliptio*	*occulta*	FL	Santa Fe River	Suwannee	SFR40
COI	*Elliptio*	*occulta*	FL	Santa Fe River	Suwannee	SFR42
ND1	*Elliptio*	*occulta*	FL	Santa Fe River	Suwannee	SFR42
COI	*Elliptio*	*occulta*	FL	Santa Fe River	Suwannee	SFR43
ND1	*Elliptio*	*occulta*	FL	Santa Fe River	Suwannee	SFR43
COI	*Elliptio*	*occulta*	FL	Santa Fe River	Suwannee	SFR48
ND1	*Elliptio*	*occulta*	FL	Santa Fe River	Suwannee	SFR48
COI	*Elliptio*	*occulta*	FL	Santa Fe River	Suwannee	SFR5
ND1	*Elliptio*	*occulta*	FL	Santa Fe River	Suwannee	SFR5
COI	*Elliptio*	*occulta*	FL	Santa Fe River	Suwannee	SFR7
ND1	*Elliptio*	*occulta*	FL	Santa Fe River	Suwannee	SFR7
COI	*Elliptio*	*occulta*	FL	Santa Fe River	Suwannee	SFR8
ND1	*Elliptio*	*occulta*	FL	Santa Fe River	Suwannee	SFR8
COI	*Elliptio*	*occulta*	FL	Santa Fe River	Suwannee	SFR9
ND1	*Elliptio*	*occulta*	FL	Santa Fe River	Suwannee	SFR9

Table 2. Continued

Marker	**Genus**	**Species**	**State**	**Waterbody**	**Basin**	**I.D.**
COI	*Elliptio*	*pullata*	FL	Chipola River	Apalachicola	MG12
ND1	*Elliptio*	*pullata*	FL	Chipola River	Apalachicola	MG12
COI	*Elliptio*	*pullata*	FL	Chipola River	Apalachicola	MG14
ND1	*Elliptio*	*pullata*	FL	Chipola River	Apalachicola	MG14
COI	*Elliptio*	*pullata*	FL	Chipola River	Apalachicola	MG15
ND1	*Elliptio*	*pullata*	FL	Chipola River	Apalachicola	MG15
COI	*Elliptio*	*pullata*	FL	Chipola River	Apalachicola	MG8
ND1	*Elliptio*	*pullata*	FL	Chipola River	Apalachicola	MG8
COI	*Elliptio*	*pullata*	FL	Chipola River	Apalachicola	MG9
ND1	*Elliptio*	*pullata*	FL	Chipola River	Apalachicola	MG9
COI	*Elliptio*	*spinosa*	GA	Altamaha River	Altamaha	Altamaha_001
ND1	*Elliptio*	*spinosa*	GA	Altamaha River	Altamaha	Altamaha_001
COI	*Elliptio*	*spinosa*	GA	Altamaha River	Altamaha	Altamaha_002
ND1	*Elliptio*	*spinosa*	GA	Altamaha River	Altamaha	Altamaha_002
COI	*Elliptio*	*spinosa*	GA	Altamaha River	Altamaha	Altamaha_003
ND1	*Elliptio*	*spinosa*	GA	Altamaha River	Altamaha	Altamaha_003
COI	*Elliptio*	*spinosa*	GA	Altamaha River	Altamaha	Altamaha_004
ND1	*Elliptio*	*spinosa*	GA	Altamaha River	Altamaha	Altamaha_004
COI	*Elliptio*	*spinosa*	GA	Altamaha River	Altamaha	Altamaha_005
ND1	*Elliptio*	*spinosa*	GA	Altamaha River	Altamaha	Altamaha_005
COI	*Elliptio*	*spinosa*	GA	Altamaha River	Altamaha	Altamaha_006
ND1	*Elliptio*	*spinosa*	GA	Altamaha River	Altamaha	Altamaha_006
COI	*Elliptio*	*spinosa*	GA	Altamaha River	Altamaha	Altamaha_007
ND1	*Elliptio*	*spinosa*	GA	Altamaha River	Altamaha	Altamaha_007
COI	*Elliptio*	*spinosa*	GA	Altamaha River	Altamaha	Altamaha_008
ND1	*Elliptio*	*spinosa*	GA	Altamaha River	Altamaha	Altamaha_008
COI	*Elliptio*	*steinstansana*	NC	Fishing Creek	Tar	B297
ND1	*Elliptio*	*steinstansana*	NC	Fishing Creek	Tar	B297
COI	*Elliptio*	*steinstansana*	NC	Fishing Creek	Tar	B300
ND1	*Elliptio*	*steinstansana*	NC	Fishing Creek	Tar	B300
COI	*Elliptio*	*steinstansana*	NC	Fishing Creek	Tar	B301
ND1	*Elliptio*	*steinstansana*	NC	Fishing Creek	Tar	B301
COI	*Elliptio*	*steinstansana*	NC	Fishing Creek	Tar	B624
ND1	*Elliptio*	*steinstansana*	NC	Fishing Creek	Tar	B624
COI	*Elliptio*	*steinstansana*	NC	Fishing Creek	Tar	B626
ND1	*Elliptio*	*steinstansana*	NC	Fishing Creek	Tar	B626
COI	*Elliptio*	*steinstansana*	NC	Fishing Creek	Tar	B628
ND1	*Elliptio*	*steinstansana*	NC	Fishing Creek	Tar	B628
COI	*Elliptio*	*steinstansana*	NC	Fishing Creek	Tar	B629
ND1	*Elliptio*	*steinstansana*	NC	Fishing Creek	Tar	B629

Table 2. Continued

Marker	**Genus**	**Species**	**State**	**Waterbody**	**Basin**	**I.D.**
COI	*Elliptio*	*steinstansana*	NC	Fishing Creek	Tar	B631
ND1	*Elliptio*	*steinstansana*	NC	Fishing Creek	Tar	B631
COI	*Elliptio*	*steinstansana*	NC	Fishing Creek	Tar	B638
ND1	*Elliptio*	*steinstansana*	NC	Fishing Creek	Tar	B638
COI	*Elliptio*	*steinstansana*	NC	Fishing Creek	Tar	B640
ND1	*Elliptio*	*steinstansana*	NC	Fishing Creek	Tar	B640
COI	*Elliptio*	*steinstansana*	NC	Fishing Creek	Tar	F392
ND1	*Elliptio*	*steinstansana*	NC	Fishing Creek	Tar	F392
COI	*Elliptio*	*steinstansana*	NC	Fishing Creek	Tar	F896
ND1	*Elliptio*	*steinstansana*	NC	Fishing Creek	Tar	F896
COI	*Elliptio*	*steinstansana*	NC	Fishing Creek	Tar	F898
ND1	*Elliptio*	*steinstansana*	NC	Fishing Creek	Tar	F898
COI	*Elliptio*	*steinstansana*	NC	Fishing Creek	Tar	NOTCH
ND1	*Elliptio*	*steinstansana*	NC	Fishing Creek	Tar	NOTCH
COI	*Elliptio*	*steinstansana*	NC	Fishing Creek	Tar	TRSM6-2A
ND1	*Elliptio*	*steinstansana*	NC	Fishing Creek	Tar	TRSM6-2A
COI	*Elliptio*	*steinstansana*	NC	Neuse River	Tar	TRSM7-16
ND1	*Elliptio*	*steinstansana*	NC	Neuse River	Tar	TRSM7-16
COI	*Elliptio*	*steinstansana*	NC	Fishing Creek	Tar	TRSM7-3A
ND1	*Elliptio*	*steinstansana*	NC	Fishing Creek	Tar	TRSM7-3A
COI	*Elliptio*	*steinstansana*	NC	Fishing Creek	Tar	TRSM7-3B
ND1	*Elliptio*	*steinstansana*	NC	Fishing Creek	Tar	TRSM7-3B

Table 3. Sequences obtained from Genbank. Author of publication in which sequence was used is provided. I.D.'s (individual identifiers) and geographic information were obtained from the literature. Geographic information is only provided for the *Elliptio sensu stricto* group.

Marker	**Genus**	**Species**	**Basin**	**Genbank**	**I.D.**	**Author**
COI	*Elliptio*	*angustata*	Santee-Cooper	EU448167	AUM9725	Gangloff et al. unpublished
COI	*Elliptio*	*angustata*	Pee Dee	EU448166	AUM9741A	Gangloff et al. unpublished
COI	*Elliptio*	*arca*	Mobile	AY654995	UAUC498	Campbell et al 2005
COI	*Elliptio*	*arctata*	Mobile	EU448168	AUM9400	Gangloff et al. unpublished
COI	*Elliptio*	*arctata*	Apalachicola	EU448170	AUM9662	Gangloff et al. unpublished
COI	*Elliptio*	*arctata*	Cape Fear	EU448169	AUM9719	Gangloff et al. unpublished
COI	*Elliptio*	*complanata*	Apalachicola	EU448172	AUM9682	Gangloff et al. unpublished
COI	*Elliptio*	*complanata*	York	EU448183	AUM9706A	Gangloff et al. unpublished
COI	*Elliptio*	*complanata*	James	EU448173	AUM9711	Gangloff et al. unpublished
COI	*Elliptio*	*complanata*	James	EU448174	AUM9712B	Gangloff et al. unpublished
COI	*Elliptio*	*complanata*	James	EU448175	AUM9713A	Gangloff et al. unpublished
COI	*Elliptio*	*complanata*	James	EU448176	AUM9713B	Gangloff et al. unpublished
COI	*Elliptio*	*complanata*	James	EU448177	AUM9713C	Gangloff et al. unpublished
COI	*Elliptio*	*complanata*	Santee-Cooper	EU448180	AUM9729	Gangloff et al. unpublished
COI	*Elliptio*	*complanata*	Santee-Cooper	EU448181	AUM9730	Gangloff et al. unpublished
COI	*Elliptio*	*complanata*	Neuse	EU448179	AUM9757C	Gangloff et al. unpublished
COI	*Elliptio*	*congaraea*	Pee Dee	EU448187	AUM9740	Gangloff et al. unpublished
COI	*Elliptio*	*congaraea*	Neuse	EU448186	AUM9763	Gangloff et al. unpublished
COI	*Elliptio*	*congaraea*	Neuse	EU448186	AUM9862	Gangloff et al. unpublished
COI	*Elliptio*	*crassidens*	Mississippi	EU377567	UAM3527	Gangloff et al. unpublished
COI	*Elliptio*	*crassidens*	Mississippi	AY613820	UAUC1493	Gangloff et al. unpublished

Table 3. Continued

Marker	Genus	Species	Basin	Genbank	I.D.	Author
COI	*Elliptio*	*crassidens*	Mobile	DQ383428	UAUC3150	Gangloff et al. unpublished
COI	*Elliptio*	*dilatata*		KF035280	Ed 01	Inoue et al. 2013
COI	*Elliptio*	*folliculata*	Pee Dee	EU448189	AUM9749	Gangloff et al. unpublished
COI	*Elliptio*	*folliculata*	Pee Dee	EU448189	AUM9749	Gangloff et al. unpublished
COI	*Elliptio*	*icterina*	York	EU448198	AUM9708	Gangloff et al. unpublished
COI	*Elliptio*	*icterina*	Pee Dee	EU448193	AUM9744B	Gangloff et al. unpublished
COI	*Elliptio*	*icterina*	Neuse	EU448191	AUM9861A	Gangloff et al. unpublished
COI	*Elliptio*	*mcmichaeli*	Choctawhatchee	EU448199	AUM9467	Gangloff et al. unpublished
COI	*Elliptio*	*mcmichaeli*	Choctawhatchee	EU377572	UAM3516	Gangloff et al. unpublished
COI	*Elliptio*	*mcmichaeli*	Choctawhatchee	EU377573	UAUC3088	Campbell and Lydeard 2012
COI	*Elliptio*	*nasutilus*	Pee Dee	EU448201	AUM9745B	Gangloff et al. unpublished
COI	*Elliptio*	*pullata*	Mobile	EU377571	A56	Gangloff et al. unpublished
COI	*Elliptio*	*purpurella*	Mobile	EU377574	UAUC3569	Gangloff et al. unpublished
COI	*Elliptio*	*waccamawensis*	Pee Dee	EU448202	AUM9746A	Gangloff et al. unpublished
ND1	*Elliptio*	*angustata*	Santee-Cooper	EU448204	AUM9725	Gangloff et al. unpublished
ND1	*Elliptio*	*angustata*	Pee Dee	EU448203	AUM9741A	Gangloff et al. unpublished
ND1	*Elliptio*	*arctata*	Mobile	EU448205	AUM9400	Gangloff et al. unpublished
ND1	*Elliptio*	*arctata*	Apalachicola	EU448206	AUM9662	Gangloff et al. unpublished
ND1	*Elliptio*	*arctata*	Mobile	JF326440	UAUC3496	Campbell and Lydeard 2012
ND1	*Elliptio*	*buckleyi*	Withlacoochee	EU448207	AUM14923	Gangloff et al. unpublished
ND1	*Elliptio*	*complanata*	Apalachicola	EU448208	AUM9401	Gangloff et al. unpublished
ND1	*Elliptio*	*complanata*	Apalachicola	EU448209	AUM9682	Gangloff et al. unpublished
ND1	*Elliptio*	*complanata*	York	EU448222	AUM9706A	Gangloff et al. unpublished
ND1	*Elliptio*	*complanata*	York	EU448223	AUM9706B	Gangloff et al. unpublished
ND1	*Elliptio*	*complanata*	York	EU448224	AUM9707A	Gangloff et al. unpublished

Table 3. Continued

Marker	Genus	Species	Basin	Genbank	I.D.	Author
ND1	*Elliptio*	*complanata*	York	EU448225	AUM9707B	Gangloff et al. unpublished
ND1	*Elliptio*	*complanata*	York	EU448244	AUM9709	Gangloff et al. unpublished
ND1	*Elliptio*	*complanata*	James	EU448210	AUM9711	Gangloff et al. unpublished
ND1	*Elliptio*	*complanata*	James	EU448211	AUM9712A	Gangloff et al. unpublished
ND1	*Elliptio*	*complanata*	James	EU448212	AUM9712B	Gangloff et al. unpublished
ND1	*Elliptio*	*complanata*	James	EU448213	AUM9713A	Gangloff et al. unpublished
ND1	*Elliptio*	*complanata*	James	EU448214	AUM9713B	Gangloff et al. unpublished
ND1	*Elliptio*	*complanata*	James	EU448215	AUM9713C	Gangloff et al. unpublished
ND1	*Elliptio*	*complanata*	Santee-Cooper	EU448219	AUM9729	Gangloff et al. unpublished
ND1	*Elliptio*	*complanata*	Santee-Cooper	EU448220	AUM9730	Gangloff et al. unpublished
ND1	*Elliptio*	*complanata*	Neuse	EU448218	AUM9757C	Gangloff et al. unpublished
ND1	*Elliptio*	*complanata*	Neuse	EU448216	AUM9761A	Gangloff et al. unpublished
ND1	*Elliptio*	*complanata*	Tar	EU448217	AUM9761B	Gangloff et al. unpublished
ND1	*Elliptio*	*complanata*	Tar	EU448221	AUM9868	Gangloff et al. unpublished
ND1	*Elliptio*	*congaraea*	Pee Dee	EU448228	AUM9740	Gangloff et al. unpublished
ND1	*Elliptio*	*congaraea*	Neuse	EU448226	AUM9763	Gangloff et al. unpublished
ND1	*Elliptio*	*congaraea*	Ogeechee	EU448227	AUM9790A	Gangloff et al. unpublished
ND1	*Elliptio*	*crassidens*	Escambia	EU448229	AUM8200	Gangloff et al. unpublished
ND1	*Elliptio*	*crassidens*	Altamaha	EU448230	AUM9403	Gangloff et al. unpublished
ND1	*Elliptio*	*crassidens*	Mississippi	JN180972	UAM3527	Burlakova et al. 2012
ND1	*Elliptio*	*crassidens*	Mobile	EU380665	UAM747	Gangloff et al. unpublished
ND1	*Elliptio*	*crassidens*	Mississippi	EU380668	UAUC 1493	Gangloff et al. unpublished
ND1	*Elliptio*	*crassidens*	Mobile	AY613788	UAUC3150	Gangloff et al. unpublished
ND1	*Elliptio*	*dilatata*		KF035420	Ed 01	Inoue et al. 2013
ND1	*Elliptio*	*folliculata*	Pee Dee	EU448231	AUM9749	Gangloff et al. unpublished
ND1	*Elliptio*	*hopetonensis*	Altamaha	EU448232	AUM9404	Gangloff et al. unpublished

Table 3. Continued

Marker	Genus	Species	Basin	Genbank	I.D.	Author
ND1	*Elliptio*	*icterina*	Ochlockonee	EU448238	AUM4564	Gangloff et al. unpublished
ND1	*Elliptio*	*icterina*	Ecofina	EU448235	AUM4567	Gangloff et al. unpublished
ND1	*Elliptio*	*icterina*	Escambia	EU448234	AUM7166	Gangloff et al. unpublished
ND1	*Elliptio*	*icterina*	York	EU448243	AUM9708	Gangloff et al. unpublished
ND1	*Elliptio*	*icterina*	Santee-Cooper	EU448242	AUM9728B	Gangloff et al. unpublished
ND1	*Elliptio*	*icterina*	Pee Dee	EU448239	AUM9741B	Gangloff et al. unpublished
ND1	*Elliptio*	*icterina*	Pee Dee	EU448240	AUM9744A	Gangloff et al. unpublished
ND1	*Elliptio*	*icterina*	Pee Dee	EU448241	AUM9744B	Gangloff et al. unpublished
ND1	*Elliptio*	*icterina*	Neuse	EU448236	AUM9861A	Gangloff et al. unpublished
ND1	*Elliptio*	*icterina*	Neuse	EU448237	AUM9861B	Gangloff et al. unpublished
ND1	*Elliptio*	*mcmichaeli*	Choctawhatchee	EU448248	AUM8205	Gangloff et al. unpublished
ND1	*Elliptio*	*mcmichaeli*	Choctawhatchee	EU448247	AUM9467	Gangloff et al. unpublished
ND1	*Elliptio*	*mcmichaeli*	Choctawhatchee	JF326441	UAUC3088	Campbell and Lydeard 2012
ND1	*Elliptio*	*nasutilus*	Pee Dee	EU448249	AUM9745A	Gangloff et al. unpublished
ND1	*Elliptio*	*nasutilus*	Pee Dee	EU448250	AUM9745B	Gangloff et al. unpublished
ND1	*Elliptio*	*nasutilus*	Tar	EU448251	AUM9866	Gangloff et al. unpublished
ND1	*Elliptio*	*purpurella*	Mobile	JF326442	UAUC3569	Campbell and Lydeard 2012
ND1	*Elliptio*	*shepardiana*	Altamaha	EU44852	AUM9405	Gangloff et al. unpublished
ND1	*Elliptio*	*waccamawensis*	Pee Dee	EU448253	AUM9746A	Gangloff et al. unpublished
ND1	*Elliptio*	*icterina*	Apalachicola	EU448233	AUM14713	Gangloff et al. unpublished
ND1	*Elliptio*	*jayensis*	St. John's Bay	EU448246	AUM149151	Gangloff et al. unpublished
ND1	*Elliptio*	*pullata*	Mobile	EU380666	A56	Gangloff et al. unpublished
ND1	*Elliptio*	*pullata*	Apalachicola	EU380667	A57	Gangloff et al. unpublished
COI	*Fusconaia*	*askewi*		JN180994	Sab1	Burlakova et al 2012
COI	*Fusconaia*	*askewi*		JN180995	Sab2	Burlakova et al 2012
COI	*Fusconaia*	*askewi*		JN180998	Sab5	Burlakova et al 2012

Table 3. Continued

Marker	Genus	Species	Basin	Genbank	I.D.	Author
COI	*Fusconaia*	*askewi*		HM230367	UAM3392	Campbell and Lydeard 2012b
COI	*Fusconaia*	*barnesiana*	AY613822	UAUC1553	Campbell et al 2005	
COI	*Fusconaia*	*cerina*		AF049522	UAUC3234	Roe and Lydeard 1998
COI	*Fusconaia*	*cor*		AY654997	UAUC2606	Campbell et al 2005
COI	*Fusconaia*	*cuneolus*		AY654998	UAUC1552	Campbell et al 2005
COI	*Fusconaia*	*lananensis*		JN180987	TS129	Burlakova et al 2012
COI	*Fusconaia*	*lananensis*		JN180984	TS179	Burlakova et al 2012
COI	*Fusconaia*	*lananensis*		JN180985	TS203	Burlakova et al 2012
COI	*Fusconaia*	*masoni*		HM230371	NCSMH	Campbell and Lydeard 2012b
COI	*Fusconaia*	*ozarkensis*		HM230373	UAM3501	Campbell and Lydeard 2012b
COI	*Fusconaia*	*subrotunda*		JN181001	PA1	Burlakova et al 2012
COI	*Fusconaia*	*subrotunda*		JN181002	Pas	Burlakova et al 2012
COI	*Fusconaia*	*subrotunda*		AY613824	UAUC1554	Campbell et al 2005
ND1	*Fusconaia*	*askewi*		JN180975	Sab1	Burlakova et al 2012
ND1	*Fusconaia*	*askewi*		JN180976	Sab2	Burlakova et al 2012
ND1	*Fusconaia*	*askewi*		JN180977	Sab5	Burlakova et al 2012
ND1	*Fusconaia*	*askewi*		HM230411	UAM3392	Campbell et al 2008
ND1	*Fusconaia*	*barnesiana*		AY613791	UAUC1553	Campbell et al 2005
ND1	*Fusconaia*	*cerina*		AY613792	UAUC3234	Campbell et al. 2005
ND1	*Fusconaia*	*cor*		AY655096	UAUC2606	Campbell et al 2005
ND1	*Fusconaia*	*cuneolus*		AY655097	UAUC1552	Campbell et al 2005
ND1	*Fusconaia*	*lananensis*		JN180980	TS129	Burlakova et al 2012
ND1	*Fusconaia*	*lananensis*		JN180981	TS179	Burlakova et al 2012
ND1	*Fusconaia*	*lananensis*		JN180982	TS203	Burlakova et al 2012
ND1	*Fusconaia*	*masoni*		HM230415	NCSMH	Campbell et al 2008
ND1	*Fusconaia*	*ozarkensis*		HM230416	UAM3501	Campbell et al 2008
ND1	*Fusconaia*	*subrotunda*		JN180978	Pal	Burlakova et al 2012
ND1	*Fusconaia*	*subrotunda*		JN180979	PAs	Burlakova et al 2012
ND1	*Fusconaia*	*subrotunda*		AY613794	UAUC1554	Campbell et al 2005
COI	*Lampsilis*	*virescens*		JF326433	clip	Campbell and Lydeard 2012a
ND1	*Lampsilis*	*virescens*		JF326443	clip	Campbell and Lydeard 2012a
COI	*Obovaria*	*jacksoniana*		KF035135	Oj01	Inoue et al 2013
COI	*Obovaria*	*jacksoniana*		KF035138	Oj04	Inoue et al 2013
ND1	*Obovaria*	*jacksoniana*		KF035283	Oj01	Inoue et al 2013
ND1	*Obovaria*	*jacksoniana*		KF035286	Oj04	Inoue et al 2013
COI	*Pleurobema*	*athearni*		AY655015	UAUC3084	Campbell et al 2005

Table 3. Continued

Marker	**Genus**	**Species**	**Basin**	**Genbank**	**I.D.**	**Author**
COI	*Pleurobema*	*beadlianum*		DQ383429	Pbead1	Campbell et al 2008
COI	*Pleurobema*	*chattanoogaense*		AY655012	UAUC1621	Campbell et al 2005
COI	*Pleurobema*	*chattanoogaense*		AY613829	UAUC3194	Campbell et al 2005
COI	*Pleurobema*	*clava*		AY655013	UAUC1477	Campbell et al 2005
COI	*Pleurobema*	*collina*		AY613830	UAUC1074	Campbell et al 2005
COI	*Pleurobema*	*cordatum*		AY613831	UAUC2572	Campbell et al 2005
COI	*Pleurobema*	*decisum*		AY613832	UAUC3196	Campbell et al 2005
COI	*Pleurobema*	*furvum*		AY613833	UAUC678	Campbell et al 2005
COI	*Pleurobema*	*georgianum*		AY613834	UAUC3193	Campbell et al 2005
COI	*Pleurobema*	*gibberum*		AY613835	UAUC3319	Campbell et al 2005
COI	*Pleurobema*	*hanleyianum*		AY613836	UAUC1622	Campbell et al 2005
COI	*Pleurobema*	*hanleyianum*		AY655016	UAUC273	Campbell et al 2005
COI	*Pleurobema*	*oviforme*		AY613837	UAUC1642	Campbell et al 2005
COI	*Pleurobema*	*perovatum*		AY613838	UAUC1640	Campbell et al 2005
COI	*Pleurobema*	*pyriforme*		AY613839	A29	Campbell et al 2005
COI	*Pleurobema*	*rubellum*		AY613840	UAUC679	Campbell et al 2005
COI	*Pleurobema*	*rubrum*		AY655018	UAUC2719	Campbell et al 2005
COI	*Pleurobema*	*rubrum*		AY613841	UAUC3229	Campbell et al 2005
COI	*Pleurobema*	*sintoxia*		AY655019	UAUC1714	Campbell et al 2005
COI	*Pleurobema*	*stabile*		AY613842	Pstab1	Campbell et al 2008
COI	*Pleurobema*	*strodeanum*		AY613843	UAUC1110	Campbell et al 2005
COI	*Pleurobema*	*taitianum*		AY613844	UAUC885	Campbell et al 2005
COI	*Pleurobema*	*troschelianum*		AY613845	UAUC516	Campbell et al 2005
ND1	*Pleurobema*	*athearni*		AY655114	UAUC3084	Campbell et al 2005
ND1	*Pleurobema*	*beadlianum*		DQ385873	Pbead1	Campbell et al 2008
ND1	*Pleurobema*	*chattanoogaense*		AY655111	UAUC1621	Campbell et al 2005
ND1	*Pleurobema*	*chattanoogaense*		AY613801	UAUC3194	Campbell et al 2005
ND1	*Pleurobema*	*clava*		AY613802	UAUC1477	Campbell et al 2005
ND1	*Pleurobema*	*collina*		AY613803	UAUC1074	Campbell et al 2005
ND1	*Pleurobema*	*cordatum*		AY613804	UAUC2572	Campbell et al 2005
ND1	*Pleurobema*	*decisum*		AY613805	UAUC3196	Campbell et al 2005
ND1	*Pleurobema*	*furvum*		AY613806	UAUC678	Campbell et al 2005
ND1	*Pleurobema*	*georgianum*		AY613807	UAUC3193	Campbell et al 2005
ND1	*Pleurobema*	*gibberum*		DQ385874	UAUC3319	Campbell et al 2008
ND1	*Pleurobema*	*hanleyianum*		AY613809	UAUC1622	Campbell et al 2005
ND1	*Pleurobema*	*hanleyianum*		AY655115	UAUC273	Campbell et al 2005
ND1	*Pleurobema*	*oviforme*		AY655116	UAUC1642	Campbell et al 2005
ND1	*Pleurobema*	*perovatum*		AY613811	UAUC1640	Campbell et al 2005
ND1	*Pleurobema*	*pyriforme*		AY613812	A29	Campbell et al 2005
ND1	*Pleurobema*	*rubellum*		AY613813	UAUC679	Campbell et al 2005
ND1	*Pleurobema*	*rubrum*		AY655117	UAUC2719	Campbell et al 2005

Table 3. Continued

Marker	Genus	Species	Basin	Genbank	I.D.	Marker
ND1	*Pleurobema*	*rubrum*		AY613814	UAUC3229	Campbell et al 2005
ND1	*Pleurobema*	*sintoxia*		AY613815	UAUC1714	Campbell et al 2005
ND1	*Pleurobema*	*stabile*		AY613816	Pstab1	Campbell et al 2008
ND1	*Pleurobema*	*strodeanum*		AY613817	UAUC1110	Campbell et al 2005
ND1	*Pleurobema*	*taitianum*		AY613818	UAUC885	Campbell et al 2005
ND1	*Pleurobema*	*troschelianum*		AY613819	UAUC516	Campbell et al 2005
COI	*Reginaia*	*ebena*		KF035133	Fe01 White	Inoue et al 2013
COI	*Reginaia*	*ebena*		AY654999	UAUC71	Campbell et al 2005
ND1	*Reginaia*	*ebena*		KF035281	Fe01 White	Inoue et al 2013
ND1	*Reginaia*	*ebena*		AY655098	UAUC71	Campbell et al 2005
COI	*Strophitus*	*subvexus*		AY655021	UAUC2715	Campbell et al 2005
ND1	*Strophitus*	*subvexus*		AY655122	UAUC2716	Campbell et al 2005
COI	*Toxolasma*	*parvus*		AY655022	UAUC3331	Campbell et al. 2005
ND1	*Toxolasma*	*parvus*		AY655123	UAUC3331	Campbell et al. 2005
COI	*Villosa*	*arkansasensis*		KF035228	Va21 Mfork	Inoue et al 2013
COI	*Villosa*	*fabalis*		DQ220726	Vfab1	Zannatta and Murphy 2006
COI	*Villosa*	*villosa*		AF385109	UAUC652	Roe et al. 2001
ND1	*Villosa*	*arkansasensis*		KF035372	Va21 Mfork	Inoue et al 2013
ND1	*Villosa*	*fabalis*		DQ220723	Vfab1	Zannatta and Murphy 2006
ND1	*Villosa*	*villosa*		AY094387	UAUC652	Buhay et al. 2002

Table 4. Sequences obtained for this study. Geographic information is provided. GPS coordinates and additional metadata is available upon request. I.D.s indicate individual identifiers.

Marker	**Genus**	**Species**	**State**	**Waterbody**	**Basin**	**I.D.**
COI	*Elliptio*	*complanata*	NC	Catawba River	Catawba	12
COI	*Elliptio*	*complanata*	NC	John's River	Catawba	55
COI	*Elliptio*	*complanata*	NC	John's River	Catawba	56
COI	*Elliptio*	*complanata*	NC	Catawba River	Catawba	010b
COI	*Elliptio*	*complanata*	NC	Catawba River	Catawba	011b
COI	*Elliptio*	*complanata*	NC	John's River	Catawba	18VIII20152-1
COI	*Elliptio*	*complanata*	NC	John's River	Catawba	18VIII20152-2
COI	*Elliptio*	*complanata*	NC	Rocky Swamp	Tar	20VIII20151-2
COI	*Elliptio*	*complanata*	NC	Rocky Swamp	Tar	20VIII20151-3
COI	*Elliptio*	*congaraea*	SC	Congaree River	Congaree	M6160419-1
COI	*Elliptio*	*congaraea*	SC	Congaree River	Congaree	M6160419-3
COI	*Elliptio*	*congaraea*	SC	Congaree River	Congaree	M6160419-4
COI	*Elliptio*	*crassidens*	FL	Yellow River	Apalachicola	12VIII20152-3
COI	*Elliptio*	*dilatata*	NC	South Fork of the New River	New	TF16
COI	*Elliptio*	*dilatata*	NC	South Fork of the New River	New	TF18
COI	*Elliptio*	*fisheriana*	NC	Little Fishing Creek	Tar	19VIII20151-1
COI	*Elliptio*	*mediocris*	NC	Little Fishing Creek	Tar	19VIII20151-21
COI	*Elliptio*	*mediocris*	NC	Little Fishing Creek	Tar	19VIII20151-22
COI	*Elliptio*	*producta*	NC	Waccamaw	Lumber	12VII20151-56
COI	*Elliptio*	*producta*	NC	Waccamaw	Lumber	12VII20151-66
COI	*Elliptio*	*producta*	NC	Waccamaw	Lumber	12VII20151-67
COI	*Elliptio*	*producta*	NC	Waccamaw	Lumber	12VIII20151-57
ND1	*Elliptio*	*ahenea*	FL	Suwannee River	Suwannee	MG5VI161-30
ND1	*Elliptio*	*chipoalensis*	FL	Chipola River	Apalachicola	MG25X2015-10
ND1	*Elliptio*	*complanata*	NC	Catawba River	Catawba	12
ND1	*Elliptio*	*complanata*	NC	John's River	Catawba	55
ND1	*Elliptio*	*complanata*	NC	John's River	Catawba	56
ND1	*Elliptio*	*complanata*	NC	Catawba River	Catawba	010b
ND1	*Elliptio*	*complanata*	NC	Catawba River	Catawba	011b
ND1	*Elliptio*	*complanata*	NC	John's River	Catawba	18VIII20152-1
ND1	*Elliptio*	*complanata*	NC	John's River	Catawba	18VIII20152-2
ND1	*Elliptio*	*complanata*	NC	John's River	Catawba	18VIII20152-3
ND1	*Elliptio*	*complanata*	NC	Rocky Swamp	Tar	20VIII20151-1
ND1	*Elliptio*	*complanata*	NC	Rocky Swamp	Tar	20VIII20151-2

Table 4. Continued

Marker	**Genus**	**Species**	**State**	**Waterbody**	**Basin**	**I.D.**
ND1	*Elliptio*	*complanata*	NC	Rocky Swamp	Tar	20VIII20151-3
ND1	*Elliptio*	*complanata*	NC	George's Mill	Roanoke	2IX20151-2
ND1	*Elliptio*	*complanata*	NC	George's Mill	Roanoke	2IX20151-3
ND1	*Elliptio*	*complanata*	ME	Royal River	Casco Bay	4IX20151-2
ND1	*Elliptio*	*complanata*	ME	Penobscot River	Penobscot	5IX20151-1
ND1	*Elliptio*	*complanata*	ME	Penobscot River	Penobscot	5IX20151-2
ND1	*Elliptio*	*complanata*	ME	Penobscot River	Penobscot	5IX20151-3
ND1	*Elliptio*	*complanata*	ME	Penobscot River	Penobscot	6IX20151-1
ND1	*Elliptio*	*complanata*	ME	East Musquash Lake	St. Croix	6IX20152-1
ND1	*Elliptio*	*complanata*	NC	PeeDee River	PeeDee	7VII20154-2
ND1	*Elliptio*	*complanata*	NC	PeeDee River	PeeDee	7VII20154-3
ND1	*Elliptio*	*complanata*	NC	PeeDee River	PeeDee	8VII20152-1
ND1	*Elliptio*	*complanata*	NC	PeeDee River	PeeDee	8VII20152-3
ND1	*Elliptio*	*complanata*	NC	Deep River	Cape Fear	MG8VII20157-1
ND1	*Elliptio*	*complanata*	NC	Deep River	Cape Fear	MG8VII20157-2
ND1	*Elliptio*	*congaraea*	NC	PeeDee River	PeeDee	7VII20153-2
ND1	*Elliptio*	*congaraea*	SC	Congaree River	Congaree	M6160419-1
ND1	*Elliptio*	*congaraea*	NC	PeeDee River	PeeDee	MG6VII20153-1
ND1	*Elliptio*	*crassidens*	FL	Yellow River	Apalachicola	12VIII20152-3
ND1	*Elliptio*	*fisheriana*	NC	Little Fishing Creek	Tar	19VIII20151-1
ND1	*Elliptio*	*fisheriana*	NC	Little Fishing Creek	Tar	19VIII20151-3
ND1	*Elliptio*	*fisheriana*	NC	Chowan River	Chowan	EF160426-1trb2
ND1	*Elliptio*	*icterina*	NC	Waccamaw	Lumber	12VIII20151-117
ND1	*Elliptio*	*icterina*	NC	Waccamaw	Lumber	12VIII20151-118
ND1	*Elliptio*	*icterina*	NC	Waccamaw	Lumber	12VIII20151-119
ND1	*Elliptio*	*icterina*	NC	PeeDee River	PeeDee	7VII20152-1
ND1	*Elliptio*	*icterina*	NC	PeeDee River	PeeDee	7VII20152-2
ND1	*Elliptio*	*icterina*	NC	PeeDee River	PeeDee	7VII20152-3
ND1	*Elliptio*	*icterina*	NC	PeeDee River	PeeDee	8VII20154-1
ND1	*Elliptio*	*icterina*	NC	PeeDee River	PeeDee	8VII20154-2
ND1	*Elliptio*	*icterina*	NC	PeeDee River	PeeDee	8VII20154-3
ND1	*Elliptio*	*icterina*	NC	Deep River	Cape Fear	MG8VII20157-3
ND1	*Elliptio*	*mediocris*	NC	Little Fishing Creek	Tar	19VIII20151-21
ND1	*Elliptio*	*mediocris*	NC	Little Fishing Creek	Tar	19VIII20151-22
ND1	*Elliptio*	*producta*	NC	PeeDee River	PeeDee	8VII20151-11
ND1	*Elliptio*	*pullata*	NC	Waccamaw	Lumber	10VIII2015-1
ND1	*Elliptio*	*pullata*	NC	Waccamaw	Lumber	10VIII2015-2
ND1	*Elliptio*	*roanokensis*	NC	PeeDee River	PeeDee	7VII20151-2
ND1	*Elliptio*	*roanokensis*	NC	PeeDee River	PeeDee	7VII20151-3
ND1	*Elliptio*	*roanokensis*	NC	PeeDee River	PeeDee	7VII20151-5

Table 4. Continued

Marker	**Genus**	**Species**	**State**	**Waterbody**	**Basin**	**I.D.**
ND1	*Elliptio*	*spp*	NC	Waccamaw	Lumber	12VII20151-35
ND1	*Elliptio*	*spp*	NC	Waccamaw	Lumber	12VII20151-36
ND1	*Elliptio*	*spp*	NC	Waccamaw	Lumber	12VII20151-37
ND1	*Elliptio*	*waccamawensis*	NC	Waccamaw	Lumber	12VIII20151-103
ND1	*Elliptio*	*waccamawensis*	NC	Waccamaw	Lumber	12VIII20151-104

Table 5. Intra-specific pairwise genetic distances of all three datasets. Pairwise genetic distances calculated using maximum composite likelihood method. "--" indicates no representative of a taxa in a given dataset. "N/A" indicates only one representative of a species in a given dataset.

	COI	**ND1**	**Concatenated**
E.ahenea	--	N/A	--
E.arca	N/A	--	--
E.arctata	0.014	0.013	N/A
E.angustata	N/A	0.011	N/A
E.buckleyi	--	N/A	--
E.chipolaensis	--	N/A	--
E.complanata	0.01	0.015	0.013
E.congaraea	0.015	0.017	0.017
E.crassidens	0.007	0.008	0.007
E.dilatata	0.004	N/A	N/A
E.fisheriana	N/A	0.063	N/A
E.folliculata	N/A	N/A	N/A
E.hopetonensis	--	N/A	--
E.icterina	0.01	0.021	0.013
E.jayensis	0.006	0.02	0.012
E.mcmichaeli	0.004	0.008	N/A
E.mediocris	0.012	0.016	0.016
E.nasutilus	N/A	0.004	N/A
E.occulta	0.007	0.011	0.01
E.producta	0	N/A	--
E.pullata	0.003	0.016	0.008
E.purpurella	N/A	N/A	--
E.roanokensis	--	0.001	--
E.shepardiana	--	N/A	--
E.spinosa	0.001	0.003	0.002
E.steinstansana	0.002	0.001	0.001
E.waccamawensis	N/A	0.019	--

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
1	-	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
2	0.083	-	0.046	0.039	N/A	N/A	0.038	0.039	0.031	0.065	0.043	0.039	0.034	N/A	0.041	0.034	0.041	0.046	0.043	0.046	0.033	0.035	N/A	N/A	0.054	0.066	0.035
3	N/A	N/A	-	0.021	N/A	N/A	0.023	0.027	0.022	0.071	0.048	0.024	0.022	N/A	0.030	0.019	0.026	0.056	0.032	0.051	0.017	0.026	N/A	N/A	0.066	0.075	0.019
4	0.062	0.095	N/A	-	N/A	N/A	0.014	0.017	0.013	0.068	0.068	0.047	0.013	N/A	0.023	0.011	0.017	0.054	0.024	0.049	0.010	0.017	N/A	N/A	0.057	0.071	0.011
5	0.033	0.075	N/A	0.046	-	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
6	0.097	0.116	N/A	0.115	0.103	-	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
7	0.036	0.074	N/A	0.045	0.016	0.099	-	0.014	0.011	0.071	0.048	0.013	0.011	N/A	0.023	0.009	0.014	0.054	0.024	0.050	0.008	0.019	N/A	N/A	0.057	0.072	0.009
8	0.035	0.075	N/A	0.048	0.019	0.107	0.020	-	0.012	0.073	0.045	0.018	0.014	N/A	0.025	0.011	0.015	0.056	0.026	0.049	0.011	0.023	N/A	N/A	0.061	0.070	0.011
9	0.037	0.080	N/A	0.053	0.008	0.105	0.023	0.027	-	0.069	0.044	0.015	0.009	N/A	0.021	0.007	0.012	0.053	0.022	0.048	0.006	0.019	N/A	N/A	0.053	0.068	0.007
10	0.125	0.123	N/A	0.140	0.130	0.136	0.128	0.127	0.130	-	0.068	0.074	0.065	N/A	0.074	0.069	0.074	0.073	0.075	0.072	0.066	0.070	N/A	N/A	0.082	0.083	0.068
11	0.086	0.074	N/A	0.104	0.084	0.122	0.082	0.080	0.081	0.108	-	0.044	0.045	N/A	0.038	0.044	0.049	0.023	0.040	0.015	0.042	0.051	N/A	N/A	0.057	0.071	0.044
12	0.045	0.088	N/A	0.056	0.037	0.114	0.038	0.041	0.044	0.129	0.092	-	0.016	N/A	0.020	0.013	0.019	0.056	0.021	0.051	0.011	0.019	N/A	N/A	0.054	0.070	0.013
13	0.041	0.077	N/A	0.054	0.026	0.118	0.026	0.017	0.034	0.134	0.084	0.045	-	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
14	0.036	0.073	N/A	0.047	0.017	0.103	0.019	0.021	0.023	0.126	0.081	0.039	0.025	-	0.022	0.007	0.012	0.050	0.024	0.045	0.007	0.020	N/A	N/A	0.053	0.070	0.007
15	0.019	0.080	N/A	0.057	0.033	0.102	0.036	0.037	0.037	0.122	0.082	0.044	0.040	0.036	-	0.019	0.024	0.054	0.049	0.017	0.017	0.025	N/A	N/A	0.046	0.077	0.019
16	0.040	0.080	N/A	0.056	0.010	0.108	0.025	0.028	0.008	0.132	0.081	0.046	0.035	0.025	0.040	-	0.011	0.051	0.020	0.046	0.004	0.019	N/A	N/A	0.054	0.071	0.004
17	0.034	0.073	N/A	0.048	0.015	0.099	0.016	0.020	0.023	0.126	0.076	0.036	0.025	0.018	0.034	0.024	-	0.058	0.025	0.054	0.010	0.021	N/A	N/A	0.061	0.077	0.011
18	0.088	0.077	N/A	0.105	0.084	0.125	0.083	0.080	0.084	0.118	0.029	0.097	0.080	0.081	0.085	0.086	0.077	-	0.055	0.008	0.049	0.058	N/A	N/A	0.066	0.084	0.051
19	0.012	0.079	N/A	0.057	0.029	0.099	0.033	0.034	0.033	0.125	0.081	0.042	0.037	0.032	0.018	0.036	0.030	0.084	-	0.050	0.018	0.027	N/A	N/A	0.049	0.078	0.020
20	0.086	0.011	N/A	0.093	0.075	0.118	0.074	0.075	0.079	0.123	0.076	0.088	0.073	0.073	0.082	0.080	0.073	0.080	0.081	-	0.044	0.058	N/A	N/A	0.061	0.074	0.046
21	0.037	0.077	N/A	0.051	0.012	0.104	0.022	0.026	0.013	0.127	0.079	0.043	0.032	0.023	0.036	0.013	0.021	0.083	0.032	0.077	-	0.017	N/A	N/A	0.051	0.069	0.004
22	0.039	0.075	N/A	0.040	0.024	0.108	0.028	0.030	0.028	0.130	0.088	0.043	0.035	0.028	0.039	0.030	0.029	0.082	0.035	0.077	0.029	-	N/A	N/A	0.060	0.079	0.019
23	0.038	0.078	N/A	0.049	0.023	0.113	0.023	0.014	0.030	0.130	0.081	0.041	0.075	0.022	0.037	0.031	0.022	0.081	0.034	0.074	0.029	0.031	-	N/A	N/A	N/A	N/A
24	0.079	0.009	N/A	0.095	0.073	0.116	0.072	0.073	0.078	0.125	0.074	0.087	0.098	0.071	0.077	0.077	0.071	0.081	0.075	0.016	0.075	0.071	0.076	-	N/A	N/A	N/A
25	0.095	0.101	N/A	0.105	0.091	0.122	0.097	0.097	0.095	0.122	0.103	0.115	0.029	0.096	0.095	0.099	0.092	0.100	0.093	0.108	0.095	0.085	0.094	0.106	-	0.082	0.054
26	0.125	0.125	N/A	0.126	0.129	0.139	0.126	0.131	0.132	0.132	0.132	0.131	0.075	0.071	0.122	0.136	0.124	0.122	0.123	0.130	0.132	0.118	0.133	0.127	0.143	-	0.071
27	0.040	0.074	N/A	0.047	0.016	0.099	0.017	0.024	0.023	0.127	0.086	0.042	0.032	0.022	0.039	0.025	0.022	0.084	0.036	0.075	0.025	0.031	0.029	0.073	0.097	0.126	-

Table 6. Inter-specific pairwise genetic distances of COI and ND1 datasets. Pairwise distances were calculated using maximum composite likelihood method. COI dataset distances are presented at the top. ND1 dataset distances are presented on the bottom. Numbers on the top row and first column represent taxa as listed in Table 1.

Table 7. Inter-specific pairwise genetic distances of concatenated dataset. Pairwise distances were calculated using maximum composite likelihood method. Numbers on the top row and first column represent taxa as listed in Table 1.

	2	**4**	**7**	**8**	**9**	**10**	**11**	**12**	**14**	**15**	**16**	**17**	**18**	**19**	**21**	**25**	**26**
2																	
4	0.077																
7	0.059	0.036															
8	0.061	0.040	0.018														
9	0.059	0.041	0.018	0.020													
10	0.102	0.114	0.107	0.105	0.106												
11	0.062	0.086	0.071	0.067	0.068	0.095											
12	0.068	0.043	0.028	0.031	0.033	0.109	0.073										
14	0.055	0.038	0.014	0.017	0.014	0.102	0.067	0.029									
15	0.067	0.047	0.032	0.032	0.032	0.103	0.066	0.035	0.031								
16	0.061	0.043	0.019	0.020	0.008	0.109	0.068	0.034	0.015	0.033							
17	0.061	0.042	0.016	0.017	0.019	0.106	0.068	0.030	0.012	0.031	0.019						
18	0.068	0.089	0.075	0.072	0.075	0.104	0.029	0.083	0.070	0.076	0.074	0.074					
19	0.066	0.047	0.030	0.030	0.030	0.105	0.066	0.034	0.029	0.012	0.031	0.029	0.076				
21	0.059	0.039	0.017	0.019	0.008	0.103	0.066	0.030	0.014	0.029	0.009	0.018	0.073	0.027			
25	0.085	0.089	0.083	0.083	0.081	0.111	0.085	0.092	0.082	0.077	0.083	0.082	0.087	0.077	0.078		
26	0.102	0.109	0.106	0.107	0.109	0.117	0.111	0.109	0.105	0.107	0.110	0.108	0.114	0.108	0.108	0.122	

Table 8. Intra-group pairwise genetic distances of all three datasets. Pairwise genetic distances calculated using maximum composite likelihood method. “--“ indicates no representative of a taxa in a given dataset. “N/A” indicates only one representative of a species in a given dataset.

	ND1	**COI**	**Concatenated**
Core	0.026	0.016	0.022
Lance	0.058	0.026	0.064
E.spinosa	0.003	0.001	0.002
E.steinstansana	0.001	0.002	0.001
E.dilatata	N/A	0.004	N/A
E.chipolaensis	N/A	--	--

Table 9. Inter-group pairwise genetic distances of COI dataset. Pairwise genetic distances calculated using maximum composite likelihood method.

	Core	**Lance**	***E.spinosa***	***E.steinstansana***	***E.dilatata***
Core					
Lance	0.044				
E.spinosa	0.053	0.059			
E.steinstansana	0.074	0.073	0.082		
E.dilatata	0.072	0.071	0.082	0.083	

Table 10. Inter-group pairwise genetic distances of ND1 dataset. Pairwise genetic distances calculated using maximum composite likelihood method.

	Core	**Lance**	***E.spinosa***	***E.steinstansana***	***E.dilatata***	***E.chipolaensis***
Core						
Lance	0.076					
E.spinosa	0.143	0.104				
E.steinstansana	0.126	0.127	0.143			
E.dilatata	0.076	0.12	0.122	0.132		
E.chipolaensis	0.102	0.12	0.122	0.139	0.136	

Table 11. Inter-group pairwise genetic distances of concatenated dataset. Pairwise genetic distances calculated using maximum composite likelihood method.

	Core	**Lance**	***E.spinosa***	***E.steinstansana***	***E.dilatata***
Core					
Lance	0.059				
E.spinosa	0.08	0.087			
E.steinstansana	0.107	0.109	0.122		
E.dilatata	0.105	0.103	0.111	0.117	

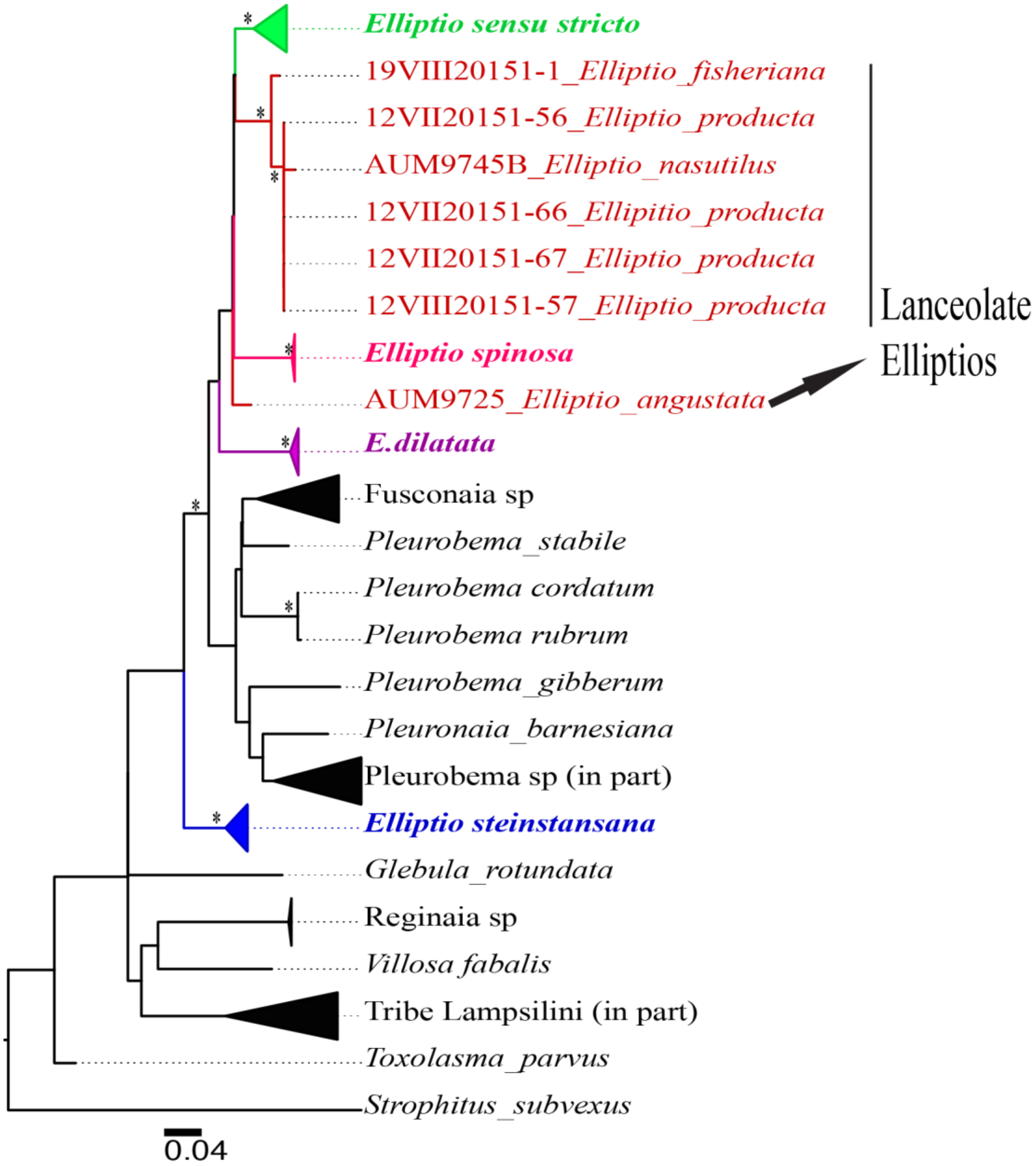

Figure 1. Maximum likelihood analysis of COI dataset. An * indicates bootstrap support >75. Scale bar indicates nucleotide substitutions per site. Dark blue represents *E. steinstansana*. Purple represents *E. dilatata*. Pink represents *E. spinosa*. Orange represents Lanceolate Elliptios. Green represents *Elliptio sensu stricto*.

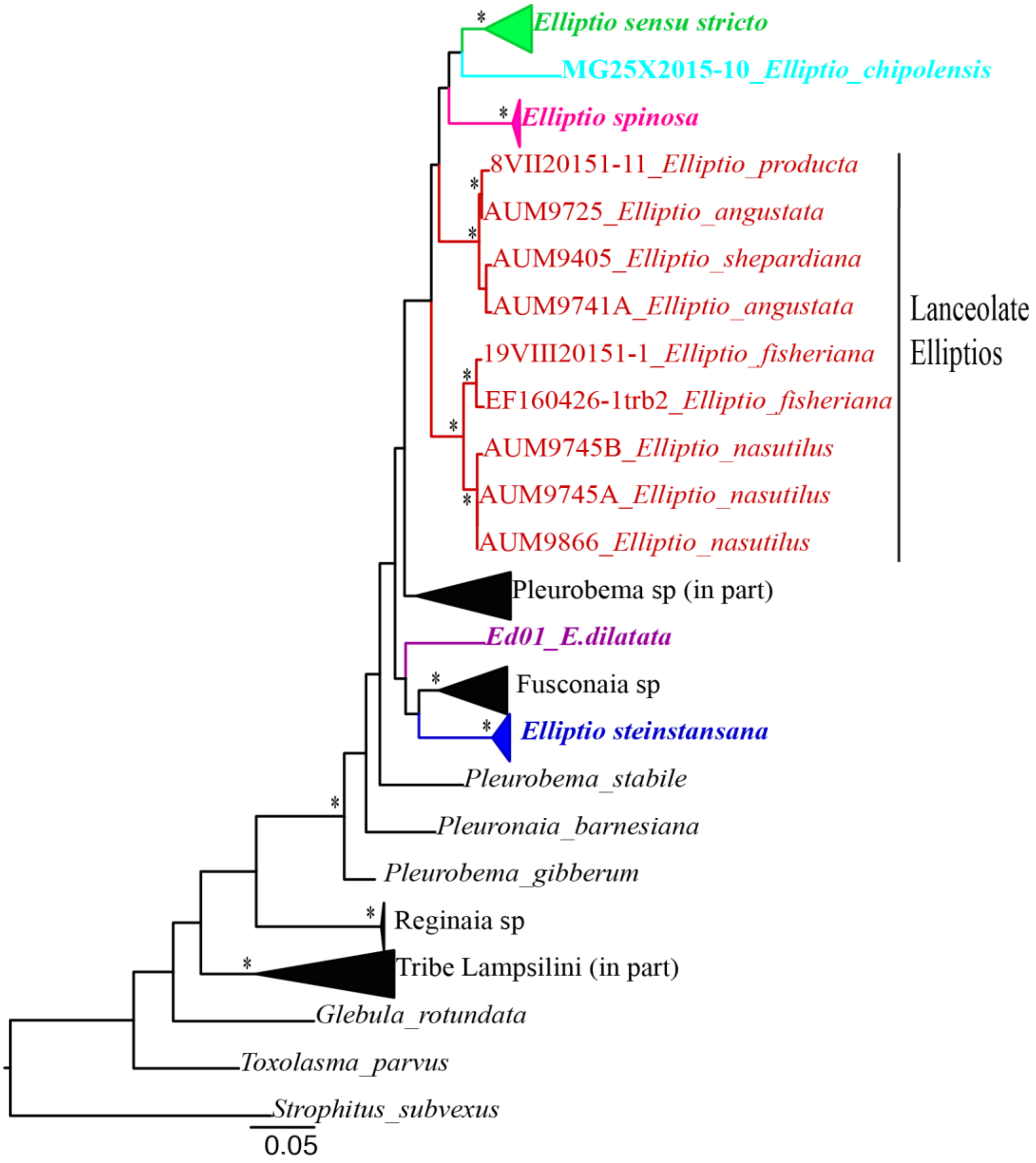

Figure 2. Maximum likelihood analysis of ND1 dataset. An * indicates bootstrap support >75. Scale bar indicates nucleotide substitutions per site. Dark blue represents *E. steinstansana*. Purple represents *E. dilatata*. Pink represents *E. spinosa*. Orange represents Lanceolate Elliptios. Green represents *Elliptio sensu stricto*. Light blue represents *E. chipolaensis*.

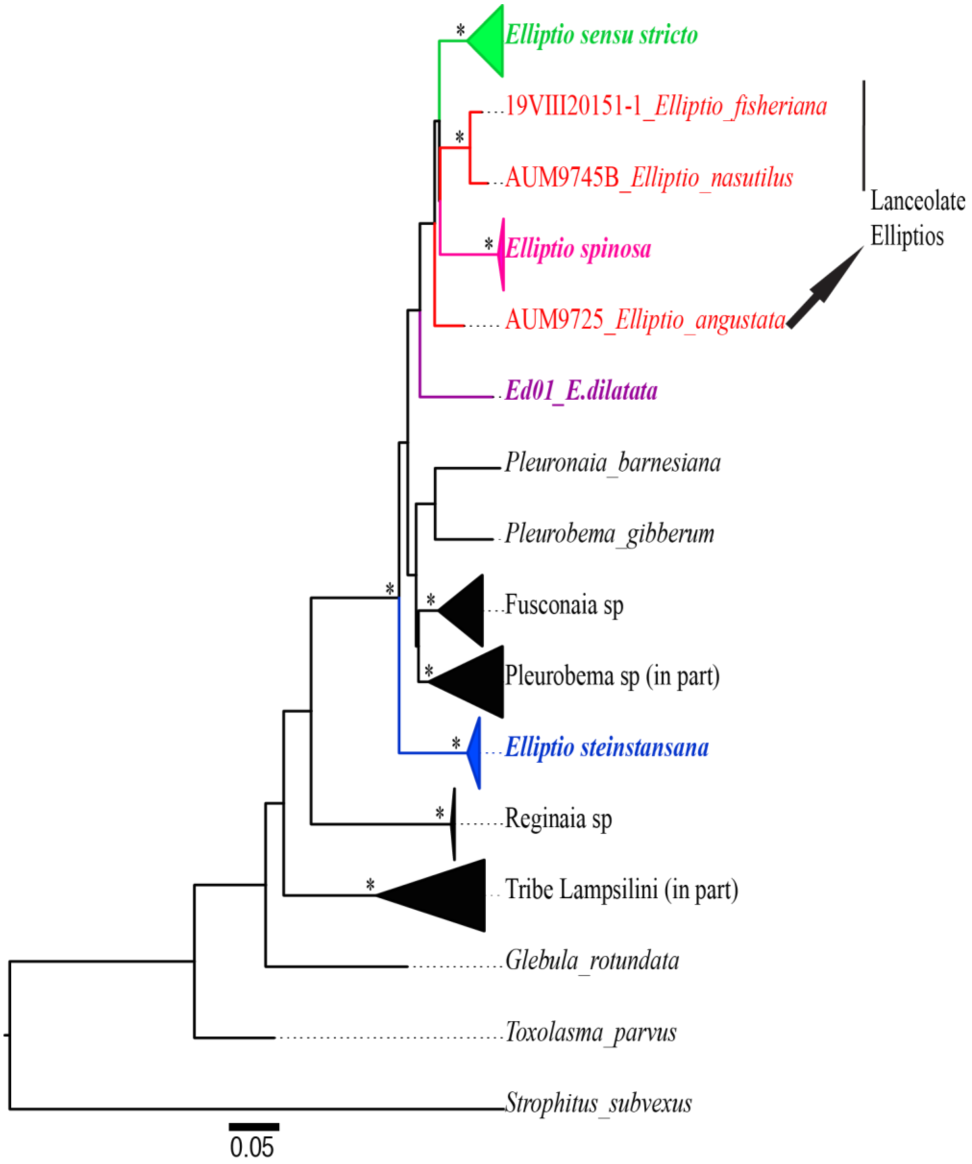

Figure 3. Maximum likelihood analysis of concatenated dataset. An * indicates bootstrap support >75. Scale bar indicates nucleotide substitutions per site. Dark blue represents *E. steinstansana*. Purple represents *E. dilatata*. Pink represents *E. spinosa*. Orange represents Lanceolate Elliptios. Green represents *Elliptio sensu stricto*.

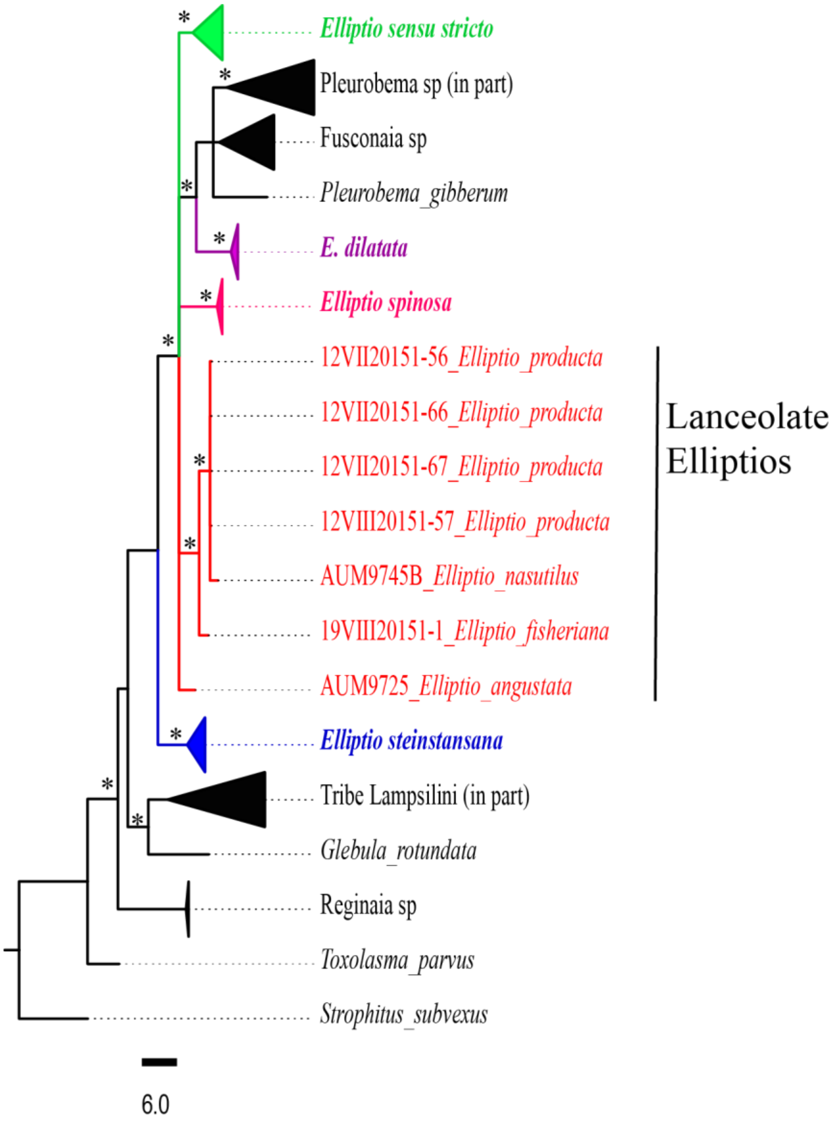

Figure 4. Bayesian inference of COI dataset. An * indicates posterior probability >0.75. Scale bar indicates nucleotide substitutions per site. Dark blue represents *E. steinstansana*. Purple represents *E. dilatata*. Pink represents *E. spinosa*. Orange represents Lanceolate Elliptios. Green represents *Elliptio sensu stricto*.

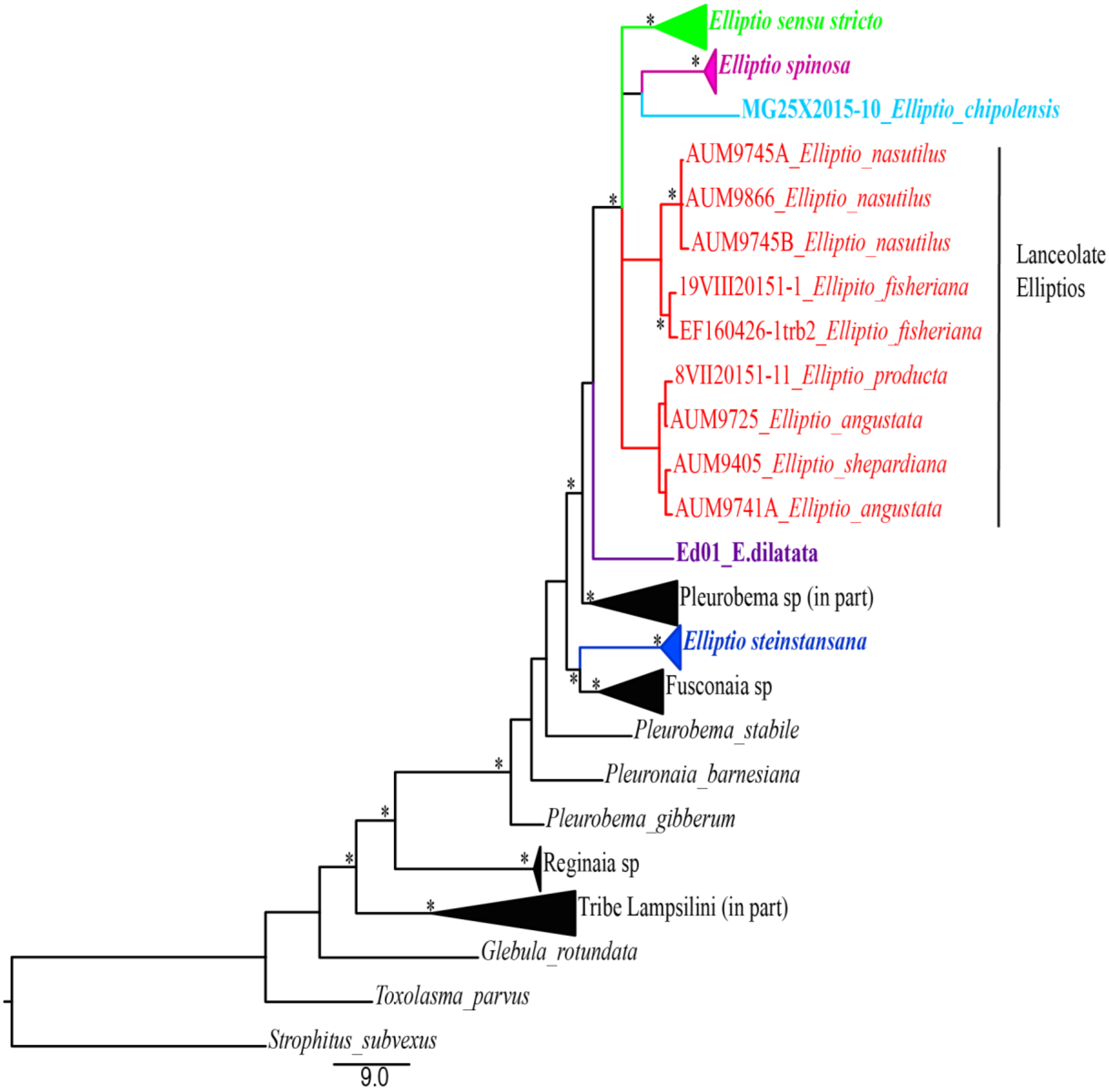

Figure 5. Bayesian inference of ND1 dataset. An * indicates posterior probability >0.75. Scale bar indicates nucleotide substitutions per site. Dark blue represents *E. steinstansana*. Purple represents *E. dilatata*. Pink represents *E. spinosa*. Orange represents Lanceolate Elliptios. Green represents *Elliptio sensu stricto*. Light blue represents *E. chipolaensis*.

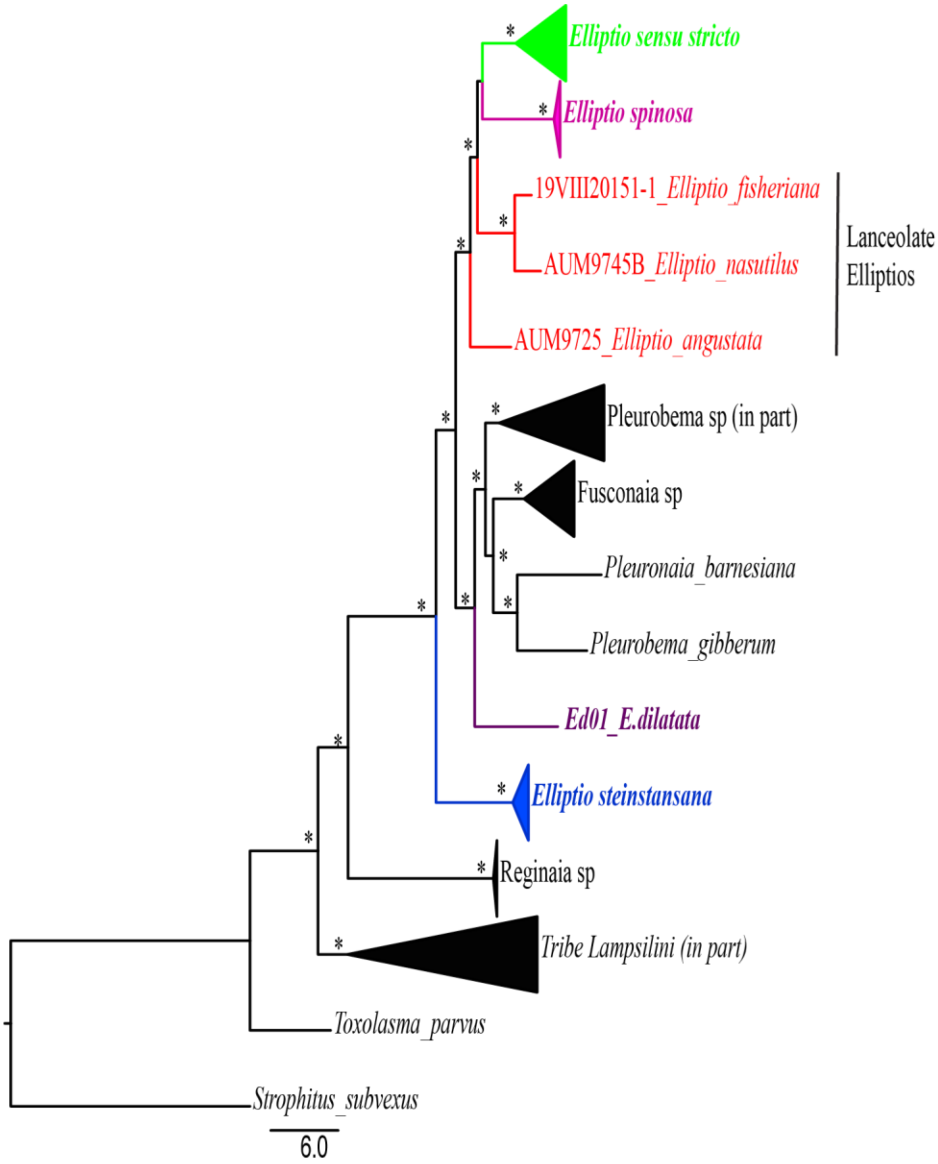

Figure 6. Bayesian inference of COI dataset. An * indicates posterior probability >0.75. Scale bar indicates nucleotide substitutions per site. Dark blue represents *E. steinstansana*. Purple represents *E. dilatata*. Pink represents *E. spinosa*. Orange represents Lanceolate Elliptios. Green represents *Elliptio sensu stricto*.

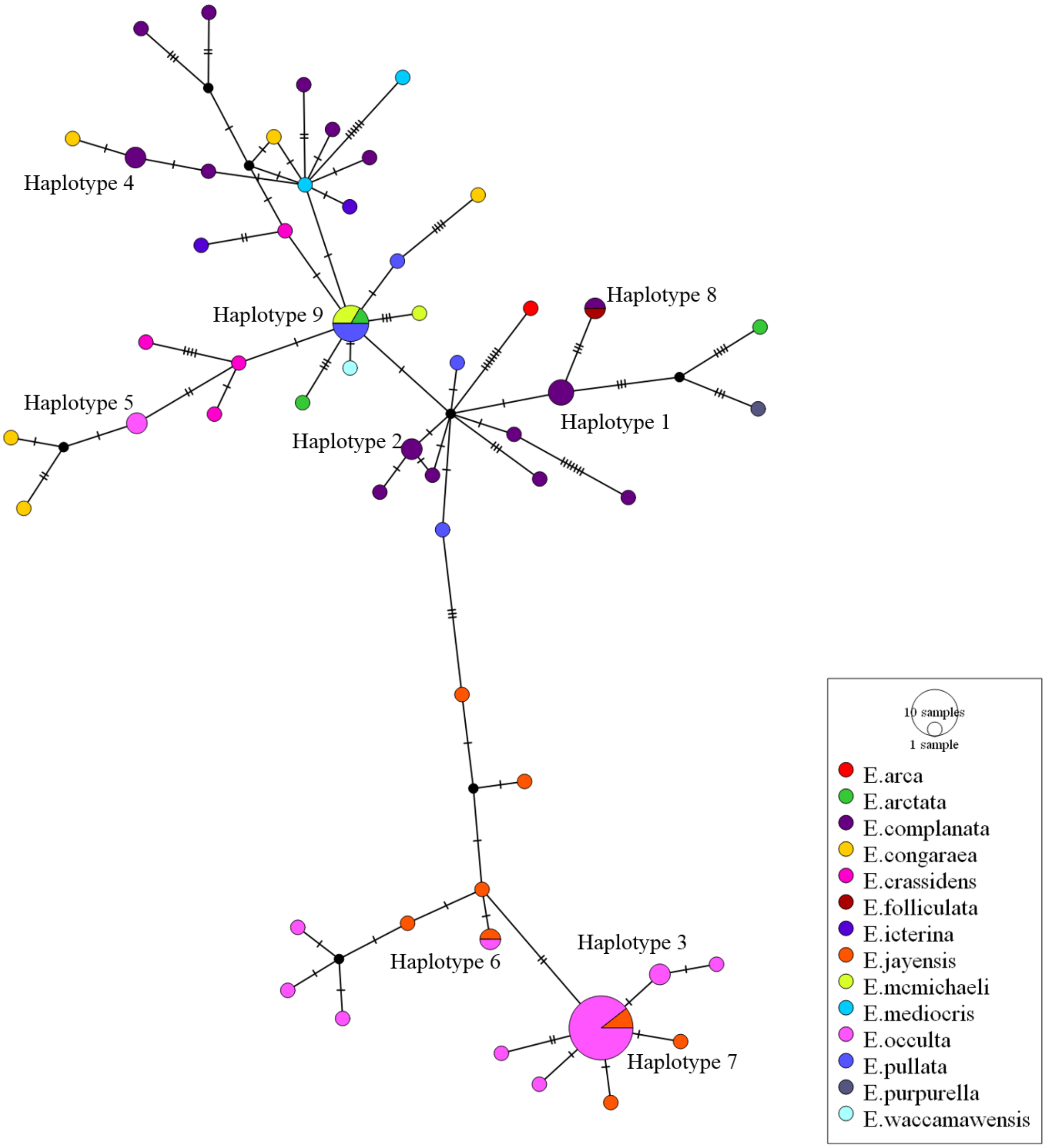

Figure 7. TCS species haplotype network of COI dataset. Circles represent haplotypes. Colors represent species. Size of circle represents number of individuals sharing a given haplotype. Filled in circles represent implied or unsampled haplotypes. Dashes haplotypes represent one nucleotide difference. Shared haplotypes referred to in text are labeled.

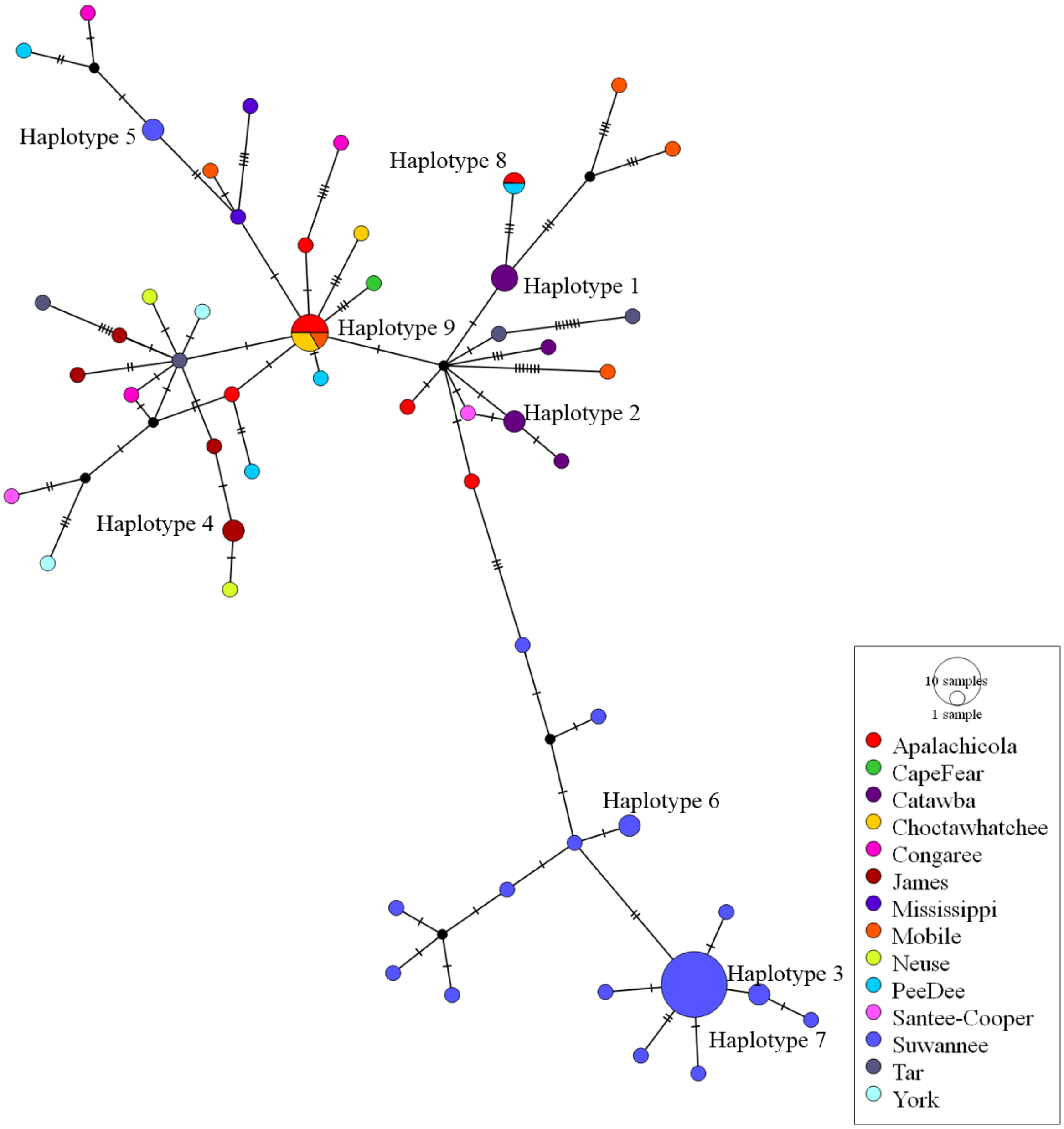

Figure 8. TCS basin haplotype network of COI dataset. Circles represent haplotypes. Colors represent basins. Size of circle represents number of individuals sharing a given haplotype. Filled in circles represent implied or unsampled haplotypes. Dashes haplotypes represent one nucleotide difference. Shared haplotypes referred to in text are labeled.

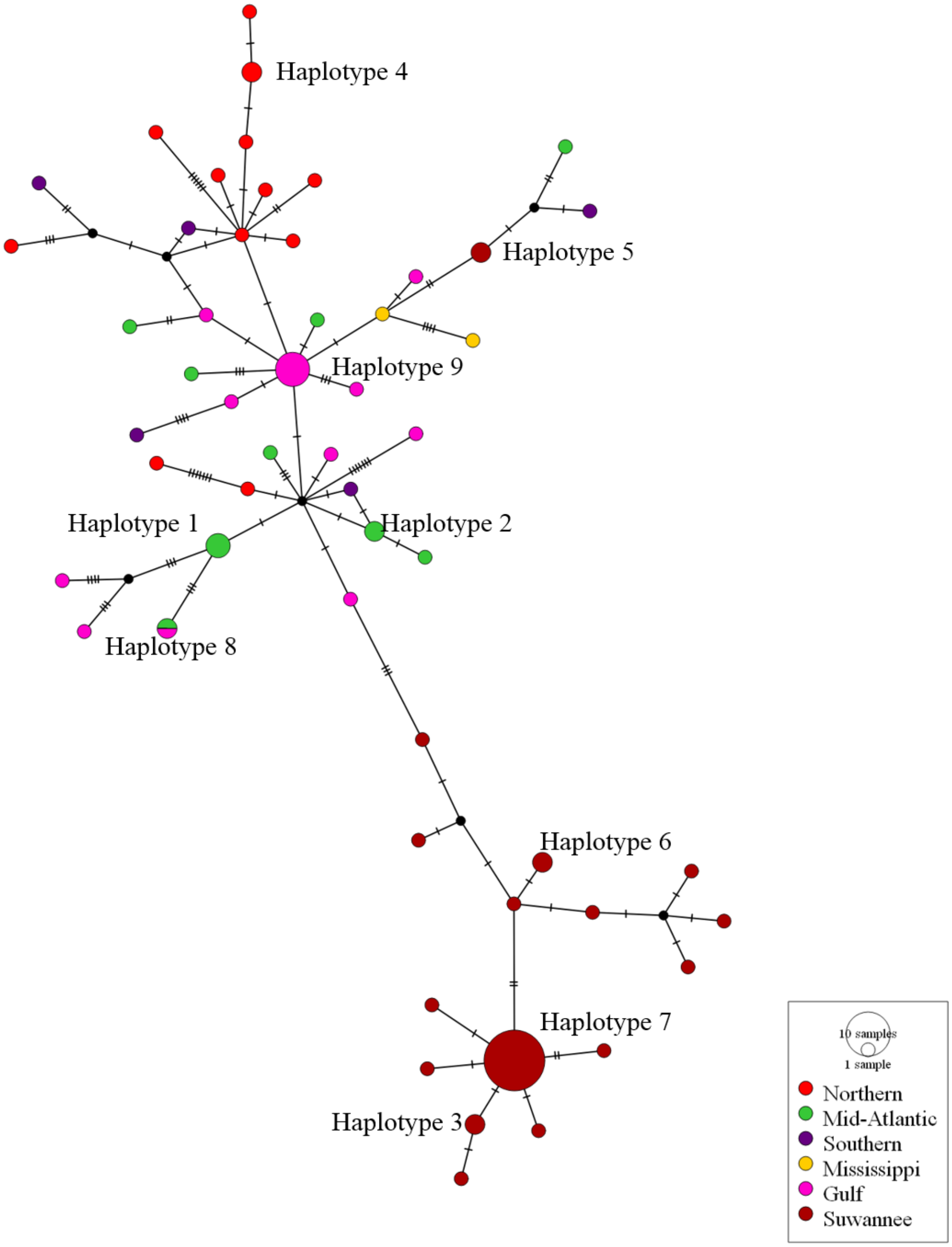

Figure 9. TCS geographic region haplotype network of COI dataset. Circles represent haplotypes. Colors represent broad geographic region. Size of circle represents number of individuals sharing a given haplotype. Filled in circles represent implied or unsampled haplotypes. Dashes haplotypes represent one nucleotide difference. Shared haplotypes referred to in text are labeled.

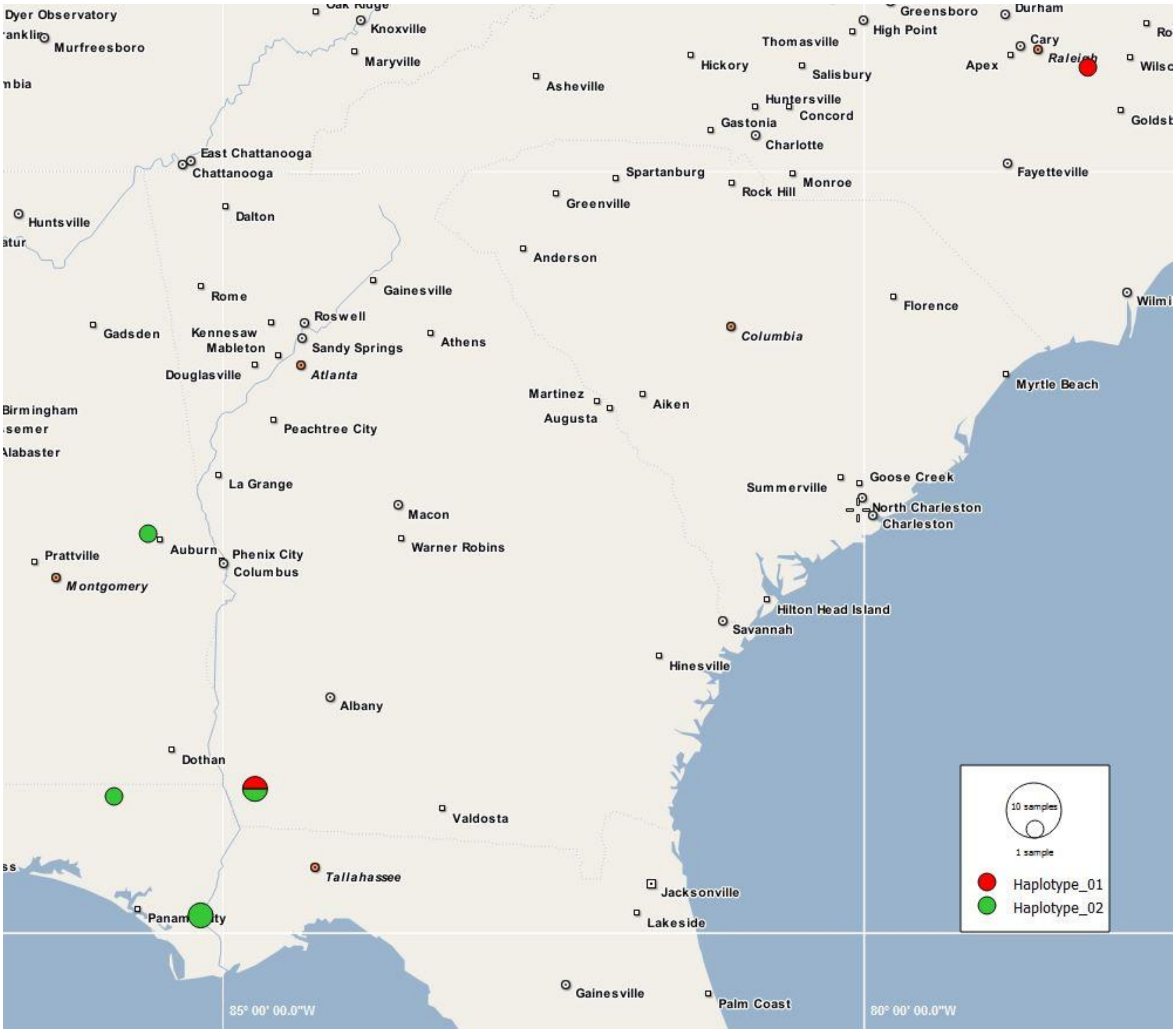

Figure 10. Map of COI dataset haplotypes shared across basins. Circles represent geographic clusters. Colors represent haplotype present in a given geographic cluster.

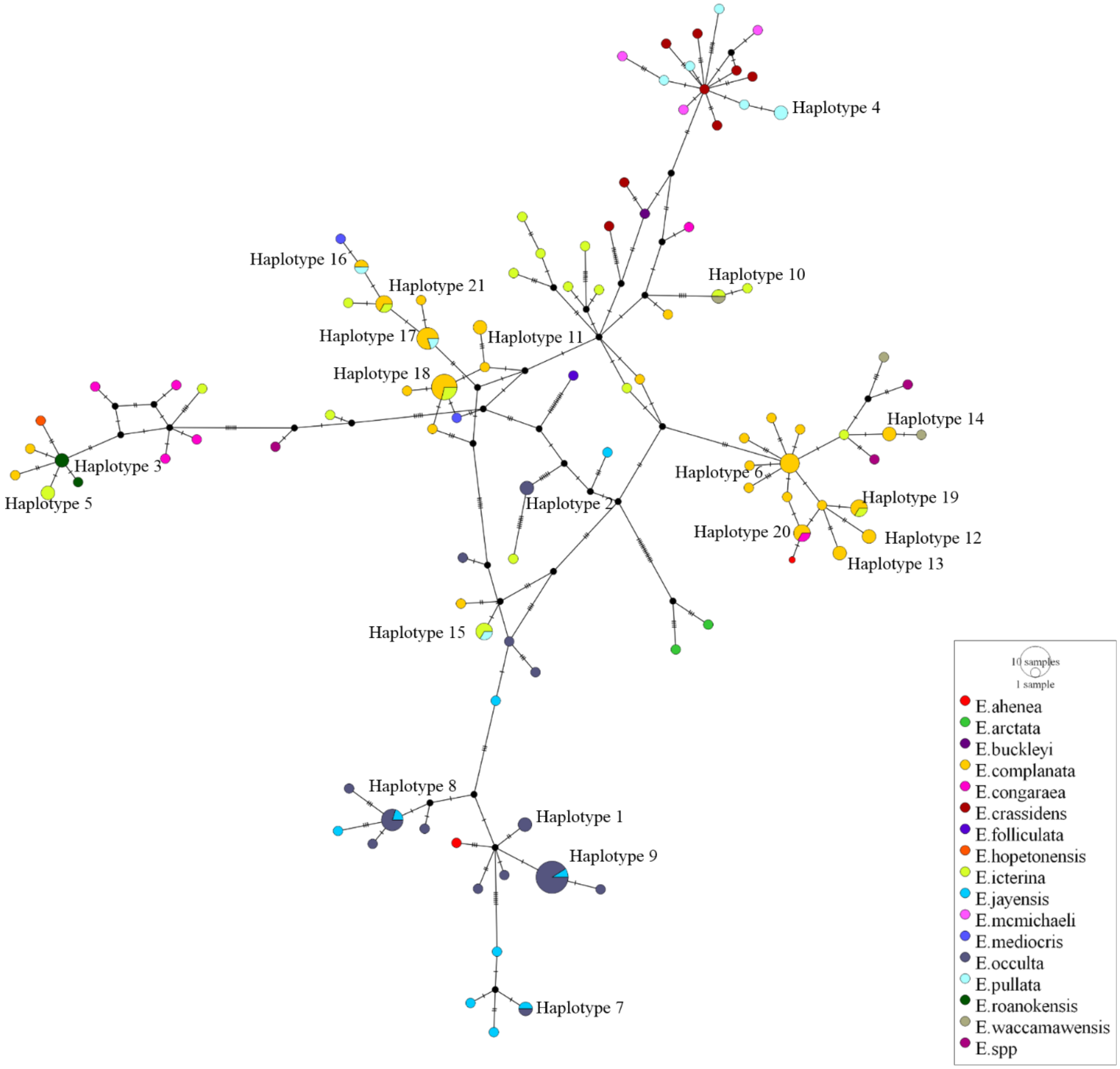

Figure 11. TCS species haplotype network of ND1 dataset. Circles represent haplotypes. Colors represent species. Size of circle represents number of individuals sharing a given haplotype. Filled in circles represent implied or unsampled haplotypes. Dashes haplotypes represent one nucleotide difference. Shared haplotypes referred to in text are labeled.

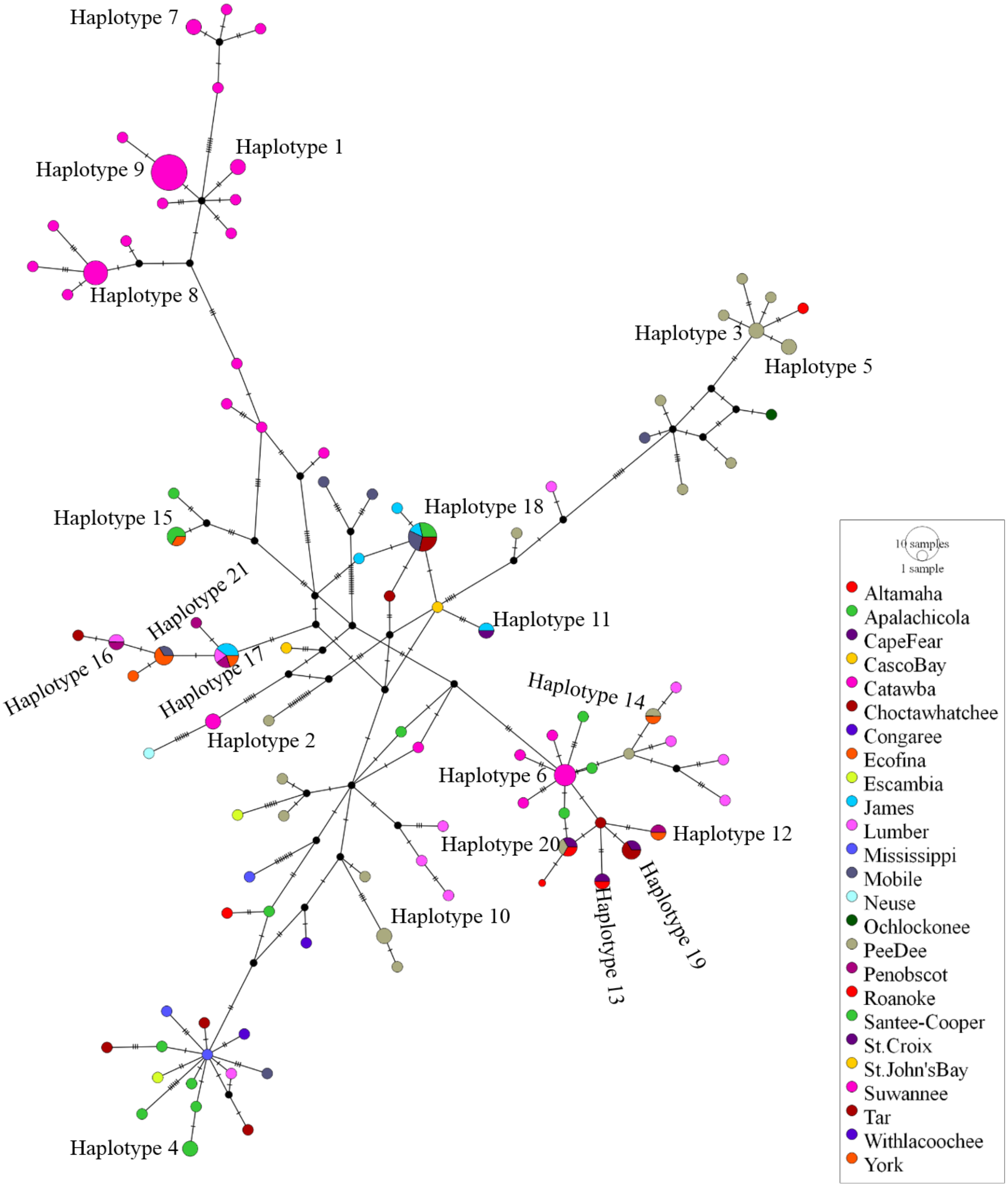

Figure 12. TCS basin haplotype network of ND1 dataset. Circles represent haplotypes. Colors represent basins. Size of circle represents number of individuals sharing a given haplotype. Filled in circles represent implied or unsampled haplotypes. Dashes haplotypes represent one nucleotide difference. Shared haplotypes referred to in text are labeled.

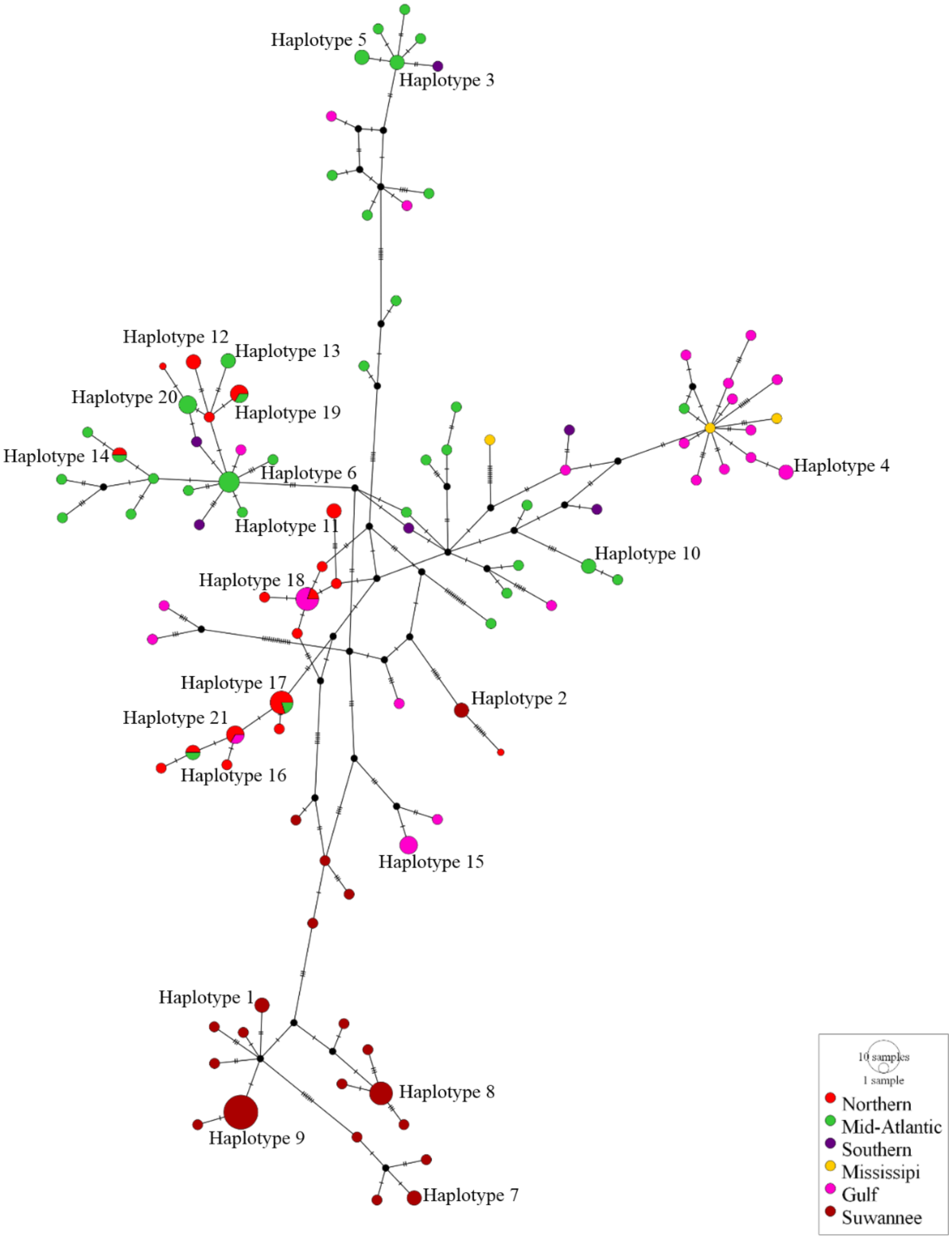

Figure 13. TCS geographic region haplotype network of ND1 dataset. Circles represent haplotypes. Colors represent broad geographic region. Size of circle represents number of individuals sharing a given haplotype. Filled in circles represent implied or unsampled haplotypes. Dashes haplotypes represent one nucleotide difference. Shared haplotypes referred to in text are labeled.

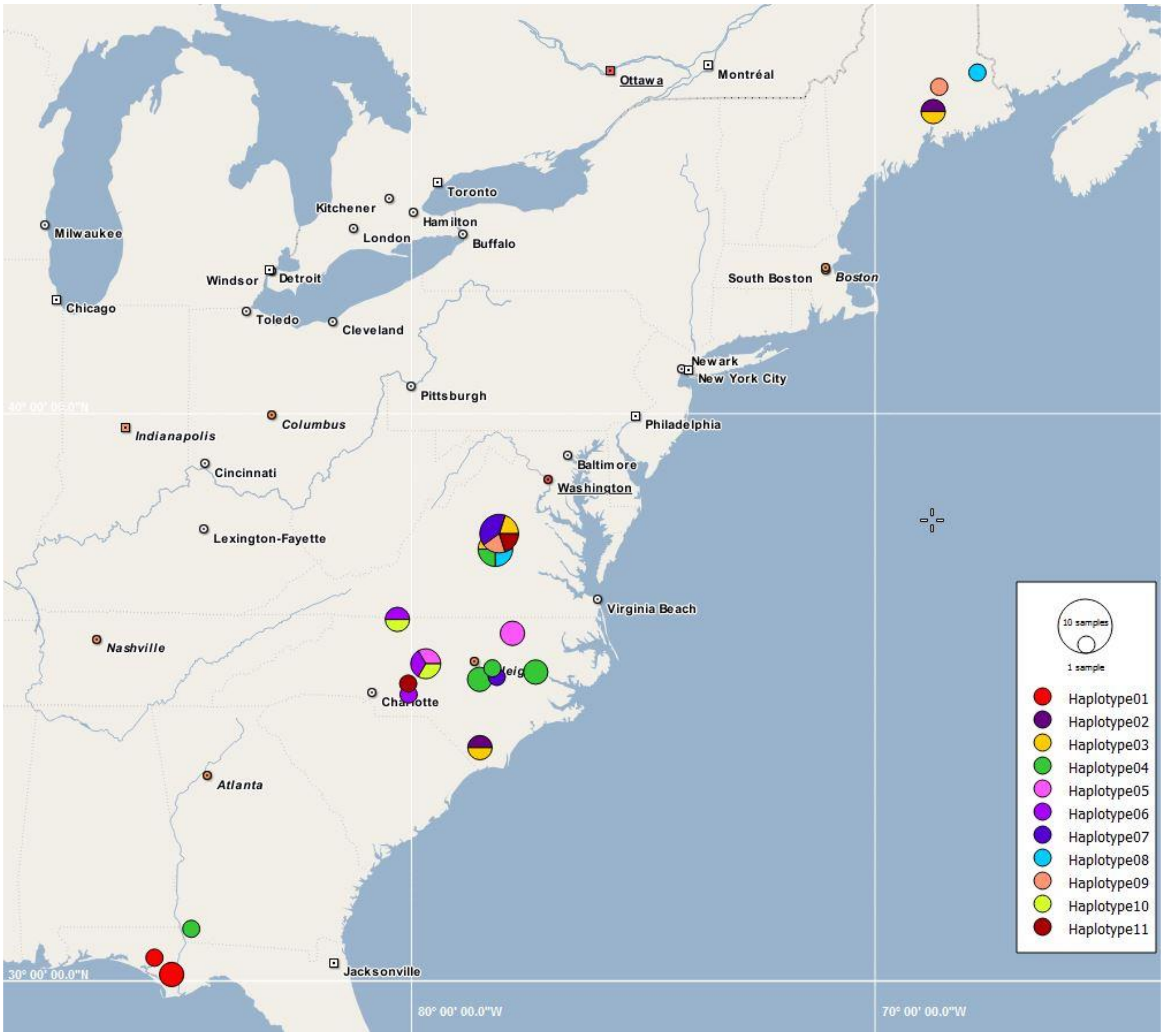

Figure 14. Map of ND1 dataset haplotypes shared across basins. Circles represent geographic clusters. Colors represent haplotype present in a given geographic cluster.

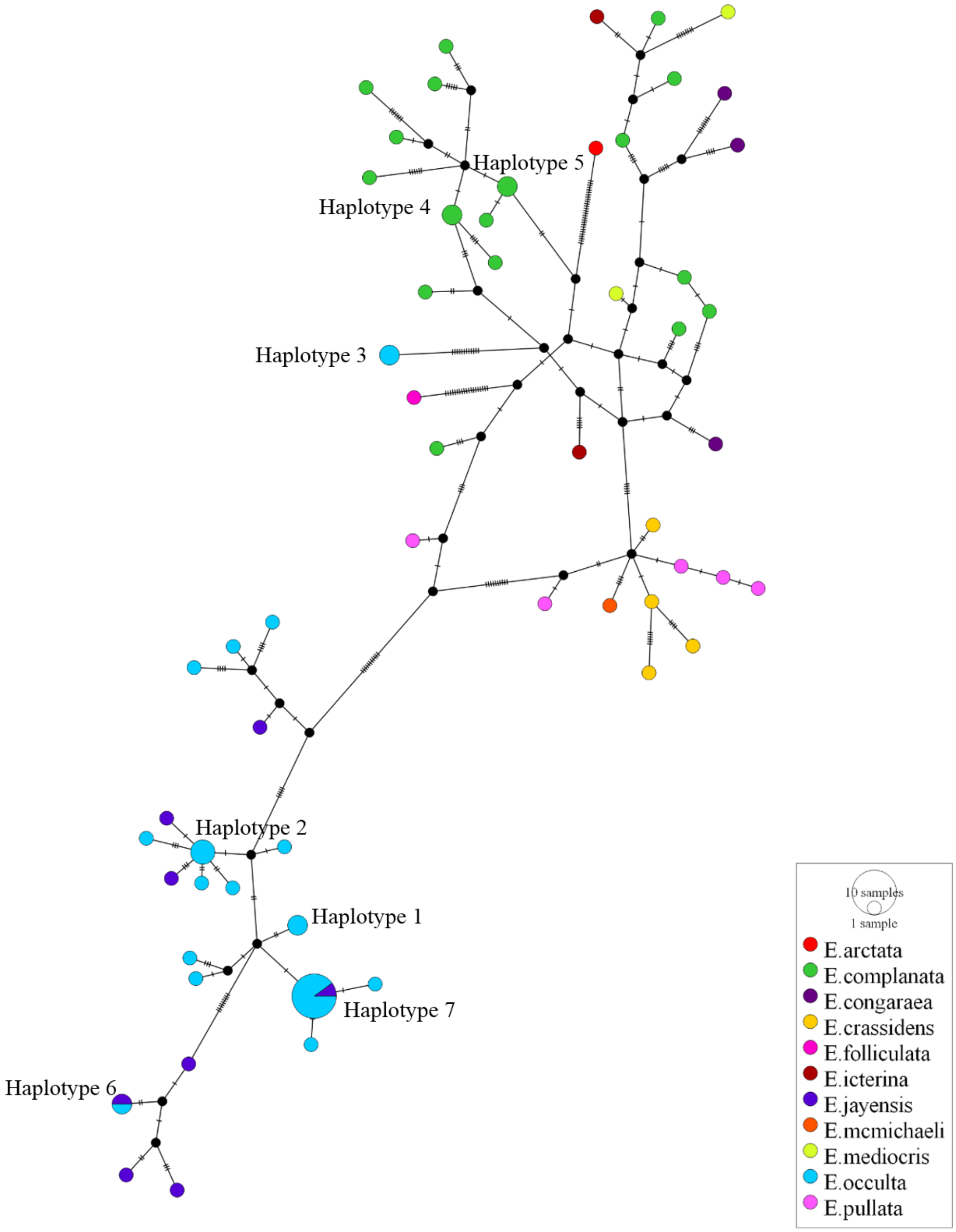

Figure 15. TCS species haplotype network of concatenated dataset. Circles represent haplotypes. Colors represent species. Size of circle represents number of individuals sharing a given haplotype. Filled in circles represent implied or unsampled haplotypes. Dashes haplotypes represent one nucleotide difference. Shared haplotypes referred to in text are labeled.

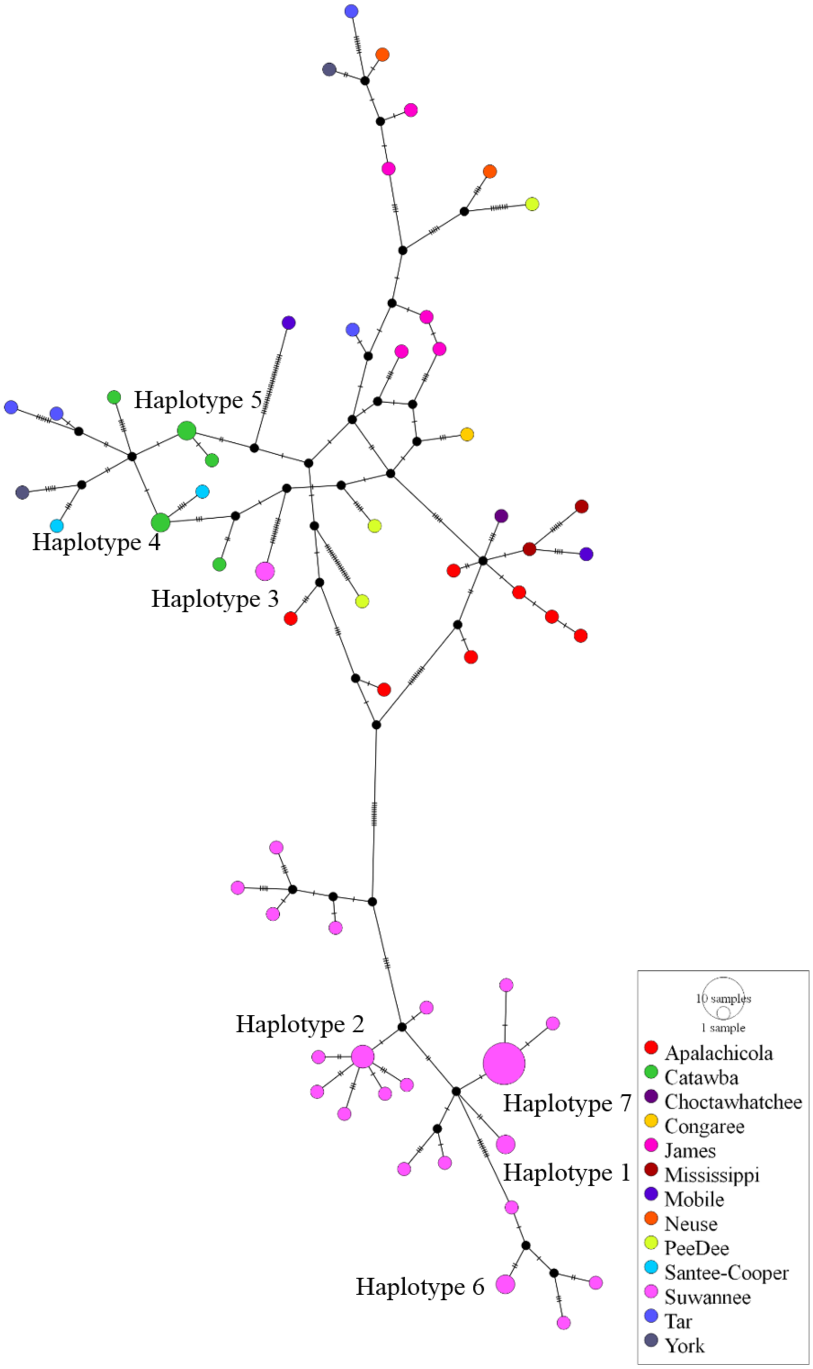

Figure 16. TCS basin haplotype network of concatenated dataset. Circles represent haplotypes. Colors represent basins. Size of circle represents number of individuals sharing a given haplotype. Filled in circles represent implied or unsampled haplotypes. Dashes haplotypes represent one nucleotide difference. Shared haplotypes referred to in text are labeled.

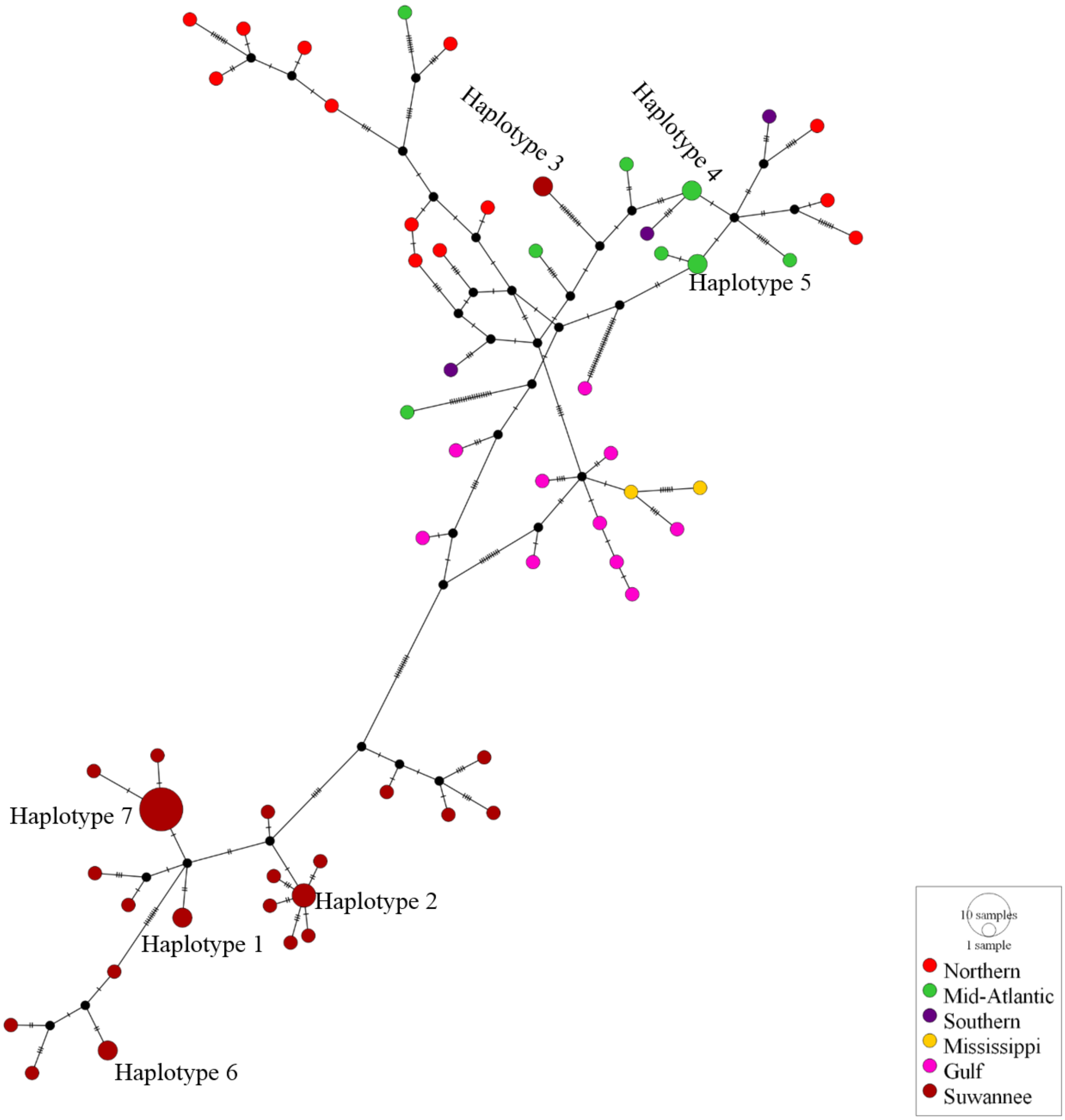

Figure 17. TCS geographic region haplotype network of concatenated dataset. Circles represent haplotypes. Colors represent broad geographic region. Size of circle represents number of individuals sharing a given haplotype. Filled in circles represent implied or unsampled haplotypes. Dashes haplotypes represent one nucleotide difference. Shared haplotypes referred to in text are labeled.

Vita

Raquel Anne Fagundo was born in Wheeling, WV, to Louann and Ramon Fagundo in 1990. She received her Bachelor of Science degree from West Liberty University in 2014 under the guidance of Dr. Zachary Loughman. She is currently employed by the North Carolina Museum of Natural Sciences as the Non-molluscan Invertebrate Collections Manager in Raleigh, NC. She currently resides near Raleigh, NC with her husband and daughter.