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Phylogeographic analysis of the threatened and endangered superconglutinate-producing mussels of the genus *Lampsilis* (Bivalvia: Unionidae)

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Abstract

Several species of freshwater unionid mussels in the genus *Lampsilis* exhibit a remarkable reproductive strategy. Female mussels of these species enclose their larvae in a minnow-like lure, called a 'superconglutinate', to attract piscivorous fishes. When a fish attempts to ingest the superconglutinate the lure ruptures and the larvae are released to parasitize the fish. Of the four species of mussel which exhibit this strategy and are endemic to the Gulf Coast drainages of the southeastern United States, three are protected under the Endangered Species Act, and one is recognized as imperilled. Phylogenetic analysis of nucleotide sequences of the mitochondrial 16S ribosomal RNA and the first subunit of the cytochrome oxidase *c* genes was conducted on 18 individual specimens representing these four species and six outgroup taxa. Phylogenetic analyses of these data support the monophyly of the superconglutinate-producing mussels, and indicates a strong geographical component to the data. The zoogeographic patterns of the four taxa included in the study are congruent with those seen in freshwater vertebrates, and are consistent with a vicariant pattern resulting from fluctuations in sea level during the Pleistocene. Despite the strong geographical structuring of the data, only one species, *Lampsilis subangulata*, was recovered as monophyletic. The authors attribute the lack of support for the monophyly of the remaining species to insufficient sequence variation and the recent origin of the ancestor of these taxa. Based on these data, any future captive breeding projects aimed at augmenting or re-establishing populations should do so only from the appropriate source populations so as to maintain the genetic integrity of these nascent species.

Keywords: fresh water, *Lampsilis*, mitochondrial DNA, phylogeny, phylogeography, Unionidae

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Introduction

The southeastern United States has been recognized as one of the most biotically diverse regions in the world (Benz & Collins 1997), particularly for aquatic organisms (Lydeard & Mayden 1995). Freshwater mussels (Bivalvia: Unionoida) are no exception, the regional fauna includes approximately 100 species, many of which are endemic (Williams & Butler 1994). Sadly, this region of the United States also holds one of the most endangered faunas in the world (Benz & Collins 1997; Master *et al.* 1998). Whereas the historical and environmental factors that contributed to the origin of this diversity are not yet entirely understood, evidence for the cause of the recent decline in diversity points towards anthropogenic factors (Neves *et al.* 1997). Unionid mussels are among the most endangered organisms in North America (Williams *et al.* 1993; Stein & Flack 1997; Master *et al.* 1998); however, very few published studies have examined the genetic diversity of freshwater mussels at or below the species level (Liu *et al.* 1996; Mulvey *et al.* 1997; Roe & Lydeard 1998; King *et al.* 1999; Lydeard *et al.* 2000; Turner *et al.* 2000). Understanding how genetic diversity of threatened or endangered organisms is distributed geographically can provide insight into factors influencing the formation and maintenance of species and is critical to their conservation.

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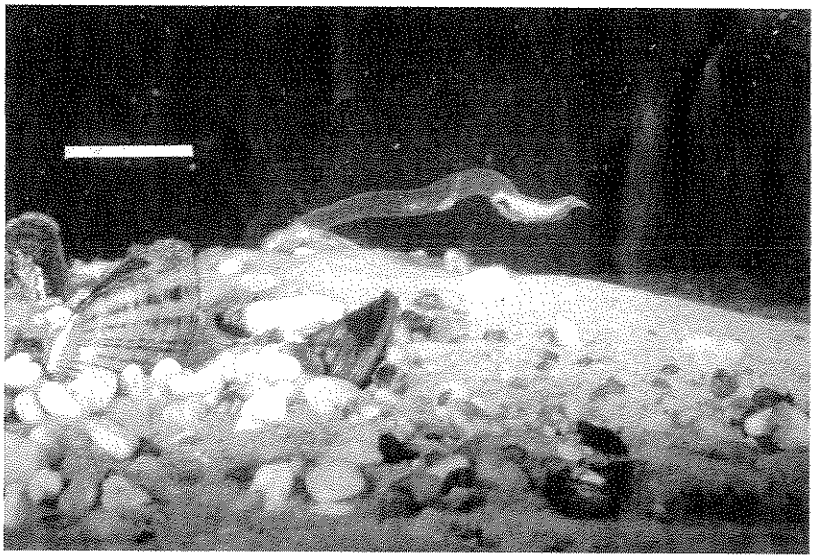

Fig. 1 Photograph of a female *Lampsilis subangulata* with attached superconglutinate lure. Several additional mussels are visible in foreground. Bar = 5 cm. From O'Brien & Brim-Box (1999), used by permission.

In most unionid species, the mature larvae, called glochidia, are obligate parasites on vertebrate hosts, typically fishes (McMahon 1993). Many species of Unionids exhibit behaviours and structures that are presumably involved in maximizing the likelihood that their larvae will encounter a suitable host (Kat 1984; Barnhart & Roberts 1997). For example, some freshwater mussels discharge glochidia in conglutinates, mucous-like packages that resemble small worms or fish larvae (e.g. Kat 1984; Hartfield & Hartfield 1996), and entice a potential host with the prospect of food.

The freshwater mussel genus *Lampsilis* includes 32 North American taxa (Turgeon *et al.* 1998), many of which use some type of lure to attract a host. In the majority of species of *Lampsilis*, a lure is formed by pigmented portions of the inner lobe of the mantle margin, which when extended mimic a small fish (Barnhart & Roberts 1997). The illusion of a swimming fish is heightened by the wave-like movements of the mantle which originate at the anterior end and move posteriorly toward the incurrent aperture. A more complete description of these mantle margins can be found in Kramer (1970).

Another distinctly different type of piscine lure has recently been described for four species of *Lampsilis*. In these taxa the glochidia are packaged in conglutinates, but because these lures are composed of many such packages they are referred to as superconglutinates (Haag *et al.* 1995). The superconglutinate mass is extruded from the excurrent aperture of the mussel in a transparent mucous tube that reaches up to 250 cm in length (Haag *et al.* 1995; Hartfield & Butler 1997). Water currents act on the mucous strand and cause the lure to mimic the swimming movements of a small fish (Fig. 1). The species known to produce superconglutinate lures are: *Lampsilis altilis* (Conrad), *L. perovalis* (Conrad), *L. australis* (Simpson) (H. Blalock-Herod, personal com.) and *L. subangulata* (I. Lea) (Haag *et al.* 1995, 1999; O'Brien *et al.* 1997).

These four species are distributed in Alabama, Florida and Georgia (Fig. 2). The historic ranges of *L. altilis* and *L. perovalis* consisted of the Alabama and Black Warrior/Tombigbee river systems that together constitute the Mobile River Basin. Both species were described from specimens collected at the same location on the Alabama River (Conrad 1834). Presently, the range of *L. altilis* is reported to be limited to portions of the Alabama River System (Haag *et al.* 1999), while *L. perovalis* is considered to be restricted to the headwaters and tributaries in the Black Warrior/Tombigbee river systems (U.S. Fish & Wildlife Service 1994). Both of these taxa are listed as threatened species by the U.S. Fish & Wildlife Service (U.S. Fish & Wildlife Service 1994). *L. australis* is known historically from the Choctawhatchee River System and the Yellow and Escambia river systems (Clench & Turner 1956), and is recognized as an imperilled species (Lydeard *et al.* 1999). *L. subangulata* has been described from the Ochlockonee River and the Apalachicola River System, which consists of the Flint and Chattahoochie rivers. The range of both of these species has been greatly reduced (Roe, KJ, personal observation; O'Brien & Brim-Box 1999), and *L. subangulata* is listed as a federally endangered species (U.S. Fish & Wildlife Service 1998).

Knowledge of phylogenetic relationships and how genetic diversity is partitioned geographically is crucial to establishing conservation priorities for unionids (Lydeard & Roe 1998; King *et al.* 1999). In this study, we use the DNA sequences of two mitochondrial genes to test the monophyly and assess the phylogeographic structure of the superconglutinate-producing mussels. We also interpret the phylogeographic patterns recovered in this study in light of the geological history of the Gulf region and compare

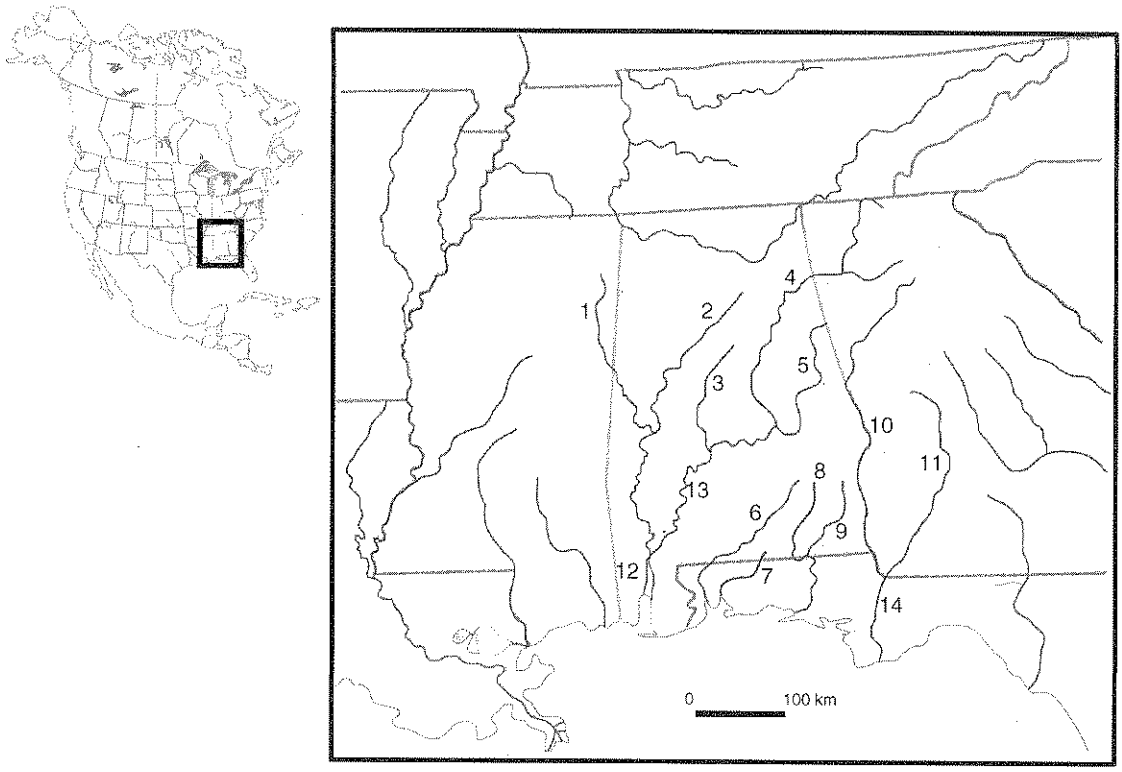

Fig. 2 Map of the southeastern United States identifying the various rivers included in this study: (1) Tombigbee; (2) Black Warrior; (3) Cahaba; (4) Coosa; (5) Tallapoosa; (6) Escambia; (7) Yellow; (8) Pea; (9) Choctawhatchee; (10) Chattahoochie; (11) Flint; (12) Mobile; (13) Alabama; (14) Apalachicola. See Table 1 for specific sample localities.

these results to zoogeographic patterns observed in other freshwater taxa in the southeastern United States.

Materials and methods

Nucleic acid isolation, polymerase chain reaction and sequencing

Specimens of the four taxa in question were obtained from throughout the respective species ranges (Table 1). Protected species were collected by permission of the U.S. Fish & Wildlife Service (permit no. SA96-31). Because of the protected status and rarity of these species, the sample sizes used in this study are necessarily small. The inclusion of additional freshwater mussel taxa is based on previous phylogenetic studies of higher order relationships, which included representatives of all lampsiline genera (K. J. Roe, unpublished PhD dissertation). Voucher specimens were deposited at The University of Alabama Unionid Collection (UAUC). Taxonomic nomenclature used follows that employed in Turgeon *et al.* (1998).

Because of the potential for amplification of non-orthologous DNA sequences, as unionid mussels exhibit bi-parental inheritance of mitochondria (Hoeh *et al.* 1996), only somatic tissues were used. Total DNA was isolated from either fresh dead, frozen, or ethanol-preserved specimens using a standard phenol–chloroform method (Palumbi *et al.* 1991). Double-stranded and single-stranded DNA was generated via the polymerase chain reaction (PCR) using the primers LCO1490 and HCO2198 (Folmer *et al.* 1994) for the first subunit of the cytochrome oxidase *c* gene (COI), and 16SarL-myt and 16SbrH-myt (Lydeard *et al.* 1996) for the 16S ribosomal RNA (rRNA) gene. PCR and manual DNA sequencing protocols follow Roe & Lydeard (1998) for the COI gene portion and Lydeard *et al.* (1996) for the 16S rRNA portion. Sequencing of both heavy and light strands for some taxa was performed using Big Dye (Perkin Elmer) terminator cycle sequencing and the products were visualized using an ABI 377 automated sequencer.

Analysis of sequence data

COI sequences were aligned by eye using the software package XESEE (Cabot & Beckenbach 1989) and 16S rDNA sequences were aligned using CLUSTALW (Thompson *et al.* 1994). Aligned matrices are available from the first author. No insertions–deletions (indels) were observed in the COI data set. Phylogenetically informative indels in the 16S data set were coded as binary characters and the resulting matrix was appended to the aligned data set. Prior to

Table 1 Localities and GenBank accession numbers of specimens used in this study

Lampsilis altilis #1 — Chewacla Creek, Tallapoosa River Drainage, Macon Co., AL. UAUC #248; AF385092, AF385116
L. altilis #2 — Shoal Creek, Coosa River Drainage, Cleburne Co., AL. UAUC #125; AF385105, AF385129
L. altilis #3 — Little Cahaba River, Cahaba River Drainage, Jefferson Co., AL. UAUC #149; AF385108, AF385132
L. altilis #4 — Fish Creek, Coosa River Drainage, Polk Co., GA. UAUC #650; AF385107, AF385131
L. altilis #5 — Conasauga River, Coosa River Drainage, Murray/Whitfield Co., GA. UAUC #651; AF385106, AF385130
L. australis #1 — West Fork of Choctawhatchee River, Barbour Co., AL. UAUC #128; AF385101, AF385125
L. australis #2 — West Fork of Choctawhatchee River, Barbour Co., AL. UAUC #130; AF385098, AF385122
L. australis #3 — Flat Creek, Pea River Drainage, Geneva Co., AL. UAUC #547; AF385097, AF385121
L. australis #4 — Shoal River, Yellow River Drainage, Okaloosa Co., FL. UAUC #643; AF385100, AF385124
L. australis #5 — Conecuh River, Escambia River Drainage, Pike Co., AL. UAUC #510; AF385099, AF385123
L. perovalis #1 — Lubbub Creek, Tombigbee River Drainage, Pickens Co., AL. UAUC #86; AF385093, AF385117
L. perovalis #2 — North River, Black Warrior River Drainage, Tuscaloosa Co., AL. UAUC #107; AF385094, AF385118
L. perovalis #3 — Sipsey River, Tombigbee River Drainage, Greene/Pickens Co., AL. UAUC #646; AF385096, AF385120
L. perovalis #4 — Brown Creek, Black Warrior River Drainage, Winston Co., AL. UAUC #648; AF385095, AF385119
L. perovalis #5 — Flannigan Creek, Black Warrior River Drainage, Lawrence Co., AL. UAUC #649; AF385091, AF385115
L. subangulata #1 — Kinchafoonie Creek, Flint River Drainage, Webster Co., GA. UAUC #133; AF385102, AF385126
L. subangulata #2 — Uchee Creek, Chattahoochee River Drainage, Russell Co., AL. UAUC #116; AF385104, AF385128
L. subangulata #3 — Whitewater Creek, Flint River Drainage, Fayette Co., GA. UAUC #645; AF385103, AF385127
L. ornata — Cahaba River, Alabama River Drainage, Bibb Co., AL. UAUC #17; AF385112, AF385136
L. ovata — Elk River, Tennessee River Drainage, Limestone Co., AL. UAUC #108; AF385111, AF385135
L. teres — Yellowleaf Creek, Coosa River Drainage, Shelby Co., AL. UAUC #6; AF385113, AF385137
Ligumia recta — Ohio River, near Louisville, KY. UAUC #89; AF385110, AF385134
Villosa villosa — Original Suwanee River campground canals, Suwanee River Drainage. Dixie Co., FL. UAUC #652; AF385109, AF385133
Obliquaria reflexa — Cahaba River, Alabama River Drainage, Bibb Co., AL. UAUC #19; AF385114, AF385138

UAUC = University of Alabama Unionid Collection.

phylogenetic analysis, the DNA sequences were examined for saturation due to multiple substitutions by plotting the number of transitions and transversions against *p*-distance values (Moritz *et al.* 1992). Sequences were tested for the presence of significant phylogenetic signal using the g^1 statistic (Hillis & Huelsenbeck 1992). Since all mitochondrial (mt) genes are nonrecombining and should be inherited as a unit, sequences from both mtDNA gene portions were combined in all analyses following the total evidence approach (Kluge 1989). This approach combines all available phylogenetic information to enhance the resolution of the resulting trees. For the purpose of recognizing species in this study, we have employed the monophyly version of the Phylogenetic Species Concept (de Queiroz & Donoghue 1990).

All phylogenetic analyses were conducted using PAUP* 4.0b4a (Swofford 1998) using three different methods: the optimality criterion of maximum parsimony (MP), the minimum evolution method using the neighbour-joining (NJ) algorithm (Saitou & Nei 1987), and the maximum-likelihood (ML) method (Felsenstein 1981). For MP analysis, searches were conducted using the heuristic search option keeping only minimal length trees. Starting trees were obtained via stepwise addition of taxa, and each search was replicated 10 times, branch swapping was implemented using the tree–bisection–reconnection (TBR) option. Minimum evolution analysis was conducted using Log/Det paralinear distances (Lake 1994), using the heuristic search option with 10 random additions of taxa, and TBR branch swapping. The model for sequence evolution for ML analysis was determined by using the program MODELTEST 3.0 (Posada & Crandall 1998). Searches were conducted under the ML criterion using the HKY 85 model (Hasegawa *et al.* 1985). Based on the results of MODELTEST, a proportion of sites were assumed to be invariable (I = 0.4850), and rates among all sites were assumed to vary according to a γ distribution (γ-shape parameter = 0.6793). Stability of internal branches of generated trees was inferred by 1000 bootstrap replicates using the 'fast stepwise addition' option for MP and NJ methods (200 replicates for ML) and by calculating branch support/decay indices (Bremer 1988) using the software package AUTODECAY (Errikson 1997) for MP analysis only. Estimates of sequence divergence were made using the HKY 85 model (Hasegawa *et al.* 1985). Alternative topologies (i.e. constraining the monophyly of a particular group) were compared statistically to the optimal trees for each analysis using the nonparametric pairwise parsimony test proposed by Templeton (1983) as implemented in PAUP* 4.0b4a. In the instance where multiple equally parsimonious trees resulted from constraining the analysis, a single representative tree was used for the Templeton test. The phylogeography of the group was investigated using area cladograms derived using Brooks Parsimony Analysis (BPA) (Wiley 1988). The resulting pattern was examined for congruence with existing area relationship hypotheses for the region.

Table 2 Pairwise genetic distances for both genes based on the HKY 85 model (Hasegawa *et al.* 1985)

	*L. al*1	*L. al*2	*L. al*3	*L. al*4	*L. al*5	*L. au*1	*L. au*2	*L. au*3	*L. au*4	*L. au*5	*L. p*1
*L. al*2	1.01%										
*L. al*3	1.51%	0.91%									
*L. al*4	1.02%	0.00%	0.91%								
*L. al*5	1.62%	0.61%	1.52%	0.61%							
*L. au*1	4.74%	4.34%	4.84%	4.37%	4.95%						
*L. au*2	4.64%	4.24%	4.74%	4.26%	4.85%	0.91%					
*L. au*3	4.84%	4.54%	5.04%	4.57%	5.15%	1.01%	0.30%				
*L. au*4	4.64%	4.24%	4.54%	4.27%	4.85%	2.42%	2.52%	2.82%			
*L. au*5	4.03%	3.43%	3.73%	3.45%	4.04%	2.72%	2.42%	2.72%	1.01%		
*L. p*1	1.51%	1.31%	2.02%	1.32%	1.82%	4.84%	4.74%	5.04%	4.44%	3.83%	
*L. p*2	1.41%	1.21%	1.92%	1.22%	1.82%	4.75%	4.65%	4.95%	4.35%	3.74%	0.40%
*L. p*3	1.51%	0.50%	1.41%	0.31%	1.11%	4.84%	4.73%	5.04%	4.74%	3.93%	1.81%
*L. p*4	1.92%	1.72%	2.42%	1.73%	2.32%	5.24%	5.14%	5.45%	4.85%	4.24%	0.91%
*L. p*5	1.51%	1.31%	2.02%	1.33%	1.92%	5.04%	4.94%	5.24%	4.74%	4.13%	0.60%
*L. sub*1	4.24%	3.84%	4.34%	3.86%	4.45%	2.32%	2.22%	2.32%	2.52%	2.62%	4.14%
*L. sub*2	4.06%	3.66%	4.16%	3.67%	4.27%	2.13%	2.03%	2.13%	2.34%	2.43%	3.96%
*L. sub*3	4.64%	4.24%	4.75%	4.17%	4.85%	2.82%	2.93%	3.03%	2.93%	3.03%	4.54%
L. ornata	8.90%	8.71%	8.91%	8.64%	9.03%	8.28%	8.38%	8.78%	8.40%	8.28%	8.80%
L. ovata	9.93%	9.33%	9.52%	9.16%	9.44%	9.41%	9.51%	9.70%	9.52%	9.41%	9.42%
L. teres	8.03%	8.25%	8.14%	7.95%	8.46%	8.33%	8.22%	8.62%	8.65%	8.22%	8.34%
L. recta	8.05%	7.86%	7.75%	7.78%	8.17%	8.65%	8.55%	8.85%	8.57%	7.94%	8.15%
V. villosa	7.26%	6.67%	6.37%	6.68%	7.18%	6.65%	6.34%	6.64%	6.47%	6.06%	6.96%
O. refl	12.16%	11.77%	12.06%	11.50%	11.97%	11.84%	12.04%	12.14%	11.86%	11.34%	11.76%

	*L. p*2	*L. p*3	*L. p*4	*L. p*5	*L. sub*1	*L. sub*2	*L. sub*3	*L. ornata*	*L. ovata*	*L. teres*	*L. recta*	*V. villosa*
*L. p*3	1.72%											
*L. p*4	0.50%	2.22%										
*L. p*5	0.51%	1.81%	1.01%									
*L. sub*1	4.04%	4.34%	4.55%	4.34%								
*L. sub*2	3.86%	4.05%	4.37%	4.16%	0.41%							
*L. sub*3	4.35%	4.74%	4.95%	4.74%	1.41%	1.02%						
L. ornata	9.02%	9.01%	9.42%	9.42%	8.50%	8.12%	8.41%					
L. ovata	9.64%	9.52%	9.74%	10.04%	9.52%	9.14%	9.43%	6.15%				
L. teres	8.25%	8.24%	8.45%	8.75%	8.33%	8.04%	8.14%	8.04%	7.33%			
L. recta	8.16%	8.15%	8.45%	8.56%	8.35%	8.18%	8.66%	8.56%	8.87%	8.51%		
V. villosa	7.18%	7.16%	7.58%	7.37%	7.17%	6.99%	7.28%	8.39%	8.79%	8.43%	7.65%	
O. refl	11.88%	12.06%	12.48%	12.27%	11.66%	11.39%	11.35%	11.54%	10.61%	11.60%	12.74%	11.23%

L. al, Lampsilis altilis; *L. au, L. australis*; *L. p, L. perovalis*; *L. sub, L. subangulata*; *L. recta, Ligumia recta*; *V. villosa, Villosa villosa*; *O. refl, Obliquaria reflexa*.

Results

Nucleotide variation and saturation

DNA sequences for 24 taxa resulted in a data matrix of 590 characters for the COI gene, and 432 (410 nucleotides + 22 indels) characters for the 16S rRNA gene, for a total of 1022 characters. The COI data set contained 169 variable sites, 92 of these were phylogenetically informative; whereas the 16S data set contained 112 variable sites, 68 of which were phylogenetically informative. Preliminary analysis of DNA sequences revealed no evidence of saturation due to multiple substitutions for either gene portion. The g_1 statistic indicates the presence of a significant phylogenetic signal ($P = 0.01$) for the combined data set (COI + 16S = −0.581541). Pair-wise comparisons of sequence divergence using the HKY 85 model (Hasegawa *et al.* 1985) are presented in Table 2. Intraspecific distances ranged from 0.00–1.62% (*Lampsilis altilis*) to 0.30–2.82% (*L. australis*). Within the superconglutinate clade, interspecific comparisons ranged from 0.31–2.42% (*L. altilis* – *L. perovalis*) to 3.74–5.45% (*L. perovalis* – *L. australis*).

Phylogenetic analysis

Analysis of combined 16S and COI data under MP resulted in 24 equally parsimonious trees of 501 steps (CI = 0.649,

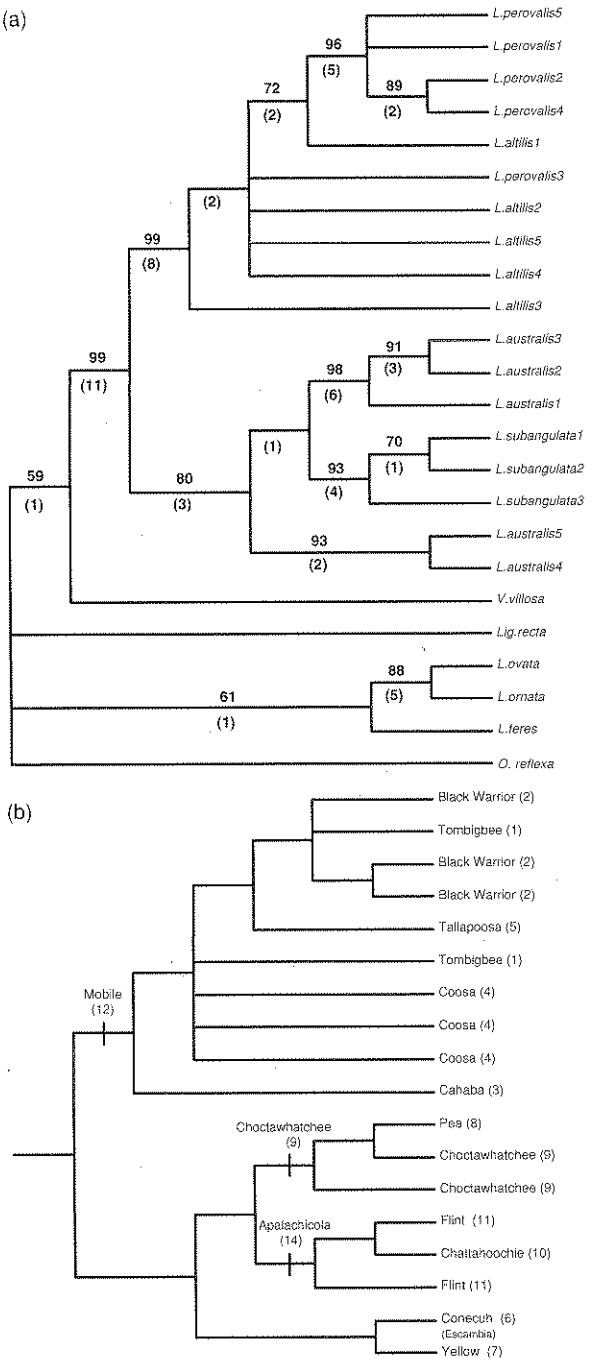

Fig. 3 (a) Strict consensus of 24 most parsimonious trees from MP analysis. Values above the nodes are bootstrap percentages, only values greater than 50% are shown. Values in parentheses below the nodes are support-decay indices. (b) Area cladogram based on superconglutinate producers generated using Brooks Parsimony Analysis. Numbers in parentheses at terminal and internal nodes correspond to river drainages in Fig. 2.

RC = 0.462) and a strict consensus of these trees is shown in Fig. 3(a). Analysis using NJ and ML resulted in less ambiguous topologies, but were entirely consistent with the results of the parsimony analysis and are not shown.

Table 3 Statistical comparison of alternate topologies using the Templeton test

Alternative hypothesis of monophyly†	No. of steps	Sign. worse	R.C.	No. of trees
Optimal tree(s)	501	—	0.462	24
L. perovalis	504	No	0.456	4
L. altilis	506	No	0.452	78
L. australis	503	No	0.458	60
Each species	507	No	0.450	20
Lampsilis	507	No	0.450	12
Each species & *Lampsilis*	513	Yes*	0.439	10

*$P < 0.05$. †Constrained trees of length equal to optimal trees are not included.

All of the superconglutinate-producing species (*L. altilis*, *L. australis*, *L. perovalis* and *L. subangulata*) were recovered as a monophyletic group in all reconstructions. The analyses place *Villosa villosa* as the sister taxon to the superconglutinate producer clade. All topologies also show two sister clades (*L. perovalis* + *L. altilis*) and (*L. australis* + *L. subangulata*). Only *L. subangulata* is consistently recovered as monophyletic, although its placement as sister group to the *L. australis* from the Pea-Choctawhatchee system renders *L. australis* paraphyletic. Trees resulting from constraining *L. australis* to be monophyletic are only two steps longer, and are not significantly worse than the optimal trees (Table 3). *L. altilis* and *L. perovalis* were never recovered as monophyletic taxa however, constraining *L. altilis* and *L. perovalis* to be monophyletic does not result in a significantly worse score (Table 3). Of the various constraint trees examined, only the tree constraining the monophyly of each superconglutinate-producing species and the genus *Lampsilis* to be monophyletic, was significantly worse ($P < 0.05$) than the optimal trees as determined by MP (Table 3).

The zoogeographic pattern produced using the superconglutinate clade depicts a monophyletic Mobile system that is sister to the Apalachicola and the remaining Gulf Coastal drainages. Within this remaining clade, the Apalachicola System was sister to the Choctawhatchee, which together were sister to the Escambia and Yellow rivers (Fig. 3b).

Discussion

Monophyly of superconglutinate producers

The results of all phylogenetic analysis support the monophyly of the four superconglutinate producers and indicate a single origin of the superconglutinate lure in the common ancestor of these species. Support/decay values for the most parsimonious solutions indicate that 11 additional steps are required to collapse the node supporting

the monophyly of this group. The support/decay index and high bootstrap values strongly support the recognition of these taxa as a natural group, and the contention of Fuller & Bereza (1973) and O'Brien & Brim-Box (1999) that the presence of the superconglutinate lure and unique gill morphology are synapomorphies that distinguish these species from other lampsiline mussels.

The results also indicate that there is a strong geographical component to the DNA data that is particularly apparent in the rivers east of the Mobile System. Lydeard *et al.* (2000) found similar geographical partitioning of genetic data in other Unionid mussels from this region. The observed lack of monophyly of the component genes of *Lampsilis altilis*, *L. australis* and *L. perovalis* could be due to any one or a combination of the following factors: (i) a lack of resolution of the terminal taxa due to inadequate sequence variation in the gene portions examined; (ii) that the recovered gene tree is evidence for introgression of mitochondrial haplotypes via hybridization; or (iii) the retention of ancestral haplotypes in several lineages. The first interpretation is supported by previous studies of invertebrates that indicate that the COI and 16S genes appear to accrue substitutions fairly slowly (Lydeard *et al.* 1996; Mulvey *et al.* 1997; Roe & Lydeard 1998). This hypothesis is also supported by the fact that when the *L. altilis*, *L. australis* and *L. perovalis* clades were constrained to be monophyletic, the resulting trees were not significantly worse than the most parsimonious trees as measured by the Templeton test.

An alternative to this interpretation is that in this instance the mtDNA gene tree is not reflective of the species tree. Historically, both *L. perovalis* and *L. altilis* were known throughout the Mobile Drainage System, and it is possible that those specimens that render *L. altilis*, *L. perovalis* and *L. australis* nonmonophyletic represent hybrids, each possessing the maternal haplotype of its sister species (Barton & Hewitt 1985). The lack of monophyly observed in *L. altilis*, *L. perovalis* and *L. australis* could also be due to the retention of ancestral haplotypes. The absence of monophyly has been noted as being a common initial stage in the divergence of population-level lineages (Neigel & Avise 1986); and the retention of ancestral mtDNA haplotypes has been invoked to explain the lack of monophyly observed in sand darters of the subgenus *Ammocrypta*, that also inhabit the Gulf region of the southeastern United States (Wiley & Hagen 1997). Resolving which of these alternatives explanations is the most likely will require the use of more rapidly evolving mitochondrial regions or microsatellites, which also would have the added benefit of providing information from multiple independent loci.

Phylogeography of superconglutinate producers

The Mobile Basin is one of the most biotically diverse freshwater systems in North America, containing many endemic species of aquatic organisms (Lydeard & Mayden 1995; Benz & Collins 1997). Gulf coastal drainages to the east of the Mobile Bay include the faunistically rich Apalachicola River System and the Escambia, Yellow and Choctawhatchee rivers. Several researchers have suggested that the distributions of species in this region are related to geological events that have altered drainage patterns (Wiley & Mayden 1985; Bermingham & Avise 1986; Swift *et al.* 1986). Examination of the area relationships indicated by taxa in this study reveals the following pattern (Fig. 3b): the Choctawhatchee River System is sister to the Apalachicola River System, which together are sister to the Yellow and Escambia rivers. All of these rivers together are sister to the Mobile System. The pattern of a split between the Mobile Basin and the rivers to the east is generally congruent with hypotheses derived from biogeographic analysis of fishes (Wiley & Mayden 1985; Swift *et al.* 1986; Kristmundsdóttir & Gold 1996; Wiley & Hagen 1997), and turtles (Lamb *et al.* 1994; Roman *et al.* 1999). All of these studies have sought to explain present day distributions of vertebrate taxa in Gulf Coastal Plain rivers using vicariant scenarios related to fluctuations in sea level during the latter part of the Cenozoic Era. The results of this study, although not strictly concordant with previously published studies of vertebrates, provides evidence of a similar phylogeographic pattern in freshwater bivalves.

Although it is impossible to determine accurately the time of origin for freshwater mussels in North America, fossil evidence indicates that unionid mussels may have been present in North America as early as the Triassic Period, at least 181 million years ago (Ma) (Haas 1969). If the common ancestor of the superconglutinate-producing species was already present in the Gulf region before the Oligocene (37–24 Ma), the receding sea level during the Oligocene or the Late Miocene (10–5 Ma) or Pleistocene (2.8 to < 0.3 Ma) would have allowed connections and subsequent dispersal between the Mobile and Apalachicola systems and the intervening Coastal Plain rivers (Donn *et al.* 1962). Using the mtDNA divergence times derived from several species of fishes, Bermingham & Avise (1986) proposed that the split between the Mobile River and the Apalachicola and the Escambia rivers occurred approximately 750 000 years ago. Such a relatively recent divergence would support a Pleistocene origin of the ancestors of the (*L. altilis* + *L. perovalis*) and (*L. australis* + *L. subangulata*) clades. A Pliocene or Pleistocene origin has been suggested for other North American freshwater mussel genera (e.g. Haas 1969; Davis *et al.* 1981), and the fluctuations in sea level during these periods could have provided avenues for dispersal and the isolating mechanism that created the phylogenetic pattern recovered in this study. A Pleistocene origin for these species is also supported by the relatively low genetic divergences observed within and between species of superconglutinate producers (Table 2).

The results of this study confirm the monophyly of the superconglutinate-producing species of the genus *Lampsilis*. Although the data presented here do not unambiguously support the monophyly of three of the four species in this clade, the phylogeographic pattern, strong geographical partitioning and low genetic divergences tend to support a relatively recent origin for these species. Further support for the recognition of these taxa as natural groups will require unionid-specific markers for more variable regions of the mitiochondrial and nuclear genomes, which are currently under development.

Conservation implications

The strong geographical structure observed in the data, combined with the apparent recent origin of these species, offer some guidance for the conservation of these taxa. All four of the superconglutinate-producing species are currently given some protection by either state or federal laws, and no captive rearing and propagation of any of these species is currently planned. The genetic data indicate that if such projects were to be instituted, care should be taken to maintain the genetic integrity of these nascent species by only augmenting existing populations, or re-establishing extirpated populations from the appropriate geographical source. In addition, monitoring of the numbers of each of these species should be conducted through regular surveys of the various watersheds. Recent surveys of freshwater mussels in the Mobile, Apalachicola, Conecuh and Pea/Choctawhatchee rivers revealed the complete absence of species (McGregor 2000a), restricted distribution of few individuals (McGregor 2000b), or found no evidence of juvenile recruitment (O'Brien & Brim-Box 1999). A likely reason for the decline of these species appears to be related to the use of visual lures to attract fish hosts. The highly specialized superconglutinate lure may make these mussels more susceptible to changes in their environment, such as increased turbidity and the absence of flowing water. The proliferation of reservoirs, which both reduce flow and increase turbidity, and increased sedimentation from the surrounding watersheds undoubtedly have had a negative impact on the effectiveness of the superconglutinate lure. Ultimately, the persistence of these species and the success of any future efforts to restock species through captive breeding will be dependent on the availability of suitable habitat throughout their respective ranges.

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