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Molecular Assessment of Three Reproducing Populations of the Clubshell Mussel (*Pleurobema clava*) Reveals Historic Hybridization With *Pleurobema cordatum*



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Introduction

- The clubshell (*Pleurobema clava*) is a federally endangered species of freshwater mussel endemic to Ohio that is currently found in fewer than ten river systems in the United States (>85% range reduction).
- Two of the USFWS's (1993) recovery goals are to establish ten viable populations and maintain any existing genetic viability.
- In order to recover the clubshell, conservation efforts including translocation and captive propagation have been used to augment existing populations.
- In order to maintain the genetic diversity and population level specificity, a molecular assessment is needed.
- In this study, we generated nuclear microsatellite data and mitochondrial (mtDNA) sequence data from three populations of clubshell to assess population structure.

Methods

- Clubshell were sampled from three reproducing populations (Fig. 1) including: Little Darby Creek, OH (N=16); St. Joseph River, MI (N=17); and the Alleghany River, PA (N = 18). Mantle tissue clips were obtained and stored in 95% ethanol.
- Seven polymorphic microsatellite loci and two mtDNA genes (CO1, ND1) were sequenced for each individual (Fig. 2).

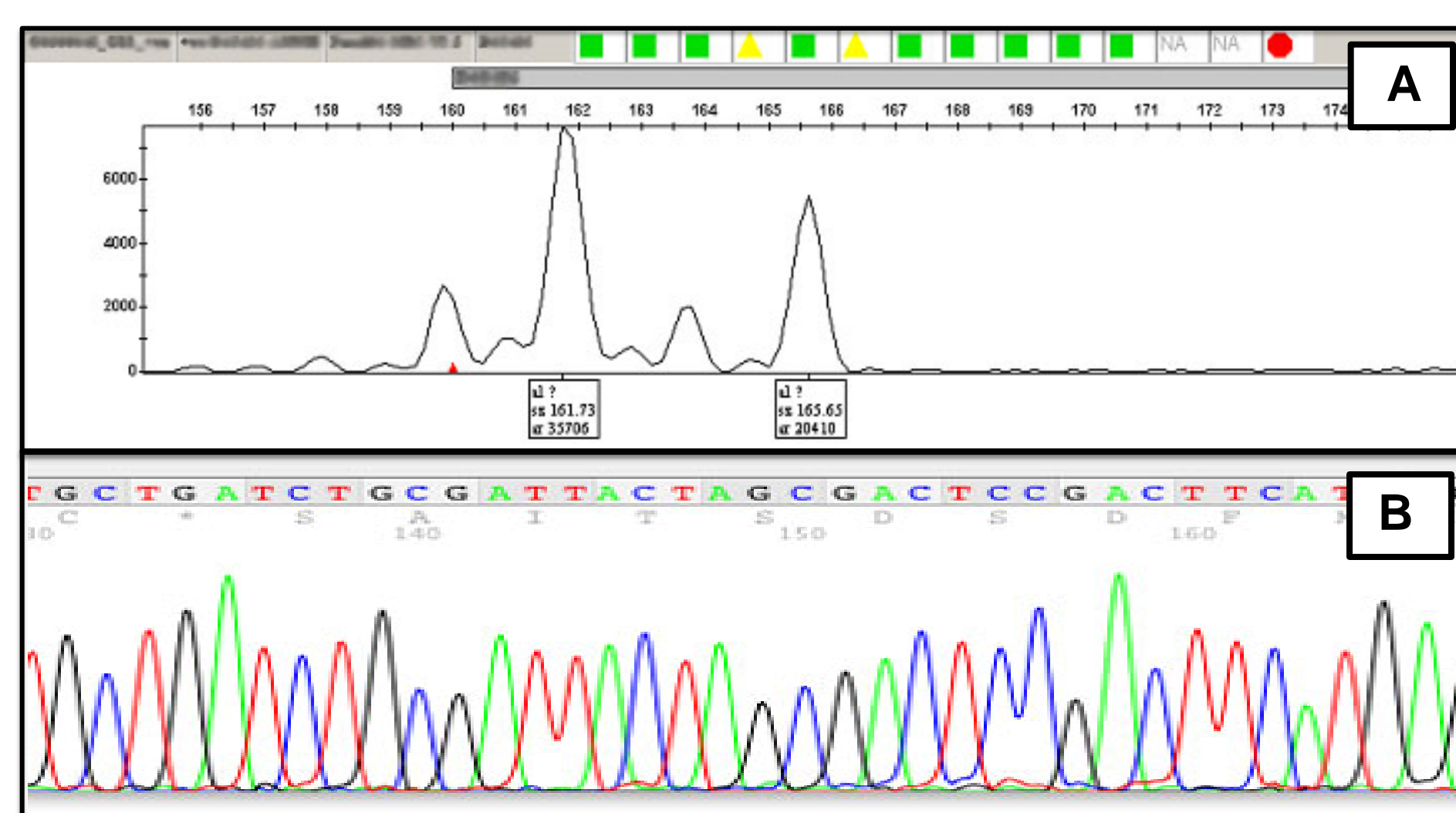


Figure 2. A. Microsatellite sample plot illustrating a heterozygote individual; B. DNA sequencing chromatogram.

- Additional sequences (26) representing various species of *Pleurobema* (Campbell & Lydeard, 2012) were obtained from Genbank.
- Phylogenetic analysis was performed using TNT (Goloboff et al., 2008) under the parsimony criterion (MP). Nodal support was determined using 10,000 jackknife replicates. Microsatellite data were analyzed using Structure (Pritchard et al., 2000) and GenePop (Raymond & Rousset, 1995). Null alleles were assessed using Micro-Checker (Oosterhout et al., 2004).

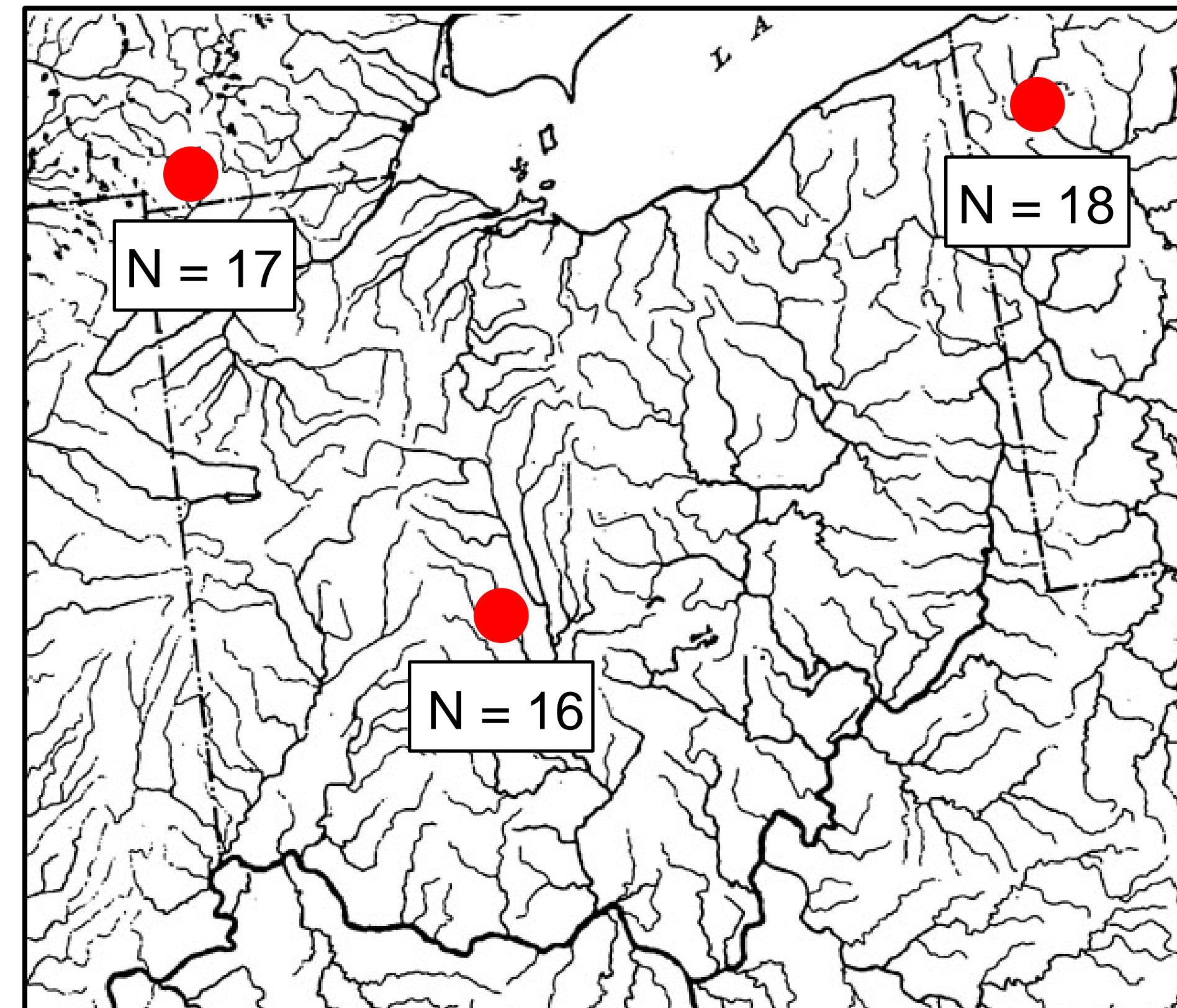


Figure 1. . Sampling locations for *Pleurobema clava*. Locations include: Big Darby Creek, OH; Alleghany River, PA; and St. Joseph River, MI.

Results

MICROSATELLITE DATA

- Structure analysis: K's 1-5, 3 runs per K, 1,000,000 iterations
- Structure Harvester (Evanno et al., 2005) identified K=2 populations (Fig 3).

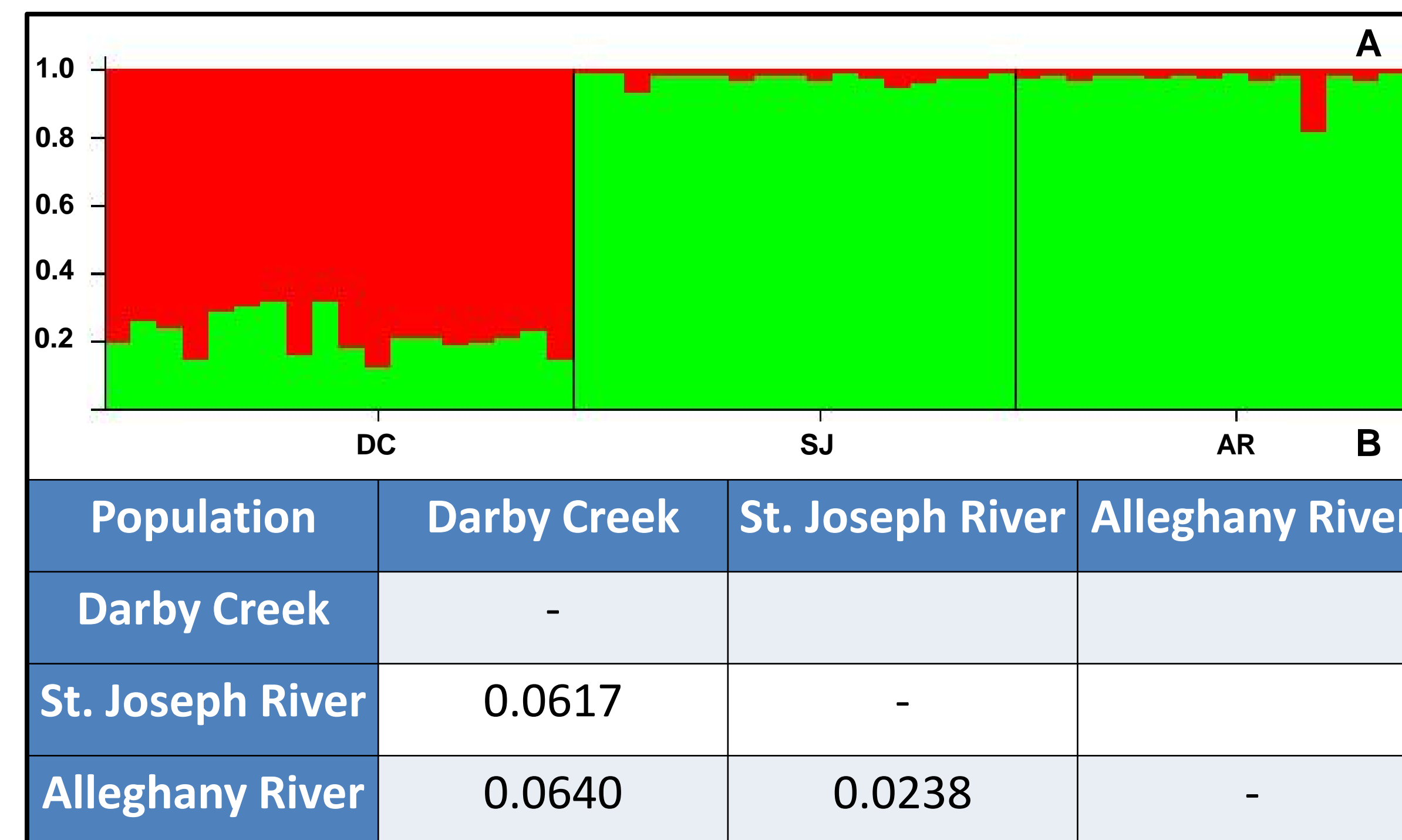


Figure 3. A. Structure Harvester analysis indicating K=2 populations; B. F_{st} values for pairwise comparison of the 3 populations (< 0.05 = low differentiation, 0.05 – 0.15 = moderate differentiation, > 0.15 = high differentiation).

MtDNA SEQUENCE DATA

- Additional sequences include: *Pleurobema* (13), *Fusconaia* (5), *Elliptio* (5), *Elliptioideus* (1), *Lexingtonia* (1), *Quincuncina* (1).
- Phylogenetic analysis produced 17 equally parsimonious trees of 617 steps.

Literature Cited

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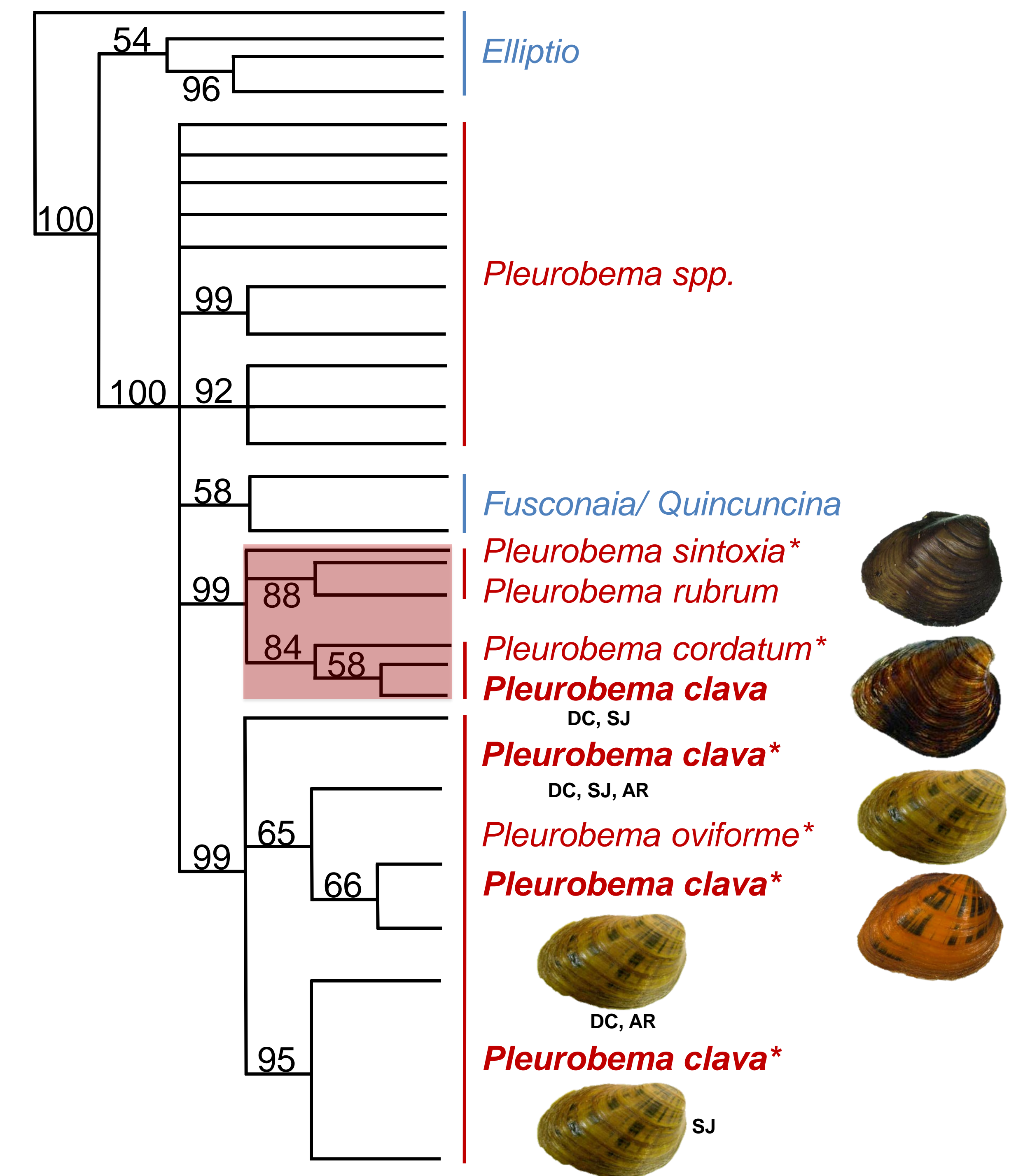


Figure 3. Phylogenetic reconstruction of *Pleurobema clava* individuals using CO1 and ND1 sequence data. Values = Jackknife support; clades with values <50 are not indicated. (*) Corresponds to an image (in sequential order); DC, SJ, AR correspond to populations.

Conclusions

Four Major Conclusions:

- Microsatellite results indicate the Little Darby Creek population is unique when compared to the St. Joseph and Alleghany River populations.
- MtDNA indicates substantial structure in clubshell populations with the St. Joseph River population the most distinct.
- Pleurobema oviforme* and *Pleurobema clava* are very similar and may represent the same species.
- MtDNA indicates historic hybridization with *Pleurobema cordatum*. The FIRST documented hybridization in freshwater mussels.