Introduction
The Pleistocene glaciation exerted a profound influence on the formation of river drainages, shaping the course of freshwater ecosystems and leaving a lasting impact on genetic diversity within aquatic species (Mayden, 1988). As vast ice sheets advanced and retreated, they sculpted landscapes, rerouting and reshaping river systems (Mayden, 1988). In the wake of glacial retreats, isolated populations of freshwater organisms, such as fish, found themselves in distinct drainage basins (Mayden, 1988). This geographic isolation allows for the possibility of genetically distinct lineages among drainage basins (Wiley and Mayden, 1985). *Ericymba dorsalis*, a northernly distributed species of fish, is a prime example. *Ericymba dorsalis* has a broad distribution exhibiting similar morphologies, but we hypothesize that the separate drainage systems will yield genetically distinct populations.

*Ericymba dorsalis*, commonly known as the Bighorn Shiner, is a species of topminnow located in small shallow streams in central North America (Lee et al., 1980). These populated streams occur throughout the tributaries of the upper Mississippi River, the Missouri River, the upper Ohio River, and the tributaries of Lake Michigan, Lake Erie, and Lake Ontario. The current discontinuous pattern of distribution of the Bighorn Shiner populations is evidence of vicariance biogeography.

Objectives:
- We generated mitochondrial cytochrome b DNA sequences for samples of *Ericymba dorsalis* collected from drainages throughout much of the species’ range.
- We used phylogenetic methods to demonstrate genetic relationships among individuals and populations.
- We tested the hypothesis that separate phylogenetic groups would correspond to separate major drainages: Missouri River, upper Mississippi, Great Lakes tributaries, Illinois River, and the Ohio River.

Methods
- DNA was extracted using Qiagen Dneasy Blood and Tissue Columns.
- Cytochrome B region was sequenced using three primers (Raley and Wood, 2002).
- Samples were sequenced using Sanger Sequencing at the Mizzou DNA Core.
- A Median Joining Network was generated using PopART v. 1.7.
- Sanger Sequences were assembled using Geneious Primer v2024.0 and phylogenies reconstructed using Maximum Likelihood with a Tamura-Nei substitution model and Nearest Neighbor Interchange in MEGA v11.0.10.

Results
Figure 1: Median Joining Network with colors corresponding to drainage systems. There were some Missouri River haplotypes present in the Illinois River drainage, accounting for the red bubbles within the Mississippi River drainage cluster.

Figure 2: ML phylogeny. A genetic divergence of 1.3% to 1.6% was found among the clades of the Missouri River, Upper Mississippi River, and the Illinois River, respectively. The divergence of the Great Lakes and Allegheny clades from the other western clades ranged from 4.3% to 5.3%.

Figure 3: Sampling locations overlaid on a Missouri River drainage map. The color of each sample location corresponds with the phylogenetic groupings in the ML tree (Figure 2). The correlation between clade assignment and drainage of origin affirms the hypothesis that the major clades correspond to the major drainages, with some evidence of mixing in the lower Illinois River drainage.

Discussion
- Our analyses support separate phylogenetic clades corresponding to major drainage basins throughout the *E. dorsalis* distribution. The genetic divergence between Missouri, Mississippi, and Illinois River clades is consistent with Pleistocene isolation of those populations.
- Divergence between the three western clades, the Great Lakes clade, and the Allegheny River clade suggest early Pleistocene or Pliocene divergence for the latter two clades. This level of divergence may require reassessment of the species status of these phylogenetic groups.
- Previous phylogenetic research on *Ericymba dorsalis* came to the conclusion of three distinct species with one species in the Allegheny River drainages, one located in the Plate River, and another species located in the rest of the species’ distribution (Page and Burr, 1991). Our results support the genetic distinctiveness of the Allegheny, but not the Plate River populations.
- These results provide a definitive assessment of drainage relationships based on a single mitochondrial gene. Future work should corroborate these results with nuclear genome phylogenetic reconstruction as well as a review of morphological variation among drainages.

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