



Assessing Phylogenetic Relationships and Testing Speciation Mechanisms in Darter Fish (genus: *Etheostoma*) in the United States



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Abstract

This study used molecular methods to determine the relationships of species in a group of fishes (Genus *Etheostoma*, subgenus *Doration*) that appear to be an excellent example of speciation by sexual selection. Species in this group differ mostly in male breeding color, which is a sexual ornament. There is a suggested phylogenetic hypothesis of this subgenus based on morphology and breeding colors (Layman and Mayden, 2012). However, molecular techniques had not been used. A phylogeny based on molecular (DNA) data is critical in modern biology. The primary method used was mitochondrial cytochrome b sequencing, since it has been shown to support relationships among darters. Phylogenetic clusters were also compared to geographical clusters to determine whether clusters were associated with geography. It is important to estimate a phylogenetic hypothesis of the subgenus *Doration* in order to track the evolutionary history of the group and determine the mechanisms of speciation.

Background

- Darters are one of the most diverse groups of North American freshwater fish
- Nearly every species is characterized by unique coloration
- Isolation can be maintained based on visual signals, coloration, and mate preferences
- Sexual ornaments are physical characteristics that serve as decorative functions to attract mates

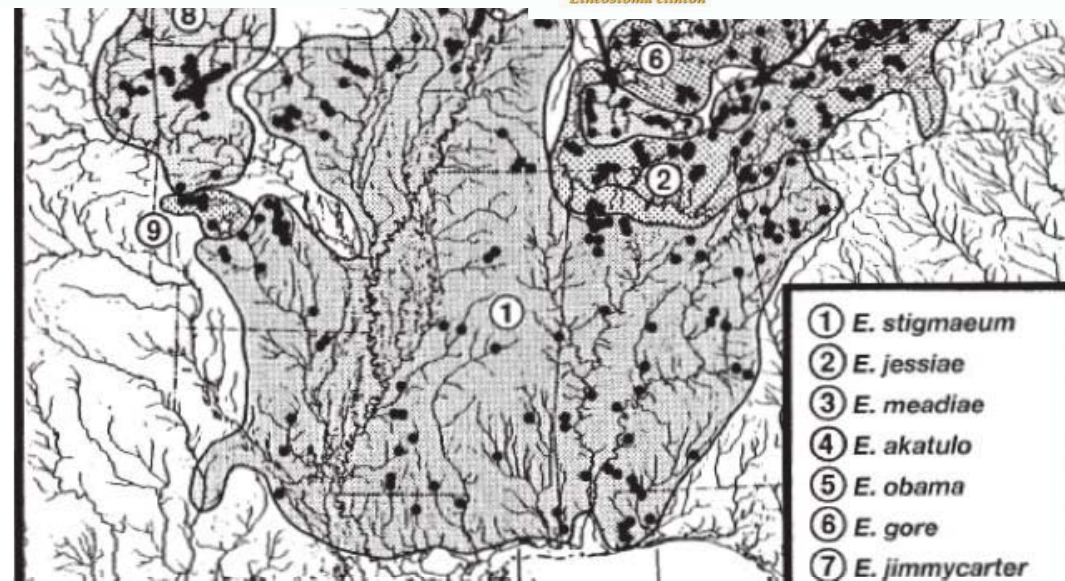


Figure 1: Distribution and proposed species names (Presidential Darters) of the stigmaeum complex of darters based on morphological characteristics (Layman and Mayden, 2012)

Methods

Obtain Samples

- Ethanol preserved and frozen samples from collaborators at St. Louis University
- Ethanol preserved samples from previous lab collections
- Fish collections of *E. obama* and *E. jessiae* in April 2016



Figure 2: Collection sites for *E. obama* and *E. jessiae* at Little River, Maryville, TN (left) and Duck River, Shelbyville, TN (right), respectively.

Genomic Analysis

- Isolate DNA from fin clippings using Qiagen DNeasy Tissue Kit
- PCR - 1040 bp of cytochrome b
- Primers
 - cytbF: GTGACTTGAAAAACCACCGTTG
 - cytbR: CTCCATCTCCGTTTACAAGAC
- Confirm PCR using gel electrophoresis
- Mitochondrial cytochrome b gene sequencing using GeneWiz

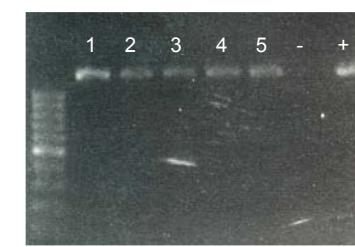


Figure 3: Confirmation of PCR through the presence of the cytb band at ~1000bp.

Phylogenetic Analysis

- Construct phylogeny (MEGA v5.2) using previously sequenced cytb samples from GenBank and DNA extractions

Results and Discussion

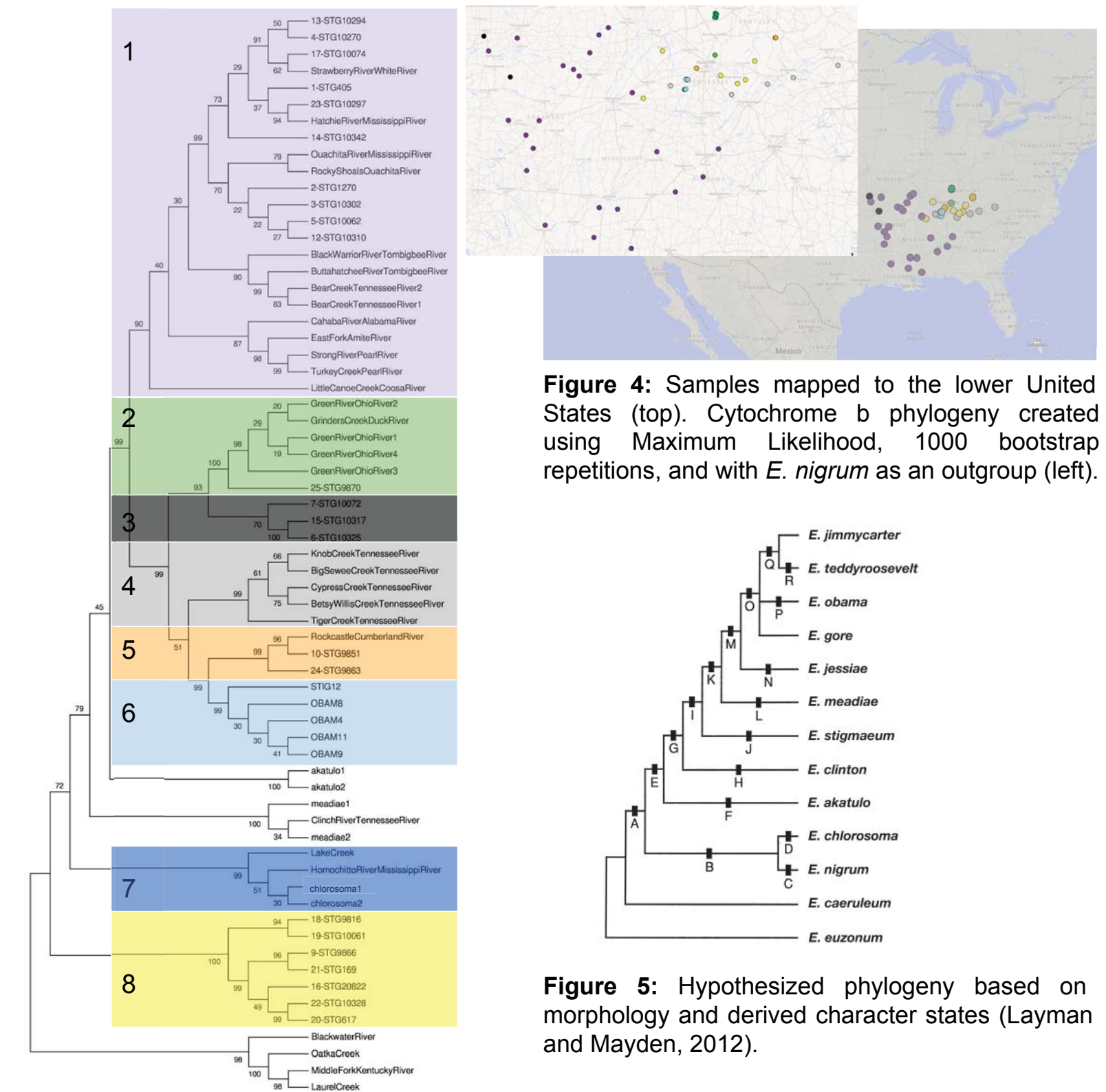


Figure 4: Samples mapped to the lower United States (top). Cytochrome b phylogeny created using Maximum Likelihood, 1000 bootstrap repetitions, and with *E. nigrum* as an outgroup (left).

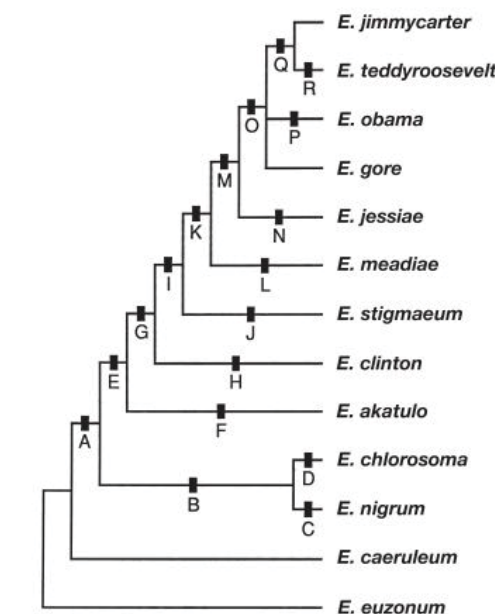


Figure 5: Hypothesized phylogeny based on morphology and derived character states (Layman and Mayden, 2012).

Future Directions

- Dichotomous mate trials
- AFLP and ddRADseq
- Paternity Test
 - 5 males and 5 females of *E. obama* and *E. jessiae* placed in a tank and allowed to spawn for several days
 - Collect and extract DNA from fertilized eggs
 - Microsatellite analysis

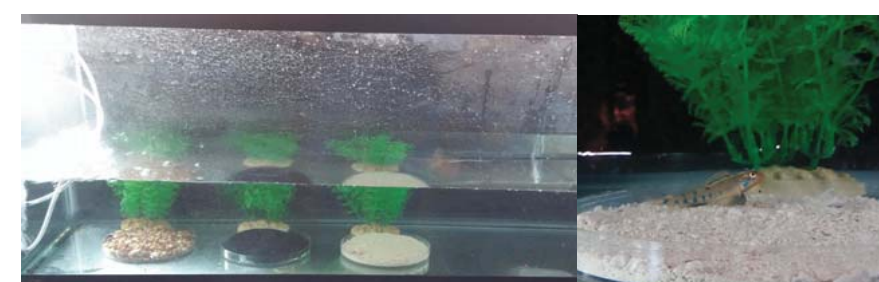


Figure 6: 60" long tank with choices of gravel, black sand, and white sand substrate allowed for the fish to freely spawn.

Summary

- Molecular clusters match geographical clusters
- Proposed clusters
 - 1 *E. stigmaeum*
 - 2 *E. jimmycarter*
 - 3 *E. teddyroosevelt*
 - 4 *E. jessiae*
 - 5 *E. gore*
 - 6 *E. obama*
 - 7 *E. chlorosoma*
 - 8 Caney Fork
- Molecular phylogeny is similar to that of the proposed phylogeny based on derived character states

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