Crayfish Size and Molecular Identification of *Faxonius durelli* (Decapoda: Cambaridae) in Giles County, Tennessee

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Introduction

Habitat destruction due to development is one of the top threats to crayfish populations, thus in many cases, it creates a restricted home range (Hobbs 1989). Other threats such as disease and invasive species also pose a threat to populations of crayfish (Taylor et al. 2007). Because of their position in the food chain, crayfish can be considered a keystone species (Larson and Olden 2011), as well as an indicator species for water quality (Kuklina et al. 2013). Morphological observation is usually the main way to identify crayfish species, however external characteristics usually tend to be similar in almost all species, therefore this can make it hard to identify and classify species based on morphological structure (Fetzner and Crandall 2014). One method used to alleviate problems with species identification is the use of genetic analysis. Further research on genetic analysis will provide robust crayfish identification for this region. Population dynamics of crayfish should be monitored in order to determine if conservation efforts would be necessary.

Methods

Specimen Collection

- Crayfish were collected using hand-held seines at 3 sites along a stream that traverse Giles County, Tennessee (Fig. 1).
- For each individual, we recorded gender, carapace and total length (mm) using a hand-held caliper, and weight (g) using a digital scale.
- We removed a claw from specimen at each site, then stored in 70% ethanol at -20°C

DNA Extraction, PCR Amplification, and Sequencing

- DNA was extracted from 50 mg of tissue, followed by the manufacturer's protocol of an E.Z.N.A. Tissue DNA Kit (Omega. U.S.A.).
- Mitochondrial cytochrome oxidase subunit 1 (COI) was amplified using the following universal primers (Folmer et al. 1994).
- Aliquots from PCR amplification were checked by 1.7% agarose gel electrophoresis.
- PCR products were enzymatically purified with 10 µl ExoSAP.
- PCR products were sequenced in both directions at Eurofins Genomics (Kentucky, U.S.A.).

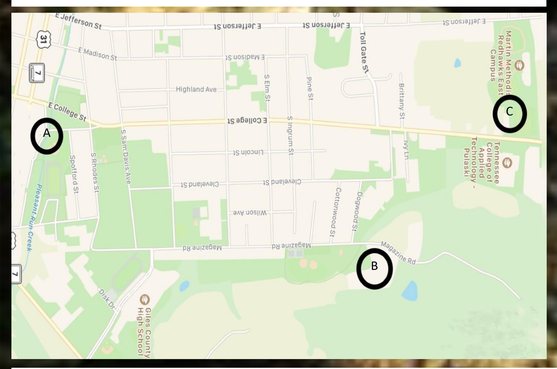

Fig. 1. Site locations of crayfish specimen collection for molecular analysis, and morphological measurement during Fall 2020 at Giles County, Tennessee. (A) Pleasant Run (B) Magazine Road (C) East Campus

Fig. 2 Photographs of the crayfish *Faxonius durelli* located in streams of Giles County, Tennessee

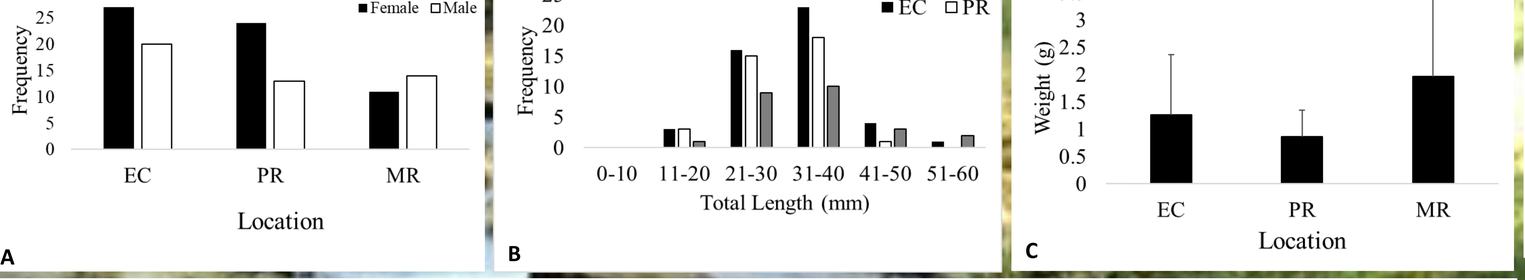

Fig. 3. Crayfish measurements at East Campus (EC), Pleasant Run (PR), and Magazine Road (MR) illustrating (A) gender frequency at sites (B) Frequency of total length (mm) and (C) Mean Weight (g) (Error bars indicate standard deviation, * represents significant difference, ANOVA, $p < 0.05$)

Results

- Female crayfish were only slightly higher at EC and PR (Fig. 3A).
- Most crayfish had total length ranging between 21-40 mm at sites (Fig. 3B)
- Crayfish weighed significantly larger at MR (ANOVA, F (2,108) = 7.95, p < 0.05) (Fig. 3C).
- Morphological observation identified orange tips, followed by a black band at the tips of all chelae (Fig. 2).
- Molecular analysis identified crayfish at all sites to be *Faxonius durelli* (Fig. 4).

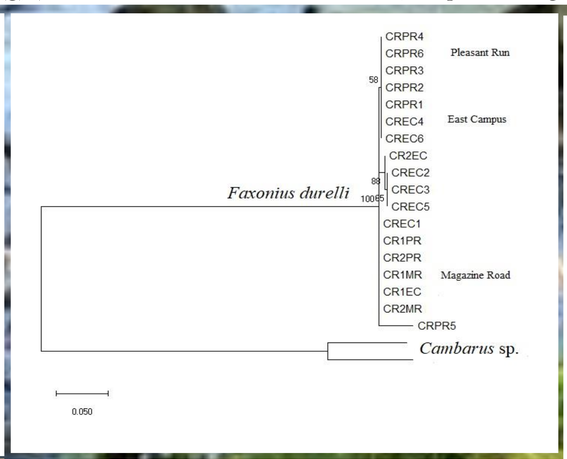

Fig. 4 Maximum Likelihood phylogenetic tree illustrating samples analyzed using molecular techniques with specimen from EC, PR, and MR shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches.

Acknowledgements

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Discussion

Collecting data on morphology and molecular analysis to determine species identification located at streams of Giles County, Tennessee is important in determining ecosystem characteristics (Kuklina et. al 2013).

Faxonius durelli (Bouchard & Bouchard 1995) is not an uncommon species, however Giles County is just one hour south of Nashville, where there is a protected crayfish species, *Orconectes shoupi* (Bizwell & Mattingly 2010). It is important to identify species located in the stream system throughout Tennessee, so that a record is maintained on local and statewide species distribution.

Morphological observations of crayfish chelae identified an orange tip, followed by a black band, is an important characteristic in determining the species *Faxonius durelli*.

Further molecular analysis supports and clarifies the identification of *Faxonius durelli*.

Conclusion

Although determining crayfish density and size distribution in streams at Giles County was relatively easy, we have finally determined one of the species at these locations using molecular identification. We have identified two other species, however a lack of informative data were unable to be used.

Our immediate goal is to continue to collect density and morphological data in order to determine if there are more species present other than *Faxonius durelli*.

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