A Comparative Survey of Morphology and Genetic Analysis of *Faxonius* sp. Crayfish in Giles County, Tennessee

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ABSTRACT

Crayfish populations are highly imperiled in North America, with threats such as habitat fragmentation, poor water quality, and increased distribution of invasive species. Morphological features used in determining crayfish species continue to be challenging, hence the need for genetic analysis to determine crayfish species at three creeks in southern Tennessee. An E.Z.N.A. tissue kit was used to extract the DNA, followed by polymerase chain reaction (PCR) analysis and sequencing. Hand-held seines were used to collect crayfish and measurements determined gender, carapace length (CL-mm), total length (TL-mm), and weight (g) for the spring and fall of 2019. Crayfish TL in spring 2019 differed significantly among sites (ANOVA,$F(2,81)=5.671$; $p<0.05$). In fall of 2019 TL differed scientifically. Weight and CL did not differ significantly among sites ($p>0.05$) respectively. Genetic analysis provided further insight to species identification.

INTRODUCTION

The purpose of this study was to survey crayfish size and morphology at three creeks in Giles County, Tennessee and to also determine the genetic sequencing of the species. Following collections, we identified gender, measured carapace length (CL-mm), total length (TL-mm), and weight (g) for each crayfish. The "kick method" was used to collect the crayfish into a hand-held seine at each site. This research will be conducted annually in order to determine if the morphology of the *local crayfish species* is changing, or if the population size is decreasing, increasing, or remaining constant. Though many species have been identified in Tennessee, there has not been research conducted on the population dynamics of crayfish in the Giles County, Tennessee, region. Morphological observation is usually the main way to identify crayfish species, but the characteristics usually tend to be similar in almost all species (Fetzner and Crandall 2014). This can make it hard to identify exactly which species it is based on morphological structure. However, genetic analysis will assist identification. Habitat destruction due to development is one of the top threats of crayfish, creating a restricted home range (Black and Russ 2015). Other threats such as disease and invasive species also pose a threat to populations of crayfish (Black and Russ 2015). 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). Due to these factors, the population dynamics of crayfish should be monitored in order to determine if conservation efforts would be necessary. Further research on genetic analysis will provide robust crayfish identification for this region.

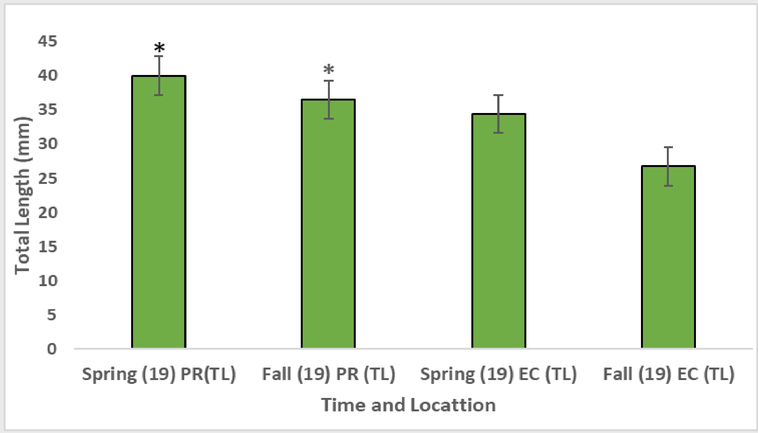

Figure 1. Mean total length (mm) at East Campus (EC) and Pleasant Run Creek (PR). Same year (2019), different seasons (Spring, Fall). Asterisks * represents significance in each season.

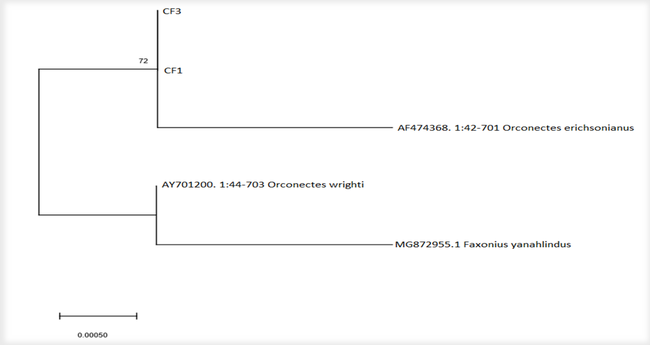

Figure 2. Crayfish Phylogeny from the products of the PCR

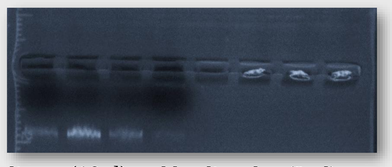

Figure 3.Gel after PCR aliquot (10µl) and loading dye (2 µl) went through electrophoresis.

METHODS

- ***Location***

Crayfish data were collected from Pulaski, Tennessee. Sites: Martin Methodist College's East Campus (35°11'37" N, 87°0'27"W), Pleasant Run Creek (35°11'54" N, 87°01'52" W), Magazine Road Park (35°11'19" N, 87°0'59" W). We collected morphological data at each site for 2 hours.

- ***Collecting Data***

Crayfish were captured using the "kick method" to direct the crayfish into a hand-held seine held at a 45° angle . One collector would hold the seine while the other members would stand two meters away to graze the bottom of the creek floor to stir up the crayfish. Once collected in the seine, we transferred the crayfish into a bucket filled halfway with water. We documented the gender, total length (TL mm), carapace length (CL mm), and weight (g) for each crayfish. We used a hand-held caliper to measure the total length and carapace length in millimeters. A lid was used to hold the crayfish on a digital scale to measure the weight in grams. Each crayfish was returned to the stream after the data was recorded individually.

- ***Statistical Analysis***

An ANOVA test was used to determine if there were any significant differences in mean total length (mm), mean carapace length (mm), or mean weight (g) between the three sites. A T-test was then used to determine which site(s) had the significant difference.

- ***Genetic Analysis***

DNA was extracted from a claw of the crayfish using the E.Z.N.A. tissue DNA Kit (Omega-Biotek, Model: D3396778-9). The manufacturer's protocol was used to extract crayfish DNA. Primers for the mitochondrial marker cytochrome oxidase subunit 1 (CO1) was used (Bloom et al. 2018). Primers that have previously been used in other studies (Folmer et al. 1994; Sinniger et al. 2005) were used in this experiment: LCO1490 (5'-ggtcaacaaatcataaagatattgg- 3') and HC02198 (5'-taaacttcagggtgaccaaaaaatca- 3'), these were used to amplify the CO1 gene (Bloom et al. 2018). The Thermocycler conditions that were mentioned in Bloom et al. (2018) were followed: 35 cycles of 30 s at 94°C, 30 s at 53 °C, and 90 s at 72 °C that occurs at initial denaturation step of 3 min at 94 °C. The product of the PCR was place in agarose gel (1%) and went through electrophoresis at 100 volts for 30 min. Each well contained 2µl loading dye and 10µl of PCR aliquot. Amplified products were sent to Eurofins Genomics LLC for DNA sequencing. MEGA-X was used to edit sequences prior to BLAST analysis.

RESULTS

There were no significant differences in crayfish mean weight ($t=2.0$, $p > 0.05$) or mean carapace length ($t= 2.0$, $p>0.05$) in the spring of 2019. There was a significant difference in the mean total length at Pleasant Run creek and East Campus ($t= 2.0$, $p< 0.05$) in the spring of 2019. There were no significant differences in the size of the carapace ($t=2.0$, $p>0.05$) in fall 2019. There was significant difference in the total length at Pleasant run compared to East Campus ($t=2.71$, $p<0.05$) in the fall of 2019.

Spring 2019- The mean total length for East Campus measured 34.4 mm ± 2.3 (Mean ± SE). The mean total length for Pleasant Run creek was 40.0 mm ± 2.2. The mean total length at Magazine Road was 36.0 mm ± 2.9.

Fall 2019- The mean total length for Pleasant Run creek was 36.52 mm ± 3.36 (Mean ± SE). The mean total length for Mt. Pleasant Run was 33.08 mm ± 1.85. The mean total length for East Campus creek was 26.73 mm ± 3.12.

Genetic Analysis- Two out of the four crayfish that were sent for DNA sequencing were identified as the Reticulate Crayfish (*Orconectes erichsonianus*). The other two were considered to be a different type of species, but did not match completely.

DISCUSSION

We have successfully completed two seasons in the same year of crayfish collections. One site in the spring of 2019 showed a significant difference in mean total length and then the same site showed significant difference in the fall of 2019. This tells us that the population at pleasant run, that showed significant difference in both seasons, has the largest crayfish from the three sites. This could mean the other sites may have habitat degradation which implies that there could be siltation, agricultural runoff or pollution, however further research will need to be conducted to insure that is the cause. Annual studies will need to be continued to determine changes in population dynamics at each site. The genus of crayfish that was identified, was thought to have been *Faxonius* sp. from morphological characteristics, however molecular analysis showed otherwise. Also, the location at which the survey was conducted has been known to have microbial, pesticide and herbicide contamination (City of Pulaski Water System Water Quality Report, 2017). In addition, the Nashville crayfish, *Faxonius shoupi*, is classified as endangered on the IUCN Red List (Nashville Crayfish Conservation). Since the crayfish of our focal study is the *Faxonius* sp., the insight of the DNA analysis and annual population dynamics is needed to determine the status of the crayfish. Also, because of the results of the DNA analysis there is still need for genetic testing to determine how many different species of crayfish that are in the Giles County area.

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