SOUTH CAROLINA STATE REPORT (SCDNR Marine Resources Division)

Gulf and South Atlantic Regional Panel on Invasive Species
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Investigating hybridization between the invasive red swamp crayfish (*Procambarus clarkii*) and its sister species the eastern red swamp crayfish (*Procambarus troglodytes*).

The red swamp crayfish, *Procambarus clarkii*, which is invasive to South Carolina, is nested within the subgenus *Scapulicambarus*, which it shares with only four other species, including the native eastern red swamp crayfish, *Procambarus troglodytes*, which Busack (1989) showed to be the species most closely related to *P. clarkii*. The eastern red swamp crayfish, *P. troglodytes* is the most abundant native crayfish species in South Carolina, where much of its range overlaps with known locations of invasive *P. clarkii*. Hybridization is common among crayfish species, however, the majority of the research to assess hybridization of non-native crayfish with native species has focused on the genus *Faxonius* (Perry *et al*., 2001; Arcella *et al*., 2014), with little data currently available for hybridization within the genus *Procambarus*.

Researchers at the SCDNR MRRI have been applying molecular tools to test whether hybridization is occurring within wild populations of the *Scapulicambarus* subgenus of crayfish. Baited minnow traps and dip netting were the primary techniques used to locate *P. clarkii* and *P. troglodytes*. Microsatellite markers were used to genotype *P. clarkii*, *P. troglodytes* and any potential hybrids collected in the field. The resulting genotypes were subjected to the model-based Bayesian clustering methods implemented in STRUCTURE to estimate and visualize potential shared ancestry that would be expected if hybridization is occurring between these two species.

Of the 17 loci that consistently amplify in *P. clarkii*, 6 also amplify and bin reliably in *P. troglodytes* (Table 1). A total of 259 samples, 127 *P. clarkii* and 132 *P. troglodytes*, are included in the final STRUCTURE analysis estimating shared ancestry between the two species (Figure 1). Our results show that no individuals included in these analyses were hybrids and there is no indication of recent or rampant hybridization between *P. clarkii* and *P. troglodytes* at any of the sampling locations. Loci Pcl-12 and Pcl-64 were particularly informative as indicators for hybridization as the 2 or 3 alleles that occur in *P. troglodytes* are ubiquitous in *P. troglodytes* and completely absent in *P. clarkii*. If hybridization were occurring, we would expect to see introgression of alleles between species at these loci.

Table 1. Microsatellite loci and number of alleles found all samples for both *P. troglodytes* (Ptr) and *P. clarkii* (Pcl).

Locus Name	Number of Alleles (Ptr)	Number of Alleles (Pcl)
Pcl-12	2	23
Pcl-34	5	8
Pcl-70	24	22
Pcl-53	34	29
Pcl-64	3	20
Pcl-79	39	15

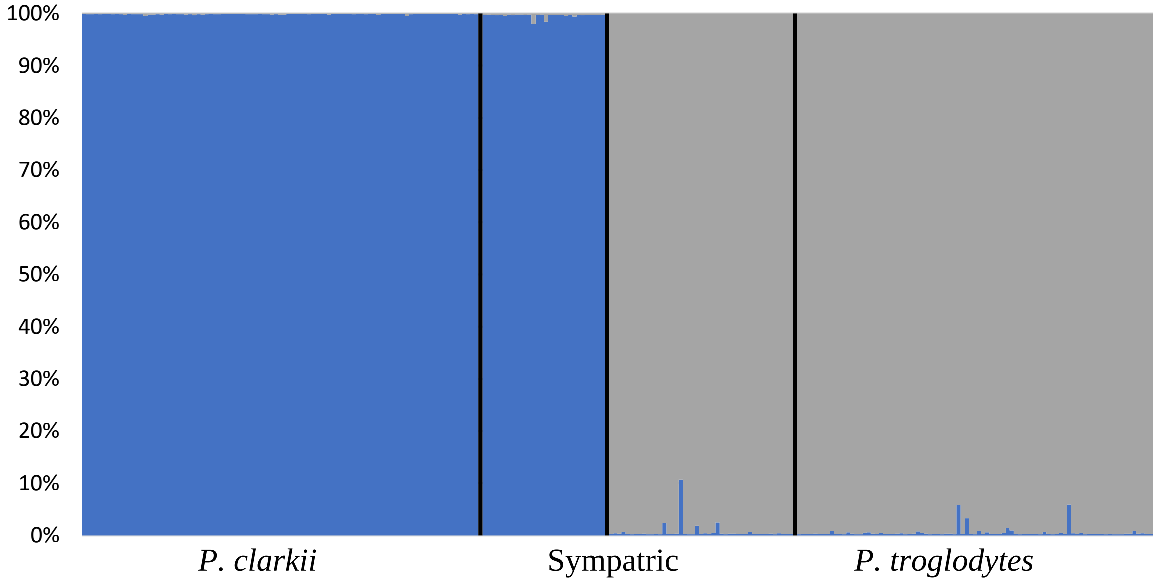

Figure 1. STRUCTURE bar plot results estimating and visualizing shared ancestry between *P. clarkii* (blue bars) and *P. troglodytes* (grey bars). Allopatric samples are shown on the right and left ends of the plot, while sympatric samples are located in the middle of this figure.

Assessing potential transmission pathways for the transmission of white spot syndrome virus (WSSV) to native crustaceans.

White spot syndrome virus (WSSV) is highly pathogenic (Escobedo-Bonilla *et al.*, 2008), infects many crustacean species, and was recently associated with both wild and farmed red swamp crayfish, *Procambarus clarkii* in Louisiana. Since Louisiana exports live *P. clarkii* to South Carolina (SC), the potential presence of WSSV in these specimens raises concerns over WSSV threats to commercially- and recreationally-important native crustacean species in SC, such as the white shrimp (*Penaeus setiferus*) and blue crab (*Callinectes sapidus*) that are known to be susceptible to WSSV. Researchers are conducting experimental trials to investigate the potential pathways of WSSV transmission from *P. clarkii* to estuarine crustaceans.

One such trial was conducted between December 7 – December 16, 2022. The objective of this trial was to evaluate potential pathways of interspecific transmission from *P. clarkii* to *P. setiferus* via cohabitation with infected crayfish (no direct contact) and ingestion of previously infected crayfish tissue. The four experimental treatments (each with 7 replicates) were as follows: (1) ingestion of control (uninfected) tissue; (2) ingestion of infected (WSSV) tissue; (3) cohabitation with control (seawater) injected crayfish; and (4) (3) cohabitation with infected (WSSV) crayfish. This trial produced results that showed positive transmission of WSSV to shrimp through the ingestion of infected tissue but failed to display transmission via cohabitation. These results were presented at the 115th Annual Meeting of National Shellfisheries Association held in Baltimore, MD on March 26-30, 2023.

Assessing abundance trends for non-native portunid crabs (family Portunidae).

Commercial and recreational crabbers have increasingly reported the occurrence of invasive portunid crabs in South Carolina. This includes the Indo-Pacific swimming crab, *Charybdis hellerii* and the bocourt swimming crab, *Callinectes bocourti*. To manage any potential ecological and fisheries impacts, researchers with SCDNR MRRI's Shellfish Research Section are interested in understanding the distribution and occurrence of these invasive portunid species. Portunid crabs are often difficult to identify at the juvenile stage leading to a lack of life history information for many portunid species in this age class. Therefore, researchers at the MRRI are using a combination of morphological and genetic approaches to facilitate greater taxonomic resolution for juvenile portunid species. Specimens were collected and retained from the SCDNR Estuarine Trawl Survey, which includes 26 statewide sampling locations. Sampling for this project has been completed, resulting in the collection of over 700 juvenile portunids from the *Callinectes, Arenaeus,* and *Achelous* genera. Genetic samples have been sent for analysis and 178 samples have been sequenced at the COI mitochondrial gene. Data analyses are ongoing.

References

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