Breaking the Mississippi River Barrier: Range Extensions of *Faxonius palmeri* Subspecies and Overlap with Rare Crayfish Species in Louisiana

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Abstract - Stream community surveys from 2008 to 2015 in the central Louisiana river basins (Red, Calcasieu, Vermillion-Teche, Mermentau, Ouachita, and Atchafalaya rivers) have resulted in new distributional data for 2 subspecies of *Faxonius palmeri* (Lowland Painted Crayfish). We also report unidentified *Faxonius* specimens appearing in streams of the northern Calcasieu River basin as well as a stream crossing the Mermentau-Calcaiseu River basin boundary, which warrant further investigation as to their identity. We determined that *F. palmeri* cannot be assumed to be separated along basin and river boundaries, and that the Mississippi River is probably not a biogeographic barrier for *F. p. palmeri*. We believe further species overlaps caused by range expansions of *F. palmeri* subspecies with endemic species of state conservation interest are likely.

Introduction

The southeastern United States contains the country's highest crayfish diversity with ~320 species represented (Taylor et al. 2007). However, biological data and distributional information for many crayfish species are incomplete (Crandall and Buhay 2008, Richman et al. 2015). Outside of the state's commercially important crayfish, *Procambarus clarkii* (Girard) (Red Swamp Crayfish), Louisiana crayfishes are poorly studied, and much of the state's research on crayfish ecology is limited to infrequent species updates and descriptions (Penn 1952, Walls 2009). Thus, there is an on-going need for contemporary research data to compliment conservation planning aiming to address ecological and anthropogenic pressure on native species (see Holcomb et al. 2015).

Faxonius palmeri (Faxon) (Lowland Painted Crayfish) is a geographically broadly distributed complex ranging from Arkansas River basin in Kansas (Ghedotti 1998) to the Flint River basin in Georgia (Sargent et al. 2011). The complex is comprised of *F. p. palmeri* (Faxon) (Gray Speckled Crayfish), *F. p. longimanus* (Faxon) (Western Painted Crayfish), and *F. p. creolanus* (Creaser) (Creole Painted Crayfish); all 3 members are part of the Louisiana crayfish fauna (Hobbs1974, Walls 2009). However, despite weak morphological resolution among the members of the complex, the genetic justifications for membership have yet to be determined. The group is phylogenetically classified within the *Virilis* section of crayfishes (Fetzner

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1996) and includes species such as *Faxonius maletae* Walls (Kisatchie Painted Crayfish), *Faxonius blacki* Walls (Calcasieu Painted Crayfish), and *Faxonius hathawyi* Penn (Teche Painted Crayfish), all of which are of conservation interest in Louisiana (Fitzpatrick 1987, Holcomb et al. 2015). Penn (1957) separates the complex geographically into 2 major subspecies groups divided at the Mississippi River boundary, creating an eastern Louisiana group (*F. p. palmeri* and *F. p. creolanus*) and a western Louisiana group (*F. p. longimanus*). The 2 subspecies groups are further partitioned along basin and river system boundaries. *Faxonius p. longimanus* currently occupies the largest range and is common in streams of the Sabine River, Calcasieu River, Ouachita River, and Red River basins. The ranges of *F. p. palmeri* and *F. p. creolanus* are separated along the Thompson Creek boundary, east of the Mississippi River, with *F. p. creolanus* being the more widespread species that extends eastward through to Mississippi. The range of *F. p. palmeri* is bounded on the west by the Mississippi River and Thompson Creek on the east, and until publication of this article, had the smallest reported range among the Lowland Painted Crayfish types in Louisiana. Recent collections by Walls (2009) failed to find any significant deviation from this original geographic grouping.

It is not understood whether the Mississippi River plays a biogeographic role as a dispersal barrier to crayfishes in Louisiana even though it is an important barrier separating congeners among numerous invertebrate and vertebrate taxa (Barr and Chapin 1981, Douglas 1974, Kaller et al. 2013, Robison 1986). Penn (1952) diagnosed a single form I male museum specimen from Bayou Teche, west of the Mississippi River, as *F. p. creolanus*, but subsequently described this specimen as *F. p. longimanus* based on geography (Penn 1957). Although only 1 form I male was collected from Bayou Teche, 16 total *F. palmeri* complex specimens were collected there as well as 1 individual from the Atchafalaya River basin (ARB; Penn 1957). However, subsequent collections failed to confirm permanent residence of eastern Louisiana *F. palmeri* subspecies in any drainages west of the Mississipi River (Walls 2009).

Herein, we provide an important update on the distribution of the Louisiana Lowland Painted Crayfish complex, which has been associated with species displacements in one US state and thus deserves greater research attention (e.g., Sargent et al. 2011). In short, we update the ranges of *F. p. longimanus* and *F. p. palmeri* to include stream systems east of the Mississippi River and stream systems of the Oachita River, Mermentau River, Vermillion-Teche River, and Atchafalaya River basins west of the Mississippi River. Our data also show that the new ranges of *F. p. longimanus* abut more closely with the documented range of the narrowly distributed *F. hathawayi* in the Cocodrie Lake stream network; this finding raises concerns about potential range overlap and may warrant monitoring.

Methods

We compiled specimen data presented herein from samples taken during various studies at multiple ecological scales (individual streams, stream systems, and river basins) from 2008 to 2015 (Bonvillain 2012, Budnick 2015, Kaller et al. 2013, Lott

2015). These data represent compiled collection data from fieldwork in the ARB, Red River, Mermentau River, Vermillion-Teche River, Ouachita River, Sabine River, and Calcasieu River basins (Fig. 1). Efforts utilized a variety of active and passive methods including backpack and boat-based electrofishing, baited traps, dipnets, and seines. We drew maps of known species' ranges in ArcMAP™ (ESRI® ArcGIS®, version 10.3.1) and adapted from maps by Walls (2009). We processed land-cover data for Louisiana from the 2011 version of the National Land Cover Database (Homer et al. 2015). For this report, we developed new projections of species ranges based on the new recorded presences and assumed continuous extensions of species ranges based on visual examination of stream network

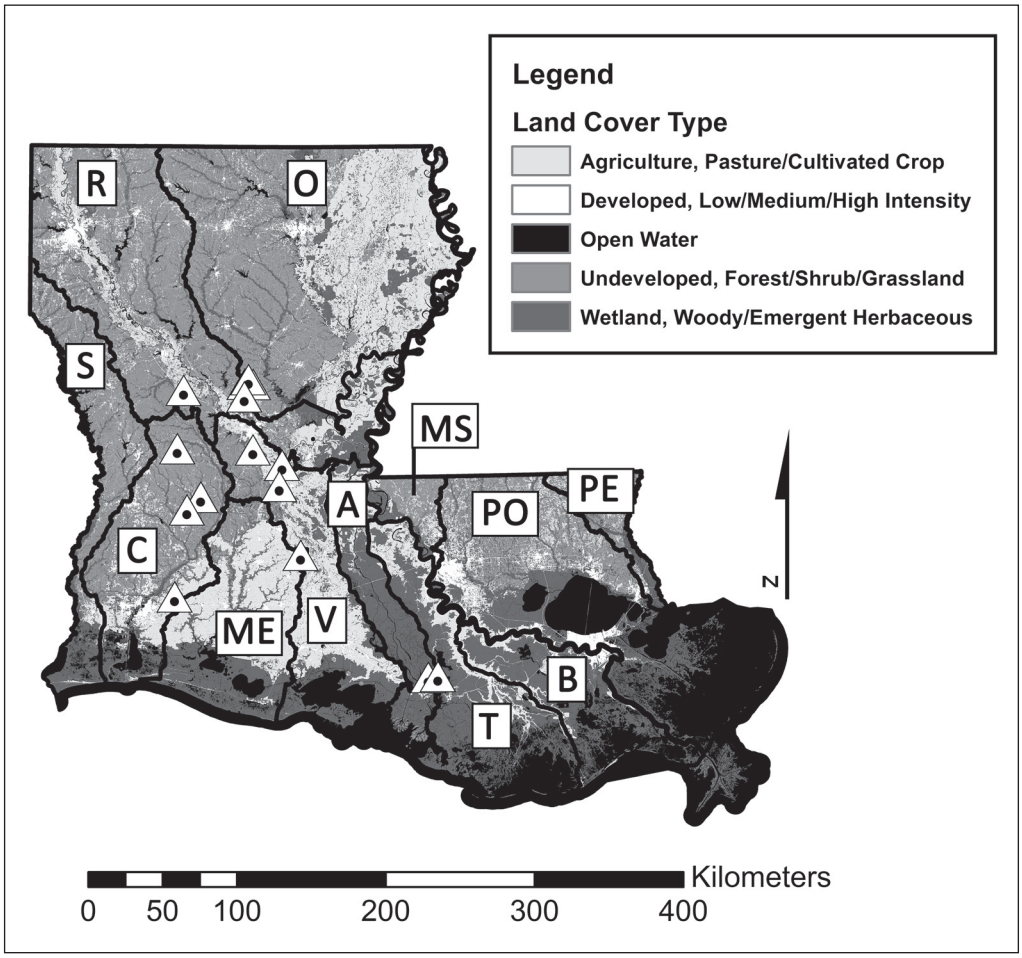

Figure 1. Louisiana land-cover map showing the distribution of crayfish collection sites (triangles) from various research projects conducted from 2008 to 2015 that procured *Faxonius palmeri* specimens. Land-cover data sourced from the 2011 National Land Cover Database (Homer et al. 2015). Thick black lines indicate major river basin boundaries, denoted also by the following letters: A = Atchafalaya, B = Barataria, C = Calcasieu, ME = Mermentau, MS = Mississippi River, O = Ouachita, PE = Pearl, PO = Pontchartrain, R = Red River, S = Sabine, T = Terrebonne, V = Vermillion-Teche.

topology (i.e., patterns of stream connectivity and relative locations of sites within the network). Because diversity (species recorded and number of individuals) and morphology data (carapace length, abdominal length, wet weight, mass) are generally sparse in the literature, especially for Louisiana, we provide these data in Supplemental Table S1 (available online at https://www.eaglehill.us/SENAonline/suppl-files/s19-1-S2532-Budnick-s1, and for Bioone subscribers, at https://dx.doi.org/10.1656/S2532.s1).

We confirmed specimen identification with morphological examination of sexually mature form I male gonopods. Although we could not identify some specimens to subspecies level (because no form I males were obtained at some sites), diagnosis of some Lowland Painted Crayfish subspecies was possible in some specimens after examining historical distribution data in combination with morphological features of form II male and female specimens (e.g., red abdominal bands are indicative of members of *F. p. palmeri*, *F. p. creolanus*, and *Faxonius hobbsi* Penn [Pontchartrain Painted Crayfish], but blue bands are distinctive in the other *Faxonius* species). Collected specimens were preserved in 95% ETOH and deposited at the School of Renewable Natural Resources, Louisiana State University. Genetic confirmation of *F. p. palmeri* specimens from Bayou Plaquemine Brule was provided by James Fetzner from Carnegie Museum of Natural History, Pittsburgh, PA.

Results

Faxonius palmeri longimanus and unknown *Faxonius* species occurrences

Between 2013 and 2014, Calcaiseu River basin samples procured 29 *F. p. longimanus* specimens from streams of the Ouiska Chitto Creek watershed (Ten Mile Creek and Six Mile Creek), as well as 5 unidentified *Faxonius* specimens from School House Creek in the headwaters of the Cypress Bayou river system, and 1 unidentified *Faxonius* specimen from Bayou Arceneaux (Supplemental Table S1, available online at https://www.eaglehill.us/SENAonline/suppl-files/s19-1-S2532-Budnick-s1, and for Bioone subscribers, at https://dx.doi.org/10.1656/S2532.s1). Samples in the Red River basin made with active gears (dipnets and backpack electrofishers) produced no *F. p. longimanus*, but collections with minnow traps yielded 3 *F. p. longimanus* specimens from Gray Creek (Fig. 2A). We did not collect any *F. p. longimanus* in the Mermentau River, Vermillion-Teche River, Sabine River, and ARB, although historical records indicate this species may occur in Ouachita River basin streams (Walls 2009). Land cover of Calcasieu River basin streams is dominated by undeveloped land cover and shrub vegetation (~51% of total basin area; Fig. 1), with agricultural impacts limited to mainly *Pinus taeda* L. (Loblolly Pine) silviculture (~25%) and row crop and hay cultivation (~15%). Stream sites within the basin containing *F. p. longimanus* were mainly located within areas surrounded by evergreen forest cover. We usually found our specimens in artificial rock piles and concrete riprap under bridges rather than under other material (e.g., wood, leaf litter, submerged vegetation); however, comparable habitat data across studies were not available to make quantitative comparisons to determine if such habitats represent optimal preference.

We were unable to determine whether the individuals collected at School House Creek and Bayou Arceneaux were *F. p. longimanus* or instead *F. hathawayi*, *F. blacki*, or their intergrade; all 3 are also known to inhabit the Calcasieu River (Walls 2009). We could not reliably identify these *Faxonius* individuals because (1) the samples produced females only, (2) did not produce males with mature gonopods or males that were large enough to have developed gonopod morphology that could clearly differentiate them from other species, or (3) their overall color patterns matched those described for both *F. palmeri* and *F. hathawayi*. Regardless of the exact identity, these represented individuals of the genus occurring outside known-documented range boundaries. Further, we collected another unknown female *Faxonius* specimen from Bayou Derbonne in the Red River system that could not be confidently diagnosed for similar reasons, particularly owing to its close proximity to the Cotile Lake population of *F. maletae*. The frequency at which we found *Faxonius* individuals across the samples from the western Louisiana drainages suggested a general range extension of the genus *Faxonius* (Fig. 2b).

Faxonius palmeri palmeri and unknown *Faxonius* species occurrences

We recorded 107 *F. p. palmeri* individuals in streams of the Vermillion-Teche River basin and 2 sites in the southeastern ARB (Fig. 2b). In streams of the Vermillion-Teche River basin, we found specimens in Bayou Joe Marcel (15 individuals), the Bayou Boeuf-Cocodrie Lake diversion canal (75 individuals), and Bayou Plaquemine-Brusly west of Opelousas, LA (3 individuals, including 2 gravid

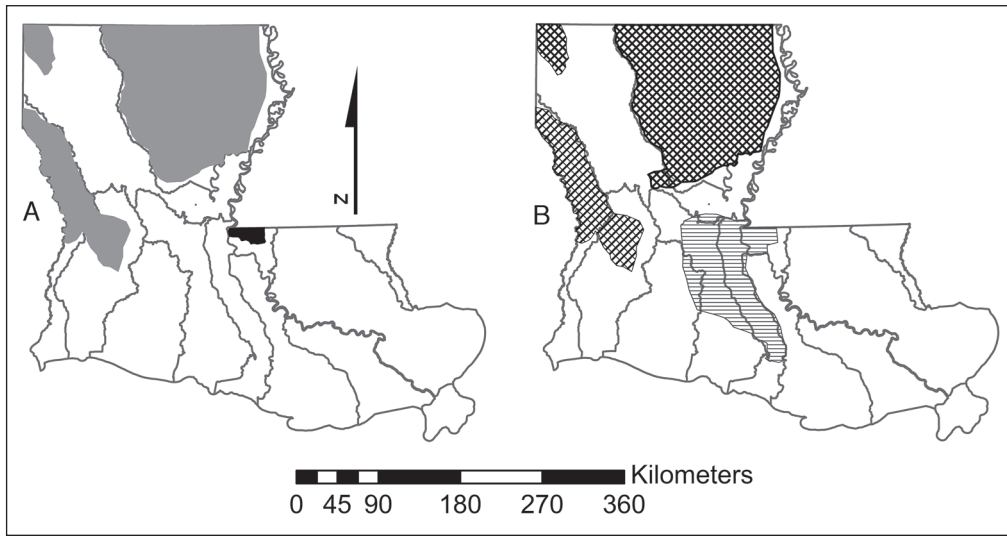

Figure 2. Distribution of *Faxonius palmeri longimanus* (Western Painted Crayfish) and *Faxonius palmeri palmeri* (Gray Speckled Crayfish) among the major Louisiana river basins (lines). (A) Historical range of *F. p. longimanus* (solid gray) and *F. p. palmeri* (solid black) (B) Suggested new ranges of *F. p. longimanus* (crosshatches) and *F. p. palmeri* (horizontal lines) based on stream network connectivity and new collection data. Note that the new range of *F. p. longimanus* extends the limits of the Ouachita River Basin group to overlap into the lower Red River Basin at Gray's Creek.

females). We also recorded 1 unidentified female *Faxonius* individual from Bayou Rouge. In the ARB, we recorded 13 *F. palmeri* specimens (subspecies unclear) from Little Bayou Jessie near the Atchafalaya River and a single individual from Bayou Grosbeak, a distributary of the Gulf Intracoastal Waterway. A form I male was not collected at either site, but specimens had red abdominal bands consistent with that of *F. p. palmeri*. No confirmed *F. p. palmeri* were collected in the Red River, Mermentau River, Ouachita River, Sabine River, and Calcasieu River basins. Therefore, we extended the potential range of *F. p. palmeri* to encompass both the eastern and western sides of the Mississippi River, occurring in streams within the lower Mississippi River, Atchafalaya River, Vermillion-Teche River, and Mermentau River basins (Fig. 2b).

The Vermillion Teche River and Mermentau River basins where *F. p. palmeri* were collected are dominated by row crop and pasture cover (~48% of total basin area; Fig. 1) with substantial cover by woody wetland and emergent herbaceous vegetation (~34%). However, *F. p. palmeri* may occupy streams with greater forest coverage based on their historic range in the Mississippi River basin streams and, thus, appear to occur in a variety of stream conditions. Similar to our collections of *F. p. longimanus*, we often had better success detecting *F. p. palmeri* under artificial rocks and construction material (e.g., concrete slabs) usually associated with bridges and culverts. Interestingly, in sites that greatly lacked hard cover such as woody debris piles (e.g., the Bayou Plaquemine-Bruly stream site), *F. p. palmeri* were only encountered under such anthropogenic material, which has also been reported for other Louisiana aquatic taxa (e.g., Alford 2014). In general, sample localities which contained *F. p. palmeri* varied greatly in their characteristics, including lightly channelized streams with piles of submerged wood, moderate to dense macrophyte stands, and presumably higher dissolved oxygen owing to nearby permanently flowing channels as opposed to the lower dissolved oxygen levels common for floodplain and lentic ARB habitats (Pasco et al. 2016). Because of the new presence of *F. p. palmeri* in streams west of the Mississippi River, the large spatial area bounded by these sites, and the discovery of 2 egg-bearing females with viable offspring (i.e., offspring were successfully reared when females were brought to laboratory), we were confident that these records did not represent transitory individuals and were evidence of an established population.

Discussion

In this study, we have presented data that updated the ranges of 2 *F. palmeri* subspecies, *F. p. palmeri* and *F. p. longimanus*. Most importantly, we showed the *F. palmeri* assemblage is not discretely separated by drainage boundary, as previously assumed and now clearly overlaps the ranges of several species of importance. Our findings underscore a common problem facing many studies of crayfish diversity patterns in that species originally absent from historical records suddenly become apparent in new samples at the same localities. Inadequate sampling methods probably serve as the primary reason for the discrepancies. However, given the unique geography and topology of Louisiana's river systems, natural and anthropogenic

dispersal are plausible contributing factors. We recommend that a primary research focus should be quantifying *F. p. palmeri* rate of dispersal across the Mississippi River boundary and identifying the mechanisms of dispersal. Whether the ARB itself serves as corridor for dispersal is also worth exploring, given that the 5000-km^2 ARB has been modified by the US Army Corps of Engineers to receive 30% of the combined volumes of the Mississippi River and Red River and divert floodwaters to the Gulf of Mexico to prevent downstream flooding (Reuss 2004).

We believe our data highlights the priority to determine the extent of undersampling of the *F. palmeri* subspecies group within stream drainages. Walls (2009) remains the most complete and up-to-date report on all crayfish distributions in Louisiana, but the data are based on contemporary and historical data collected with protocols likely not effective at sampling local communities; this limitation likely contributed to the discrepancies that we report. In other words, we believe recent and historical surveys of crayfish communities simply missed these subspecies. Development of effective crayfish sampling protocols is ongoing, although Budnick et al. (2018) tested a combined gear protocol (backpack electrofishers and dipnets) in Louisiana streams that showed usefulness for documenting species presence patterns at stream-drainage scales. Considering the diversity of aquatic habitats types in the ARB that preclude the use of active stream sampling protocols for crayfish, trapping is the preferred and recommended sampling method. However, an analogous protocol for sampling diversity of crayfish communities in the ARB is still needed. Given Penn's (1957) historical data from Bayou Teche and the Atchafalaya River, as well as our recent findings, which included berried *F. p. palmeri* female specimens, we conclude that we sampled undocumented and established *F. p. palmeri* subpopulations west of the Mississippi River.

Summary

Management or conservation planning with respect to *Faxonius* species in Louisiana should not consider the *Faxonius palmeri* subspecies ranges to be cleanly separated along drainage or river system boundaries. Conservatively, our collections indicate that range boundaries of *F. p. longimanus* and *F. p. palmeri* strongly abut or overlap those of 3 rare *Faxonius* species (*F. maleate* with *F. p. longimanus* in the Red River basin; *F. blacki* and *F. hathawayi* with *F. p. palmeri* in the Calcasieu River and Vermillion-Teche River basins, respectively). Fortunately, we observed no local co-occurrence (i.e., within the same stream site) among any positively identified Lowland Painted Crayfish subspecies and the endemic and rare *Faxonius* species. We cannot conclude the same for peripheral stream sites harboring unknown *Faxonius* individuals, which especially raises our concern considering that displacements of native crayfish species by range expansion of *F. palmeri* has been documented elsewhere (Sargent et al. 2011). We call for, and recommend, that future surveys of crayfish populations and communities prioritize identification and reporting of any *Faxonius* species observed in order to improve and more accurately map species' ranges.

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