MORPHOLOGICAL DIVERGENCE BETWEEN CAVE AND SURFACE POPULATIONS OF THE DIGGER CRAYFISH, *FALVICAMBARUS FODIENS* (COTTLE, 1863) (DECAPODA, CAMBARIDAE)

BY

MICHAEL S. TAYLOR¹,³, BRANDON E. BLECHLE¹ and BRAD S. POBST²

¹) Department of Biology, Southeast Missouri State University, Cape Girardeau, Missouri 63701, U.S.A.
²) Fisheries Division, Southeast Regional Office, Missouri Department of Conservation, 2302 County Park Drive, Cape Girardeau, Missouri 63701, U.S.A.

ABSTRACT

*Fallicambarus fodiens* (Cottle, 1863), the digger crayfish, is widespread across lowland woods and other wetlands of the eastern United States. More recently, *F. fodiens* was discovered in two caves located in the Perryville Karst system in southeastern Missouri. We performed multivariate analyses to explore whether morphological divergence has occurred between cave and nearby surface populations of *F. fodiens*. Our results revealed that cave individuals had significantly longer antennae relative to surface individuals, and that cave females had longer abdomens relative to surface females. Sexual dimorphism, independent of habitat, was also found. Males had larger chelae and longer antennae, and females had larger tails. The presence of morphologically distinct *F. fodiens* in caves of the Perryville Karst further increases the already high biodiversity of this karst system. The Perryville Karst is associated with urban and agricultural areas, so the cave fauna should be closely monitored to guard against a potentially detrimental impact from urban and agricultural pollution sources.

RÉSUMÉ

*Fallicambarus fodiens* (Cottle, 1863), l’écrevisse fouisseuse, est largement distribuée dans les bois de faible altitude et autres zones humides de l’est des États Unis. Plus récemment *F. fodiens* a été découverte dans deux grottes localisées dans le système karstique de Perryville dans le sud-est du Missouri. Nous avons réalisé des analyses multifactorielles pour explorer si des divergences morphologiques sont apparues entre les populations proches de *F. fodiens* des grottes et celles de la surface. Nos résultats révèlent que les individus des grottes ont significativement des antennes plus longues par rapport aux individus de surface, et que les femelles des grottes ont des abdomens plus longs que les femelles de surface. Un dimorphisme sexuel, indépendant de l’habitat, a aussi été trouvé. Les mâles ont des pinces plus larges et de plus longues antennes, et les femelles ont un abdomen plus large. La présence de *F. fodiens* morphologiquement distinctes dans les grottes karstiques de Perryville augmente encore la, déjà grande, biodiversité de ce système karstique.

³) Corresponding author; e-mail: mtaylor@semo.edu
INTRODUCTION

The freshwater crayfish genus *Fallicambarus* Hobbs, 1969 contains 18 species (Crandall & Buhay, 2008), of which 17 are endemic to the Gulf Coastal Plain of the southern United States. Only a single species, *Fallicambarus fodiens* (Cottle, 1863), occurs outside of the Coastal Plain. The range of *F. fodiens* extends from the Gulf Coast states north into the central Mississippi River and lower Ohio River drainages of Illinois, Indiana, and Ohio, and then into the Great Lakes drainages of Michigan and southern Ontario. A disjunct population ranges from Georgia and South Carolina northward along the Atlantic Coast to Virginia and Maryland (Pflieger, 1996; Guiasu, 2007). Across its range, *F. fodiens* is typically associated with seasonally flooded lowlands and woodlands, wet meadows, drainage ditches, and other wetland areas (Page, 1985; Pflieger, 1996; Guiasu, 2007). The wet soils allow *F. fodiens* to dig relatively simple burrows where it dwells during dry periods, emerging during the wet season from late fall through early spring (Norrocky, 1991; Trépanier & Dunham, 1999; Guiasu, 2007).

Little noted, however, are the populations of *F. fodiens* inhabiting Mystery Cave and Rimstone River Cave in the Perryville Karst System of Perry County, Missouri (fig. 1). The Perryville Karst has more than 650 caves (Elliot, 2007). Although most

---

Fig. 1. Location of Mystery and Rimstone River caves along Cinque Hommes Creek in Perry County, Missouri. Inset shows location of Perry County.
of the caves in this karst system have not been biologically inventoried, five of them rank among the 50 most biologically diverse caves of Missouri, and Mystery Cave is ranked third (Elliot, 2007). Mystery Cave, two other high diversity caves, and Rimstone River Cave (not a high biodiversity cave) are stream caves that resurge into Cinque Hommes Creek, which flows northeast into the Bois Brule ditch and then into the Mississippi River (fig. 1).

The cave populations of *F. fodiens* are intriguing for several reasons. First, surface populations of *F. fodiens* have never been found in Perry County, despite the presence of surface populations from nearby counties in Missouri and Illinois. Second, a faunal survey of Mystery Cave during all seasons of 1972, plus less extensive sampling of Rimstone River Cave, did not reveal *F. fodiens* (cf. Lewis, 1974). Furthermore, *F. fodiens* was not included in other faunal lists of regional caves (Peck & Lewis, 1978; Lewis et al., 2003) until one of us (BSP) collected specimens from Mystery Cave in February 2006 (Elliot, 2007). The presence of *F. fodiens* in Rimstone River Cave has not been formally reported until now. Finally, few species of crayfishes occupy both cave and surface habitats (Finlay et al., 2006).

The presence of *F. fodiens* in cave and surface habitats, together with a possible recent natural range expansion of the species into Mystery and Rimstone River caves, provide a unique opportunity to examine the early stages of morphological divergence associated with the subterranean habitat. The cave environment often leads to predictable changes of morphological features, such as enlarged chemosensory structures and reduced eye size (Jones et al., 1992). Collectively, these morphological adaptations have been termed troglomorphy (Christiansen, 1961) but similar morphological changes are also evident in other lightless environments, such as the deep-sea (Montgomery & Pankhurst, 1997).

Elliot (2007) listed *F. fodiens* as a trogloxene, which are species that must occasionally return to the surface for food (Barr & Holsinger, 1985). However, the presence of *F. fodiens* far within Mystery Cave suggests that the cave crayfish may be isolated from surface populations. Troglo Xenes are predicted to maintain high levels of gene flow with surface populations (Caccone, 1985). If gene flow is high between cave and surface populations of *F. fodiens* then the cave population should not show troglomorphic divergence. Alternatively, if gene flow is limited, then troglomorphic divergence between cave and surface populations should be evident. We explored these two alternatives through morphological comparison of cave and surface populations of *F. fodiens*.

**MATERIAL AND METHODS**

We randomly collected 33 specimens of *Fallicambarus fodiens* from Mystery Cave and six specimens from Rimstone River Cave between August 2007 and
January 2008. All specimens were placed immediately in 95% ethanol. A small tissue sample was excised from the abdomen of each specimen and stored at \(-80^\circ\text{C}\) for future genetic analyses. Surface specimens from the vicinity of Mystery and Rimstone River caves were not found after approximately 15 hours of search effort in the Cinque Hommes Creek drainage. We obtained from the Illinois Natural History Survey (INHS) an additional 22 adult specimens collected from 15 different localities in southern Illinois to represent the closest available surface populations. A single Perry County, Missouri specimen collected in 1993 was available from the INHS, but the exact locality was not recorded so the specimen was not included in this study. We measured the 60 total specimens with dial callipers to the nearest 0.1 mm. We chose 13 morphological characters that represent overall body morphology and potential troglomorphic traits: carapace length, width, and depth; abdomen length and width; telson length; eye length and width; antenna length; antennal scale width; chela length and width; and merus length of the first walking leg. The longest antenna and largest chela were measured for each individual.

We quantified morphological variation of the individuals by computing a principal components analysis (PCA) on the variance–covariance matrix of the natural log-transformed values for the 13 morphological characters. This type of PCA typically summarizes most of the size-related variation in the first principal component (PC 1) (Thorpe, 1988; James & McCulloch, 1990). Components beyond the first were used to represent relatively size-independent variation. We followed the PCA with a two-way multivariate analysis of variance (MANOVA) to determine whether the morphological variation captured by the principal components showed significant differences between cave and surface populations in morphometric space. We included habitat and sex as the two independent variables in the MANOVA model to account for morphological variation by habitat and by sex (Stein et al., 1977; Boyd & Page, 1978). We then used post-hoc analysis of variance (ANOVA with Tukey’s honestly significant difference test) to assess whether the characters that accounted for the greatest amount of variation on the second and third principal component axes (PC 2 and PC 3, respectively) differed significantly between habitats or between sexes. Statistical analyses were performed with SAS v9.1.3 (SAS Institute Inc., Cary, NC, 2003). Summary statistics are presented as the mean ± standard error.

RESULTS

PC 1 accounted for the majority of the overall variation, and all character loadings had the same sign, consistent with interpretation of PC 1 as a size-related
Table I

Eigenvector coefficients and percent variation of the first three principal components extracted from a PCA of the variance–covariance matrix derived from cave and surface populations of *Fallicambarus fodiens* (Cottle, 1863). Post-hoc ANOVA was performed on bolded characters. Superscript letters indicate significant differences (*p* < 0.05) between habitats (h) or sexes (s).

<table>
<thead>
<tr>
<th>Character</th>
<th>PC 1</th>
<th>PC 2</th>
<th>PC 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carapace Length</td>
<td>0.2568</td>
<td>0.1375</td>
<td>0.0638</td>
</tr>
<tr>
<td>Carapace Height</td>
<td>0.2734</td>
<td>0.1099</td>
<td>0.0479</td>
</tr>
<tr>
<td>Carapace Width</td>
<td>0.2481</td>
<td>0.1220</td>
<td>0.0894</td>
</tr>
<tr>
<td><strong>Abdomen Length</strong></td>
<td>0.2836</td>
<td><strong>0.3029</strong></td>
<td><strong>0.4896</strong></td>
</tr>
<tr>
<td><strong>Abdomen Width</strong></td>
<td>0.2277</td>
<td><strong>0.2143</strong></td>
<td>0.2148</td>
</tr>
<tr>
<td><strong>Telson Length</strong></td>
<td>0.2583</td>
<td><strong>0.2592</strong></td>
<td>0.2606</td>
</tr>
<tr>
<td>Eye Length</td>
<td>0.0837</td>
<td>0.1084</td>
<td>−0.0039</td>
</tr>
<tr>
<td>Eye Width</td>
<td>0.0901</td>
<td>0.1076</td>
<td>−0.0738</td>
</tr>
<tr>
<td><strong>Antenna Length</strong></td>
<td>0.3343</td>
<td>−0.8329</td>
<td><strong>0.3503</strong></td>
</tr>
<tr>
<td>Antenna Width</td>
<td>0.1411</td>
<td>−0.0930</td>
<td>0.0729</td>
</tr>
<tr>
<td><strong>Chela Length</strong></td>
<td>0.4575</td>
<td>−0.1306</td>
<td><strong>0.4737</strong></td>
</tr>
<tr>
<td><strong>Chela Width</strong></td>
<td>0.3863</td>
<td>0.0789</td>
<td><strong>0.5227</strong></td>
</tr>
<tr>
<td>Merus Length</td>
<td>0.3057</td>
<td>0.0158</td>
<td>−0.0277</td>
</tr>
<tr>
<td>Eigenvalue</td>
<td>0.4319</td>
<td>0.0484</td>
<td>0.0236</td>
</tr>
<tr>
<td>Variation (%)</td>
<td>81.78</td>
<td>9.17</td>
<td>4.46</td>
</tr>
<tr>
<td>Cumulative Variation (%)</td>
<td>81.78</td>
<td>90.96</td>
<td>95.42</td>
</tr>
</tbody>
</table>

PC 2 contrasted the dimensions of the abdomen and telson with antenna length. PC 3 contrasted abdomen length and antenna length with dimensions of the chelae (table I). A plot of PC 2 vs PC 3 showed that individuals could be differentiated both by habitat and by sex (fig. 2). Cave individuals tended to have longer antennae than surface individuals, and cave females tended to have longer abdomens than surface females. Independent of habitat, males tended to have longer antennae, larger chelae, and smaller tails than females. A MANOVA of the first three principal components confirmed the significant morphological differences between habitats and between sexes, but interaction of habitat and sex was insignificant (table II).

Post-hoc analysis of the most divergent characters (table I, fig. 2) between cave and surface populations showed that cave individuals had significantly longer antennae (48.0 mm ± 1.54) than surface individuals (32.3 mm ± 2.10; *p* < 0.0001). Cave females had significantly longer abdomens (27.6 mm ± 0.97) than surface females (24.5 mm ± 0.97; *p* < 0.05). Remaining significant morphological divergence was due to sexual dimorphism (table III). Females had longer and wider abdomens, and longer telsons than males. Males had longer antennae and longer chelae than females. Although chela width also loaded heavily on PC 3, a significant difference was not found between sexes or between habitats.
DISCUSSION

Our PCA results revealed clear morphological divergence within *Fallicambarus fodiens*. Most character variation was due to sexual dimorphism, evident in the significantly longer chelae for males and larger tails (abdomen and telson) for females. Similar sexual dimorphism has been observed for numerous species of crayfishes (Boyd & Page, 1978; Grandjean et al., 1997; Streissl & Hödl, 2002). Larger chela size in male crayfish has been linked to intrasexual fighting and for manipulating females during copulation (Stein, 1976; Grandjean et al., 1997; Schroeder & Huber, 2001). The role of the chela as a secondary sexual character

TABLE II

Results from a two-way multivariate analysis of variance of the first three principal components extracted from a PCA of the variance–covariance matrix derived from cave and surface populations of *Fallicambarus fodiens* (Cottle, 1863)

<table>
<thead>
<tr>
<th>Source</th>
<th>Exact $F$ $(d.f. = 3, 55)$</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>25.0318</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Habitat</td>
<td>19.8892</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Habitat * Sex</td>
<td>0.7898</td>
<td>0.5049</td>
</tr>
</tbody>
</table>
Mean size (mm ± standard error) of sexually dimorphic characters for males and females of *Fallicambarus fodiens* (Cottle, 1863), independent of habitat. Statistical significance was determined by post-hoc analysis of variance with Tukey’s honestly significant difference test.

<table>
<thead>
<tr>
<th>Character</th>
<th>Male</th>
<th>Female</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abdomen Length</td>
<td>23.0 ± 0.83</td>
<td>26.0 ± 0.83</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Abdomen Width</td>
<td>10.2 ± 0.33</td>
<td>11.5 ± 0.33</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Telson Length</td>
<td>9.8 ± 0.36</td>
<td>11.2 ± 0.36</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Antenna Length</td>
<td>46.9 ± 2.09</td>
<td>38.2 ± 2.09</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Chela Length</td>
<td>24.7 ± 1.36</td>
<td>20.6 ± 1.36</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

In *F. fodiens* remains to be tested specifically but large size resulting from frequent male–male combat seems unlikely. Intrasexual aggression was observed to be very low for similar-sized males (Guia¸su et al., 2005), and multiple males and females have been found within a single burrow (Norrocky, 1991). The larger tail size of the females may allow them to carry more or larger eggs (Streissl & Hödl, 2002) but, to our knowledge, this hypothesis has not been specifically tested for crayfishes.

Antenna length was the primary character that differed between cave and surface habitats. Antennae were significantly longer in the cave population. The enlarged chemosensory structure presumably reflects an increased reliance by crayfish on chemosensory cues in the lightless cave environment. The dependence on chemosensory cues is often accompanied by reduced eye size (Christiansen, 1961; Jones et al., 1992; Montgomery & Pankhurst, 1997) but we did not detect any significant difference in eye size between cave and surface individuals.

An especially interesting result was the post-hoc discovery that cave females had significantly longer abdomens \( (p < 0.05) \) compared to surface females, although abdomen width \( (p = 0.17) \) and telson length \( (p = 0.052) \) did not differ between the two habitats. If tail size does correlate with the number of eggs carried by the female, then longer abdomens may suggest that cave *F. fodiens* have higher fecundity than surface females. Several lines of evidence lead us to this hypothesis. First, Mystery and Rimstone River caves are stream caves so the habitat is always wet. The consistently wet, cool cave environment is therefore similar to the fall and winter breeding conditions of surface crayfish, and both male and female *F. fodiens* are commonly observed in cave streams during all seasons (BSP, pers. obs.). Second, the caves have a large amount of allochthonous nutrient input from stream and sinkhole sources. Leaf litter and other debris are abundant in both caves. Third, the cave streams support a rich invertebrate fauna (Lewis, 1974), so nutrient sources for *F. fodiens* are apparently abundant. The combination of constant breeding conditions and abundant nutrients may therefore provide more reproductive opportunities for cave individuals. In contrast, surface individuals
remain in their burrows during the summer, emerging primarily during extended wet periods and during the fall and winter (Williams et al., 1974; Norrocky, 1991), which may reduce breeding opportunities. This hypothesis is contrary to evidence from many cave species that have reduced fecundity compared to closely related surface species due to limited resource availability in typical cave environments (Poulson, 1963; Culver, 1982; Peck, 1986). Cave species may offset reduced fecundity by producing larger and presumably more energy-rich eggs (Poulson, 1963; Peck, 1986), so the longer abdomens of female *F. fodiens* may correspond to larger eggs rather than more eggs. These alternative hypotheses warrant further study.

The limited morphological divergence between cave and surface populations of *F. fodiens* is consistent with a recent natural range expansion of Mystery and Rimstone River caves. Previous faunal surveys of Mystery and other caves in this region (Lewis, 1974; Peck & Lewis, 1978; Lewis et al., 2003) did not note the presence of *F. fodiens*. Lewis (1974) surveyed the terrestrial and aquatic fauna of Mystery Cave across all seasons of 1972. He sampled 23 stations in the same section of Mystery Cave where we collected the specimens studied here. Crayfish may have been present but not observed by Lewis, but we consider this unlikely. The streams that run through this section of Mystery Cave are narrow, shallow, and clear in many places, and crayfish are easily sighted. Further, crayfish burrows have been found in Mystery Cave (BSP, pers. obs.) but whether these were dug by *F. fodiens* or another species is currently unknown. We therefore think that the absence of *F. fodiens* from Lewis’s (1974) faunal list reflects an absence of *F. fodiens* from at least this section of the cave, although we cannot rule out the prior presence of *F. fodiens* from elsewhere in the cave. More recently, one specimen of *F. fodiens* was collected from Mystery Cave in October 1991 and three specimens were collected from the mouth of Rimstone River Cave in July 1992 by B. Burr (Southern Illinois University, Carbondale), and subsequently deposited at the National Museum of Natural History (USNM 260064 and USNM 260389, respectively). Since 2006, multiple observations of many individuals in Mystery Cave (BSP, pers. obs.) suggest that the population of *F. fodiens* is stable.

Assuming surface populations are eventually discovered near the region of the caves, we can then assess whether gene flow occurs between cave and surface populations. If gene flow is high, then the observed morphological divergence of antennae may be a plastic phenotypic response to a lightless environment. Alternatively, if gene flow is low or absent, then troglomorphic differentiation would suggest rapid evolutionary adaptation of the cave population. This would have important conservation and taxonomic implications for the cave populations of *F. fodiens*. Caves of the Perryville Karst system, including Mystery and Rimstone River caves, are home to at least four endemic species (Elliot, 2007). The
discovery of *F. fodiens* in Mystery and Rimstone River caves further increases the faunal importance of the Perryville Karst system, especially if gene flow between cave and surface populations is limited.

Cave species are often species of special conservation concern. More than half of the imperiled or vulnerable animal species in the United States are subterranean (Buhay et al., 2006) and as much as 95% of these subterranean species are similarly imperiled or vulnerable (Culver et al., 2000). Most of the Perryville Karst system, including Mystery and Rimstone River caves, is associated with the City of Perryville and surrounding agricultural lands. Urban and agricultural run-off into sinkholes and other karst water sources could potentially jeopardize the endemic fauna in the high biodiversity caves. Therefore, these caves should be closely monitored to determine whether pollution from urban and agricultural sources is negatively affecting the cave fauna.

ACKNOWLEDGEMENTS

We thank L. Taylor of the Missouri Department of Conservation for field assistance and discussions about the Perryville Karst, C. Taylor of the Illinois Natural History Survey for the loan of crayfish specimens, J. Robins for valuable statistical assistance, and R. DiStefano and H. Schubert for comments that improved this manuscript. This research was supported by a Graduate Research Funding Committee Grant to MST from Southeast Missouri State University.

LITERATURE CITED


First received 8 September 2009.
Final version accepted 10 June 2010.