



Extension of the geographical range of the primary burrowing crayfish *Cambarus strigosus* Hobbs, 1981 (Decapoda: Astacidea: Cambaridae) in South Carolina

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ABSTRACT

Knowledge of the geographical distribution for many primary burrowing crayfishes is often limited to information presented in species descriptions or field notes associated with epigeal crayfish surveys. *Cambarus strigosus* Hobbs, 1981, a rare primary burrowing crayfish, was collected as part of a survey of the Sumter National Forest, South Carolina (SC), USA. Prior to this discovery, *C. strigosus* appeared to be endemic to mid-reaches of the Broad River Drainage in Georgia (GA). We conducted additional field sampling in SC and GA and used mitochondrial DNA (mtDNA) to compare the relative genetic distance between individuals across all populations in an effort to determine the identity of the specimens collected. Genetic data indicated that the crayfish collected in SC was *C. strigosus*, with one shared haplotype between SC and GA populations. Our genetic results exhibited geographic structuring and indicated that SC populations have a specific haplotype unique to them, providing strong evidence that SC populations are not introduced. Two additional SC records of *C. strigosus* from 1983 housed in the US National Museum were examined and determined to be *C. strigosus*. With these records, we hypothesized that like in GA, the distribution of *C. strigosus* in SC is limited to riparian habitats associated with the Savannah River Drainage. While the range of this species has been extended, it is still limited to small portions of this drainage. Thus, further sampling to determine the true extent of *C. strigosus* distribution within SC along with conservation efforts are needed to protect this globally rare species.

KEYWORDS: Crustacea, genetic distance, lean crayfish, mtDNA, museum records, range expansion, Savannah River Drainage

INTRODUCTION

Crayfishes have received concerted survey efforts across eastern and central North America over the past two decades (Taylor *et al.*, 2019). Several species, most of which are epigeal species that live in perennial waterways, have had significant range extensions as a result of these efforts (Loughman *et al.*, 2018; Glon *et al.*, 2018, 2022). Nonetheless, crayfishes occur in numerous habitat types (e.g., riparian zones, wetlands, caves; Hobbs, 1981), where it is often difficult to collect and document crayfishes (Loughman *et al.*, 2018). Species inhabiting riparian zones and wetland environments with active hydroperiods have evolved mechanisms to follow groundwater levels as they retreat underground during dry seasons. These species are referred to as secondary or primary burrowers (hereafter referred to as burrowing crayfishes; Hobbs, 1981). Collecting burrowing crayfishes can be difficult, time intensive, are often limited due to time and funding, and

requires investigators to learn the intricacies of burrow excavation. These difficulties have resulted in large knowledge gaps for many burrowing crayfishes.

The extent of our knowledge for many burrowing species is limited to natural history notes included in species descriptions. Another problem is that investigators often spend considerable time identifying the species in question. Recent genetic work indicates that cambarid crayfishes likely exhibit cryptic diversity that can only be captured through genetic techniques (Hurt *et al.*, 2019; Glon *et al.*, 2022; Thoma *et al.*, 2023) that warrants the use of these methods to confirm identification.

Documentation of new populations of burrowing crayfishes is still occurring, with discoveries made yearly. This increased effort has resulted in the description of several undescribed species (Schuster *et al.*, 2015; Glon *et al.*, 2019, 2020a; Loughman *et al.*, 2019). In addition to the description of new taxa,

burrowing crayfishes have had significant range extensions noted over the past decade. Loughman *et al.* (2018) noted *Craerinus fodiens* (Cottle, 1863) in northwestern Pennsylvania for the first time in 2016, while Glon *et al.* (2022) confirmed that *Lacunicambarus nebrascensis* (Girard, 1852) (formerly *Cambarus diogenes*) still occurred in the Rocky Mountain foothills of Wyoming, where it was thought to be extirpated. *Cambarus monongalensis* Ortmann, 1905, an iconic species due to its blue coloration, was first documented in Ohio in 2020 (Glon *et al.*, 2020b). Another example of the discovery of a new population occurred in the Long Cane Ranger District, Sumter National Forest (SNF), South Carolina (SC) in 2020. During an intensive survey to determine the presence of *Distocambarus* Hobbs, 1981 in, a burrowing *Cambarus* species was encountered in a lowland field managed for wildlife. This crayfish was identified as *Cambarus strigosus* Hobbs, 1981, the lean crayfish, following Hobbs (1981). According to state and federal records and reports, as well as peer-reviewed literature, *C. strigosus* appeared to be endemic to the mid-reaches of the Broad River Drainage in Georgia (GA). Collection of *C. strigosus* in SNF they represented a significant range extension for this poorly known species. Subsequent surveys of SNF bottomland habitats along the Savannah River confirmed additional *C. strigosus* colonies, which warranted an effort to determine 1) if the specimens belonged to *C. strigosus* or to an undescribed species, and 2) if *C. strigosus* had been collected before our efforts and documented in SC.

MATERIALS AND METHODS

Field collections

Collections associated with the discovery of *C. strigosus* in SC occurred February 29, 2020, in the Long Cane Ranger District of SNF, McCormick County, SC (Table 1; Fig. 1B). Crayfish were collected by excavating burrows by hand. Burrows were excavated until burrow chambers or water was reached. If water

was reached before reaching the chamber, burrows were plunged vigorously, and time was afforded to allow crayfish to respond. Any crayfish present would normally rise to the surface within 1–5 min following plunging, at which point it was captured.

Because there was only one *C. strigosus* mitochondrial COI sequence publicly available in the National Center of Biotechnology Information (NCBI) GenBank database, additional field collections were made in spring of 2022 from *C. strigosus* current range in GA and the Long Cane Ranger District in SNF, McCormick and Edgefield counties, SC (Fig. 1A, B) to confirm that collected SC specimens belong to this species. Individuals of *C. strigosus* were collected between February and April 2022 via hand excavations from one site in GA and six sites in SC (Table 1). The GA site, six miles from the type locality of the species, was a field along a tributary to the Broad River (Hobbs, 1981). The type locality was sampled, but no *C. strigosus* were collected. Sites in SC were all within the SNF and included three field and three forest sites. We recorded crayfish identification, sex, reproductive form, and total carapace length (TCL) immediately after sampling. We preserved legs in 95% ethanol for genomic analyses. We also preserved voucher specimens in 70% ethanol for future morphometric analyses and deposited the specimens in the North Carolina Museum of Natural Sciences, Raleigh, NC.

Collection of genetic data

We extracted genomic DNA from crayfish-leg tissue using a DNeasy Blood and Tissue Kit (Qiagen, Germantown, MD, USA), following the manufacturer's recommendations. We amplified a portion of the mitochondrial cytochrome oxidase subunit I (COI) gene via polymerase chain reaction, using primers LCO1490 and HCO2198 (Folmer *et al.*, 1994).

Amplifications of polymerase chain reactions were performed in a final volume of 15 μ l containing 1.5 μ l genomic DNA, 3.0 μ l 5 $\times$ buffer (Promega, Madison, WI, USA), 1.2 μ l MgCl₂ (25 mM, Promega), 2.5 μ l deoxynucleotides (1.25 μ M, Promega), 0.75 μ l bovine serum albumin (10 mg μ l⁻¹; New England Biolabs,

Table 1. Locations, habitat, elevation, and number of individuals of *Cambarus strigosus* collected during 2020 (*) and 2022 in South Carolina and Georgia, USA.

Latitude	Longitude	River Basin	County	State	Habitat	Elevation (m)	Number of individuals
*33.6056	-82.11906	Savannah	McCormick	South Carolina	tilled field	60.0	3
33.60171	-82.114133	Savannah	McCormick	South Carolina	riparian forest	60.0	16
33.60184	-82.107638	Savannah	Edgefield	South Carolina	riparian forest	61.0	2
33.60167	-82.108987	Savannah	Edgefield	South Carolina	tilled field	60.4	5
33.60193	-82.107596	Savannah	Edgefield	South Carolina	riparian forest	59.4	1
33.60162	-82.11394	Savannah	McCormick	South Carolina	riparian forest	64.0	9
33.60193	-82.107596	Savannah	Edgefield	South Carolina	riparian forest	59.4	8
33.60172	-82.109063	Savannah	Edgefield	South Carolina	tilled field	54.0	5
33.60165	-82.108662	Savannah	Edgefield	South Carolina	tilled field	60.0	9
33.60184	-82.107638	Savannah	Edgefield	South Carolina	riparian forest	61.0	17
33.6016	-82.114089	Savannah	McCormick	South Carolina	riparian forest	64.0	11
33.60167	-82.108987	Savannah	Edgefield	South Carolina	tilled field	60.0	2
33.60165	-82.108662	Savannah	Edgefield	South Carolina	tilled field	60.0	3
33.6016	-82.114089	Savannah	McCormick	South Carolina	riparian forest	64.0	4
33.97087	-82.863995	Broad	Oglethorpe	Georgia	field/roadside ditch	124.0	15

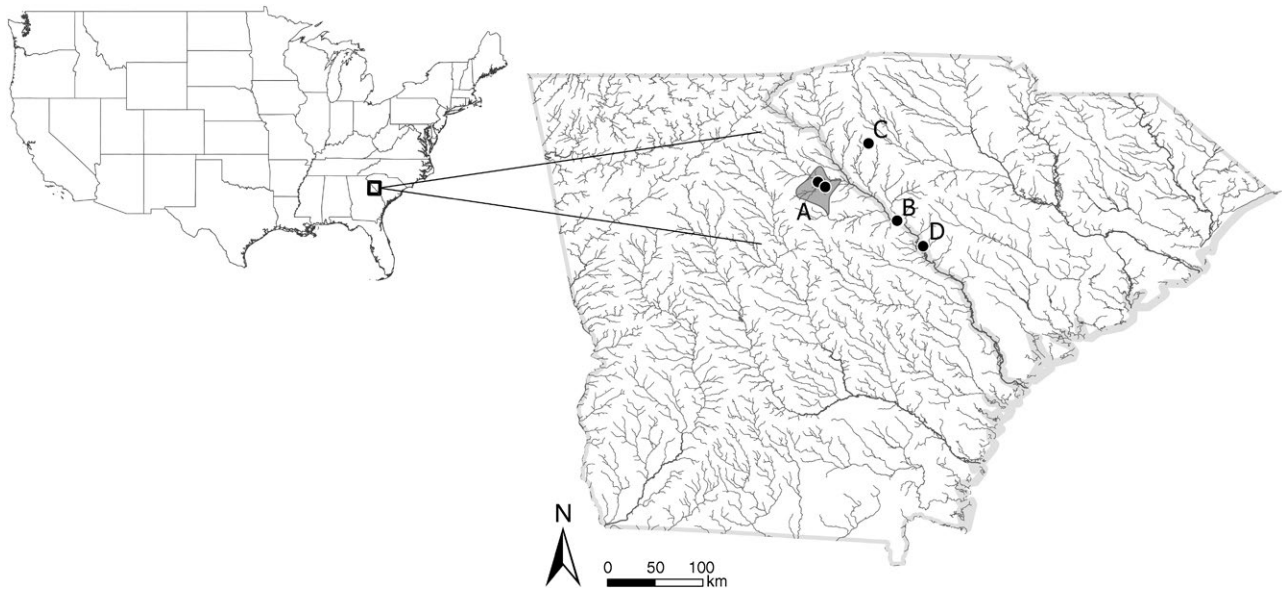


Figure 1. Study area with sampling locations and historic sites represented by dots, and known range of *Cambarus strigosus* represented by a gray polygon. Georgia sites (A), all sites sampled on the Sumter National Forest (B), and historic SC samples (C, D).

Ipswich, MA, USA), 4.4 μ l dH₂O, 0.15 μ l Go-Taq DNA polymerase (5U μ l⁻¹, Promega), and 0.75 μ l of each primer (10 μ M). Thermocycling conditions were 95 °C for 2 min (1 cycle), 95 °C (denaturing) for 30 s, 50 °C (annealing) for 30 s, 72 °C for 1 min (35 cycles), and a final extension at 72 °C for 2 min (1 cycle). To assess the quality and estimate the size (in base pairs (bp)) of amplified products, we used agarose gel electrophoresis and compared amplified products to a 100-bp ladder. Amplified products were purified using ExoSAP-IT® (Affymetrix, Santa Clara, CA, USA) and sequenced on an Applied Biosystems 3730xl DNA Analyzer at Azenta Life Sciences (Burlington, MA, USA). Sequence chromatograms were manually edited, aligned, and assessed for quality by translating DNA to amino acids to confirm the absence of premature stop codons, using MEGA v.11 (Tamura *et al.*, 2021). All sequences generated in this study are available from NCBI GenBank under accession numbers PP555610–PP555634 (Supplementary material Table S1). We also used publicly available mitochondrial COI sequence data from the NCBI GenBank database.

Sequence analyses

We compared the relative genetic distance between *C. strigosus* collected in GA and SC using two approaches to verify the identity of the collected material. We used Mega v.11 to assess pairwise nucleotide differences between each specimen using the maximum likelihood method at uniform rates, with gaps and missing data treated as pairwise deletions (Beerli & Felsenstein, 2001; Tamura *et al.*, 2021). Additionally, we assessed pairwise nucleotide differences between SC and GA specimens by using the same methods stated above. We then estimated phylogenetic relationships among haplotypes using statistical parsimony networks (Clement *et al.*, 2000) calculated using PopART v.1.7 (Leigh & Bryant, 2015). Haplotype networks can better illustrate genetic divergence within species than do bifurcating phylogenetic trees, especially when multiple haplotypes are derived from a single ancestral sequence (Templeton *et al.*, 1992).

Additional specimen determination

We examined of museum collections and digital records to further verify the identity of the specimens collected: digital databases for the North Carolina Museum of Natural Sciences, United States National Museum, Eastern Kentucky University crayfish collection, Illinois Natural History Survey crustacean collection, South Carolina Heritage Program Database, West Liberty University astacology collection, as well as the Illinois Natural History Surveys American Crayfish Atlas website (<https://americancrayfishatlas.web.illinois.edu/>).

RESULTS

Field collections

We collected one female and two juveniles of *C. strigosus* in 2020 from SC (Fig 2), and 92 (7 reproductive (form I) males, 20 non-reproductive (form II) males, 41 adult females, 24 juveniles) also from SC and 15 (1 form I male, 5 form II males, 9 females) from GA. We used the species description by Hobbs (1981) for identification. Diagnostic characters included the noticeable slender appearance of *C. strigosus* as compared to congeners *C.* within the coastal plain region, a single pair of fixed spines on the uropod and coloration (Fig. 3).

Genetics

We obtained mitochondrial COI sequences from 26 individuals of *C. strigosus*, 16 from SC and 10 from GA (1 sequence from NCBI, GenBank accession number JX514445.1; Brienholt *et al.*, 2012), with a final alignment of 481 bp. The alignment contained 33 polymorphic sites and 12 haplotypes. Haplotype diversity (Hd) was 0.86 and nucleotide diversity (π) 0.023. Pairwise nucleotide distance ranged 0.000–0.094 (median 0.02). The mean pairwise nucleotide distance between groups was 0.047, within groups 0.013 and 0.028 for SC and GA, respectively. The haplotype network for *C. strigosus* displayed strong geographic



Figure 2. *Cambarus strigosus* showing the species unique, diagnostic color pattern. Photograph by Z. Graham, West Liberty University.

structure indicating limited dispersal and gene flow (Fig. 4). Five haplotypes were unique to SC, six to GA. Nonetheless, one SC haplotype showed only a three-mutation difference from the most common GA haplotype. Additionally, there was one shared haplotype between *C. strigosus* individuals from GA ($N = 1$) and SC ($N = 8$), indicating that gene flow occurred between SC and GA *C. strigosus* populations.

Additional *C. strigosus* records

Two SC records for *C. strigosus* were documented in 1983. Both records were retrieved through use of the INHS Crayfish Atlas tool housed in the United States National Museum, but we were unable to retrieve either record. USNM 206336 specimen was collected in a seepage area in Abbeville County, SC (Fig. 1C) and USNM 206261 from a bottomland swamp in Aiken County, SC (Fig. 1D). Both records were collected by P.H. Carlson and identified as *C. strigosus* in 1983 by H. Hobbs Jr. We studied the USNM specimens and determined to be *C. strigosus* based on the diagnostic features provided by Hobbs (1981). These records proved to be the first published for *C. strigosus* from SC.

DISCUSSION

Crayfishes have been recognized since the mid-1990s as one of the most imperiled taxa on our planet (Taylor et al., 1996). Since then, additional global (Richman et al., 2015), national (Taylor et al., 2007, 2019), and regional (southeastern USA; Loughman & Fetzner, 2015; Elkins et al., 2019) reviews have supported such assumption. A major driver of imperilment for cambarid crayfishes is their high level of endemism (Taylor et al., 2007, Loughman & Fetzner, 2015, Richman et al., 2015, Taylor et al., 2019). Endemics have long been identified by conservation biologists to suffer higher levels of imperilment than wide-ranging species (Caldecott et al., 1996). Determining if

endemic taxa are as narrowly distributed as they appear justifies survey efforts not only for crayfishes, but for other understudied taxa as well.

Given their limited dispersal abilities, many burrowing crayfishes are classic examples of narrow endemic taxa. It is important to determine if their perceived endemism is real and not an artifact of under-sampling or lack of reporting. *Cambarus strigosus* was described by Hobbs (1981) from GA in the early 1980s from specimens collected from five sites in Elbert, Oglethorpe, and Wilkes counties. Skelton (2016) increased the number of sites to 16 in the same counties. Based on these results, *C. strigosus* appeared to be endemic to GA. Unfortunately, *C. strigosus* collections in SC from the 1980s were not reported to state or federal agencies, or published in a report or journal, and hence these records were overlooked. Our search of historical records, along with our collections and subsequent genetics results, show that *C. strigosus* is not limited to three GA counties, and has a potentially wider range in SC than it does in GA.

One explanation for *C. strigosus* presence in SC is anthropogenic introduction. Burrowing crayfishes have been translocated by humans and established extralimital populations in the mid-Atlantic US (Foltz et al., 2018, Allison et al., 2021). Our genetic results showed geographic structuring and indicated that SC populations have specific haplotypes unique to them. These results suggest that the SC population is not undergoing founder effects or bottleneck, which is common in introduced populations where a new colony is started from a few members of the original population leading to low genetic diversity (Wilson et al., 1999; Astanei et al., 2005; Herborg et al., 2007). Additionally, mtDNA markers are substantially more sensitive to founder effects and bottlenecks because of a fourfold lower effective population size compared with nuclear DNA markers (Birky et al. 1983). Thus, the haplotype structure of the SC *C. strigosus* population provides strong evidence

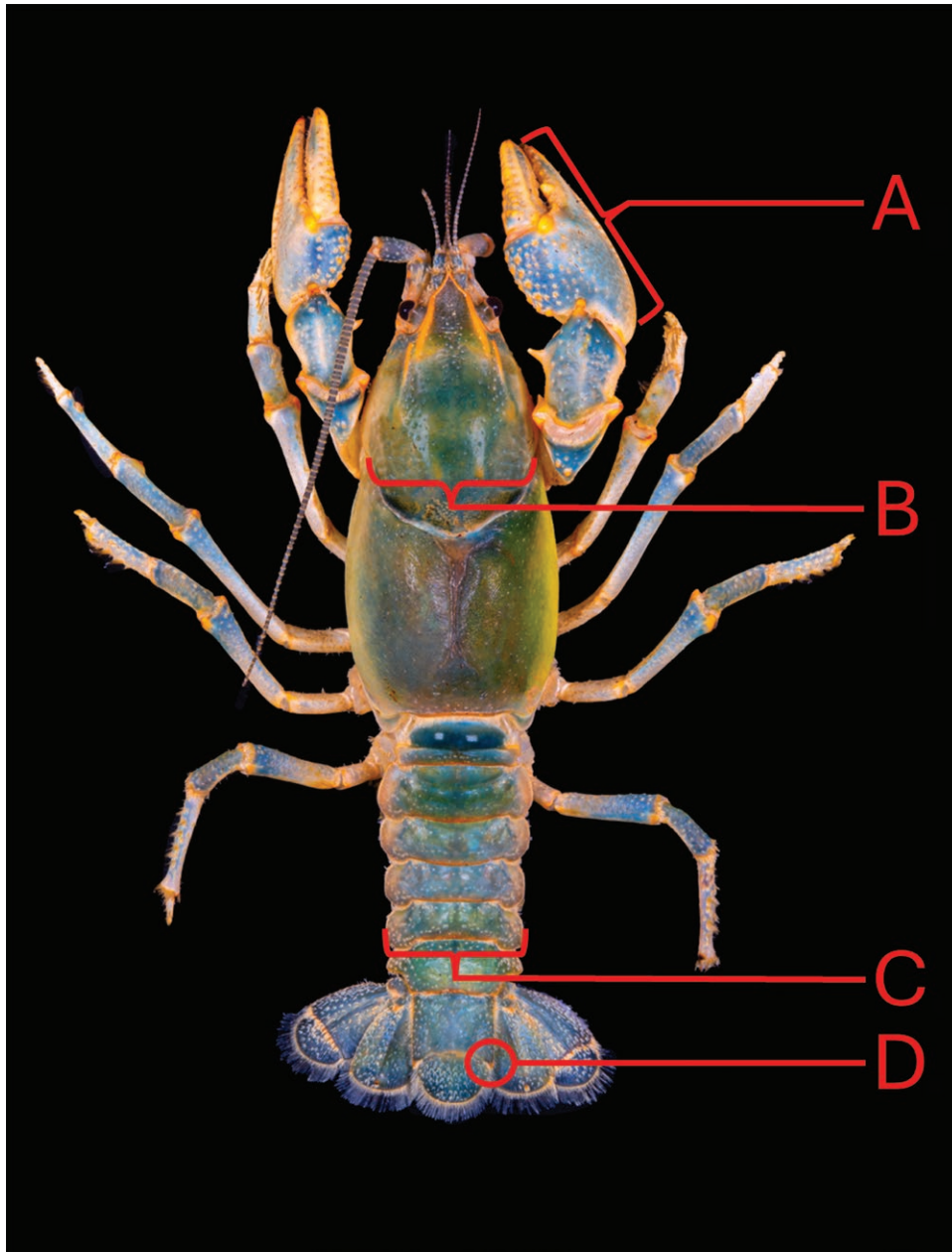


Figure 3. Morphological characters used to identify *Cambarus strigosus* specimens in South Carolina; all characters from Hobbs (1981). Slender chelae (A) and narrow carapace (B) and abdomen (C) as compared to sympatric species; scabrous telson with single pair of fixed spines (D). Photograph by G. Schuster, Eastern Kentucky University.

that these populations SC are native and not introduced. We suggest that, like in GA, the SC distribution of *C. strigosus* is limited to riparian habitats associated with the Savannah River. Bottomland swamps are notoriously difficult to sample for crayfishes and often are under-sampled when compared to stream crossings, roadside wetlands, and roadside ditches. All current SC records of *C. strigosus* are from open fields and bottomland habitats. Sampling extensive tracts of bottomland habitat often requires permission from landowners and the US Forest Service which complicates field investigations. We are currently unsure if *C. strigosus* occurs in roadside ditch habitats, but no specimens have so far been collected from these environments.

Roadside ditches are easier to sample, and therefore much more accessible to biologists once collection permits are obtained. Field sampling throughout the region by ZCB and ZJL for species of *Distocambarus*, which prefer open habitats as well as anthropogenic habitats like roadside ditches. These crews sampled roadside ditches within 100 m of the Abbeville County record of *C. strigosus*, but individuals were missed perhaps because of the preference of bottomland hardwoods habitats by the species.

Understanding the geographical range of species is particularly important for the conservation and protection of imperiled taxa. State record publications serve as documents that can be used by both state and federal-level biologists to justify

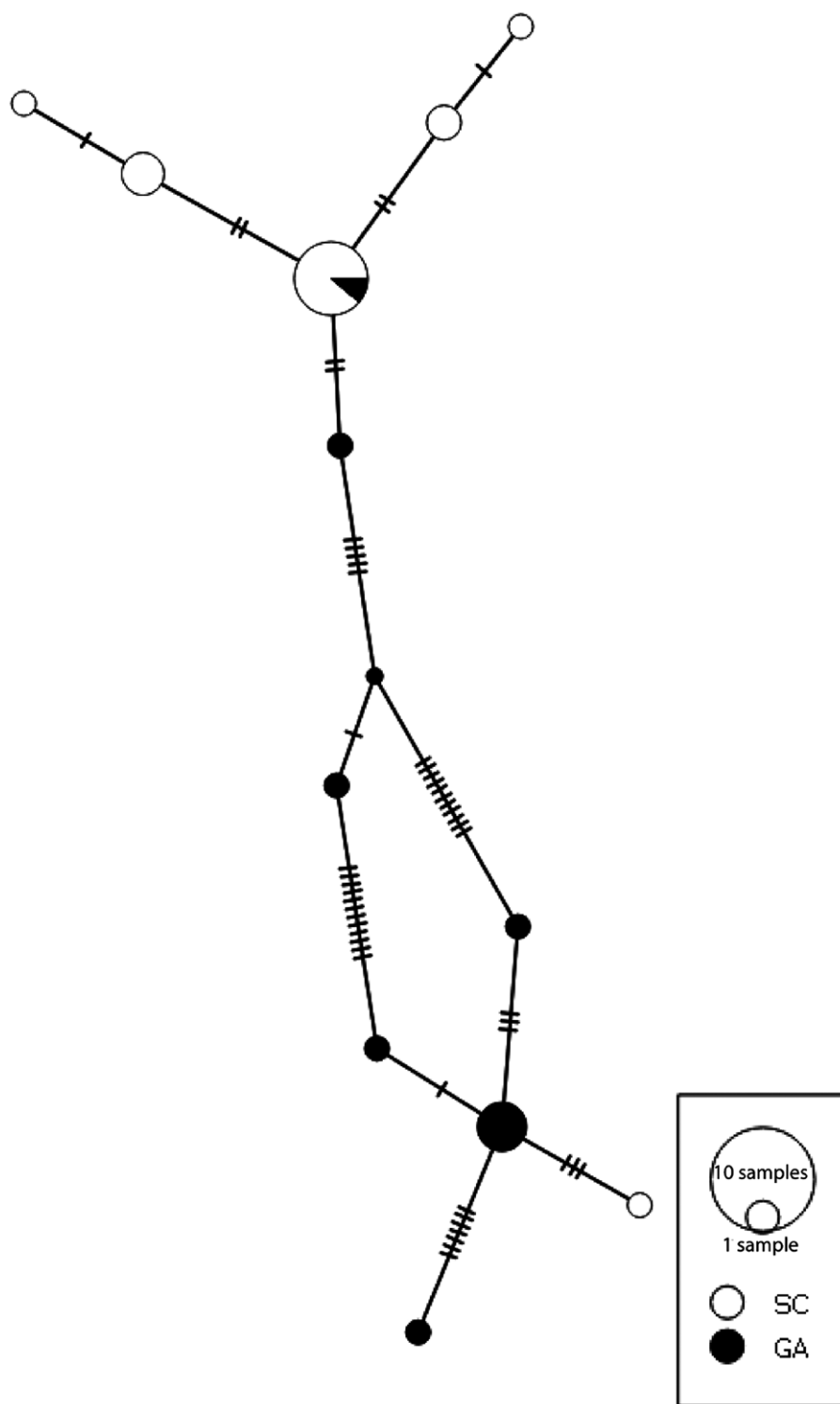


Figure 4. *Cambarus strigosus* ($N = 26$) haplotype network based on 481-bp mitochondrial COI sequence alignments. Each circle represents a unique haplotype. Lines connecting haplotypes indicate a single mutational difference between haplotypes, with dashes along lines representing the number of mutational differences, each dash corresponding to one mutation. The size of each circle is proportional to the frequency with which that haplotype was sampled. SC, South Carolina samples; GA, Georgia samples.

immediate conservation attention. While publications of this nature do not normally carry high impact factors, and often do not help to increase the h-index scores of authors, their utility in conservation can be immense.

An example of conservation efforts due to a particular published report of the range extension of a crayfish species is the short communication of [Loughman et al. \(2018\)](#) documenting the burrowing crayfish *C. fodiens* in Pennsylvania. This report

showed that *C. fodiens* was one of the rarest crayfishes in Pennsylvania, which resulted in making funds available for additional distributional studies (Allison et al., 2022) that ultimately led biologists to list the species as a state-endangered species (Lieb, 2022). As a result, it

Our study will hopefully lead attention for conservation efforts for *C. strigosus* in SC. The species has been identified by SNF biologists at the federal level as a species worthy of tracking (K. Whalen (USFS), personal communications). Dedicated surveys should occur outside of Forest Service lands to determine the actual distribution of *C. strigosus* in SC. At least some *C. strigosus* populations will need to be considered for protection and studied given the presence of the species in SNF.

SUPPLEMENTARY MATERIAL

Supplementary material is available at *Journal of Crustacean Biology* online.

S1 Table. GenBank database accession numbers for each sequence and state where collected.

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