Subterranean Biology 32: 43–59 (2019)
doi: 10.3897/subtbiol.32.38280
http://subtbiol.pensoft.net

SHORT COMMUNICATION

A peer-reviewed open-access journal
Subterranean Biology
Published by The International Society for Subterranean Biology

Discovery of a new population of the federally endangered Alabama Cave Shrimp, *Palaemonias alabamae* Smalley, 1961, in northern Alabama

Matthew L. Niemiller[1], Thomas Inebnit[2], Amata Hinkle[3], Bradley D. Jones[4], Mark Jones[3], Joseph Lamb[5], Nathaniel Mann[6], Benjamin Miller[7], Jennifer Pinkley[3], Stephen Pitts[3], Kayla N. Sapkota[8], Michael E. Slay[9]

1 *Department of Biological Sciences, The University of Alabama in Huntsville, Huntsville, Alabama 37899, USA* **2** *Arkansas Ecological Services Field Office, U.S. Fish and Wildlife Service, Conway, Arkansas 72032, USA* **3** *Southeastern Cave Conservancy, Inc., Signal Mountain, Tennessee 37377, USA* **4** *Birmingham Grotto, Trussville, Alabama 35173, USA* **5** *Department of Biological and Environmental Sciences, Alabama A&M University, Huntsville, Alabama 35762, USA* **6** *Spencer Mountain Grotto, Spencer, Tennessee 38585, USA* **7** *Lower Mississippi Gulf Water Science Center, U.S. Geological Survey, Nashville, Tennessee 37211, USA* **8** *Department of Business, Arkansas State University at Beebe, Beebe, Arkansas 72012, USA* **9** *Arkansas Field Office, The Nature Conservancy, Little Rock, Arkansas 72205, USA*

Corresponding author: *Matthew L. Niemiller* (matthew.niemiller@uah.edu; cavemander17@gmail.com)

Academic editor: *Oana Moldovan* | Received 15 July 2019 | Accepted 16 August 2019 | Published 18 September 2019

http://zoobank.org/F06D3388-E96D-465C-A426-A316017AA5C5

Citation: Niemiller ML, Inebnit T, Hinkle A, Jones BD, Jones M, Lamb J, Mann N, Miller B, Pinkley J, Pitts S, Sapkota KN, Slay ME (2019) Discovery of a new population of the federally endangered Alabama Cave Shrimp, *Palaemonias alabamae* Smalley, 1961, in northern Alabama. Subterranean Biology 32: 43–59. https://doi.org/10.3897/subtbiol.32.38280

Abstract

The Alabama Cave Shrimp *Palaemonias alabamae* Smalley, 1961 is a federally endangered cave shrimp endemic to just four cave systems within and near the greater Huntsville metropolitan area in Madison County, Alabama USA. It is one of two described atyid cave shrimp in the Interior Low Plateau karst region. Here we report the discovery of a new population of *P. alabamae* from the Fern Cave system in western Jackson County, Alabama. We observed four cave shrimp in August 2018 in an isolated pool in the base-level stream passage of the longest cave system in Alabama. Two cave shrimp were observed during a subsequent survey in July 2019: one in the same isolated pool and a second shrimp in a pool in the main stream passage. Morphological and genetic analyses confirm that this population is closely allied with other populations in Madison County. This new population expands the known distribution of the species into a new county and watershed (Lower Paint Rock River). The potential exists to discover additional populations in Paint Rock River valley and other nearby regions.

Keywords
Atyidae, conservation, crustacean, Decapoda, distribution, range extension

Introduction

In the United States, over 1,350 troglobionts and stygobionts (i.e., terrestrial and aquatic cave-obligate species) have been described, and many additional taxa await formal description (Niemiller et al. 2019). However, our knowledge on the distribution, abundance, life history, and ecology of most subterranean biodiversity is grossly inadequate (Mammola et al. 2019). These biodiversity knowledge shortfalls are especially pronounced for invertebrate species (e.g., Cardoso et al. 2011; Niemiller et al. 2018), severely hampering our ability to accurately assess, manage, and conserve this unique fauna. For example, the Wallaean shortfall refers to the lack of knowledge regarding the geographical distribution of species (Lomolino 2004). Our knowledge on species' distributions is intimately correlated with spatial and temporal variation in surveying effort (Hortal et al. 2008, 2015; Boakes et al. 2010). Such variation is widespread in subterranean environments where some regions, such as Mammoth Cave system in central Kentucky and cave systems in the Valley and Ridge of Virginia and West Virginia, have received considerably more attention from biospeleologists than many other regions (e.g., Holsinger et al. 1976, 2013; Holsinger and Culver 1988; Fong et al. 2007; Culver and Hobbs III 2017).

Most cave systems have not been adequately surveyed or studied, and many regions have been grossly underrepresented. For example, less than 7% of caves in Tennessee, the most cave-rich state in United States, have been surveyed for biological resources and even fewer have been repeatedly and comprehensively bioinventoried (Niemiller and Zigler 2013). Moreover, most troglobionts and stygobionts are endemic to a single site or known from very few (<5) cave systems (Christman et al. 2005; Deharveng et al. 2009; Niemiller and Zigler 2013) in a small geographical region (i.e., short-range endemism; Harvey 2002) and inherently assumed to be at higher extinction risk. However, uncertainly often exists whether such species are truly rare or if presumed rarity reflects inadequate surveying effort historically.

The Alabama Cave Shrimp, *Palaemonias alabamae* Smalley, 1961, is a stygobitic atyid shrimp endemic to just four cave systems (six caves) in and around the greater Huntsville area in Madison County, Alabama, USA (Figure 1). It is one of two described stygobitic shrimp in the Interior Low Plateau karst region in the eastern United States; the other is the Kentucky Cave Shrimp, *P. ganteri* Hay, 1902, from the Mammoth Cave system in central Kentucky. *Palaemonias alabamae* was officially listed as endangered under the authority of the Endangered Species Act of 1973 in 1988 (USFWS 1988) but critical habitat has not been designated to date. A recovery plan for

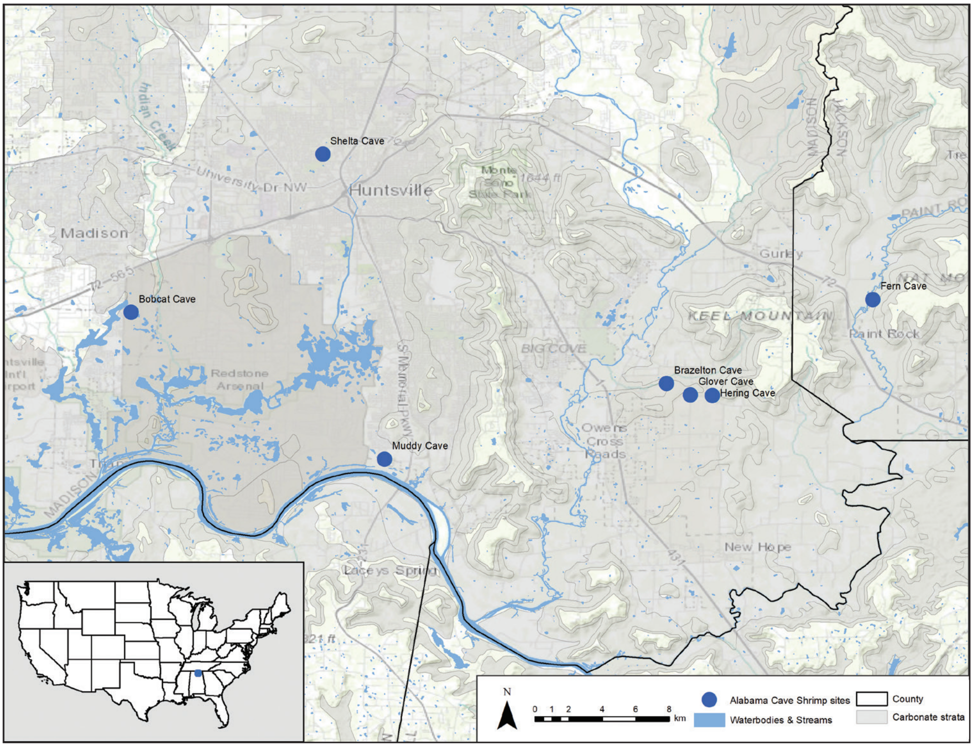

Figure 1. Distribution of the Alabama Cave Shrimp (*Palaemonias alabamae*) in Madison and Jackson counties, Alabama, USA. Carbonate strata are depicted in gray. Alabama Cave Shrimp sites are shown as blue dots.

the species was published in 1997 (USFWS 1997), and the last 5-year review was published in 2016 (USFWS 2016). *Palaemonias alabamae* is State Protected in Alabama under 22–2-.98 (Invertebrate Species Regulation) and a Priority 1 Species (Highest Conservation Concern) under the State Wildlife Action Plan (Alabama Department of Conservation and Natural Resources 2015). This cave shrimp has been assessed as Endangered B1ab(iii) on the IUCN Red List (De Grave and Rogers 2013) and Imperiled-Vulnerable (G2G3) (Critically Imperiled [S1] in Alabama) by NatureServe (NatureServe 2019).

Palaemonias alabamae was first discovered at Shelta Cave (Alabama Cave Survey no. AMD4) in northwestern Huntsville, Madison County, Alabama by Thomas Poulson in 1958 and described by Smalley (1961). It was not until 1973 that *P. alabamae* were discovered at a second cave system when William Torode observed shrimp at Bobcat Cave (AMD1283) located 13.3 km southwest of Shelta Cave on Redstone Arsenal, a military installation operated by the U.S. Army (Cooper and Cooper 1974; Torode 1974). Another two decades passed before an additional population was discovered in the Hering-Glover-Brazelton cave system (Alabama Cave Survey nos. AMD6, AMD54, and AMD337, respectively) located 24.0 km southeast of Shelta Cave in southeastern Madison County (Rheams et al. 1992, 1994; McGregor et al. 1994). Finally, a fourth population was discovered in November 2005, when a single cave shrimp was collected by Bernie Kuhajda and confirmed by DNA analysis from Muddy Cave (AMD1095) in southern Madison County (USFWS 2016). More recent surveys have confirmed that all but the Shelta Cave population are extant (McGregor et al. 2015; USFWS 2016; Niemiller et al., unpublished data). Cave shrimp have not been observed at Shelta Cave since 1973 despite periodic surveys since then (e.g., Lee 1987; Hobbs and Bagley 1989; Moser and Rheams 1992; Rheams et al. 1992; McGregor et al. 1994; Cooper and Cooper 2011; USFWS 2016; Niemiller et al. unpublished data).

Here, we report on the discovery of a population of *P. alabamae* from the Fern Cave system (AJK597) located in the Paint Rock River watershed in western Jackson County, Alabama. This discovery represents the first new locality for this federally endangered cave shrimp in 14 years and just the fifth population discovered to date, extending the geographic range of *P. alabamae* into a new hydrological basin. In addition, this new discovery suggests that this endangered species may be found in other cave systems in the Paint Rock watershed and perhaps further east along the Tennessee River.

Methods

Study site

The Fern Cave system is located along the eastern edge of the Paint Rock River Valley on Nat Mountain in western Jackson County between the cities of Huntsville and Scottsboro. It is the largest cave system in Alabama with over 25 km of passages developed primarily in the Mississippian-aged Bangor and Monteagle Limestone formations (Pinkley 2014). Seven entrances are known, four of which are located on the 199-acre Fern Cave National Wildlife Refuge. The other entrances are owned by the Southeastern Cave Conservancy, Inc. The largest winter colony of federally endangered Gray Bats *Myotis grisescens* (Howell, 1909) hibernate in portions of Fern Cave (Martin 2007).

Biological survey

As part of a two-year study implemented by the Inventory & Monitoring Branch of the U.S. Fish & Wildlife Service to biologically inventory the Fern Cave system comprehensively, four of us (MLN, BJ, JL, and NM) on 25 August 2018 entered the Davidson Entrance (aka Sump Entrance) located at the base of Nat Mountain at an elevation of ca. 187.5 m (615 ft). The Davidson Entrance directly accesses the "Bottom Cave" section of the Fern Cave system. For much of the year, this section of the cave is inaccessible or only accessible via cave diving due to high water levels. During our visit, water levels were low enough to permit entry through a low airspace swim and crawl for ca. 40 m leading to the main stream passage that can be traversed for several hundred meters to a sump. We searched aquatic habitats using time-constrained visual surveys with headlamps and recorded all fauna observed. A cave shrimp was captured using a handheld dipnet in a shallow, isolated pool in the hands-and-knees crawl between the low-airspace pool near the entrance and the main stream passage. The retained specimen was photographed and then preserved in 100% ethanol for morphological and genetic analyses. We returned the following summer and conducted a second survey on 13 July 2019.

DNA extraction, PCR, and sequencing

We extracted genomic DNA from three pleopods using the Qiagen DNEasy Blood & Tissue Extraction Kit following the manufacturer's protocol. A 539-bp fragment of the mitochondrial 16S ribosomal DNA locus was amplified via PCR using primers *16Sar* and *16Sbr* (Simon et al. 1991). This marker exhibits significant genetic variation and has been used in past phylogenetic and population genetic studies of crustaceans (e.g., Zakšek et al. 2007; von Rintelen et al. 2012). In addition, 16S sequences are available on GenBank for *P. alabamae* and the undescribed species in Colbert and Lauderdale counties for comparison (GenBank accession nos. FN995378–FN995383). PCR amplicons were visualized via gel electrophoresis and then sent to Eurofins, Inc. (Louisville, Kentucky) for Sanger sequencing in both directions. The 16S sequence generated in this study was accessioned into GenBank (GenBank accession no. MN097157).

Phylogenetic analyses

Forward and reverse sequences were trimmed at the ends based on quality and assembled into contigs in ChromasPro v2.1.8 (Technelysium Pty Ltd, South Brisbane, Australia) then aligned using MUSCLE (Edgar 2004) in MEGA X version 10.0.5 (Kumar et al. 2018). We constructed a 16S gene genealogy using Bayesian analysis in MrBayes v3.2.7 (Ronquist and Huelsenbeck 2003). The best-fit model of nucleotide substitution was determined using corrected Akaike's Information Criterion (AICc) implemented in the R package *phangorn* v2.5.3 using the function 'modelTest' (Schliep 2011). Outgroup taxa included the atyid shrimp *Atyaephyra desmarestii* (Millet, 1831) (JX469087), *Dugastella marocana* Bouvier, 1912 (FN995363), and *D. valentina* (Ferrer Galdiano, 1924) (FN995365) (von Rintelen et al. 2012). Posterior probabilities were estimated using two independent runs, six Markov chains, and temperature profiles at the default setting of 0.2 for 10 million generations, sampling every 1,000 generations. Random trees were employed at the beginning of each Markov chain, and a molecular clock was not enforced. Stationarity was determined by examining the average standard deviations. We assumed stationarity was achieved if the average standard deviation was < 0.005. The first 25% of trees (5,000 trees) of each run were discarded as burn-in. The remaining trees from the stationarity distribution were sampled to generate a 50% majority-rule consensus tree.

Geographic range size determination

We calculated two measures of geographic range size for *P. alabamae* in the web-based program GeoCAT (Bachman et al. 2011; available at http://geocat.kew.org/): extent of occurrence (EOO, also referred to as range extent) and area of occupancy (AOO). Both metrics are used in IUCN Red List and NatureServe conservation assessments. EOO was calculated as a minimum convex hull, while a grid size of 2 km (4 km^2) was used to estimate AOO (Faber-Langendoen et al. 2009; IUCN 2010).

Results and discussion

After wading and swimming through the low-airspace pool and passage near the Davidson entrance of the Fern Cave system for ca. 40m on 25 August 2018, we climbed onto a mudbank in a passage that leads to the main cave stream. We searched an isolated pool measuring ca. 5 m long, 2 m at its widest point, and water depth up to 25 cm, with mud/silt substrate and a few larger rocks. We encountered a substantial diversity of life in this pool, including a large cave shrimp (*Palaemonias* sp.). The shrimp was observed at the edge of the pool in 4 cm of water. This cave shrimp was captured and retained as a voucher by recommendation of USFWS personnel to confirm species identification (Figure 2). Although shallow, visibility throughout much of the pool was poor due to silt suspended in the water column. This section of the cave is flooded during most of the year. Aquatic life in this and other pools likely were stranded when water levels receded. We surveyed the remainder of aquatic habitat along the main stream passage until reaching the sump but did not encounter additional shrimp. On our exit of the cave, we stopped and searched again at this pool (Figure 3) and observed three additional cave shrimp ca. 12–18 mm total length measured from the rostrum to end of the uropod. These shrimp were photographed only and not captured.

Two cave shrimp were observed during a return trip the following summer on 13 July 2019. One shrimp was observed in the same isolated pool, which was slightly larger in surface area (ca. 6 m long and 2.5 m wide) due to higher water levels. A second shrimp was observed in a quiet pool ca. 0.4 m in depth with silt/sand substrate and scattered cobble in the cave stream. This pool is just downstream of the junction of the passage with the previously referenced isolated pool and the main cave stream.

We identified the specimen from Fern Cave morphologically as *Palaemonias alabamae* by the presence of degenerate eyes, lack of pigmentation, and a long rostrum with eight dorsal and one ventral spine. In the original description of *P. alabamae* by Smalley (1961), the merus of the third pereiopod lacks a distal spine; however, this spine is present in the Fern Cave specimen. The female specimen measured 29.9 mm in total length (tip of rostrum to end of uropod). The specimen was cataloged into the Auburn University Museum of Natural History (AUM 45503). Molecular analysis confirmed that the Fern Cave population was closely related

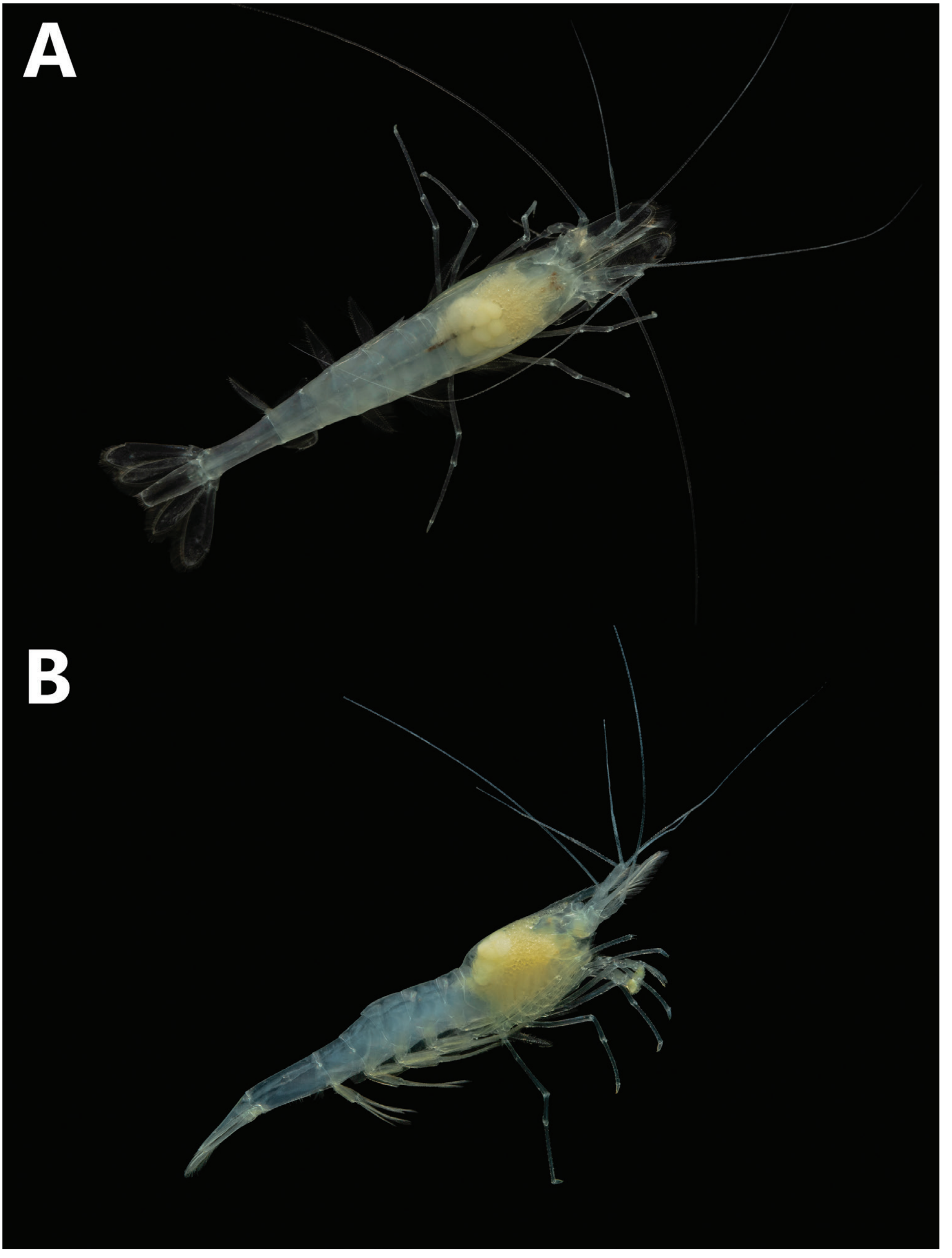

Figure 2. Photographs of the cave shrimp from Fern Cave, Jackson County, Alabama in life: dorsal (**A**) and lateral views (**B**).

to other populations of *P. alabamae* in Madison County (i.e., Hering and Bobcat caves), with 0.4–0.6% uncorrected sequence divergence at the 16S locus between Fern Cave and these two caves (Figure 4).

Figure 3. Joe Lamb and Bradley Jones searching for cave shrimp in an isolated pool near the Davidson Entrance to Fern Cave system on 25 August 2018.

Ecological associates

Palaemonias alabamae co-occurs with several other species at Fern Cave, including Southern Cave Crayfish *Orconectes australis* Rhoades, 1941, *Caecidotea bicrenata* (Steeves, 1963), Southern Cavefish *Typhlichthys subterraneus* Girard, 1859, Cavespring Crayfish *Cambarus tenebrosus* Hay, 1902, and Banded Sculpin *Cottus carolinae* Gill, 1861. *Typhlichthys subterraneus* is thought to be a significant predator of *P. alabamae* (Cooper and Cooper 2011) based on an observation in Shelta Cave in 1966 when a large *T. subterraneus* regurgitated an adult cave shrimp (Cooper 1974, 1975; Cooper and Cooper 1974). *Cottus carolinae* are seasonably abundant in many cave systems in the Interior Low Plateau and Appalachian Valley (Poly and Boucher 1996; Niemiller et al. 2006, 2016) and have been documented to feed on stygobitic prey (e.g., *Caecidotea* isopods in Mammoth Cave, Lisowski 1983), but no reports of predation on *P. alabamae* are known. Another potential predator of cave shrimp is the Tennessee Cave Salamander *Gyrinophilus palleucus* McCrady, 1954, a large stygobitic, neotenic salamander endemic to the Interior Low Plateau karst region of central Tennessee, northern Alabama, and northwestern Georgia (Cooper 1968, Cooper and Cooper 1968; Miller and Niemiller 2008, 2012; Huntsman et al.

2011). *Gyrinophilus palleucus* are generalist predators that co-occur with *P. alabamae* at Shelta and Bobcat caves (Cooper and Cooper 2011) but are yet to be reported from the Hering-Glover-Brazelton and Fern cave systems.

Distribution and range size

At the time of federal listing in 1988, *P. alabamae* was known from two cave systems in Madison County (USFWS 1988): Shelta (type locality) and Bobcat caves (Table 1; Figure 1). Both caves are located in the Indian Creek watershed (USGS HUC10 0603000205) on the Tennessee River basin. Rheams et al. (1992, 1994) extended the distribution 24 km to the southeast into the Lower Flint River watershed (USGS HUC10 0603000204) when cave shrimp were discovered in October 1991 in Glover and Hering caves. Hering Cave is located at the base of Keel Mountain, an outlier of the Cumberland Plateau, in southeastern Madison County. The stream that issues from the spring entrance of Hering Cave flows on the surface for ca. 180-m into the upper entrance of Glover Cave during higher-flow conditions. Cave shrimp also were observed in nearby Brazelton Cave in November 1991 (Rheams et al. 1992, 1994) and later confirmed in November 1994 by McGregor et al. (1994). All three caves are hydrologically connected (Jones and Varnedoe 1968; Graham 1969; Rheams et al. 1992, 1994). Bernie Kuhajda discovered the fourth population at Muddy Cave located 18.4 km south of Shelta Cave in southern Madison County near the banks of the Tennessee River (USGS HUC10 0603000209) (USFWS 2016). Cave shrimp have not been observed from the type locality at Shelta Cave since 1973 (Cooper and Cooper 2011; USFWS 2016). We observed cave shrimp at Fern, Hering, and Bobcat caves in 2018–2019 (this study; Niemiller et al., unpublished data).

Our discovery at Fern Cave extends the distribution of *P. alabamae* into the Lower Paint Rock River watershed (USGS HUC10 0603000202) and into western Jackson County, 9.7 km to the northeast of the nearest population (Hering-Glover-Brazelton cave system) (Figure 1). The Fern Cave system is developed in the carbonate strata along the western escarpment of Nat Mountain. Including the new population at Fern Cave, the EOO of *P. alabamae* is expanded from 251.8 km^2 to 363.2 km^2 and AOO from 24 km^2 to 28 km^2. However, if Shelta Cave is presumed to be extirpated (Cooper and Cooper 2011; USFWS 2016), then EOO and AOO are estimated at 195.4 km^2 and 24 km^2, respectively. Although estimated range size is larger, no change in conservation status is recommended; *P. alabamae* was last assessed as Endangered B1ab(iii) under IUCN Red List criteria (De Grave and Rogers 2013).

There are a few possible explanations as to why so few populations of *P. alabamae* have been reported even though the species has been known to science for almost 60 years and is distributed within and adjacent to a major metropolitan area with extremely high recreational and scientific caving activity. First, *P. alabamae* may in fact be quite rare with an extremely restricted distribution. Many troglobionts and stygobionts are considered short-range endemics (sensu Harvey 2002) with small ranges and re-

Table 1. Confirmed occurrences of *Palaemonias alabamae* in Madison and Jackson counties, Alabama.

Cave	ACS no.	County	Geological formation	Watershed	First observed	Last observed
Shelta Cave	AMD4[a]	Madison	Tuscumbia Limestone and Fort Payne Chert undifferentiated	Indian Creek	1958	1973
Bobcat Cave	AMD1283	Madison	Tuscumbia Limestone	Indian Creek	1973	2019
Hering Cave	AMD6[b]	Madison	Monteagle Limestone	Lower Flint River	1991	2018
Glover Cave	AMD54[b]	Madison	Monteagle Limestone	Lower Flint River	1991	1998
Brazelton Cave	AMD337[b]	Madison	Monteagle Limestone	Lower Flint River	1991	1998
Muddy Cave	AMD1095	Madison	Tuscumbia Limestone	Tennessee River-Wheeler Lake	2005	2012
Fern Cave	AJK597	Jackson	Monteagle Limestone	Lower Paint Rock	2018	2019

[a] type locality;
[b] hydrologically connected.

ported from a single or few cave systems (Christman et al. 2005; Niemiller and Zigler 2013; Niemiller et al. 2017). In Alabama alone, several species have more restricted ranges than *P. alabamae*, such as three cave crayfishes (Cooper and Cooper 1997a,b; Buhay and Crandall 2009), a cavefish (Cooper and Kuehne 1974), and several cave pseudoscorpions (Muchmore and Chamberlain 1995; Muchmore 1996) that are all single-site endemics.

Another hypothesis to explain the low number of *P. alabamae* occurrences relates to detectability. Caves and groundwater ecosystems are extremely difficult for humans to access, survey, and study. Caves that are large enough to permit human entry and exploration as well as sinkholes, springs, and wells represent mere windows into a more expansive subterranean realm. And while many caves, springs, and other points of survey of subterranean biology exist within and near the distribution of *P. alabamae* in northern Alabama, most of these sites have not been adequately sampled. An undescribed species of cave shrimp closely related to *P. alabamae* is known from three caves to the west along the Tennessee River in Colbert and Lauderdale counties (Kuhajda and Mayden 2001; USFWS 2016; B. Kuhajda, pers. comm.). *Palaemonias alabamae* exhibits 1.7–2.3% divergence from populations of this undescribed species (Figure 4). Caves in this region of the Highland Rim have not received the attention by cave biologists historically as cave systems to the east in Jackson, Madison, and Marshall counties.

Moreover, cave systems that have been repeatedly visited by biologists may harbor *P. alabamae* populations that are not consistently observed each trip. For example, Muddy Cave had been visited on several occasions previously without reliable cave shrimp observations (Kuhajda 2004; USFWS 2016). Both Hering and Glover caves are well-known caves that had been visited periodically for decades by cave biologists before the species was discovered in the early 1990s (e.g., Rhoades 1941; Woods and Inger 1957; Hobbs and Barr 1960; Peck 1983, 1986). Dr. John Cooper, who studied *P. alabamae* at Shelta Cave (Cooper 1975) and coauthored the most comprehensive paper on the ecology and life history of the species (Cooper and Cooper 2011), did not observe cave shrimp at Hering and Glover caves during the 1960s (Cooper and Cooper 2011; J. Cooper, pers. comm.).

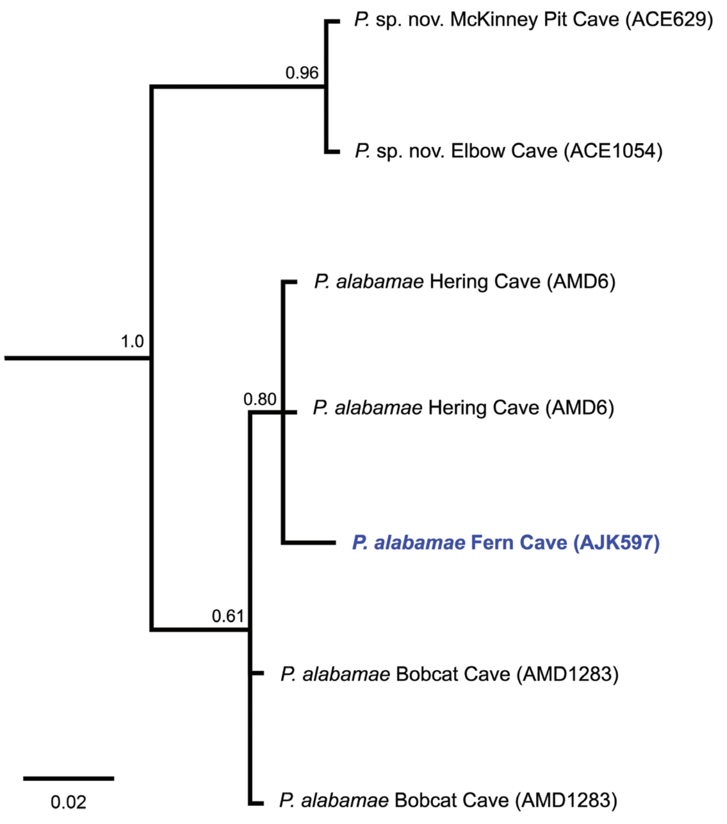

Figure 4. Bayesian phylogram showing the relationships among the new Fern Cave population and other populations of *P. alabamae* and *P.* sp. nov. in Alabama inferred from the mitochondrial 16S ribosomal RNA locus. Posterior probabilities are to the left of the corresponding node.

Low detection may be related to population dynamics or temporal variation of groundwater habitat extent, quality, and accessibility for surveys where *P. alabamae* occurs. Population dynamics of *P. alabamae* are unknown; however, some caves, such as Muddy Cave where very few shrimp have ever been observed, may represent sinks that are periodically extirpated but recolonized from currently unknown or inaccessible (to humans) source populations. Water levels in all caves where *P. alabamae* has been reported are highly variable. For example, aquatic habitat at Shelta Cave largely is reduced to shallow pools during summer and autumn but can fill to 6–8 m or more with higher precipitation during late winter and spring months restricting access to upper levels of the cave only (Cooper 1975; Hobbs and Bagley 1989). Likewise, passages where cave shrimp have been observed are inaccessible during high-water periods at Bobcat Cave, the Hering-Glover-Brazelton cave system, Muddy Cave, and Fern Cave. Consequently, almost all observations of cave shrimp have occurred during periods of low precipitation and low water levels (Cooper 1975; Rheams et al. 1992, 1994; McGregor et al. 1994, 2015; Cooper and Cooper 2011).

The dynamic hydrology of these cave systems has made it difficult to effectively assess and characterize *P. alabamae* habitat. At most sites, cave shrimp are observed in silt or mud-bottomed, isolated pools with little to no flow that persist through dry seasons or remain once the water table has lowered after late winter-spring rainfall (Rheams et al. 1992, 1994; McGregor et al. 1994; Cooper and Cooper 2011). *Palaemonias alabamae* also has been observed in shallow, lotic pools ranging from silt and mud to gravel and cobble substrate in the main stream at Hering Cave (Rheams et al. 1992, 1994; McGregor et al. 1994; Cooper and Cooper 2011; Niemiller et al., unpublished data). In Fern Cave, cave shrimp were observed in the isolated pool perched above the main stream but also in a shallow pool in the main stream. We searched several additional pools in the main cave stream similar in depth and substrate to those in Hering Cave but did not observe any additional cave shrimp. Based on observations of mud stains and debris on passage walls, water levels in the passage where cave shrimp were observed can fluctuate at least 3 m above the water levels observed during our 2018 and 2019 surveys. In those instances, the pool where cave shrimp were observed is hydrologically connected with the main stream. We anticipate additional cave shrimp will be observed in the main cave stream leading up to the sump and in isolated pools and main cave stream beyond the sump. Cave shrimp habitat beyond the sump is only accessible through cave diving the sump or by accessing "Bottom Cave" through highly technical, vertical passages from upper level entrances.

The discoveries of *P. alabamae* at Muddy Cave in 2005 and at Fern Cave renew optimism that additional populations may be discovered in the future. The recovery plan for *P. alabamae* identified two criteria for downlisting the species from endangered to threatened (USFWS 1997): identification and protection of reproductively viable populations in five groundwater basins (or aquifers) and demonstratable reproductive viability for all five populations over a 20-year period. Reproductively viable populations are known from just two cave systems (Bobcat and Hering-Glover-Brazelton) and potentially Fern Cave. The Shelta Cave population has been extirpated, and the status of the Muddy Cave population is unknown. However, several caves that are developed in the same geological formation with permanent or seasonal pools near the local water table exist in the Flint and Paint Rock river watersheds. Many of these caves have not been comprehensively surveyed for biological resources. We recommend a new survey initiative of caves that have not been surveyed in the past as well as revisiting Muddy and Fern caves to determine if these populations are reproductively viable.

Acknowledgements

We thank the USFWS staff at Wheeler National Wildlife Refuge, particularly Rob Hurt, Emory Hoyle, and Ricky Ingram, as well as David Richardson from the Refuges Inventory and Monitoring (I&M) Branch, for allowing access and providing logistical support. We also thank the Alabama Field Office of the Nature Conservancy for providing local facilities to serve as fieldhouse for the multi-year study of Fern Cave fauna. Funding for this project was provided by the USFWS Refuges I&M Branch, Arkansas Field Office of The Nature Conservancy, Cave Conservancy Foundation (MLN), and The University of Alabama in Huntsville. This work was permitted by the Alabama Department of Conservation and Natural Resources under scientific collection permit no. 2018061776268680 and the Southeastern Cave Conservancy, Inc. under research permit no. 1015.

References

Alabama Department of Conservation and Natural Resources (2015) Alabama's Wildlife Action Plan 2015-2025. Alabama Division of WIldlife and Freshwater Fisheries, Department of Conservation and Natural Resources, Montgomery, Alabama, USA, 473 pp.

Bachman S, Moat J, Hill AW, de Torre J, Scott B (2011) Supporting Red List threat assessments with GeoCAT: geospatial conservation assessment tool. Zookeys 150: 117–126. https://doi.org/10.3897/zookeys.150.2109

Boakes EH, McGowan PJK, Fuller RA, Chang-qing D, Clark NE, O'Connor K, Mace GM (2010) Distorted views of biodiversity: spatial and temporal bias in species occurrence data. PLOS Biology 8: e1000385. https://doi.org/10.1371/journal.pbio.1000385

Buhay JE, Crandall KA (2009) Taxonomic revision of cave crayfish in the genus *Cambarus*, subgenus *Aviticambarus* (Decapoda: Cambaridae) with descriptions of two new species, *C. speleocoopi* and *C. laconensis*, endemic to Alabama, USA. Journal of Crustacean Biology 29: 121–134. https://doi.org/10.1651/08-3089.1

Cardoso P, Erwin TL, Borges PAV, New TR (2011) The seven impediments in invertebrate conservation and how to overcome them. Biological Conservation 144: 2647–2655. https://doi.org/10.1016/j.biocon.2011.07.024

Christman MC, Culver DC, Madden M, White D (2005) Patterns of endemism of the eastern North American cave fauna. Journal of Biogeography 32: 1441–1452. https://doi.org/10.1111/j.1365-2699.2005.01263.x

Cooper JE (1968) The salamander *Gyrinophilus palleucus* in Georgia, with notes on Alabama and Tennessee populations. Journal of the Alabama Academy of Science 39: 182–185.

Cooper JE (1974) Biological studies in Shelta Cave, Alabama. Proceedings of the 5th International Congress of Speleology, Stuttgart (1969), Bd. 4, B1/1–8.

Cooper JE (1975) Ecological and behavioral studies in Shelta Cave, Alabama, with emphasis on decapod crustaceans. PhD Thesis, University of Kentucky, Lexington, 364 pp.

Cooper JE, Cooper MR (1968) Cave-associated herpetozoa II: salamanders of the genus *Gyrinophilus* in Alabama caves. National Speleological Bulletin 30: 19–24.

Cooper JE, Cooper MR (1974) Distribution and ecology of troglobitic shrimp of the genus *Palaemonias* (Decapoda: Atyidae). Association of Southeastern Biologist Bulletin 21: 48 pp.

Cooper JE, Cooper MR (1997a) A new species of troglobitic crayfish of the genus *Cambarus*, subgenus *Aviticambarus* (Decapoda: Cambaridae), endemic to White Spring Cave, Alabama. Proceedings of the Biological Society of Washington 110: 608–616.

Cooper JE, Cooper MR (1997b) A new troglobitic crayfish of the genus *Orconectes*, subgenus *Orconectes* (Decapoda: Cambaridae), endemic to Shelta Cave, Huntsville, Alabama. Journal of Caves and Karst Studies 59: 119–127.

Cooper JE, Cooper MR (2011) Observations on the biology of the endangered stygobiotic shrimp *Palaemonia alabamae*, with notes on *P. ganteri* (Decapoda: Atyidae). Subterranean Biology 8: 9–20. https://doi.org/10.3897/subtbiol.8.1226

Cooper JE, Kuehne RA (1974) *Speoplatyrhinus poulsoni*, a new genus and species of subterranean fish from Alabama. *Copeia* 1974: 486–493. https://doi.org/10.2307/1442539

Culver DC, Hobbs III HH (2017) Biodiversity of Mammoth Cave. In: Hobbs III HH, Olsen RA, Winkler EG, Culver DC (Eds) Mammoth Cave, Cave and Karst Systems of the World. Springer, 227–234. https://doi.org/10.1007/978-3-319-53718-4_15

De Grave S, Rogers C (2013) *Palaemonias alabamae*. The IUCN Red List of Threatened Species 2013: e.T15887A788932. https://doi.org/10.2305/IUCN.UK.2013-1.RLTS.T15887A788932.en [25 March 2019]

Deharveng L, Stoch F, Gibert J, Bedos A, Galassi D, Zagmajster M, Brancelj A, Camacho A, Fiers F, Martin P, Giani N, Magniez G, Marmonier P (2009) Groundwater biodiversity in Europe. Freshwater Biology 54: 709–726. https://doi.org/10.1111/j.1365-2427.2008.01972.x

Edgar R (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acid Research 32: 1792–1797. https://doi.org/10.1093/nar/gkh340

Faber-Langendoen D, Master L, Nichols J, Snow K, Tomaino A, Bittman R, Hammerson G, Heidel B, Ramsay L, Young B (2009) NatureServe conservation status assessments: methodology for assigning ranks. NatureServe, Arlington.

Fong DW, Culver DC, Hobbs III HH, Pipan T (2007) The invertebrate cave fauna of West Virginia, 2nd edn. West Virginia Speleological Survey, 167 pp.

Graham D (1969) Glover Sink Cave. In: Wilson J (Ed.) Alabama caving ’68–’69. Special publication Huntsville Grotto, National Speleological Society, Huntsville, 10–11 [corrected].

Harvey MS (2002) Short-range endemism among the Australian fauna: some examples from non-marine environments. Invertebrate Systematics 16: 555–570. https://doi.org/10.1071/IS02009

Hobbs HH III, Bagley FM (1989) Shelta Cave management plan. Unpublished report prepared by Biological Subcommittee of the Shelta Cave Management Committee, Huntsville, 78 pp.

Hobbs HH, Barr TC (1960) The origins and affinities of the troglobitic crayfishes of North America (Decapoda, Astacidae). I. The genus *Cambarus*. The American Midland Naturalist 64: 12–33. https://doi.org/10.2307/2422890

Holsinger JR, Culver DC (1988) The invertebrate cave fauna of Virginia and a part of eastern Tennessee: zoogeography and ecology. Brimleyana 14: 1–162.

Holsinger JR, Culver DC, Hubbard Jr DA, Orndorff WD, Hobson CS (2013) The invertebrate cave fauna of Virginia. Banisteria 42: 9–56.

Holsinger JR, Baroody RA, Culver DC (1976) The invertebrate cave fauna of West Virginia. Bulletin 7. West Virginia Speleological Survey, 86 pp.

Hortal J, de Bello F, Diniz-Filho JAF, Lewinsohn TM, Lobo JM, Ladle RJ (2015) Seven shortfalls that beset large-scale knowledge of biodiversity. Annual Review of Ecology and Evolutionary Systematics 46: 523–549. https://doi.org/10.1146/annurev-ecolsys-112414-054400

Hortal J, Jimenez-Valverde A, Gomez JF, Lobo JM, Baselga A (2008) Historical bias in biodiversity inventories affects the observed environmental niche of the species. Oikos 117: 847–858. https://doi.org/10.1111/j.0030-1299.2008.16434.x

Huntsman BM, Venarsky MP, Benstead JP, Huryn AD (2011) Effects of organic matter availability on the life history and production of a top vertebrate predator (Plethodontidae: *Gyrinophilus palleucus*) in two cave streams. Freshwater Biology 56: 1746–1760. https://doi.org/10.1111/j.1365-2427.2011.02609.x

IUCN (2010) Guidelines for using the IUCN Red List categories and criteria, version 8.1. Prepared by the Standards and Petitions Subcommittee in March 2010.

Jones WB, Varnedoe WW (1968) Caves of Madison County, Alabama. Circular 52. Geological Society of Alabama, Division of Economic Geology, Tuscaloosa, 177 pp.

Kuhajda BR (2004) Survey of biological resources in Muddy Cave, Madison County, Alabama. Unpublished report. Volkert 7 Associates, Inc., Mobile, 10 pp.

Kuhajda BR, Mayden RL (2001) Status of the federally endangered Alabama cavefish, *Speloplatyrhinus poulsoni* (Amblyopsidae), in Key Cave and surrounding caves, Alabama. Environmental Biology of Fishes 62: 215–222. https://doi.org/10.1023/A:1011817023749

Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. Molecular Biology and Evolution 35: 1547–1549. https://doi.org/10.1093/molbev/msy096

Lee DM (1987) Shelta Cave survey. Proceedings of the 1987 annual convention, National Speleological Society, 34 pp.

Lisowski EA (1983) Distribution, habitat, and behavior of the Kentucky cave shrimp *Palaemonias ganteri* Hay. Journal of Crustacean Biology 3: 88–92. https://doi.org/10.2307/1547855

Lomolino MV (2004) Conservation biogeography. In: Lomolino MV, Heaney LR (Ed.) Frontiers of Biogeography. New Directions in the Geography of Nature, Sinauer, Sunderland, 293–296.

Mammola S, Cardoso P, Culver DC, Deharveng L, Ferreira RL, Fiser C, Galassi DMP, Griebler C, Halse S, Humphreys WF, Isaia M, Malard F, Martinez A, Moldovan OT, Niemiller ML, Pavlek M, Reboleria ASPS, Souza-Silva M, Teeling EC, Wynne JJ, Zagmajster M (2019) Scientists' warning on the conservation of subterranean ecosystems. BioScience: biz064. https://doi.org/10.1093/biosci/biz064

Martin CO (2007) Assessment of the population status of the gray bat (*Myotis grisescens*). U.S. Army Corps of Engineers Report ERDC/EL TR-07-22. 106 pp.

McGregor SW, O'Neil PE, Bearden RA (2015) Water quality and biological monitoring in Bobcat and Matthews caves, Redstone Arsenal, Alabama, 1990–2015. Open-file Report 1513, Geological Survey of Alabama, Ecosystems Investigations Program, Tuscaloosa, 42 pp.

McGregor SW, Rheams KF, O'Neil PE, Moser PH, Blackwood R (1994) Geologic, hydrologic, and biologic investigations in Arrowwood, Bobcat, Matthews, and Shelta Caves and selected caves, Madison County, Alabama. Geological Survey of Alabama. Report prepared for the U.S. Fish and Wildlife Service, Jackson, 82 pp.

Miller BT, Niemiller ML (2008) Distribution and relative abundance of Tennessee cave salamanders (*Gyrinophilus palleucus* and *Gyrinophilus gulolineatus*) with an emphasis on Tennessee populations. Herpetological Conservation and Biology 3: 1–20.

Miller BT, Niemiller ML (2012) *Gyrinophilus palluecus*. Catalogue of American Amphibians and Reptiles 884.1.

Moser PH, Rheams KF (1992) Hydrogeologic investigations of Shelta and Bobcat Caves and adjoining areas, Madison County, Alabama. Alabama Geological Survey, 86 pp. [unpublished report]

Muchmore WB (1996) The genus *Tyrannochthonius* in the eastern United States (Pseudoscorpionida: Chthoniidae). Part II. More recently discovered species. Insecta Mundi 10: 153–168.

Muchmore WB, Chamberlin JC (1995) The genus *Tyrannochthonius* in the eastern United States (Pseudoscorpionida: Chthoniidae). Part I. The historical taxa. Insecta Mundi 9: 249–257.

NatureServe (2019) NatureServe explorer: an online encyclopedia of life [web application]. Version 7.1. NatureServe, Arlington, Virginia. http://explorer.natureserve.org [25 March 2019]

Niemiller ML, Glorioso BM, Miller BT (2006) An eastern and subterranean maximum size record of the banded sculpin, *Cottus carolinae*, from Tennessee. Journal of the Tennessee Academy of Science 81: 41–43.

Niemiller ML, Taylor SJ, Bichuette ME (2018) Conservation of cave fauna, with an emphasis on Europe and the Americas. In: Moldovan OT, Kovac L, Halse S (Eds) Cave Ecology. Springer, 451–478. https://doi.org/10.1007/978-3-319-98852-8_22

Niemiller ML, Taylor SJ, Slay ME, Hobbs III HH (2019) Diversity patterns in the United States and Canada. In: Culver DC, White WB, Pipan T (Eds) Encyclopedia of Caves, 3rd edn. Elsevier, 163–176. https://doi.org/10.1016/B978-0-12-814124-3.00018-2

Niemiller ML, Zigler KS (2013) Patterns of cave biodiversity and endemism in the Appalachians and Interior Plateau of Tennessee, USA. PLoS ONE 8: e64177. https://doi.org/10.1371/journal.pone.0064177

Niemiller ML, Zigler KS, Ober KA, Carter ET, Engel AS, Moni G, Philips TK, Stephen CDR (2017) Rediscovery and conservation status of six short-range endemic *Pseudanophthalmus* cave beetles (Carabidae: Trechini). Insect Conservation and Diversity 10: 495–501. https://doi.org/10.1111/icad.12263

Niemiller ML, Zigler KS, Stephen CDR, Carter ET, Paterson AT, Taylor SJ, Engel AS (2016) Vertebrate fauna in caves of eastern Tennessee within the Appalachians karst region, USA. Journal of Cave and Karst Studies 78: 1–24. https://doi.org/10.4311/2015LSC0109

Peck SB (1983) Experimental hybridizations between populations of cavernicolous *Ptomaphagus* beetles (Coleoptera: Leiodidae: Cholevinae). The Canadian Entomologist 115: 445–452. https://doi.org/10.4039/Ent115445-5

Peck SB (1986) Evolution of adult morphology and life-history characters in cavernicolous *Ptomaphagus* beetles. Evolution 40: 1021–1030. https://doi.org/10.1111/j.1558-5646.1986.tb00569.x

Pinkley JE (2014) Fern Cave, The Discovery, Exploration, and History of Alabama's Greatest Cave. Blue Bat Books, Taft, 371 pp.

Poly WJ, Boucher CE (1996) Nontroglobitic fishes in caves: Their abnormalities, ecological classification and importance: American Midland Naturalist 136: 187–198. https://doi.org/10.2307/2426644

Rheams KF, Moser PH, McGregor SW (1992) Geologic, hydrologic, and biologic investigations in Arrowwood, Bobcat, Matthews, and Shelta Caves and selected caves, Madison County, Alabama. Geological Survey of Alabama. Report prepared for the U.S. Fish and Wildlife Service, Jackson, 262 pp.

Rheams KF, Moser PH, McGregor SW (1994) Hydrogeologic and biologic factors related to the occurrence of the Alabama cave shrimp (*Palaemonias alabamae*), Madison County, Alabama. Geological Survey of Alabama, Bulletin 161, Tuscaloosa, 147 pp.

Rhoades R (1941) Notes on some crayfishes from Alabama caves, with the description of a new species and a new subspecies. Proceedings of the United States National Museum 91: 141–148. https://doi.org/10.5479/si.00963801.91-3129.141

Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572–1574. https://doi.org/10.1093/bioinformatics/btg180

Schliep K (2011) phangorn: phylogenetic analysis in R. Bioinformatics 27: 592–593. https://doi.org/10.1093/bioinformatics/btq706

Simon C, Franke A, Martin A (1991) The polymerase chain reaction: DNA extraction and amplification. In: Hewitt GM, Johnson AWB, Young JPW (Eds) Molecular Techniques in Taxonomy, Springer, 329–355. https://doi.org/10.1007/978-3-642-83962-7_22

Smalley AE (1961) A new cave shrimp from southeastern United States (Decapoda, Atyidae). Crustaceana 3: 127–130. https://doi.org/10.1163/156854061X00626

Torode B (1974) Bobcat Cave, Madison County, Alabama. Huntsville Grotto Newsletter 15: 62–63.

U.S. Fish and Wildlife Service (USFWS) (1988) Endangered and threatened wildlife and plants; endangered status of the Alabama cave shrimp, *Palaemonias alabamae*. Federal Register 53: 34696–34698.

USFWS (1997) Recovery Plan for The Alabama cave shrimp (*Palaemonias alabamae*). Atlanta, 59 pp.

USFWS (2016) Alabama cave shrimp (*Palaemonias alabamae*) 5-year review. Summary and evaluation. Daphne, 21 pp.

Von Rintelen K, Page, TJ, Cai Y, Roe K, Stelbrink B, Kuhajda BR, Iliffe TM, Hughes J, von Rintelen T (2012) Drawn to the dark side: a molecular phylogeny of freshwater shrimps (Crustacea: Decapoda: Caridea: Atyidae) reveals frequent cave invasions and challenges current taxonomic hypotheses. Molecular Phylogenetics and Evolution 63: 82–96. https://doi.org/10.1016/j.ympev.2011.12.015

Woods LP, Inger RF (1957) The cave, spring, and swamp fishes of the family Amblyopsidae of central and eastern United States. American Midland Naturalist 58: 232–256. https://doi.org/10.2307/2422371

Zakšek V, Sket B, Trontelj P (2007) Phylogeny of the cave shrimp *Troglocaris*: evidence of a young connection between Balkans and Caucasus. Molecular Phylogenetics and Evolution 42: 223–235. https://doi.org/10.1016/j.ympev.2006.07.009