Invertebrate and Vertebrate Cave Fauna Records for the Appalachian Valley and Ridge

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Abstract

Of the >50,000 caves reported in the United States, nearly 1,140 cave-restricted animals (troglobionts) have been described. One of the most biodiverse karst areas in the United States is the area near the shared boundaries of Tennessee, Alabama, and Georgia (TAG) in the Appalachian Valley and Ridge (AVR) ecoregion. Large sampling gaps in the AVR likely indicate considerable undiscovered biodiversity. From 2013 to 2016, survey teams conducted visual encounter surveys in 95 unique AVR caves, including 64 caves in eastern Tennessee, seven caves in northeastern Alabama, and 24 caves in northwestern Georgia. In Tennessee, most of the caves had never been comprehensively bioinventoried, and some counties were without records for obligate cave fauna. Our effort doubled the knowledge of troglobiont distribution in Tennessee caves alone, simply by increasing the number of caves with new troglobiont records. To date, at least 80 distinct troglobiont species have been identified from caves in the AVR. Species descriptions are ongoing. Among the invertebrates, pseudoscorpions represent some of the most significant terrestrial records. New distribution records now exist for 15 terrestrial and aquatic taxa, and we discovered potentially nine undescribed species, including two species of aquatic hydrobiid snails, five pseudoscorpions, a harvestman, and an annelid. We identified new locations for the federally endangered Gray Bat (Myotis grisescens) and for the only cave-adapted vertebrate in the AVR of Tennessee, the Berry Cave Salamander (Gyrinophilus gulolineatus). We also discovered a new population of Southern Cavefish (Typhlichthys subterraneus) in the AVR of northwestern Georgia, which represents a significant range extension for this species. Collectively, this study emphasizes the importance of bioinventories in understanding the distribution of cave fauna in karst regions. Previous assumptions about the lack of troglobiont or stygobiont diversity in the AVR of TAG were largely due to enduring sampling gaps.

Keywords: biodiversity, cave, Appalachian, invertebrate, vertebrate, sampling gaps

1. Introduction

Over 1,138 cave-restricted species and subspecies from 112 families and 239 genera have been described in the more than 50,000 caves in the United States alone (Hobbs 2012). Karst regions in the United States comprise approximately 20% of the land surface, and 30% of all these caves occur in the states of Tennessee, Alabama, and Georgia, referred collectively to as TAG. TAG has two of the most biodiverse regions, the Interior Low Plateau and the Appalachians (Culver and Pipan 2009; Hobbs 2012). Considerable research has been conducted for over a century to assess cave-restricted fauna in TAG (e.g., Hay 1902; Barr 1961; Lewis 1982; Holsinger and Peck 1971; Peck 1989, 1995; Niemiller and Zigler 2013). Nearly all of the troglobionts (terrestrial obligate cave species) and stygobionts (aquatic obligate cave species) possess conspicuous traits uniquely associated with life in perpetual darkness and limited food resources (Culver and Pipan 2009). Tennessee currently ranks 2nd, with 170 species behind Texas (with 201 species) for the highest richness of obligate subterranean species in the United States. Also on the list, Alabama is 3rd (148 species) and Georgia is 10th (45 species).

The Appalachian Valley and Ridge (AVR) province extends from southeastern Quebec to northwestern Georgia and northeastern Alabama. In Tennessee, the AVR is the second largest karst area after the Highland Rim of the Interior Low Plateau (ILP). The AVR is characterized by parallel ridges and valleys of sandstones with faulted and folded shales and carbonates formed during the uplift of the Appalachian Mountains to the east. The ILP is comprised of horizontal carbonates with limited faulting or folding that extend from southern Illinois and Indiana, southward through Kentucky and Tennessee, and into northern Alabama. The Cumberland Plateau escarpments in Kentucky, Tennessee, Alabama, and Georgia have some of the highest cave densities in the United States, and the Cumberland Plateau in south-central Tennessee and northeastern Alabama is considered to be a hot spot for cave biodiversity, defined by both species richness and endemism (Culver *et al.* 2006). In contrast, the biodiversity of cave species in the AVR is significantly lower than the other karst regions in TAG (Niemiller and Zigler 2013).

Rather than reflecting true reductions in biodiversity in the AVR compared to the ILP, one hypothesis has been that lower biodiversity in AVR caves is due to poorer sampling efforts. Niemiller and Zigler (2013) evaluated cave faunal records throughout Tennessee and identified several undersampled areas in the AVR, defined as 20 x 20 km grid cells. They found only 5.0% of AVR caves in TAG had any records of cave-restricted fauna (Table 1). These results suggested that much diversity had yet to be discovered. Our research focused on addressing sampling gaps by conducting biological inventories in targeted areas of the AVR. By combining these bioinventories with faunal records from previously documented species occurrences, it is now possible to begin to assess geographic extents of some species and examine spatial patterns of biodiversity and endemism, which may highlight additional sampling gaps.

2. Methods

2.1. Bioinventory Efforts

To select caves for bioinventories, we obtained cave descriptions, maps, and locations from state surveys, including the Tennessee Cave Survey (TCS; http://www.subworks.com/tcs/), the Alabama Cave survey (ACS; http://www.alabamacavesurvey.org/main/index.php), and the Georgia Speleological Survey (GSS; http://caves.org/survey/gss/GSSWebsite/Home.html). We were unable to gain access to several caves due to difficulty in finding and obtaining permission from landowners. A secondary challenge was determining if a cave was in the AVR or ILP based on state maps and descriptions.

Bioinventories involved visual encounter surveys for cave life in terrestrial, riparian, and aquatic habitats, such as entrance areas, cave walls and ceilings, mud banks, rimstone pools, and streams. These surveys traversed the cave systematically from the entrance to the farthest extent of the cave explorable by the team. Search effort included lifting rocks and other cover, as well as searching through cobbles, detritus, and organic matter. Depending on the extent of the cave system, each survey typically involved 2 to 4 surveyors (maximum 12), with a search effort of 2 to 36 person-hours per cave visit.

Permits to conduct the research and to collect specimens were obtained from each AVR state. For fishes, amphibians, and reptiles, we made a concerted effort to capture individuals either by hand or with dip nets to confirm identification and to obtain voucher photographs. We also tried to capture representatives of each invertebrate species observed as voucher specimens. Specimens were collected by hand with the aid of brushes, aspirator, or fine-meshed dip nets for aquatic taxa, and placed into 70–100% ethanol. Specimens were sorted in the lab and identified to the lowest taxonomic level possible using published taxonomic keys and species descriptions.

To put our bioinventory efforts in the context of past knowledge of cave fauna in TAG, we also searched for additional distributional records of invertebrates and vertebrates in AVR caves in the scientific literature, unpublished reports, biodiversity databases, and museum accession records. Literature sources included journals, books, proceedings, theses and dissertations, government reports, and regional caving organization newsletters. Literature searches included keyword queries of ISI Web of Science, Google Scholar, and Zoological Record. Database sources included biodiversity databases maintained by the Tennessee Natural Heritage Inventory Program (TNNH) and the Bat Population Database, v2 (http://my.usgs.gov/bpd). We also queried the VertNet database (http://www.vertnet.org), a web portal to search accessions of over 170 vertebrate museum collections from 12 countries. Institutions for which accessions include AVR specimens are Auburn University Museum of Natural History, Carnegie Museum of Natural History, Kansas University Biodiversity Institute, Museum of Vertebrate Zoology at University of California-Berkeley, North Carolina Museum of Natural Sciences, Sam Noble Oklahoma Museum of Natural History, Santa Barbara Museum of Natural History, Museum of Texas Tech University, University of Michigan Museum of Zoology, Smithsonian Institution National Museum of Natural History, and the Western Foundation of Vertebrate Zoology.

3. Results And Discussion

Knowing that <5% of caves in the AVR of Tennessee had never been biologically surveyed, we began the field work by targeting counties that did not have any records for obligate cave fauna (e.g., Loudon, Sevier, Monroe, and Jefferson counties, Tennessee). Other Tennessee counties (e.g., Carter, Grainger, Meigs, and McMinn) had very few troglobiont records from just a handful of caves. For this study, we completed 113 cave trips for 95 unique caves from 2013 through 2016, which totaled bioinventories for 64 individual caves in eastern Tennessee, seven caves in northeastern Alabama, and 24 caves in northwestern Georgia. More than 45 researchers have been involved since 2013 (a list of participants is provided at http://www.speleobiology.com/projects/vrbio/contributors.html).

Species identifications are ongoing, but currently there are >221 identified taxa found from at least one AVR cave. To date, at least 80 distinct troglobiont species from AVR caves have been identified, with the expectation that this number will increase. For Tennessee, the percentage of known troglobionts in 14 of 21 surveyed counties increased, ranging from ≤2% increase in the number of caves with troglobionts (e.g., Carter, Sevier, and Jefferson counties, Tennessee) to as much as 30.8% increase (Monroe County). For some Tennessee counties (e.g., Hamblen, Jefferson, and Loudon), new records for troglobionts were made for all bioinventoried caves. But, for other Tennessee counties (e.g., Blount, Claiborne, Grainger, Hawkins, and Meigs), no new records were added. Collectively, the average increase in the number of caves with troglobionts was 8.0%, from 43 new bioinventories from the 2,255 known caves in the Tennessee AVR. This doubles the current knowledge of troglobiont distribution in AVR caves in Tennessee, and we anticipate a similar increase in knowledge for Alabama and Georgia.

3.1. Invertebrates

Identification of invertebrate specimens is ongoing. At present we have identified 163 taxa. This includes: two flatworms (potentially five after identifications are complete), four annelids, 14 crustaceans (potentially 22), 25 arachnids (18 spiders, three harvestmen, four pseudoscorpions), 16 millipedes, 10 springtails, two diplurans, 18 beetles, six flies, two moths, three crickets, and 20 snails. Seventy-seven of these taxa are considered troglobionts. These data include new distribution records for 15 terrestrial and aquatic taxa, including troglobiont spiders (*Phanetta subterranea*, *Porrhomma cavernicola*, *Nesticus dilutes*), pseudoscorpions (Hesperochernes spp.), millipedes (*Scoterpes blountensis*, Pseudotremia spp., and other genera), beetles (*Pseudanophthalmus assimilis*, *P. pallidus*, *P. rotundatus*, *P. tennesseensis*, *P. unionis*), stygobiont amphipods (*Crangonyx antennatus*), isopods (*Caecidotea richardsonae*), and terrestrial snails (*Helicodiscus barri*, *Glyphalinia specus*).

Several populations discovered may represent new species: two aquatic hydrobiid snails, four millipedes, an annelid, a harvestman, and at least five pseudoscorpions.

Pseudoscorpions: In the eastern United States, the enigmatic genus Hesperochernes comprises two species, *H. holsingeri (*Muchmore, 1994) and *H. mirabilis* (Banks, 1895). Over 150 populations from caves located south of the Ohio River have been attributed to *H. mirabilis* or to the genus with no species-level identification. In contrast, *H. holsingeri* is known from two caves in Indiana. All of these were identified by William Muchmore, the only North American pseudoscorpion taxonomist active between 1960 and 2000. He was of the opinion that *H. mirabilis* is highly variable both within populations and across its geographic range (Muchmore, 1994). The genus is currently being revised using a combination of molecular and morphological methods; accordingly, identifications at present are limited to the generic level. Published records from 1967-1971 documented Hesperochernes from 19 caves in the AVR of TAG (11 Alabama, 7 Georgia, 1 Tennessee). We resampled populations from three caves (2 Alabama, 1 Georgia), and discovered 14 new populations from our other sampling efforts (6 Alabama, 3 Georgia, 5 Tennessee). Hesperochernes is now known from 49 caves in the AVR of TAG (29 Alabama, 14 Georgia, 6 Tennessee) (Stephen *et al.*, 2016). Further sampling in the AVR is likely to uncover more populations, some of which may represent undescribed species of this widely distributed genus.

Other important pseudoscorpion discoveries include: (1) discovering new populations of a species thought to be extinct; (2) new populations of known species with restricted distributions; and (3) populations of species potentially not yet described. *Microcreagris nickajackensis* (Muchmore, 1966) is endemic to Nickajack Cave, Tennessee; it was thought to be extinct due to flooding of the cave system with the construction of Nickajack Dam in the 1960s (Lewis, 2009). Attempts to find the species in Nickajack Cave in accessible, unflooded areas of the cave were not successful. However, we discovered two populations of this presumed extinct species in nearby caves in Alabama and Tennessee. Discoveries of new populations of known species included three species in the genus Kleptochthonius. Two of these are endemic to the AVR: *K. affinis* (Muchmore, 1976), and *K. charon* (Muchmore, 1965). These species are now known from three and two caves, respectively. We also discovered *K. magnus* (Muchmore, 1966) for the first time in one cave of the AVR; previously it was known only from the ILP. Finally, populations of troglobiont pseudoscorpions in three families were discovered that may represent undescribed species. These include specimens identified as Neobisiidae from Limrock Blowing Cave, Alabama; Neobisiidae from Hooker Cave, Georgia; Olpiidae from Beech Spring Cave, Alabama; and two potentially new Tyrannochthonius (family Chthoniidae) from a cave in Georgia and a cave in Tennessee.

Millipedes: The millipede genus Scoterpes (Chordeumatida: Trichopetalidae) is comprised of 15 species that occur throughout much of the ILP and the southern AVR. In AVR, 6 species have been described, including *S. austrinus*, *S. blountensis*, *S. cf. copei*, *S. nudus*, *S. syntheticus*, and *S. willreevesi*. This project yielded several new distributional records of this genus in the AVR. New populations of *S. blountensis* were discovered in Knox and Sevier counties, Tennessee, while new populations of a likely undescribed species closely related to *S. copei* were discovered in Roane and Monroe counties, Tennessee. These populations are closely related to a population from Sensabaugh Cave in Meigs County, Tennessee, which was previously identified as *S. copei*. Over 250 specimens of other millipede taxa were collected and are currently being examined by Dr. Bill Shear. This material includes new records of the genera Pseudotremia, Oxidus, Polydesmus, Pseudopolydesmus, Cambala, and Tetracion, among others. Preliminary examinations suggest that at least four new species of Pseudotremia in east Tennessee and northeastern Alabama exist from these collections.

Beetles: Cave beetles in the genus Pseudanophthalmus (Carabidae: Trechini) constitute a large radiation of >140 taxa in the ILP and Appalachians karst regions. Previously, most species in the AVR were known from <5 caves. Bioinventories associated with this project documented eight species from 16 caves, including new distributional records for *P. assimilis* (DeKalb County, Alabama), *P. rotundatus* (Claiborne County, Tennessee), *P. tennesseensis* (Knox, Loudon, and Roane counties, Tennessee), and *P. unionis* (Campbell and Union counties, Tennessee). In addition, we also confirmed the continued existence of *P. paulus*, *P. pusillus*, and *P. wallacei*, all single cave endemics. *Pseudanophthalmus paulus* had not been observed in >50 years from Nobletts Cave in Monroe County, Tennessee. As such, all of these confirmations are significant for the IUCN Red List of Threatened Species and NatureServe conservation rank criteria for the different species. Additional material remains to be examined from Anderson and Hamilton counties, Tennessee, as well as material from northwestern Georgia.

Amphipods: At least eight species of troglobiotic crangonyctid amphipods (Amphipoda: Crangonyctidae) have been described from the AVR of TAG. We documented three species during our bioinventories: *Bactrurus angulus* (Taylor and Niemiller 2016), *Crangonyx antennatus*, and *Stygobromus mackini*. Several new distributional records of *C. antennatus* were documented. This species is one of the few troglobionts that has a wide distribution in the AVR. Additional material remains to be examined from northwestern Georgia and the southern AVR in East Tennessee, so other taxa may be documented. In addition, a possibly undescribed species may exist in Union County, Tennessee.

Snails: No previous records for aquatic cave snails existed in Tennessee prior to our investigations, and now aquatic snails have been found in four AVR caves in Tennessee (Keenan *et al.*, 2016). A new population of aquatic hydrobiid snails was discovered in Knox County in 2013. The snails are thought to belong to an undescribed species of Fontigens. Another Fontigens, possibly the same species as observed in Knox County, was observed in two other AVR counties (Roane, 1 individual; Sevier, several individuals). An unidentified aquatic snail likely belonging to the genus Antrorbis was found in Roane County, Tennessee.

Annelids: Ectosymbiotic branchiobdellidans, *Bdellodrilus illuminatus*, *Cambarincola holostomus*, and an as of yet undescribed species likely belonging to the genus Cambarincola were found colonizing the dorsal and ventral surfaces of an ovigerous (egg-bearing) crayfish (*Cambarus bartonii cavatus*) in a Knox County AVR cave (Keenan *et al.* 2014). Branchiobdellidan worms are frequently observed on surface- and cave-dwelling crayfish, but several features stand out from this particular crayfish. *B. illuminatus*, when found, occurs in low density and is locally rare despite a wide range from southeastern Canada to Alabama. *C. holostomus* is endemic to Virginia, Tennessee, and North Carolina, but prior to our observations, this species was never observed on a cave-dwelling crayfish. The high density (200+ individuals) and physical location of the branchiobdellidans, in particular high densities on the rostrum, was also distinct from previous surface- or subsurface-based observations. The observation of an undescribed species of Cambarincola suggests that there is high potential for novel ectosymbiotic species within AVR caves.

3.2. Vertebrates

Several studies have resulted from this new research. For one study, an annotated list of 54 vertebrate taxa was generated from eight years of data for only vertebrates from 56 caves in 15 counties in the AVR of eastern Tennessee and adjacent Blue Ridge Mountains in Tennessee (Niemiller *et al.* 2016a). In total, the list included 8 fishes, 19 amphibian (8 anurans and 11 salamanders), 6 reptiles, 3 birds, and 18 mammals. Three species were associated with the IUCN Red List of Threatened Species and six species were identified at risk of extinction according to the NatureServe conservation rank criteria. Two new localities for the only cave-adapted vertebrate in the AVR of east Tennessee, the Berry Cave Salamander (*Gyrinophilus gulolineatus*), were reported. Five bat species were observed in 29 caves, and there were new records for the federally endangered Gray Bat (*Myotis grisescens*). New observations of White-Nose Syndrome were made in four caves in Blount, Roane, and Union counties in Tennessee.

Another study focused on the Southern Cavefish (*Typhlichthys subterraneus*), which has one of the largest distributions of any cavefish in the world, extending throughout the ILP of Kentucky, Tennessee, Alabama, and Georgia. A population of *T. subterraneus* was discovered in Crane Cave in the South Chickamauga Creek watershed. The cave formed in the Ordovician Newala Limestone in Catoosa County, northwestern Georgia, and is clearly within the AVR. This represents a significant range extension for this stygobiotic cavefish (Zigler *et al.* 2015; Niemiller *et al.* 2016b). Genetic analysis of the new fish was undertaken to compare it to other cavefish populations in the region. The new population is genetically related to other fish from the ILP on the western margins of Lookout and Fox mountains in Dade County, Georgia, where caves are formed in Mississippian limestones, as well as to populations in the Little Sequatchie River valley in southern Marion County, Tennessee, where the caves are also in Mississippian limestone. These findings suggest that the newly discovered population shares its evolutionary history with the Dade County, Georgia and Marion County, Tennessee populations. The common ancestor must pre-date the emergence of the modern drainage divide and subsequent isolation of the Crane Cave fish, which likely happened in the Late Pliocene. The timing of these events corresponds with the estimated divergence of the TAG *T. subterraneus* populations from other lineages in the ILP, about 2.2 million years ago (Niemiller *et al.* 2016b). The potential exists to discover additional cavefish populations in other AVR caves within the same limestone units in Georgia and Tennessee.

4. Conclusions

Rather than representing a true biological signal that caves in the AVR ecoregion of Tennessee, Georgia, and Alabama had lower biodiversity than caves in the ILP, we found that less than 5% of caves in the AVR were comprehensively bioinventoried prior to 2013. This paucity of biological surveys has resulted in significant gaps in our knowledge of subterranean biodiversity and distribution compared to the ILP. From 2013–2016, surveys of 95 unique caves in the AVR added observations of >221 troglobiont taxa. This effort has substantially increased the number of biosurveyed caves and our understanding of troglobiont distribution and diversity in the AVR. For some of the troglobionts, there appears to be high rates of endemism, with only a few species having wide distribution throughout the AVR. These surveys now also provide important information for the IUCN Red List of Threatened Species and NatureServe conservation rank criteria for several species. The potential for observations of additional novel taxa, new cave records for troglobionts, and continued range extensions is still high, and continued surveys will help to fill these gaps.

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Table 1. Number of known caves from the Valley and Ridge by state (as of 2016), caves with obligate cave species records, and the number of obligate cave species known. Note: the total number of species is not the sum of the values for the three states because some species are present in more than one state.

State	Number of caves in AVR	Number of caves with obligate cave fauna records	Number of obligate cave species	% caves withobligate cave fauna records
Tennessee	1459	75	50	5.1%
Georgia	213	8	16	3.8%
Alabama	317	16	25	5.0%
Total	1989	99	73	5.0%

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