JOURNAL OF CAVE AND KARST STUDIES

December 2020
Volume 82, Number 4
ISSN 1090-6924
A Publication of the National Speleological Society

DEDICATED TO THE ADVANCEMENT OF SCIENCE, EDUCATION, EXPLORATION, AND CONSERVATION

Published By
The National Speleological Society

http://caves.org/pub/journal

Office
6001 Pulaski Pike NW
Huntsville, AL 35810 USA
Tel:256-852-1300
nss@caves.org

The *Journal of Cave and Karst Studies* , ISSN 1090-6924, CPM Number #40065056, is a multi-disciplinary, refereed journal published four times a year by the National Speleological Society. The *Journal* is available by open access on its website, or check the website for current print subscription rates. Back issues are available from the NSS office.

POSTMASTER: send address changes to the National Speleological Society Office listed above.

The Journal of Cave and Karst Studies is covered by the following ISI Thomson Services Science Citation Index Expanded, ISI Alerting Services, and Current Contents/Physical, Chemical, and Earth Sciences.

Front cover: The pseudoscorpion *Kleptochthonius griseoanus*. See Lewis et al. this issue.

Cato Holler Jr., Jonathan D. Mays, and Matthew L. Niemiller. The fauna of caves and other subterranean habitats of North Carolina, USA. *Journal of Cave and Karst Studies*, v. 82, no. 4, p. 221-260. DOI:10.4311/2019LSC0133

THE FAUNA OF CAVES AND OTHER SUBTERRANEAN HABITATS OF NORTH CAROLINA, USA

Cato Holler Jr.[1], Jonathan D. Mays[2], and Matthew L. Niemiller[3,C]

Abstract

Over 1,500 caves have been documented in North Carolina, however, cave fauna in the Blue Ridge Mountains and Piedmont regions of North Carolina have been overlooked historically compared to the cave-rich karst terrains in the Appalachian Valley and Ridge and Interior Low Plateau to the west. Here, we provide the first comprehensive faunal list of caves and other subterranean habitats in the state based on over 40 years of periodic surveys and compilation of literature, biodiversity databases, and museum records. We report 475 occurrences from 127 caves, springs, and wells in 29 counties, representing 5 phyla, 17 classes, 43 orders, 90 families, 124 genera, and at least 164 species. Vertebrate fauna comprised 32 species, including 4 fishes, 9 salamanders, 1 lizard, 4 snakes, 2 birds, and 12 mammals (8 bats). Diverse invertebrate groups included spiders (11 families and 18 genera), springtails (7 families and 9 genera), segmented worms (3 families and 8 genera), and snails (6 families and 9 genera). At least 25 taxa are troglobites/stygobites (cave obligates), including 5 species of cave flatworms, 5 cave springtails, and 5 cave amphipods. Most troglobitic/stygobitic fauna documented in this study are endemic to North Carolina. Counties with the greatest cave biodiversity include Rutherford, McDowell, Swain, Henderson, Polk, and Avery counties. Over 20 species documented are of conservation concern, including 14 troglobites and 3 federally-listed bats. Although not as diverse as adjacent states, caves and other subterranean habitats in North Carolina support a diverse community of invertebrates and vertebrates. Our review serves as a base line for future cave biological surveys in the state and highlights the importance of subterranean habitats for North Carolina biodiversity.

Introduction

Caves and associated subterranean habitats are home to a unique and taxonomically diverse assemblage of invertebrate and vertebrate organisms in North America. In addition to the more than 1,350 species that are obligate inhabitants of terrestrial and aquatic subterranean habitats in the United States (i.e., troglobites and stygobites) (Niemiller et al., 2019), hundreds of other species use caves on an occasional to semi-permanent basis. Most of this subterranean diversity is known from the ten major karst biogeographic regions defined in the United States that are associated with carbonate exposures (Culver et al., 2003; Hobbs, 2012). Several additional smaller karst regions exist, but these regions have received comparatively little attention from biospeleologists.

The fauna of caves and associated subterranean habitats in the Blue Ridge Mountains and Piedmont physiographic provinces of North Carolina has been grossly understudied compared to subterranean fauna of the cave-rich Appalachian and Interior Low Plateau karst to the west of the Appalachian Mountains in Tennessee, Alabama, Georgia, and Virginia. In contrast to most cave systems in the Appalachians and Interior Low Plateau karst regions, cave systems in North Carolina are predominately non-solutional (i.e., pseudo-karst) and granite-gneiss talus and fissure caves, in particular. A few troglobites and stygobites are known from North Carolina, including some that are endemic to the state, such as the Carolina Groundwater Amphipod (*Stygobromus carolinensis*) and Blowing Springs Cave Springtail (*Pseudosinella flatua*). The bat fauna from several caves and mines has been characterized (Boynton et al., 1992), particularly in association to winter hibernation surveys in recent years (e.g., North Carolina Wildlife Resources Commission, 2017b). However, a comprehensive, annotated list of the subterranean fauna of North Carolina has never been published.

In early 1972, after the Flittermouse Grotto of the National Speleological Society was chartered, its members initiated the North Carolina Cave Survey (NCCS) to track and catalog caves in the state. In conjunction with this statewide cave inventory, and with the suggestion and encouragement of the late Dr. John Cooper, former Director of Research and Collections at the North Carolina State Museum of Natural Sciences, a survey of North Carolina's little-known cave fauna also began. Here we report new distributional records of subterranean fauna documented during periodic surveys of caves between 1970 and 2018 conducted by the lead author and maintained by the NCCS. We also compiled occurrence records from caves and other subterranean habitats (e.g., wells, springs, and seeps) for invertebrate and vertebrate fauna of North Carolina from several sources, including published and unpublished literature, databases, and museum collections. We include these data to generate the first comprehensive faunal list from caves in the state.

[1] Director, North Carolina Cave Survey, PO Box 100, Old Fort, North Carolina 28762; hollers2@frontier.com
[2] Fish and Wildlife Research Institute, Florida Fish and Wildlife Conservation Commission, 1105 SW Williston Rd., Gainesville, Florida, 32601; jonathan.mays@myfwc.com
[3] Department of Biological Sciences, The University of Alabama in Huntsville, Huntsville, Alabama 35899; cavemander17@gmail.com, matthew.niemiller@uah.edu
[C]Corresponding author

Materials and Methods

STUDY REGION AND GEOLOGICAL SETTING

North Carolina lies in the southeastern United States bordered on the east by the Atlantic Ocean, on the south by South Carolina and Georgia, on the west by Tennessee, and on the north by Virginia. The state is divided into three physiographic regions: i) the Blue Ridge Mountains, composed of assorted igneous, metamorphic, volcanic, and sedimentary rocks of widely ranging ages; ii) the Piedmont Plateau consisting primarily of metamorphic and igneous rocks, and iii) the extensive Coastal Plain with its sands, clays, phosphates, and limestone, making up 45% of the state.

As of October 2018, the North Carolina Cave Survey (NCCS) has 1,582 caves on record (Figure 1). Some states limit the term "cave" to a specific length measure; for example, several state cave surveys require that a passage be at least 50 feet (15.2m) in length to be considered a cave. Karst regions and solution caves are not common in North Carolina, thus the NCCS recognizes the term as any naturally occurring opening within the earth capable of being entered by a human. True karst (solution) caves are limited to a few areas of the state. Madison County has three cave-bearing geological formations: Shady Dolomite, the Honaker Limestone, and a dolomite member of the Rome formation. Several significant caves have been dissolved out of the Murphy Marble in Swain County. The Cambrian age Shady Dolomite also occurs in McDowell County where numerous caves have resulted, including Linville Caverns, a well-known commercial cave. Finally, on the Coastal Plain, Eocene age limestones harbor several small solutional caves.

What the state is lacking in karst is more than made up for in its pseudokarst. Most caves in North Carolina are, in fact, pseudokarstic in origin, created not by solution but by physical processes, such as gravity-sliding producing talus and fissure caves, often in igneous and metamorphic rocks (Holler, 2019). Several of these are surprisingly extensive. Bat Cave, for example, has over 2 km of surveyed passages and represents the longest known pseudokarst cave of its type (augen-gneiss) in the world (Holler, 1981; Holler and Holler, 2009).

BIOLOGICAL INVENTORIES

The lead author (CH) conducted periodic biological inventories of caves and fissures in North Carolina from 1970 to 2018. Most of the cave surveys were in the western North Carolina counties of Avery, Burke, McDowell, Rutherford, and Swain. 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 cave streams. These surveys systematically traversed the cave from the entrance to the farthest extent of the cave explorable by the research 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 4), with a search effort of 2 to 36 person-hours per cave visit. For vertebrates (other than mammals), an effort was made to capture each individual observed either by hand or with dip nets to confirm identification and obtain voucher photographs. A concerted effort was made 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. In some instances, Berlese funnels were used to collect tiny specimens within leaves and detritus. Specimens were sorted in the laboratory and identified to the lowest taxonomic level possible using published taxonomic keys and species descriptions. Specimens of several taxonomic groups were sent to experts for identification. In some cases, identification to the species level was not possible due to collection of immature specimens or lack of taxonomic expertise for some groups.

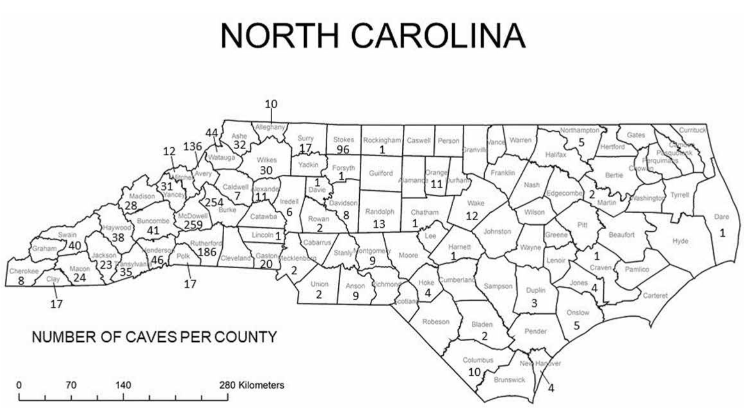

Figure 1. Distribution of the 1,582 caves by county in North Carolina. Data from the North Carolina Cave Survey (NCCS) as of October 2018.

We searched for additional distributional records of invertebrates and vertebrates in North Carolina caves in the scientific literature, unpublished reports, biodiversity databases, and museum accession records. Literature sources included peer-reviewed journals, books, proceedings, theses and dissertations, government reports, and caving organization newsletters. Searches of literature sources included keyword queries of ISI Web of Science, Google Scholar, and Zoological Record. 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. The U.S. Geological Survey Bat Population Database (Ellison et al., 2003) was queried for bat observations from caves in North Carolina. We also queried the

Symbiota Collections of Arthropods Network (SCAN) database (https://scan-bugs.org/portal/), a web portal to search over 21 million arthropod records in over 215 museum collections, and InvertEBase (http://www.invertebase.org/portal/index.php), a web portal to search 18 arthropod and mollusk collections in the United States. Institutions for which accessions included specimens collected from North Carolina include Carnegie Museum of Natural History (CMNH), Denver Museum of Nature & Science (DMNS), Field Museum of Natural History (FMNH), North Carolina Museum of Natural Sciences (NCSM), University of Florida Museum of Natural History (UF), Museum of Comparative Zoology at Harvard University, Museum of Vertebrate Zoology at University of California-Berkeley (MVZ), Royal Ontario Museum (ROM), San Diego State University Museum of Biodiversity (SDSU), Smithsonian National Museum of Natural History (USNM), and University of Michigan Museum of Zoology (UMMZ). We also searched the North Carolina Natural Heritage Program database for occurrences (https://www.ncnhp.org/data/species-community-search).

The annotated list of invertebrate and vertebrate fauna includes the scientific name, authority, ecological classification, common name, and conservation status for each species. Taxonomic nomenclature primarily followed the Encyclopedia of Life (http://www.eol.org). We used common names from published sources when available (e.g. NatureServe). We note the type locality for species described from caves or other subterranean habitats in North Carolina. Classifications of cave-associated organisms (cavernicoles) have been proposed by several authors (e.g., Barr, 1968; Sket, 2008; Culver and Pipan, 2009). We used terminology from Barr (1968) with clarification from Sket (2008) and Culver and Pipan (2009), depending on the taxa, to indicate species found in terrestrial (troglo-) versus aquatic (stygo-) habitats. The four primary ecological categories, with the abbreviations used in the fauna list below and Table 2, were troglobite or stygobite (TB or SB, respectively), troglophile or stygophile (TP or SP) (synonym: eutroglophile), trogloxene or stygoxene (TX or SX) (synonym: subtroglophile), and accidental (AC) (synonym: trogloxene, sensu Sket, 2008). Troglobites and stygobites are obligate cavernicoles with morphological, physiological, and behavioral adaptations for living in subterranean habitats and that have few to no records from surface habitats. Troglophiles and stygophiles frequent subterranean habitats and can complete their life cycles within caves but also may occur in surface habitats. Trogloxenes and stygoxenes use subterranean habitats seasonally, or for only a portion of their life cycles, but also rely significantly on surface habitats. Accidentals are species found in caves only by accident, such as by falling into a pit or being washed into a cave during a flood. We also include species that are intentionally introduced into caves by humans and would not otherwise occur there naturally in this category (e.g., trout). We also denote edaphobites (ED), species thought to be deep-soil inhabitants that may occasionally occur in caves.

The conservation status of each species, based on the International Union for Conservation of Nature (IUCN) Red List of Threatened Species (http://www.iucnredlist.org/; accessed 1 December 2019) and NatureServe (http://www.natureserve.org/; accessed 1 December 2019), is included to provide a better understanding of the distribution and biogeography of subterranean organisms in North Carolina, and to aid in the future conservation and management of this unique fauna. The status of a species according to the U.S. list of threatened and endangered species under the Endangered Species Act is included (http://www.fws.gov/endangered), as well as if a species is included on the list of rare animal species in North Carolina (LeGrand et al., 2014). In addition, we also note species designated “Species of Greatest Conservation Need” in the 2015 North Carolina Wildlife Action Plan (North Carolina Wildlife Resources Commission, 2015).

Seven IUCN Red List categories are recognized on a continuum of increasing extinction risk (International Union for the Conservation of Nature, 2012): Least Concern, Near Threatened, Vulnerable, Endangered, Critically Endangered, Extinct in the Wild, and Extinct. Two additional categories are also recognized: Data Deficient, in which a species has been evaluated, but insufficient data are available to determine a conservation rank; and Not Evaluated, in which a species has yet to be evaluated. Critically Endangered, Endangered, and Vulnerable are considered Threatened categories. Species are classified as Threatened provided they meet one of five criteria (International Union for the Conservation of Nature, 2012): (A) past, present, or projected reduction in population size over three generations; (B) small geographic range in combination with fragmentation, population decline or fluctuations; (C) small population size in combination with decline or fluctuations; (D) very small population or very restricted distribution; or (E) a quantitative analysis of extinction risk. The IUCN Red List classification and associated criteria and subcriteria are presented, if applicable. Subcriteria are detailed in International Union for the Conservation of Nature (2012). NatureServe conservation status ranks are based on a one to five scale, from most to least at risk of extinction (Faber-Langendoen et al., 2012): G1 (Critically Imperiled), G2 (Imperiled), G3 (Vulnerable), G4 (Apparently Secure), and G5 (Secure). Two additional ranks associated with extinction exist: GH (Possibly Extinct) and GX (Presumed Extinct). Finally, species that have not been assessed are assigned GNR (Unranked). A Questionable rank qualifier (?) can be used to denote uncertainty in the conservation status rank (e.g., G2?). Status ranks are assessed at three geographic scales: global (G1–5), national (N1–5), and state (S1–5). Ranks at the global and state scales are given in the text and Table 2.

ANNOTATED LIST OF INVERTEBRATE AND VERTEBRATE SUBTERRANEAN FAUNA OF NORTH CAROLINA

Phylum Platyhelminthes
Class Turbellaria
Order Tricladida
Family Planariidae
Genus *Phagocata*

***Phagocata carolinensis* Kenk, 1979 (SB) Carolina Cave Planarian**
Localities: Burke Co.: One Bat Cave (type locality)[1]; McDowell Co.: Wind Cave[2].
Conservation Status: IUCN: Not assessed; NatureServe: G1G2 (S1S2 in North Carolina).
Comments: This cave flatworm is known only from these two caves.
References: [1] Kenk (1979b); [2] Kenk (1987).

***Phagocata holleri* Kenk, 1979 (SB) Holler's Cave Planarian**
Localities: Ashe Co.: Mount Jefferson Cave (type locality)[1].
Conservation Status: IUCN: Not assessed; NatureServe: G1G2 (S1S2 in North Carolina).
Comments: This cave flatworm is known only from the type locality. Specimens have been collected from a seep within the cave. This species was named in honor of the lead author who first discovered this stygobite.
References: [1] Kenk (1979a).

***Phagocata morgani* (Stevens & Boring, 1906) (SP) Morgan's Planarian**
Localities: Avery Co.: Frying Pan Cave*; McDowell Co.: Staircase Cave*; Rutherford Co.: Bat Cave complex[1]; Wilkes Co.: Devils Den Cave*.
Conservation Status: IUCN: Not assessed; NatureServe: G4G5 (SNR in North Carolina).
Comments: This species is known from spring-runs, springs, and caves in eastern North America.
References: [1] Hertl (1981); * this study.

***Phagocata procera* Kenk, 1984 (SB) A Cave Planarian**
Localities: Burke Co.: Fifty-Fifty Fissure[2], Flatworm Fissure[2]; Jackson Co.: Cat Den Cave (type locality)[1,2]; McDowell Co.: Lake Tahoma Cave[2]; Mitchell Co.: Buckshot Cave[2]; Yancey Co.: seep on State Road 128[2], Mount Mitchell[2].
Conservation Status: IUCN: Not assessed; NatureServe: G1G2 (S1S2 in North Carolina).
Comments: This stygobite was described from Cat Den Cave in Jackson County but has been found in several caves and springs in western North Carolina.
References: [1] Kenk (1984); [2] Kenk (1987).

***Phagocata pygmaea* Kenk, 1987 (SB) A Cave Planarian**
Localities: Stokes Co.: Turtle Shell Cave (type locality)[1].
Conservation Status: IUCN: Not assessed; NatureServe: GNR (SNR in North Carolina).
Comments: This small cave flatworm is known only from the type locality.
References: [1] Kenk (1987).

***Phagocata spuria* Kenk, 1987 (SB) A Cave Planarian**
Localities: McDowell Co.: Bennett's Mill Cave (type locality)[1].
Conservation Status: IUCN: Not assessed; NatureServe: GNR (SNR in North Carolina).
Comments: This species is similar in appearance to *P. morgani* but is known only from the type locality.
References: [1] Kenk (1987).

***Phagocata* sp. (SP) A Planarian**
Localities: Ashe Co.: Mount Jefferson Cave*; Burke Co.: Crystal Worm Cave*; Flaky Lips Fissure Cave*; Henderson Co.: Stillers Cave*.
Comments: Flatworms collected from these caves were immature and could not be identified confidently to species.
References: * this study.

***Phagocata* sp.? (SP) A Planarian**
Localities: Avery Co.: Charlie's Ridge Bat Cave*; Buncombe Co.: Cedar Cliff Cave*; Mitchell Co.: Buckshot Cave*; Surry Co.: Mitchell River Cave*.

Comments: These immature flatworms resembled *Phagocata* but may be another related genus.
References: * this study.

Phylum Annelida
Class Clitellata
Order Haplotaxida
Family Acanthodrilidae
Genus *Diplocardia*

***Diplocardia caroliniana* Eisen, 1899 (ED) An Earthworm**
Localities: Macon Co.: Marie Cave[1].
Conservation Status: IUCN: Not assessed; NatureServe: GNR (SNR in North Carolina).
Comments: *Diplocardia* sp. have been reported from caves in Illinois and Missouri (Peck and Lewis, 1978).
References: [1] Reynolds (1994).

Family Lumbricidae
Genus *Aporrectodea*

***Aporrectodea trapezoides* (Duges, 1828) (ED) Southern Worm**
Localities: Macon Co.: Horse Cave[1], Marie Cave[1].
Conservation Status: IUCN: Not assessed; NatureServe: GNR (SNR in North Carolina).
Comments: This species has also been reported from caves in Georgia, Illinois, and Missouri (Peck and Lewis, 1978; Reeves and Reynolds, 1999; Reeves et al., 2000).
References: [1] Reynolds (1994).

Genus *Dendrobaena*

***Dendrobaena octaedra* (Savigny, 1826) (ED) Octagonal-tail Worm**
Localities: Macon Co.: Horse Cave[1], Marie Cave[1].
Conservation Status: IUCN: Not assessed; NatureServe: GNR (SNR in North Carolina).
Comments: This species has also been reported from a cave in Georgia (Reeves and Reynolds, 1999).
References: [1] Reynolds (1994).

Genus *Dendrodrilus*

***Dendrodrilus rubidus* (Savigny, 1826) (ED) European Earthworm**
Localities: Macon Co.: Horse Cave[1].
Conservation Status: IUCN: Not assessed; NatureServe: GNR (SNR in North Carolina).
Comments: This species has also been reported from several caves in Georgia, Illinois, Missouri, Tennessee and New Brunswick, Canada (McAlpine and Reynolds, 1977; Peck and Lewis, 1978; Reeves and Reynolds, 1999; Reeves et al., 2000).
References: [1] Reynolds (1994).

Genus *Eisenoides*

***Eisenoides lonnbergi* (Michaelsen, 1894) (ED) American Grey Soil Worm**
Localities: Macon Co.: Horse Cave[1]; Rockingham Co.: Pine Hill Cave[1].
Conservation Status: IUCN: Not assessed; NatureServe: GNR (SNR in North Carolina).
Comments: The only reported cave records of this wide-ranging, common earthworm are from North Carolina to date.
References: [1] Reynolds (1994).

Genus *Lumbricus*

***Lumbricus rubellus* Hoffmeister, 1843 (ED) Red Marshworm**
Localities: Macon Co.: Horse Cave[1].
Conservation Status: IUCN: Not assessed; NatureServe: GNR (SNR in North Carolina).
Comments: This species has also been reported from caves in Georgia and Illinois (Peck and Lewis 1978; Reeves et al. 2000).
References: [1] Reynolds (1994).

Genus *Octolasion*

***Octolasion tyrtaeum* (Savigny, 1826) (ED) Woodland White Worm**
Localities: Macon Co.: Horse Cave[1]; Rockingham Co.: Pine Hill Cave[1].
Conservation Status: IUCN: Not assessed; NatureServe: GNR (SNR in North Carolina).
Comments: This species has also been reported from caves in Georgia, Illinois, and Tennessee (Holsinger and Peck, 1971; Peck and Lewis, 1978; Lewis, 2005a), including Great Smoky Mountains National Park (Reeves, 2000b).
References: [1] Reynolds (1994).

Family Sparganophilidae
Genus *Sparganophilus*

***Sparganophilus eiseni* (Smith, 1895) (ED) American Mudworm**
Localities: Macon Co.: Horse Cave[1].
Conservation Status: IUCN: Not assessed; NatureServe: GNR (SNR in North Carolina).
Comments: This species is known from several records in the eastern United States and southeastern Canada, including North Carolina (Reynolds, 1980; McAlpine et al., 2001).
References: [1] Reynolds (1994).

Phylum Mollusca
Class Gastropoda
Order Neotaenioglossa
Family Pleuroceridae
Genus *Elimia*

***Elimia proxima* (Say, 1825) (SX) Sprite Elimia**
Localities: Rutherford Co.: McGrath Fissure*, Rumbling Bald Spring Cave*, Spring Fissure[1].
Conservation Status: IUCN: Least Concern; NatureServe: G5 (S5 in North Carolina).
Comments: This freshwater snail also has been reported from Santee Cave in Orangeburg Co., South Carolina (Reeves, 2001).
References: [1] Christ (2003); * this study.

Order Stylommatophora
Family Gastrodontidae
Genus *Zonitoides*

***Zonitoides elliotti* (Redfield, 1856) (TX) Green Gloss**
Localities: Macon Co.: Horse Cave[1]; McDowell Co.: Staircase Cave[2].
Conservation Status: IUCN: Not assessed; NatureServe: G4 (S4 in North Carolina).
References: [1] Invertebase: CMNH; [2] Invertebase: UF.

Family Haplotrematidae
Genus *Haplotrema*

***Haplotrema concavum* (Say, 1821) (TX/AC) Grayfoot Lancetooth Snail**
Localities: Davidson Co.: Boone's Cave[1].
Conservation Status: IUCN: Not assessed; NatureServe: G5 (S5 in North Carolina).
References: [1] Invertebase: FMNH.

***Haplotrema* sp. (TX/AC) A Lancetooth Snail**
Localities: Swain Co.: Blowing Springs Cave*.
Comments: A carnivorous genus that feeds on other gastropod.Two species have been documented from western North Carolina, *H. concavum* and the rarer *H. kendeighi* (Dourson, 2013).
References: * this study.

Family Helicodiscidae
Genus *Helicodiscus*

***Helicodiscus saludensis* (Morrison, 1937) (TX/AC) Corncob Snail**
Localities: Rutherford Co.: Ice Cave[1].
Conservation Status: IUCN: Not assessed; NatureServe: G1 (S1? in North Carolina).
References: [1] Invertebase: UF.

Family Polygyridae
Genus *Inflectarius*

***Inflectarius inflectus* (Say, 1821) (TX) Shagreen**

Localities: Davidson Co.: Boone's Cave[1]; Henderson Co.: Hog Rock Cave*, Middle Fork Shelter Cave no. 2*; Rutherford Co.: McGrath Fissure*, Rumbling Bald Spring Cave*, Spring Fissure[2].
Conservation Status: IUCN: Not assessed; NatureServe: G5 (S5 in North Carolina).
References: [1] Invertebase: FMNH; [2] Christ (2003), * this study.

Genus *Mesodon*

***Mesodon andrewsae* Binney, 1879 (AC) Balsam Globe**

Localities: Polk Co.: World's Edge Fissure*.
Conservation Status: IUCN: Not assessed; NatureServe: G3 (S2S3 in North Carolina); Listed as a Species of Greatest Conservation Need in North Carolina (North Carolina Wildlife Resources Commission, 2015).
References: * this study.

***Mesodon thyroidus* (Say, 1816) (AC) Whitelip Globe**

Localities: Rutherford Co.: Devil's Smokehouse Cave[1].
Conservation Status: IUCN: Not assessed; NatureServe: G5 (S5 in North Carolina).
References: 1 Invertebase: NCSM.

***Mesodon* sp. (AC) A Globe**

Localities: Madison Co.: Anthodite Cave[1].
Comments: This occurrence may be *M. andrewsae*, *M. thyroidus*, or another species.
References: [1] Invertebase: UF.

Genus *Patera*

***Patera perigrapta* (Pilsbry, 1894) (TX) Engraved Bladetooth**

Localities: Henderson Co.: Hog Rock Cave*; Madison Co.: French Broad Cave[1]; Shutin Creek Cave[1], Polk Co.: Beast Cave[1], Rutherford Co.: Bat Cave[2], Rumbling Bald Cave[1], White Root Shelter*.
Conservation Status: IUCN: Not assessed; NatureServe: G5 (S5? in North Carolina).
References: [1] Invertebase: UF; [2] Invertebase: NCSM; * this study.

Family Zonitidae
Genus *Glyphyalinia*

***Glyphyalinia carolinensis* (Cockerell, 1890) (TX/AC) Spiral Mountain Glyph**

Localities: McDowell Co.: Staircase Cave[1].
Conservation Status: IUCN: Not assessed; NatureServe: G4 (S4 in North Carolina).
References: [1] Invertebase: UF.

***Glyphyalinia* sp. (TX/AC) A Glyph**

Localities: Rutherford Co.: Western Talus Tunnel*.
References: * this study.

Genus *Mesomphix*

***Mesomphix andrewsae* (Pilsbry, 1895) (TX/AC) Mountain Button**

Localities: Swain Co.: Lost Nantahala Cave[1].
Conservation Status: IUCN: Not assessed; NatureServe: G3G4 (S3S4 in North Carolina).
References: [1] Invertebase: UF.

***Mesomphix latior* (Pilsbry, 1900) (TX/AC) Broad Button**

Localities: Swain Co.: Lost Nantahala Cave[1].
Conservation Status: IUCN: Not assessed; NatureServe: G3G4 (S2S3 in North Carolina); Listed as a Species of Greatest Conservation Need in North Carolina (North Carolina Wildlife Resources Commission, 2015).
References: [1] Invertebase: UF.

Mesomphix subplanus **(Binney, 1842) (TX/AC) Flat Button**
Localities: Rutherford Co.: Bat Cave[1].
Conservation Status: IUCN: Not assessed; NatureServe: G3G4 (S3S4 in North Carolina).
References: [1] Invertebase: NCSM.

Phylum Arthropoda
Subphylum Chelicerata
Class Arachnida
Order Araneae
Family Agelenidae
Genus *Agelenopsis*

Agelenopsis **sp. (TX/AC) A Grass Spider**
Localities: Polk Co.: Little Warrior Mountain Cave[1]; Rutherford Co.: Rumbling Bald Cave[1].
References: [1] Gaddy (1986b).

Genus *Coras*

Coras **sp. (TX) A Funnel Weaver Spider**
Localities: Henderson Co.: Hog Rock Cave[2]; Polk Co.: Little Warrior Mountain Cave[1]; Rutherford Co.: Bat Cave[1], Cane Creek Mountain Cave[2], Rumbling Bald Cave[1], Sliding Rock Cave*, Western Talus Tunnel[2]; Yancey Co.: Celo Cave*.
References: [1] Gaddy (1986b); [2] Christ (2003); * this study.

Genus *Wadotes*

Wadotes bimucronatus **(Simon, 1898) (TX/AC) Hackledmesh Weaver Spider**
Localities: Swain Co.: Blowing Springs Cave*
Conservation Status: IUCN: Not assessed; NatureServe: Not assessed.
References: * this study.

Wadotes **sp. (TX/AC) A Weaver Spider**
Localities: Rutherford Co.: Bat Cave complex[1].
References: [1] Hertl (1981).

Undetermined Genus

Undetermined species (TX/AC) A Weaver Spider
Localities: Henderson Co.: Devils Kitchen Cave[1]; Rutherford Co.: A-Frame Fissure[1], Moonshiners Cave*, Rumbling Bald Cave[1].
References: [1] Christ (2003); * this study.

Family Antrodiaetidae
Genus *Antrodiaetus*

Antrodiaetus unicolor **(Hentz, 1842) (TX) Collar Door Spider**
Localities: McDowell Co.: Wind Cave*; Rutherford Co.: Rumbling Bald Cave*, Western Talus Tunnel[1].
Conservation Status: IUCN: Not assessed; NatureServe: GNR (SNR in North Carolina).
Comments: This is a burrowing species; most records were of wandering adult males.
References: [1] Christ (2003); * this study.

Family Araneidae
Genus *Mangora*

Mangora maculata **(Keyserling, 1865) (TX/AC) Green-legged Orbweaver**
Localities: Rutherford Co.: Western Talus Tunnel[1].
Conservation Status: IUCN: Not assessed; NatureServe: GNR (SNR in North Carolina).
References: [1] Christ (2003).

Family Cybaeidae
Genus *Calymmaria*

Calymmaria persica **(Hentz, 1847) (TX) A Dwarf Sheet Spider**
Localities: Caldwell Co.: Nats Cave*; Rutherford Co.: Bat Cave*.

Conservation Status: IUCN: Not assessed; NatureServe: GNR (SNR in North Carolina).
Comments: This is one of over 30 known species of *Calymmaria*, all from North America.
References: * this study.

Family Hypochilidae
Genus *Hypochilus*

***Hypochilus coylei* Platnick, 1987 (TX) A Lampshade Weaver**
Localities: Polk Co.: Little Warrior Mountain Cave[2]; Rutherford Co.: Bat Cave[2], Breakdown Cave[1], Rumbling Bald Cave[2], Sliding Rock Cave*.
Conservation Status: IUCN: Not assessed; NatureServe: G3? (S3? in North Carolina).
Comments: This species lives an average of two years and feeds on other spiders and crickets.
References: [1] SCAN: SDSU; [2] Gaddy (1986b); * this study.

***Hypochilus pococki* Platnick, 1987 (TX) Pocock's Lampshade-web Spider**
Localities: Avery Co.: Elk River Cave[2]; McDowell Co.: Linville Caverns[1]; Transylvania Co.: Snake Den no. 2 Cave*.
Conservation Status: IUCN: Not assessed; NatureServe: G4G5 (SNR in North Carolina).
References: [1] SCAN: DMNS; [2] SCAN: SDSU; * this study.

***Hypochilus* sp. (TX) A Lampshade Weaver**
Localities: Henderson Co.: Devils Kitchen Cave[1]; Madison Co.: French Broad Cave*; Polk Co.: Garnet Shelter Cave[1], World's Edge Cave[1]; Rutherford Co.: Breakdown Cave*, Cane Creek Mountain Cave[1], Moonshiners Cave*, Western Talus Tunnel[1].
Comments: The records represent collections of immatures or visual observations only that could not be identified to species.
References: [1] Christ (2003); * this study.

Family Linyphiidae
Genus *Microneta*

***Microneta viaria* (Blackwell, 1841) (TX/AC) Small Sheetweb Weaver**
Localities: Polk Co.: World's Edge Fissure[1].
Conservation Status: IUCN: Not assessed; NatureServe: Not assessed.
References: [1] Christ (2003).

Genus *Origanates*

***Origanates rostratus* (Emerton, 1882) (TX/AC) A Sheetweb Weaver**
Localities: Henderson Co.: Stillers Cave*.
Conservation status: IUCN: Not assessed; NatureServe: GNR (SNR in North Carolina).
References: * this study.

Genus *Phanetta*

***Phanetta subterranea* (Emerton, 1875) (TB) Subterranean Sheetweb Spider**
Localities: McDowell Co.: Lake Tahoma Cave*; Rutherford Co.: Breakdown Cave*, White Root Shelter[1]; Yancey Co.: Cooper's Cave*.
Conservation status: IUCN: Not assessed; NatureServe: G5 (SNR in North Carolina).
Comments: Like many linyphiids, this species is likely more common in North Carolina caves than records indicate but easily overlooked due to its small size.
References: [1] Christ (2003); * this study.

Genus *Porrhomma*

***Porrhomma cavernicola* (Keyserling, 1886) (TB) Appalachian Cave Spider**
Localities: Swain Co.: Blowing Springs Cave*, Lost Nantahala Cave*.
Conservation status: IUCN: Not assessed; NatureServe: G5 (SNR in North Carolina).
Comments: This wide-ranging troglobite is known from several caves in the Appalachian Valley and Ridge and Interior Low Plateau (Fong et al., 2012; Niemiller et al., 2013).
References: * this study.

Undetermined Genus

Undetermined species (TX/AC) A Sheetweb Spider
Localities: Swain Co.: Blowing Springs Cave*.
Comments: The specimen was immature.
References: * this study.

Family Nesticidae
Genus *Nesticus*

***Nesticus brimleyi* Gertsch, 1984 (TB) A Cave Cobweb Spider**
Localities: Polk Co.: Little Warrior Mountain Cave[1,4,5,*]; Rutherford Co.: A-Frame Fissure[7], Bat Cave complex[2,3,5], Breakdown Cave[3,6], Ice Cave[3], Moonshiners Cave*, Rumbling Bald Cave (type locality)[3,5], Rumbling Bald Mountain Cave[7], Rumbling Bald Spring Cave*, Sliding Rock Cave[7].
Conservation status: IUCN: Not assessed; NatureServe: G1G2 (S1? in North Carolina).
Comments: This troglobite is endemic to North Carolina.
References: [1] NCNHP; [2] Hertl (1981); [3] Gertsch (1984); [4,5] Gaddy (1986a,b); [6] Hedin (1997b); [7] Christ (2003); * this study.

***Nesticus carolinensis* Gertsch, 1984 (TP) Linville Caverns Spider**
Localities: McDowell Co.: Lime Kiln Cave*, Linville Caverns (type locality)[1–4], Staircase Cave*.
Conservation status: IUCN: Not assessed; NatureServe: G1? (S1 in North Carolina).
Comments: Gertsch (1984) considered this species a troglophile despite being known only from caves in McDowell County.
References: [1] NCNHP; [2] Gertsch (1984); [3,4] Hedin (1997a,b); * this study.

***Nesticus cooperi* Gertsch, 1984 (TP) Lost Nantahala Cave Spider**
Localities: Swain Co.: Blowing Springs Cave[1–3], Lost Nantahala Cave (type locality)[2,3].
Conservation status: IUCN: Not assessed; NatureServe: G1 (S1 in North Carolina).
Comments: Although described from a cave, this species is also known from surface records.
References: [1] NCNHP; [2] Gertsch (1984); [3] Hedin (1997b).

***Nesticus mimus* Gertsch, 1984 (TP) A Cobweb Spider**
Localities: Caldwell Co.: Burnt Field Branch Cave*.
Conservation status: IUCN: Not assessed; NatureServe: G2 (S2? in North Carolina).
Comments: This species also is known from surface collections in southwestern Virginia and northwestern North Carolina (Gertsch, 1984).
References: * this study.

***Nesticus* sp. (TP) A Cobweb Spider**
Localities: Madison Co.: Mine Hollow Cave*; McDowell Co.: Wind Cave*; Yancey Co.: Celo Cave*.
Comments: Immatures from these caves could not be identified to species.
References: * this study.

Family Pisauridae
Genus *Dolomedes*

***Dolomedes* sp. (TX) A Fishing Spider**
Localities: Avery Co.: Charlie's Ridge Bat Cave*.
Comments: Fishing spiders are observed not infrequently around the entrances and twilight zones of caves. This individual was observed crawling along the floor of a pool pursuing an undescribed species of *Stygobromus* amphipod.
References: * this study.

Family Tetragnathidae
Genus *Meta*

***Meta ovalis* (Gertsch, 1933) (TP) Cave Orbweaver**
Localities: Avery Co.: Cranberry Mines*; Jackson Co.: Kitchen Cave*; McDowell Co.: Lime Kiln Cave*, Linville Caverns*, Linville Indian Cave*, Pseudo-Saltpetre Cave*, Staircase Cave*, Wind Cave*; Polk Co.: Little Warrior Mountain Cave[1-3]; Rutherford Co.: Bat Cave complex[1,3], Breakdown Cave*, Ice Cave*, Moonshiners Cave*, Rumbling Bald Cave[3], Rumbling Bald Spring Cave*, Sliding Rock Cave*; Swain Co.: Blowing Springs Cave*, Lost Nantahala Cave*; Transylvania Co.: Snake Den no. 2 Cave*; Yancey Co.: Celo Cave*, Cooper's Cave*.

Conservation status: IUCN: Not assessed; NatureServe: GNR (SNR in North Carolina).
Comments: This species is widely distributed in caves in North Carolina and throughout its range. It is also commonly reported from culverts, basements, and other dark recesses.
References: [1] Hertl (1981); [2,3] Gaddy (1986a,b); * this study.

Family Theridiidae
Genus *Cryptachaea*

***Cryptachaea rupicola* (Emerton, 1882) (TX) A Cobweb Spider**
Localities: Rutherford Co.: Breakdown Cave*, Rumbling Bald Cave*.
Conservation status: IUCN: Not assessed; NatureServe: GNR (SNR in North Carolina).
References: * this study.

Genus *Parasteatoda*

***Parasteatoda tepidariorum* (Koch, 1841) (TX) Common House Spider**
Localities: Madison Co.: Anthodite Cave*, French Broad Cave*, Shutin Creek Cave*; McDowell Co.: Linville Indian Cave*.
Conservation status: IUCN: Not assessed; NatureServe: GNR (SNR in North Carolina).
References: * this study.

Genus *Tidarren*

***Tidarren sisyphoides* (Walckenaer, 1841) (TX/AC) Tent Cobweb Spider**
Localities: Polk Co.: Little Warrior Mountain Cave[1,2]; Rutherford Co.: Bat Cave[2], Rumbling Bald Cave[2].
Conservation status: IUCN: Not assessed; NatureServe: Not assessed.
References: [1,2] Gaddy (1986a,b).

Undetermined Genus

Undetermined species (TX/AC) A Cobweb Spider
Localities: Henderson Co.: Devils Kitchen Cave[1].
References: [1] Christ (2003).

Family Theridiosomatidae
Genus *Theridiosoma*

***Theridiosoma gemmosum* (Koch, 1877) (TX) A Ray Orbweaver**
Localities: Caldwell Co.: Nats Cave*; McDowell Co.: Staircase Cave*.
Conservation status: IUCN: Not assessed; NatureServe: GNR (SNR in North Carolina).
References: * this study.

Order Opiliones
Family Phalangiidae
Genus *Leiobunum*

***Leiobunum bicolor* (Wood, 1870) (TX) A Harvestman**
Localities: McDowell Co.: Lime Kiln Cave*, Linville Caverns*, Staircase Cave*; Rutherford Co.: McGrath Fissure[1].
Conservation status: IUCN: Not assessed; NatureServe: Not assessed.
Comments: This genus is known to overwinter, often in large aggregations, in caves.
References: [1] Christ (2003); * this study.

***Leiobunum* sp. (TX) A Harvestman**
Localities: Avery Co.: Cranberry Mines*; Swain Co.: Lost Nantahala Cave*; Transylvania Co.: Snake Den no. 2 Cave*; Yancey Co.: Celo Cave*, Isom Mica Mine*.
Comments: These records may be *L. bicolor*.
References: * this study.

Family Phalangodidae
Genus *Bishopella*

***Bishopella laciniosa* (Crosby & Bishop, 1924) (TP) Bishop's Harvestman**
Localities: McDowell Co.: Linville Caverns[1].
Conservation status: IUCN: Not assessed; NatureServe: Not assessed.
Comments:
References: [1] SCAN: SDSU.

***Bishopella* sp. (TP) A Harvestman**
Localities: Swain Co.: Blowing Springs Cave*, Lost Nantahala Cave*.
Comments: These records are probably *B. laciniosa*.
References: * this study.

Family Travuniidae
Genus *Theromaster*

***Theromaster brunneus* (Banks, 1902) (TX) A Harvestman**
Localities: Henderson Co.: Devils Kitchen Cave[3]; McDowell Co.: Linville Caverns[1]; Polk Co.: Garnet Shelter Cave[3]; Rutherford Co.: Bat Cave complex[1,2].
Conservation status: IUCN: Not assessed; NatureServe: Not assessed.
Comments: This armored harvestman is collected occasionally from caves.
References: [1] SCAN: SDSU; [2] Hertl (1981); [3] Christ (2003).

Order Pseudoscorpiones
Family Chthoniidae
Genus *Kleptochthonius*

***Kleptochthonius* sp. (TP/TX) A Pseudoscorpion**
Localities: Henderson Co.: Devil's Kitchen Cave*.
References: * this study.

Undetermined Genus

Undetermined species (TX/AC) A Pseudoscorpion
Localities: Swain Co.: Blowing Springs Cave*.
References: * this study.

Family Neobisiidae
Genus *Microcreagris*

***Microcreagris* sp. (TP/TX) A Pseudoscorpion**
Localities: Polk Co.: Zig-Zag Fissure*.
References: * this study.

Subclass Acari
Order Mesostigmata
Family Arctacaridae
Undetermined Genus

Undetermined species (TX/AC) A Mite
Localities: Rutherford Co.: Bat Cave complex[1].
References: [1] Hertl (1981).

Family Parasitidae
Undetermined Genus

Undetermined species (TX/AC) A Mite
Localities: Henderson Co.: Devils Kitchen Cave[1]; Rutherford Co.: Block Creep Cave[1].
References: [1] Christ (2003).

Family Parholaspididae
Undetermined Genus

Undetermined species (TX/AC) A Mite
Localities: Henderson Co.: Devils Kitchen Cave[1].
References: [1] Christ (2003).

Order Sarcoptiformes
Family Lohmanniidae
Genus *Lohmannia*

***Lohmannia* sp. (TX) A Mite**
Localities: Rutherford Co.: Bat Cave complex[1].
References: [1] Hertl (1981).

Family Phthiracaridae
Genus *Steganacarus*

***Steganacarus* sp. (TX) A Mite**
Localities: Rutherford Co.: Bat Cave complex[1].
References: [1] Hertl (1981).

Order Trombidiformes
Family Bdellidae
Undetermined Genus

Undetermined species (TP/TX) A Mite
Localities: Rutherford Co.: Block Creep Cave[1].
References: [1] Christ (2003).

Family Rhagidiidae
Undetermined Genus

Undetermined species (TP) A Mite
Localities: Polk Co.: Garnet Shelter Cave[1], World's Edge Cave[1].
References: [1] Christ (2003).

Subphylum Hexapoda
Class Collembola
Order Entomobryomorpha
Family Entomobryidae
Genus *Entomobra*

***Entomobrya quadrilineata* Bueker, 1939 (AC) A Springtail**
Localities: Rutherford Co.: Block Creep Cave*.
Conservation Status: IUCN: Not assessed; NatureServe: Not assessed.
Comments: This well-pigmented and eyed springtail is occasionally collected in caves.
References: * this study.

Genus *Pseudosinella*

***Pseudosinella collina* Wray, 1952 (TP) A Springtail**
Localities: Polk Co.: Garnet Shelter Cave[1].
Conservation Status: IUCN: Not assessed; NatureServe: GNR (SNR in North Carolina).
Comments: This troglophilic springtail is known from several cave and surface records in the eastern United States.
References: [1] Christ (2003).

***Pseudosinella flatua* Christiansen & Bellinger, 1996 (TB) Blowing Springs Cave Springtail**
Localities: Swain Co.: Blowing Springs Cave (type locality)[1,2].
Conservation Status: IUCN: Not assessed; NatureServe: G1G2 (S1S2 in North Carolina).
Comments: This troglobite is known only from the type locality.
References: [1] Christiansen and Bellinger (1996); [2] SCAN: MCZ.

***Pseudosinella gisini carolina* Christiansen & Bellinger, 1996 (TB) Carolina Cave Springtail**
Localities: Rutherford Co.: McGrath Fissure (type locality)[1,2], Rumbling Bald Cave[2].
Conservation Status: IUCN: Not assessed; NatureServe: G3G4T1T2 (S1S2 in North Carolina).
Comments: This subspecies is known only from these two caves.
References: [1] Christ (2003); [2] Christiansen and Bellinger (1996).

***Pseudosinella orba* Christiansen, 1961 (TB) A Cave Springtail**
Localities: Polk Co.: World's Edge Cave[1].
Conservation Status: IUCN: Not assessed; NatureServe: G3G4 (SU in North Carolina).
Comments: This species also is known from caves in Kentucky, Tennessee, Virginia, and West Virginia.
References: [1] Christiansen Grinnell Database.

***Pseudosinella pecki* Christiansen & Bellinger, 1980 (TB) Peck's Cave Springtail**
Localities: Swain Co.: Hot Pit Cave[1].
Conservation Status: IUCN: Not assessed; NatureServe: G2G3 (SNR in North Carolina).
Comments: This troglobite is known from caves in several states in the eastern United States (Christiansen and Bellinger, 1980; Reeves et al., 2000).
References: [1] Christiansen Grinnell Database.

***Pseudosinella vespera* Christiansen & Bellinger, 1996 (TB) Bat Cave Springtail**
Localities: Rutherford Co.: Bat Cave (type locality)[1–4]; Swain Co.: Blowing Springs Cave[3].
Conservation Status: IUCN: Not assessed; NatureServe: G2 (S1S2 in North Carolina).
Comments: This troglobite is known from these two North Carolina caves as well as a cave in Bland Co., Virginia.
References: [1] Christiansen Grinnell Database; [2] Hertl (1981); [3] Christiansen and Bellinger (1996), [4] SCAN: MCZ.

***Pseudosinella* sp. nov.? (TB) A Springtail**
Localities: Rutherford Co.: White Root Shelter[1].
Comments: This record may represent a new species or one of the described troglobitic taxa.
References: [1] Christ (2003).

Family Isotomidae
Genus *Folsomia*

***Folsomia candida* Willem, 1902 (TP) White Springtail**
Localities: Swain Co.: Blowing Springs Cave*.
Conservation Status: IUCN: Not assessed; NatureServe: GNR (SNR in North Carolina).
References: * this study.

Family Lepidocyrtidae
Genus *Lepidocyrtus*

***Lepidocyrtus* sp. (TX) A Springtail**
Localities: Henderson Co.: Devils Kitchen Cave[1], Hog Rock Cave[1]; Polk Co.: World's Edge Cave[1]; Rutherford Co.: Sliding Rock Cave[1], White Root Shelter[1].
References: [1] Christ (2003).

Family Tomoceridae
Genus *Pogonognathellus*

***Pogonognathellus bidentatus* (Folsom, 1913) (TP) A Springtail**
Localities: Caldwell Co.: Burnt Field Branch Cave*; McDowell Co.: Lake Tahoma Cave*; Polk Co.: Little Warrior Mountain Cave*, World's Edge Cave[2]; Rutherford Co.: Bat Cave complex[1]; Rimstone Skull Cave[2], Sliding Rock Cave[2]; Swain Co.: Blowing Springs Cave*.
Conservation Status: IUCN: Not assessed; NatureServe: Not assessed.
References: [1] Hertl (1981); [2] Christ (2003); * this study.

***Pogonognathellus flavescens* (Tullberg, 1871) (TP) A Springtail**
Localities: Polk Co.: World's Edge Cave[1]; Swain Co.: Blowing Springs Cave*.
Conservation Status: IUCN: Not assessed; NatureServe: Not assessed.
References: [1] Christ (2003); * this study.

***Pogonognathellus* sp. (TP) A Springtail**
Localities: Rutherford Co.: Middle Bat Cave (Bat Cave complex)*.
References: * this study.

Order Poduromorpha
Family Onychiuridae
Genus *Onychiurus*

***Onychiurus similis* Folsom, 1917 (TP) A Springtail**
Localities: Swain Co.: Blowing Springs Cave*.
Conservation Status: IUCN: Not assessed; NatureServe: Not assessed.
References: * this study.

***Onychiurus* sp. nov.? (TP) A Springtail**
Localities: Henderson Co.: Devils Kitchen Cave[1].
Comments: This record may represent an undescribed species.
References: [1] Christ (2003).

***Onychiurus* sp. (TP) A Springtail**
Localities: Henderson Co.: Devils Kitchen Cave[1].
References: [1] Christ (2003).

Genus *Paronychiurus*

***Paronychiurus ramosus* (Folsom, 1917) (TP) A Springtail**
Localities: Polk Co.: Garnet Shelter Cave[1], Little Warrior Mountain Cave*.
Conservation Status: IUCN: Not assessed; NatureServe: Not assessed.
References: [1] Christ (2003); * this study.

Order Symphypleona
Family Arrhopalitidae
Genus *Arrhopalites*

***Arrhopalites* sp. (TP) A Springtail**
Localities: Swain Co.: Blowing Springs Cave*.
Comments: This specimen was collected from the surface of a cave pool, but unfortunately was lost in the taxonomic lab before species could be determined.
References: * this study.

Family Katiannidae
Genus *Sminthurinus*

***Sminthurinus* sp. nov.? (TP) A Springtail**
Localities: Polk Co.: World's Edge Shelter*.
Comments: This record may represent an undescribed species (K. Christensen, pers. comm.).
References: * this study.

Class Entognatha
Order Diplura
Family Campodeidae
Genus *Litocampa*

***Litocampa* sp. (TB) A Cave Bristletail**
Localities: Henderson Co.: Hog Rock Cave[1], Stillers Cave*; Madison Co.: Mine Hollow Cave*, Shut-In Creek Cave*; McDowell Co.: Lime Kiln Cave*, Staircase Cave*, Wind Cave*; Polk Co.: Little Warrior Mountain Cave*, Zig-Zag Fissure[1]; Rutherford Co.: McGrath Fissure[1]; Swain Co.: Blowing Springs Cave*, Flowstone Cave*, Lost Nantahala Cave*, Sand Cave*.
Comments: *Litocampa* diplurans are common in caves in Interior Low Plateau and Appalachians karst regions. Cave records from North Carolina may include multiple undescribed species.
References: [1] Christ (2003); * this study.

Family Japygidae
Undetermined Genus

Undetermined species (TP/TX) A Two-pronged Bristletail
Localities: Polk Co.: Zig-Zag Fissure[1].
Comments: This family is reported rarely from caves in the eastern United States.
References: [1] Christ (2003).

Class Insecta
Order Coleoptera
Family Carabidae
Genus *Scaphinotus*

***Scaphinotus andrewsi montanus* (Valentine, 1935) (TX/AC) A Ground Beetle**
Localities: Avery Co.: Davis Moonshine Cave*.
Conservation Status: IUCN: Not assessed; NatureServe: Not assessed.
Comments: This large beetle was observed dragging its preferred food (a snail) across the cave floor.
References: * this study.

Family Coccinellidae
Undetermined Genus

Undetermined species (AC) A Ladybug
Localities: Rutherford Co.: Rumbling Bald Cave*.
References: * this study.

Family Staphylinidae
Genus *Lathrobium*

***Lathrobium* sp. (TX) A Rove Beetle**
Localities: Jackson Co.: Balsam Cave*.
References: * this study.

Genus *Leptusa*

***Leptusa cribratula* (Casey, 1906) (TX) A Rove Beetle**
Localities: Henderson Co.: Stillers Cave*.
Conservation Status: IUCN: Not assessed; NatureServe: GNR (SNR in North Carolina).
References: * this study.

Undetermined Genus

Undetermined species (TX/AC) A Rove Beetle
Localities: McDowell Co.: Linville Caverns*.
References: * this study.

Order Diptera
Family Culicidae
Genux *Culex*

***Culex* sp. (TX) A Mosquito**
Localities: McDowell Co.: Linville Caverns*.
Comments: Mosquitoes often overwinter in caves.
References: * this study.

Family Heleomyzidae
Undetermined Genus

Undetermined species (TX) A Sunfly
Localities: Swain Co.: Blowing Springs Cave*.
Comments: Heleomyzid flies are common in caves, particularly in winter. The two most common species in caves in the eastern United States are *Amoebaleria defessa* and *Oecothea specus*.
References: * this study.

Family Limoniidae
Genus *Chionea*

***Chionea scita* Walker, 1848 (TX) A Crane Fly**
Localities: Rutherford Co.: Amazing Bat Cave[1].
Conservation Status: IUCN: Not assessed; NatureServe: Not assessed.
Comments: Crane flies are observed on occasion in the twilight zones of caves.
References: [1] Gaddy (1986a).

***Chionea* sp. (TX) A Crane Fly**
Localities: Rutherford Co.: Bat Cave complex[1].
Comments: Crane flies are observed on occasion in the twilight zones of caves.
References: [1] Hertl (1981).

Family Muscidae
Undetermined Genus

Undetermined species (TX/AC) A House Fly
Localities: Rutherford Co.: Bat Cave complex[1].
References: [1] Hertl (1981).

Family Mycetophilidae
Genus *Orfelia*

***Orfelia fultoni* (Fisher, 1940) (TX) Foxfire Fly**
Localities: Burke Co.: Conley Cove Rockhouse*, Glow Worm Grotto*; McDowell Co.: Lake Tahoma Cave*.
Conservation Status: IUCN: Not assessed; NatureServe: Not assessed.
Comments: This bioluminescent fly was found near the entrances and twilight zones of these three caves.
References: * this study.

Undetermined Genus

Undetermined species (TX) A Hump-backed Fly
Localities: Swain Co.: Blowing Springs Cave*.
Comments: Mycetophilid flies are reported frequently from caves.
References: * this study.

Family Psychodidae
Undetermined Genus

Undetermined species (TX/AC) A Moth Fly
Localities: Rutherford Co.: Bat Cave complex[1].
References: [1] Hertl (1981).

Family Sciaridae
Undetermined Genus

Undetermined species (TP/TX) A Dark-winged Fungus Gnat
Localities: Rutherford Co.: Bat Cave complex[1].
Comments: Sciarid flies are reported frequently from caves.
References: [1] Hertl (1981).

Order Ephemeroptera
Family Heptageniidae
Genus *Stenonema*

***Stenonema* sp. (AC) A Mayfly**
Localities: Onslow Co.: Brinson's Rockhouse Cave*.
References: * this study.

Order Hemiptera
Family Cicadellidae
Genus *Erythroneura*

***Erythroneura* sp. (AC) A Leafhopper**
Localities: Rutherford Co.: Block Creep Cave[1].
References: [1] Christ (2003).

Family Reduviidae
Genus *Empicoris*

***Empicoris* sp. (AC) A Thread-legged Bug**
Localities: Rutherford Co.: Block Creep Cave[1].
References: [1] Christ (2003).

Order Hymenoptera
Family Braconidae
Undetermined Genus

Undetermined species (AC) A Parasitoid Wasp
Localities: Rutherford Co.: Bat Cave complex[1].
References: [1] Hertl (1981).

Family Formicidae
Undetermined Genus

Undetermined species (AC) An Ant
Localities: Rutherford Co.: Dusty Bat Cave*.
References: * this study.

Order Lepidoptera
Family Erebidae
Genus *Scoliopteryx*

***Scoliopteryx libatrix* (Linnaeus, 1758) (TX) Herald Moth**
Localities: Ashe Co.: Three Top Mountain Wildcat Den*; Avery Co.: Cranberry Mines*; Burke Co.: Cardboard Cave*; Henderson Co.: Cloven Cliffs Cave*, Devils Kitchen Cave*; McDowell Co.: Bennett's Mill Cave*; Rutherford Co.: Breakdown Cave*; Stokes Co.: Scoliopteryx Column Cave*.
Conservation Status: IUCN: Not assessed; NatureServe: G5 (SNR in North Carolina).
Comments: This species commonly overwinters in caves in eastern North America.
References: * this study.

Order Neuroptera
Family Myrmeleontidae
Undetermined Genus

Undetermined species (AC) An Antlion
Localities: Henderson Co.: Indian Cave*.
References: * this study.

Order Orthoptera
Family Rhaphidophoridae
Genus *Ceuthophilus*

***Ceuthophilus* sp. (TX) A Camel Cricket**
Localities: McDowell Co.: Staircase Cave*, Limekiln Cave*.
Comments: These records are likely *C. gracilipes*.
References: * this study.

Genus *Euhadenoecus*

Euhadenoecus adelphus Hubbell & Norton, 1978 (TX) A Cave Cricket
Localities: Macon Co.: Granite City Cave*; Rutherford Co.: Bat Cave complex[1,2]; Swain Co.: Blowing Springs Cave*.
Conservation Status: IUCN: Not assessed; NatureServe: GNR (SNR in North Carolina).
Comments: Cave crickets were commonly observed in many caves in North Carolina. However, only confirmed records are presented.
References: [1] Hubbell and Norton (1978), [2] Hertl (1981), * this study.

Euhadenoecus puteanus (Scudder, 1877) (TX) Puteanus Camel Cricket
Localities: Madison Co.: Campbells Vegetable Cave[2]; McDowell Co.: Linville Caverns[1,2]; Swain Co.: cave in Indian Ridge near Judson[2].
Conservation Status: IUCN: Not assessed; NatureServe: Not assessed.
Comments: Cave crickets were commonly observed in many caves in North Carolina. However, only confirmed records are presented.
References: [1] Petrie (1942), [2] Hubbell and Norton (1978).

Euhadenoecus sp. (TX) A Cave Cricket
Localities: Madison Co.: Anthodite Cave*; McDowell Co.: Staircase Cave*; Rutherford Co.: Breakdown Cave*, Moonshiners Cave*; Transylvania Co.: Snake Den no. 2 Cave*; Yancey Co.: Celo Cave*.
Comments: Cave crickets were commonly observed in many caves in North Carolina. However, only confirmed records are presented. These records are either *E. adelphus* or *E. puteanus*.
References: * this study.

Order Zygentoma
Family Lepismatidae
Undetermined Genus

Undetermined species (TX/AC) A Silverfish
Localities: McDowell Co: Wind Cave*; Rutherford Co.: Breakdown Cave*, Rumbling Bald Cave*; Swain Co.: Blowing Springs Cave*.
References: * this study.

Subphylum Crustacea
Class Malacostraca
Order Amphipoda
Family Crangonyctidae
Genus *Stygobromus*

Stygobromus araeus (Holsinger, 1969) (SB) Tidewater Interstitial Amphipod
Localities: Gates Co.: seepage spring near Merchants Mill Pond[1,2].
Conservation Status: IUCN: Vulnerable D2 (Inland Water Crustacean Specialist Group 1996a); NatureServe: G3 (SU in North Carolina).
Comments: This small groundwater amphipod also is known from several seepage springs and seep-fed streams in the Coastal Plain of Virginia (Culver et al., 2012).
References: [1] Terwilliger (1991); [2] Culver et al. (2012).

Stygobromus carolinensis Holsinger, 1978 (SB) Carolina Groundwater Amphipod
Localities: Yancey Co.: seeps on W side of State Rd 128, 2.4 km N of Blue Ridge Parkway (type locality)[1].
Conservation Status: IUCN: Not assessed; NatureServe: G1 (S1 in North Carolina).
Comments: This small groundwater amphipod is known only from the type locality.
References: [1] Holsinger (1978).

Stygobromus cf. _carolinensis_ (SB) A Cave Amphipod
Localities: Wilkes Co.: Harrold Mountain Cave*.
Comments: Specimens from Harrold Mountain Cave may be *S. carolinensis* or an undescribed species.
References: * this study.

Stygobromus indentatus **(Holsinger, 1967) (SB) Tidewater Amphipod**
Localities: Nash Co.: shallow well at Bailey[1,2].
Conservation Status: IUCN: Vulnerable D2 (Inland Water Crustacean Specialist Group, 1996b); NatureServe: G3 (SU in North Carolina).
Comments: This relatively large groundwater amphipod also is known from several seepage springs and wells in the Coastal Plain of Maryland and Virginia (Culver et al., 2012).
References: [1] Holsinger (1978); [2] Culver et al. (2012).

Stygobromus **nov. sp. A (SB) A Cave Amphipod**
Localities: Avery Co.: Black Rocks Mystery Cave*, Charlie's Ridge Bat Cave*, Frying Pan Cave*; Burke Co.: Odell Cave*; Henderson Co.: Stillers Cave*; McDowell Co.: Lake Tahoma Cave*, Linville Caverns*, Roadside Surprise Cave*, Staircase Cave*, Wind Cave*; Mitchell Co.: Buckshot Cave*.
Comments: This undescribed species is the most wide-ranging groundwater amphipod in North Carolina.
References: * this study.

Stygobromus **nov. sp. B (SB) A Cave Amphipod**
Localities: Jackson Co.: seep below Jones Knob[1].
Conservation Status: IUCN: not assessed; NatureServe: GNR (S1? in North Carolina).
Comments: This undescribed groundwater amphipod is known only from a seep in Jackson County.
References: [1] NCNHP.

Stygobromus **sp. (SB) A Cave Amphipod**
Localities: Buncombe Co.: Cedar Cliff Cave*; McDowell Co.: Roadside Surprise Cave*, Staircase Cave*; Polk Co.: Little Warrior Mountain Cave*.
Comments: These records represent collections of immatures or females that could not be identified to species.
References: * this study.

Order Decapoda
Family Cambaridae
Genus *Cambarus*

Cambarus asperimanus **Faxon, 1914 (SX) Mitten Crayfish**
Localities: McDowell Co.: Staircase Cave[1].
Conservation Status: IUCN: Least concern; NatureServe: G4 (S4 in North Carolina).
References: [1] NCSM.

Cambarus bartonii **(Fabricius, 1798) (SP) Common Crayfish**
Localities: McDowell Co.: Linville Caverns[1,2], Staircase Cave[1].
Conservation Status: IUCN: Least concern; NatureServe: G5 (S5 in North Carolina).
Comments: This species is common in caves in the Appalachian Valley and Ridge (Fong et al., 2012).
References: [1] NCSM; [2] SCAN: USNM.

Cambarus latimanus **(LeConte, 1856) (SX) Variable Crayfish**
Localities: Jones Co.: small limestone cave along NC 41 0.9 miles west of Comfort; Onslow Co.: small limestone cave off SR 1222 2 miles NNE of Catherine Lake[1].
Conservation Status: IUCN: Least concern; NatureServe: G5 (S5 in North Carolina).
References: [1] NCSM.

Genus *Procambarus*

Procambarus acutus **(Girard, 1852) (SX) White River Crayfish**
Localities: Onslow Co.: small limestone cave off SR 1222 2 miles NNE of Catherine Lake[1].
Conservation Status: IUCN: Least concern; NatureServe: G5 (S5 in North Carolina).
References: [1] NCSM.

Order Isopoda
Family Armadillidiidae
Genus *Armadillidium*

Armadillidium vulgare **Latreille, 1804 (TP) Common Woodlouse**
Localities: Henderson Co.: Hog Rock Cave[1]; Rutherford Co.: Tank Bug Cave*.

Conservation Status: IUCN: Not assessed; NatureServe: GNR (SNR in North Carolina).
Comments: This species is native to Europe but has been introduced throughout North America and is often observed in caves.
References: [1] Christ (2003), * this study.

Family Asellidae
Genus *Caecidotea*

***Caecidotea carolinensis* Lewis & Bowman, 1977 (SB) Bennett's Mill Cave Isopod**
Localities: McDowell Co.: Bennetts Mill Cave (type locality)[1–3].
Conservation Status: IUCN: Not assessed; NatureServe: G2G3 (SH in North Carolina; Listed as Endangered in North Carolina (NCWRC 2017).
Comments: This stygobite also has been collected from Parlar Cave in Orangeburg Co., South Carolina (Reeves, 2000a) suggesting that this species may be more widespread than currently known.
References: [1] NCNHP; [2] SCAN: USNM; [3] Lewis and Bowman (1977).

Family Trichoniscidae
Genus *Miktoniscus*

***Miktoniscus linearis* (Patience, 1908) (TP) A Terrestrial Isopod**
Localities: McDowell Co.: Linville Caverns[1,2].
Conservation Status: IUCN: Not assessed; NatureServe: Not assessed.
Comments: This species was described in England; however, Schultz (1964) compared three specimens collected from Linville Caverns with specimens redescribed by Vandel (1950) and noted that they were identical.
References: [1] SCAN: USNM; [2] Schultz (1964).

Class Maxillopoda
Order Cyclopoida
Family Cyclopidae
Genus *Cyclops*

***Cyclops* sp. (SP/SX) A Copepod**
Localities: McDowell Co.: Staircase Cave*.
References: * this study.

Genus *Diacyclops*

***Diacyclops crassicaudis brachycercus* (Kiefer, 1929) (SP) A Copepod**
Localities: Chatham Co.: well 200 yards south of the Orange-Chatham county line[1]; well 12 miles east of Pittsboro[1].
Conservation Status: IUCN: Not assessed; NatureServe: Not assessed.
Comments: This wide-ranging species in North America is occasional sampled from groundwater habitats (Reid, 2004; Lewis and Reid, 2007).
References: [1] Yeatman (1943).

***Diacyclops jeanneli putei* (Yeatman, 1943) (SB) Carolina Well Copepod**
Localities: Orange Co.: well 2.7 miles from the Wilson Zoological Laboratory on US 501 (type locality)[1].
Conservation Status: IUCN: Not assessed; NatureServe: G3G4 (SH in North Carolina); Listed as Special Concern in North Carolina (NCWRC, 2017).
Comments: This subspecies is only known from North Carolina.
References: [1] Yeatman (1943).

***Diacyclops navus* (Herrick, 1882 (SP) A Copepod**
Localities: Chatham Co.: well 12 miles east of Pittsboro[1]; well 2 mi from US 501 between Pittsboro and Moncure[1]; well 2.5 north of US 1 near New Elam Christian Church[1].
Conservation Status: IUCN: Not assessed; NatureServe: Not assessed.
Comments: This species also has been collected from groundwater habitats in Indiana (Lewis and Reid, 2007).
References: [1] Yeatman (1943).

Diacyclops nearcticus **(Kiefer, 1934) (SP) A Copepod**
Localities: Chatham Co.: well 1.5 miles north of Fearrington[1].
Conservation Status: IUCN: Not assessed; NatureServe: G3G4 (SNR in North Carolina).
Comments: This species ranges throughout the eastern and central United States. It also has been collected from groundwater habitats in Florida, Indiana, Kentucky, and Tennessee (Strayer and Reid, 1999; Bruno et al., 2000; Reid, 2004; Lewis and Reid, 2007).
References: [1] Yeatman (1943).

Genus *Eucyclops*

Eucyclops serrulatus **(Fischer, 1851) (SX) A Copepod**
Localities: Orange Co.: well 2.7 miles from the Wilson Zoological Laboratory on US 501[1].
Conservation Status: IUCN: Not assessed; NatureServe: Not assessed.
Comments: This species also is known from Mammoth Cave in Kentucky (Kofoid, 1900).
References: [1] Yeatman (1943).

Genus *Microcyclops*

Microcyclops rubellus **(Lilljeborg, 1901) (SP) A Copepod**
Localities: Chatham Co.: well 12 miles east of Pittsboro[1].
Conservation Status: IUCN: Not assessed; NatureServe: Not assessed.
Comments: This species also is known from groundwater habitats in Illinois, Indiana, Kentucky, and Tennessee (Strayer and Reid, 1999; Lewis and Reid, 2007).
References: [1] Yeatman (1943).

Subphylum Myriapoda
Class Chilopoda
Order Lithobiomorpha
Family Lithobiidae
Genus *Watobius*

Watobius anderisus **Chamberlin, 1911 (AC) A Centipede**
Localities: Henderson Co.: Hog Rock Cave[1]; Rutherford Co.: McGrath Fissure[1], Western Talus Tunnel[1].
Conservation Status: IUCN: Not assessed; NatureServe: Not assessed.
References: [1] Christ (2003).

Order Scolopendromorpha
Family Scolopocryptopidae
Genus *Scolopocryptops*

Scolopocryptops **sp. (AC) A Centipede**
Localities: Rutherford Co.: Western Talus Tunnel[1].
Conservation Status: na.
References: [1] Christ (2003).

Class Diplopoda
Order Chordeumatida
Family Cleidogonidae
Genus *Pseudotremia*

Pseudotremia fracta nantahala **Hoffman, 1981 (TX) A Millipede**
Localities: Swain Co.: Blowing Springs Cave (type locality)[1].
Conservation Status: IUCN: Not assessed; NatureServe: Not assessed.
Comments: This pigmented species is known only from the type locality and from Cliff Ridge near Blowing Springs.
References: [1] Hoffman (1981).

Pseudotremia shelleyi **Lewis, 2009 (TB) Shelley's Cave Millipede**
Localities: McDowell Co.: Lime Kiln Cave[3]; Rutherford Co.: Bat Cave complex[1,3]; Rumbling Bald Cave (type locality)[3,4]; Rumbling Bald Spring Cave[3,4].
Conservation Status: IUCN: Not assessed; NatureServe: Not assessed.

Comments: This is the only troglobitic *Pseudotremia* in North Carolina. Several troglobitic *Pseudotremia* are known from the Appalachians and Interior Low Plateau karst regions (Lewis, 2000, 2005b, 2009; Shear, 2008, 2011).
References: [1] Hertl (1981); [2] Shelley (2000); [3] Lewis (2009), [4] NCSM.

***Pseudotremia* sp. (TP/TX) A Millipede**
Localities: Avery Co.: Cranberry Mines[2]; Rutherford Co.: A-Frame Fissure[3], Bat Cave complex[1,2]; Sliding Rock Cave[3]; Madison Co.: Anthodite Cave[2].
Comments: Specimens from these caves could not be identified to species.
References: [1] Hertl (1981); [2] Shelley (2000); [3] Christ (2003).

Family Trichopetalidae
Genus *Nannopetalum*

***Nannopetalum vespertilio* Shear, 2003 (TB) Bat Cave Millipede**
Localities: Rutherford Co.: Bat Cave (type locality)[1].
Conservation Status: IUCN: Not assessed; NatureServe: GNR (SNR in North Carolina).
Comments: This troglobite is known only from the type locality and is the smallest known trichopetalid millipede measuring under 4 mm in total length.
References: [1] Shear (2003).

Order Julida
Family Parajulidae
Genus *Ptyoiulus*

***Ptyoiulus* sp. (TX/AC) A Millipede**
Localities: Rutherford Co.: Western Talus Tunnel*.
Comments: This genus is known from several surface records in North Carolina (Shelley, 2000).
References: * this study.

Order Polydesmida
Family Macrosternodesmidae
Genus *Chaetaspis*

***Chaetaspis* sp. (TX/AC) A Millipede**
Localities: Swain Co.: Lost Nantahala Cave[1].
Comments: This genus is known primarily from surface records in North Carolina (Shelley, 2000).
References: [1] Shelley (2000).

Family Polydesmidae
Genus *Pseudopolydesmus*

***Pseudopolydesmus* sp. (TX/AC) A Millipede**
Localities: Rutherford Co.: Western Talus Tunnel[1].
Comments: This may be one of four species *Pseudopolydesmus* known primarily from surface records in North Carolina (Shelley, 2000).
References: [1] Christ (2003).

Order Spirostreptida
Family Cambalidae
Genus *Cambala*

***Cambala annulata* (Say, 1821) (TX) A Millipede**
Localities: Henderson Co.: Hog Rock Cave[1].
Conservation Status: IUCN: Not assessed; NatureServe: G5 (SNR in North Carolina).
Comments: This species is known primarily from surface records in several counties in North Carolina (Shelley, 2000).
References: [1] Christ (2003).

***Cambala hubrichti* Hoffman, 1958 (TX) A Millipede**
Localities: Rutherford Co.: Bat Cave complex[1].

Conservation Status: IUCN: Not assessed; NatureServe: G5 (SNR in North Carolina).
Comments: This species is known primarily from surface records in several counties in North Carolina (Shelley, 2000).
References: [1] Hertl (1981).

Cambala sp. (TX) A Millipede
Localities: McDowell Co.: Linville Caverns*; Rutherford Co.: McGrath Fissure[1]; Swain Co.: Blowing Springs Cave*.
Comments: These records may be either *C. annulata* or *C. hubrichti*.
References: [1] Christ (2003); * this study.

Class Symphyla
Order Cephalostigmata
Family Scutigerellidae
Genus _Scutigerella_

Scutigerella sp. (TP/TX) A Symphylan
Localities: Henderson Co.: Devils Kitchen Cave[1]; Rutherford Co.: Western Talus Tunnel[1].
Comments: These soil-dwelling arthropods are not well represented in cave collections.
References: [1] Christ (2003).

Phylum Chordata
Subphylum Vertebrata
Class Actinopterygii
Order Cypriniformes
Family Cyprinidae
Genus _Rhinichthys_

Rhinichthys cataractae (Valenciennes, 1842) (AC) Longnose Dace
Localities: McDowell Co.: Linville Caverns*.
Conservation Status: IUCN: Least Concern; NatureServe: G5 (S5 in North Carolina).
Comments: This species also has been reported from caves in Minnesota and West Virginia (Dearolf, 1956; Schmidt, 1994; Poly, 2001).
References: * this study.

Order Salmoniformes
Family Salmonidae
Genus _Oncorhynchus_

Oncorhynchus mykiss (Walbaum, 1792) (AC) Rainbow Trout
Localities: McDowell Co.: Linville Caverns*.
Conservation Status: IUCN: Not assessed; NatureServe: G5 (SNA in North Carolina).
Comments: Trout were purposefully stocked at Linville Caverns by the owners.
References: * this study.

Genus _Salmo_
Salmo trutta Linnaeus, 1758 (AC) Brown Trout
Localities: McDowell Co.: Linville Caverns*.
Conservation Status: IUCN: Least Concern; NatureServe: G5 (SNA in North Carolina).
Comments: Trout were purposefully stocked at Linville Caverns by the owners.
References: * this study.

Genus _Salvelinus_

Salvelinus fontinalis (Mitchill, 1814) (AC) Brook Trout
Localities: McDowell Co.: Linville Caverns*.
Conservation Status: IUCN: Not assessed; NatureServe: G5 (S5 in North Carolina); Listed as a Species of Greatest Conservation Need in North Carolina (North Carolina Wildlife Resources Commission, 2015).
Comments: Trout were purposefully stocked at Linville Caverns by the owners.
References: * this study.

Class Amphibia
Order Caudata
Family Plethodontidae
Genus *Aneides*

***Aneides caryaensis* Patton et al., 2019 (TX) Green Salamander**
Localities: Henderson Co.: Green Salamander Shelter*.
Conservation Status: IUCN: Near Threatened (Hammerson 2004); NatureServe: G3G4 (S2S3 in North Carolina); Listed as Threatened in North Carolina (North Carolina Wildlife Resources Commission, 2017a); Listed as a Species of Greatest Conservation Need in North Carolina (North Carolina Wildlife Resources Commission, 2015). Conservation statuses are for *A. aeneus*, as this species was described last year and has not yet been assessed officially.
Comments: This species is a member of the Green Salamander (*A. aeneus*) species complex and was described recently (Patton et al. 2019). *Aneides aeneus* has been observed infrequently in the twilight zone and entrance area of caves throughout its range (Dearolf, 1956; Osbourn, 2005; M.L. Niemiller, unpublished data). We believe this occurrence to represent a new site for this newly described salamander endemic to Hickory Nut Gorge.
References: * this study.

Genus *Desmognathus*

***Desmognathus monticola* Dunn, 1916 (TX/AC) Seal Salamander**
Localities: McDowell Co.: Linville Caverns*.
Conservation Status: IUCN: Least Concern; NatureServe: G5 (S5 in North Carolina).
Comments: Like other *Desmognathus*, *D. monticola* has been infrequently reported from caves throughout its range (Garton et al., 1993; Pauley, 1993; Osbourn, 2005).
References: * this study.

***Desmognathus* sp. (TX/AC) A Dusky Salamander**
Localities: Rutherford Co.: Rumbling Bald Spring Cave*, White Root Shelter*.
Comments: *Desmognathus* salamanders are infrequently reported from caves in the Blue Ridge Mountains and adjacent Appalachian Valley and Ridge, with most records from the entrance or twilight zones (Wallace, 1984, 2003; Garton et al., 1993; Osbourn, 2005; Niemiller et al., 2016).
References: * this study.

Genus *Eurycea*

***Eurycea wilderae* Dunn, 1920 (TX/AC) Blue Ridge Two-lined Salamander**
Localities: McDowell Co.: Linville Caverns*.
Conservation Status: IUCN: Least Concern; NatureServe: G5 (S5 in North Carolina).
Comments: *Eurycea wilderae* has been reported from a few caves in eastern Tennessee, including Great Smoky Mountains National Park (Wallace, 1984, 2003; Dodd et al., 2001; Niemiller et al., 2016).
References: * this study.

Genus *Gyrinophilus*

***Gyrinophilus porphyriticus* (Green, 1827) (TP) Spring Salamander**
Localities: Rutherford Co.: White Root Shelter*.
Conservation Status: IUCN: Least Concern; NatureServe: G5 (S5 in North Carolina).
Comments: This species is common in caves in the Appalachian Valley and Ridge and Blue Ridge Mountains (Dearolf, 1956; Brandon, 1966; Cooper and Cooper, 1968; Wallace, 1984, 2003; Garton et al., 1993; Dodd et al., 2001; Osbourn, 2005; Miller and Niemiller, 2008; Miller et al., 2008; Niemiller and Miller, 2009; Niemiller et al., 2010).
References: * this study.

Genus *Plethodon*

***Plethodon cinereus* (Green, 1818) (TX/AC) Eastern Red-backed Salamander**
Localities: McDowell Co.: Linville Caverns*.
Conservation Status: IUCN: Least Concern; NatureServe: G5 (S5 in North Carolina).
Comments: This species also has been reported from caves in Indiana, Maryland, Virginia, and West Virginia (Dearolf, 1956; Garton et al., 1993; Osbourn, 2005).
References: * this study.

***Plethodon cylindraceus* (Harlan, 1825) (TX) White-spotted Slimy Salamander**
Localities: McDowell Co.: Linville Caverns*; Rutherford Co.: Breakdown Cave*, Rimstone Skull Cave*.
Conservation Status: IUCN: Least Concern; NatureServe: G5 (S5 in North Carolina).
Comments: *Plethodon cylindraceus* is a member of the Slimy Salamander (*P. glutinosus*) species complex. A few members of this complex, such as. *P. glutinosus* and *P. albagula*, are common in caves throughout their respective ranges (Garton et al., 1993; Buhlmann, 2001; Trauth et al., 2004; Taylor and Mays, 2006; Taylor et al., 2015; Niemiller et al., 2016).
References: * this study.

***Plethodon metcalfi* Brimley, 1912 (TX) Southern Gray-cheeked Salamander**
Localities: Polk Co.: Little Warrior Mountain Cave[1].
Conservation Status: IUCN: Least Concern; NatureServe: G4 (S5 in North Carolina).
Comments: *Plethodon metcalfi* is a member of the *P. jordani* species complex. Gaddy (1986a) observed this species (reported as *P. jordani*) at the entrance to Little Warrior Mountain Cave. Highton and Peabody (2000) removed *P. metcafi* from synonymy with *P. jordani*.
References: [1] Gaddy (1986a).

***Plethodon yonahlossee* Dunn, 1917 (TX) Yonahlossee Salamander**
Localities: McDowell Co.: Limekiln Cave*, Linville Caverns*, Wind Cave*; Rutherford Co.: Bat Cave complex[2,3], Breakdown Cave*, Rumbling Bald Cave*; Watauga Co.: cave along Howard's Creek[1].
Conservation Status: IUCN: Least Concern; NatureServe: G4 (S4 in North Carolina); Listed as a Species of Greatest Conservation Need in North Carolina (North Carolina Wildlife Resources Commission, 2015).
Comments: *Plethodon yonahlossee* from the Bat Cave area in Rutherford County are a unique variant, commonly referred to as the Crevice Salamander. This species is often found in rock crevices (Beane et al., 2010; Niemiller and Reynolds, 2011).
References: [1] VertNet: UF; [2] NCSM; [3] Holland (1981); * this study.

Genus *Pseudotriton*

***Pseudotriton ruber* (Latreille, 1801) (TP) Red Salamander**
Localities: McDowell Co.: Linville Caverns*.
Conservation Status: IUCN: Least Concern; NatureServe: G5 (S5 in North Carolina).
Comments: This species has been reported from several caves in the Interior Low Plateau and Appalachian Valley and Ridge to the west of the study area where it is frequently found in the twilight zone (Buhlmann, 2001; Osbourn, 2005; Camp and Jensen, 2007; Godwin, 2008; Miller et al., 2008; Niemiller and Miller, 2009; Niemiller and Reeves, 2014; Niemiller et al., 2016). *Pseudotriton ruber* has been documented to breed in caves (Miller and Niemiller, 2005; Niemiller et al., 2006; Miller et al., 2008).
References: * this study.

Class Reptilia
Order Squamata
Suborder Serpentes
Family Colubridae
Genus *Thamnophis*

***Thamnophis sirtalis* (Linnaeus, 1766) (AC) Common Gartersnake**
Localities: Wake Co.: Raleigh Mine[1].
Conservation Status: IUCN: Least Concern; NatureServe: G5 (S5 in North Carolina).
Comments: This species also has been reported from caves in Pennsylvania and Tennessee (Dearolf, 1956; Niemiller et al., 2016).
References: [1] VertNet: USNM.

Family Dipsadidae
Genus *Diadophis*

***Diadophis punctatus* (Linnaeus, 1766) (AC) Ring-necked Snake**
Localities: Burke Co.: Holy Moley Cave*.
Conservation Status: IUCN: Least Concern; NatureServe: G5 (S5 in North Carolina).

Comments: This species has been reported on occasion from caves (Brode, 1958; Cliburn and Middleton, 1983; Pauley, 1993; Niemiller et al., 2016).
References: * this study.

Family Viperidae
Genus *Agkistrodon*

***Agkistrodon contortrix* (Linnaeus, 1766) (AC) Copperhead**
Localities: Burke Co.: Copperhead Cave*; Davidson Co.: New Boone's Cave*.
Conservation Status: IUCN: Least Concern; NatureServe: G5 (S5 in North Carolina).
Comments: This species is observed on occasion around cave entrances (Dearolf, 1956; Pauley, 1993; Dodd et al., 2001; Niemiller et al., 2016).
References: * this study.

Genus *Crotalus*

***Crotalus horridus* Linnaeus, 1758 (AC) Timber Rattlesnake**
Localities: McDowell Co.: Crotalus Shelter*; Polk Co.: Rattlesnake Fissure*.
Conservation Status: IUCN: Least Concern; NatureServe: G4 (S3 in North Carolina); Listed as Special Concern in North Carolina (North Carolina Wildlife Resources Commission, 2017a); Listed as a Species of Greatest Conservation Need in North Carolina (North Carolina Wildlife Resources Commission, 2015).
Comments: This species is observed on occasion around cave entrances (Dearolf, 1956; Garton et al., 1993; Osbourn, 2005).
References: * this study.

Suborder Lacertilia
Family Dactyloidae
Genus *Anolis*

***Anolis carolinensis* (Voigt, 1832) (AC) Green Anole**
Localities: Rutherford Co.: Rumbling Bald Cave*.
Conservation Status: IUCN: Least Concern; NatureServe: G5 (S5 in North Carolina).
Comments: Lizards, including anoles, are not regularly encountered in caves, and all records are suspected to be accidental occurrences.
References: * this study.

Class Aves
Order Passeriformes
Family Corvidae
Genus *Corvus*

***Corvus corax* Linnaeus, 1758 (TX/AC) Common Raven**
Localities: Yancey Co.: Cooper's Cave*.
Conservation Status: IUCN: Least Concern; NatureServe: G5 (S3 in North Carolina); Listed as a Species of Greatest Conservation Need in North Carolina (North Carolina Wildlife Resources Commission, 2015).
Comments: We observed a pair of Common Ravens nesting at a skylight entrance to Cooper's Cave in March 2006.
References: * this study.

Family Tyrannidae
Genus *Sayornis*

***Sayornis phoebe* (Latham, 1790) (TX) Eastern Phoebe**
Localities: Avery Co.: Shelter of the Hands*; McDowell Co.: Staircase Cave*; Yancey Co.: Cooper's Cave*; Watauga Co.: Boone Rockhouse*.
Conservation Status: IUCN: Least Concern; NatureServe: G5 (S5 in North Carolina).
Comments: *Sayornis phoebe* is common throughout the eastern United States and is frequently encountered nesting on ledges in the entrances and twilight zone of caves (Dearolf, 1956; Garton et al., 1993; Fong et al., 2012; Niemiller et al., 2013, 2016; Slay et al., 2016).
References: * this study.

Class Mammalia
Order Carnivora
Family Procyonidae
Genus *Procyon*

***Procyon lotor* (Linnaeus, 1758) (TX) Raccoon**

Localities: McDowell Co.: Wind Cave*.

Conservation Status: IUCN: Least Concern; NatureServe: G5 (S5 in North Carolina).

Comments: Raccoons are known to enter caves throughout the range of the species (Dearolf, 1956; Garton et al., 1993; Niemiller et al. 2016). The authors recall observing raccoon scat in several North Carolina caves.

References: * this study.

Order Chiroptera
Family Vespertilionidae
Genus *Corynorhinus*

***Corynorhinus rafinesquii* Handley, 1955 (TX) Rafinesque's Big-eared Bat**

Localities: Swain Co.: Eagle Creek Mine[1], Sugar Fork Mine[1]; Yancey Co.: Cooper's Cave*.

Conservation Status: IUCN: Least Concern; NatureServe: G3G4 (S3 in North Carolina); Listed as Threatened in North Carolina (North Carolina Wildlife Resources Commission, 2017a); Listed as a Species of Greatest Conservation Need in North Carolina (North Carolina Wildlife Resources Commission, 2015).

Comments: This species frequents caves throughout its range, primarily during winter. Populations in the western part of the state associated with mines and occasionally caves are considered *C. r. rafinesquii*.

References: [1] Bat Population Database; * this study.

***Corynorhinus townsendii virginianus* Handley, 1955 (TX) Virginia Big-eared Bat**

Localities: Avery Co.: Black Coral Cave[1], Black Rock Cliffs Cave[1–3], Black Rock Mystery Cave[1], Pilot Knob Cave no. 1[1], Thunder Hole[1], Tom Terrific Cave[1].

Conservation Status: IUCN: Least Concern; NatureServe: G3G4T2 (S1 in North Carolina); Listed as Endangered in North Carolina (North Carolina Wildlife Resources Commission, 2017a); Listed as Endangered under the U.S. Endangered Species Act; Listed as a Species of Greatest Conservation Need in North Carolina (North Carolina Wildlife Resources Commission, 2015).

Comments: This federally endangered species is rare in North Carolina where it has been reported from caves and mines at high elevations in the western part of the state. The IUCN Red List status rank reflects the conservation status of the species and not this subspecies.

References: [1] Boynton et al. (1992); [2] VertNet: MVZ; [3] Bat Population Database.

Genus *Eptesicus*

***Eptesicus fuscus* (Beauvois, 1796) (TX) Big Brown Bat**

Localities: Avery Co.: Cranberry Mines*; Jackson Co.: Kitchen Cave[1]; Madison Co.: French Broad Cave*; McDowell Co.: Linville Caverns*; Rutherford Co.: Rumbling Bald Cave*, Sliding Rock Cave*; Transylvania Co.: Snake Den no. 2 Cave*; Yancey Co.: Cooper's Cave*, Isom Mica Mine*.

Conservation Status: IUCN: Least Concern; NatureServe: G5 (S5 in North Carolina).

Comments: *Eptesicus fuscus* is encountered frequently in caves throughout its range, particularly in winter, where it often roosts alone or in small clusters on ledges or in crevices.

References: [1] VertNet: MVZ; * this study.

Genus *Myotis*

***Myotis leibii* (Audubon & Bachman, 1842) (TX) Eastern Small-footed Bat**

Localities: Avery Co.: Black Rock Cliffs Cave[1,3]; Jackson Co.: Kitchen Cave[2]; McDowell Co.: Wind Cave[1]; Rutherford Co.: Bat Cave[1,3], Breakdown Cave[1], Rumbling Bald Cave[1]; Swain Co.: Blowing Springs Cave[1], Flowstone Cave*, Lost Nantahala Cave[1]; Yancey Co.: Cooper's Cave[1]; Isom Mica Mine*.

Conservation Status: IUCN: Endangered A4bce (Solari, 2018a); NatureServe: G4 (S2 in North Carolina); Listed as Special Concern in North Carolina (North Carolina Wildlife Resources Commission, 2017a); Listed as a Species of Greatest Conservation Need in North Carolina (North Carolina Wildlife Resources Commission, 2015).

Comments: This species is known primarily from mountainous areas in the western part of the state. The conservation status of *M. leibii* was elevated recently to Endangered due to impacts from White-nose Syndrome among other factors (Solari, 2018a).
References: [1] Boynton et al. (1992); [2] VertNet: MVZ; [3] Bat Population Database, * this study.

***Myotis lucifugus* (LeConte, 1831) (TX) Little Brown Bat**
Localities: Avery Co.: Black Rock Cliffs Cave[2], Cranberry Mines*; Jackson Co.: Kitchen Cave[1]; McDowell Co.: Linville Caverns*, Wind Cave*; Rutherford Co.: Bat Cave[1], Moonshiners Cave*, Sliding Rock Cave*; Swain Co.: Blowing Springs Cave*; Yancey Co.: Celo Cave*, Isom Mica Mine*.
Conservation Status: IUCN: Endangered A3be (Solari, 2018b); NatureServe: G3 (S2 in North Carolina); Listed as a Species of Greatest Conservation Need in North Carolina (North Carolina Wildlife Resources Commission, 2015).
Comments: This species is known from all physiographic regions in North Carolina but is known primarily from mountainous areas in the western part of the state. Due to population declines associated with White-nose Syndrome, *M. lucifugus* is now considered Endangered by IUCN. Population declines have been particularly severe in North Carolina (North Carolina Wildlife Resources Commission, 2017b; O'Keefe et al., 2019). Declines also have been noted for some eastern Tennessee populations (Flock, 2013, 2014). This species is under review by USFWS for potential listing.
References: [1] VertNet: MVZ; [2] VertNet: NCSM; * this study.

***Myotis septentrionalis* (Trovessart, 1897) (TX) Northern Long-eared Bat**
Localities: Avery Co.: Black Rock Cliffs Cave[1,3], Cranberry Mines*; Rutherford Co.: Bat Cave[1], Rumbling Bald Cave[3]; Swain Co.: Blowing Springs Cave*; Transylvania Co.: Snake Den no. 2 Cave*; Yancey Co.: Celo Cave*, Isom Mica Mine*.
Conservation Status: IUCN: Near Threatened (Solari, 2018c); NatureServe: G1G2 (S2 in North Carolina); Listed as Threatened in North Carolina (North Carolina Wildlife Resources Commission, 2017a); Listed as a Species of Greatest Conservation Need in North Carolina (North Carolina Wildlife Resources Commission, 2015); Listed as Threatened under the U.S. Endangered Species Act.
Comments: *Myotis septentrionalis* is one of the bat species most impacted by White-nose Syndrome. The species was listed as Threatened under the Endangered Species Act, effective 4 May 2015 (USFWS, 2015). It is considered uncommon in the mountainous areas of western North Carolina where significant declines have been documented (North Carolina Wildlife Resources Commission, 2017b; O'Keefe et al., 2019).
References: [1] Boynton et al. (1992); [2] VertNet: MVZ; [3] VertNet: NCSM; * this study.

***Myotis sodalis* Miller & Allen, 1928 (TX) Indiana Bat**
Localities: Jackson Co.: Kitchen Cave[2,3]; Madison Co.: Anthodite Cave[1]; McDowell Co.: Linville Caverns[1], Wind Cave[1]; Rutherford Co.: Bat Cave[1–5]; Swain Co.: Blowing Springs Cave[1], Hewitt Station Mine[2], Lost Nantahala Cave[1].
Conservation Status: IUCN: Near Threatened (Arroyo-Cabrales and Ospina-Garces, 2016); NatureServe: G2 (S1S2 in North Carolina); Listed as Endangered in North Carolina (NCWRC, 2017); Listed as a Species of Greatest Conservation Need in North Carolina (North Carolina Wildlife Resources Commission, 2015); Listed as Endangered under the U.S. Endangered Species Act.
Comments: Two caves and one mine in North Carolina are considered priority sites for this federally endangered bat (USFWS, 2007). Records from Madison, McDowell and Swain counties (other than Hewitt Station Mine) by Boynton et al. (1992) have not been confirmed and are based on questionable identifications. Like many other cave-hibernating bat species, populations of *M. sodalis* have suffered declines due to White-nose Syndrome. *Myotis sodalis* was never common in North Carolina hibernacula before the arrival of White-nose Syndrome but now are rarely encountered (Katherine Caldwell, North Carolina Wildlife Resources Commission, personal communication in O'Keefe et al., 2019).
References: [1] Boynton et al. (1992); [2] USFWS (2007); [3] VertNet: MVZ; [4] VertNet: NCSM; [5] VertNet: UMMZ.

Genus *Perimyotis*

***Perimyotis subflavus* (Cuvier, 1832) (TX) Tri-colored Bat**
Localities: Avery Co.: Black Rock Cliffs Cave[2], Cranberry Mines*; Jackson Co.: Kitchen Cave*; Madison Co.: Anthodite Cave*; McDowell Co.: Lime Kiln Cave*, Linville Caverns[3,4], Pseudo-Saltpetre Cave*, Wind Cave*; Onslow Co.: Brinson's Rockhouse Cave[2]; Rutherford Co.: Bat Cave[1,4,5]; Breakdown Cave*, Halloween Haven*, Moonshiners Cave*, Rumbling Bald Cave*, Sliding Rock Cave*; Swain Co.: Blowing Springs Cave[2]; Transylvania Co.: Snake Den no. 2 Cave*; Yancey Co.: Celo Cave*, Cooper's Cave*, Isom Mica Mine*.
Conservation Status: IUCN: Vulnerable A3bce (Solari, 2018d); NatureServe: G2G3 (S3 in North Carolina); Listed as a Species of Greatest Conservation Need in North Carolina (North Carolina Wildlife Resources Commission, 2015).

Comments: *Perimyotis subflavus* was quite common in caves of North Carolina before White-nose Syndrome spread into the region but have experienced significant declines in the Southern Appalachians (O'Keefe et al., 2019). Its conservation status was recently elevated to Vulnerable due to impacts from White-nose Syndrome. In addition, *P. subflavus* is under review by USFWS for potential listing.
References: [1] VertNet: MVZ; [2] VertNet: NCSM; [3] VertNet: ROM; [4] VertNet: UMMZ; [5] Bat Population Database; * this study.

Order Didelphimorphia
Family Didelphidae
Genus *Didelphis*

***Didelphis virginiana* (Kerr, 1792) (AC) Virginia Opossum**
Localities: McDowell Co.: Holler's Potato Hole no. 2*; Rutherford Co.: Moonshiners Cave*, Rumbling Bald Cave*.
Conservation Status: IUCN: Least Concern; NatureServe: G5 (S5 in North Carolina).
Comments: Opossums have been reported from caves in the eastern United States (Dearolf, 1956; Cliburn and Middleton, 1983; Niemiller, unpublished data), but caves are thought to be much less important to this species compared to *Procyon lotor*.
References: * this study.

Order Rodentia
Family Cricetidae
Genus *Neotoma*

***Neotoma floridana haematoreia* Howell, 1934 (TX) Southern Appalachian Woodrat**
Localities: McDowell Co.: Lime Kiln Cave*, Wind Cave*; Rutherford Co.: Moonshiners Cave*, Rumbling Bald Cave[1]; Yancey Co.: Celo Cave*.
Conservation Status: IUCN: Least Concern; NatureServe: G5T4Q (S3S4 in North Carolina); Listed as a Species of Greatest Conservation Need in North Carolina (North Carolina Wildlife Resources Commission, 2015).
Comments: Nests and scat are common in North Carolina caves. The IUCN Red List status rank reflects the conservation status of the species and not this subspecies.
References: [1] VertNet: NCSM; * this study.

Family Dipodidae
Genus *Napaeozapus*

***Napaeozapus insignis* (Miller, 1891) (TX/AC) Woodland Jumping Mouse**
Localities: Henderson Co.: Middle Fork Shelter Cave no. 2*.
Conservation Status: IUCN: Least Concern; NatureServe: G5 (S4 in North Carolina).
Comments: Mice are not uncommon in caves, although few records for this particular species are known.
References: * this study.

Summary of bioinventory and literature records

We compiled occurrence records from 127 caves and other subterranean habitats in 29 counties in North Carolina from the literature and biological surveys. In total, this dataset includes occurrences for at least 5 phyla, 17 classes, 43 orders, 90 families, 124 genera, and 164 species (Table 1; Figure 2). Twenty-nine taxa are aquatic, whereas 135 taxa are terrestrial. Diverse invertebrate groups included spiders (Order Araneae; 11 families, 18 genera, 22 species), springtails (Class Collembola; seven families, nine genera, 15 species), copepods (Class Maxillopoda; one family, four genera, seven species), clitellate worms (Class Clitellata; three families, eight genera, eight species), and snails (Class Gatropoda; six families, nine genera, 12 species. The most common invertebrate species included the Cave Orbweaver (*Meta ovalis*) (21 occurrences from eight counties), a cave-obligate dipluran (*Litocampa* sp.) (15 occurrences from six counties), a cave cobweb spider (*Nesticus brimleyi*) (11 occurrences from two counties), and an undescribed species of *Stygobromus* amphipod (11 occurrences from five counties). Thirty-two vertebrate taxa were documented, including four fishes, nine salamanders, one lizard, four snakes, two birds, and 12 mammals. Mammal diversity was dominated by bats (eight species), with Tri-colored bats (*Perimyotis subflavus*) documented most frequently (20 occurrences). Most of the vertebrates reported to date in North Carolina have been previously documented from caves in adjacent states (e.g., Buhlmann, 2001; Fong et al., 2012; Niemiller et al., 2016).

At least 25 taxa are considered cave obligates, i.e., troglobites and stygobites (Table 2), with the greatest troglobitic/stygobitic species richness observed in *Phagocata* flatworms (five species), *Pseudosinella* springtails (five species), and *Stygobromus* amphipods (three described species and at least two undescribed taxa). Three of the 25 cave-ob-

Table 1. Taxonomic diversity of invertebrates and vertebrates from caves and other subterranean habitats in North Carolina.

Taxon	No. of Orders	No. of Families	No. of Genera	No. of Taxa	No. of Troglobites
Phylum Platyhelminthes					
Class Turbellaria	1	1	1	6	5
Phylum Annelida					
Class Clitellata	1	3	8	8	0
Phylum Arthropoda					
Class Arachnida	6	23	30	34	3
Class Chilopoda	2	2	2	2	0
Class Collembola	3	7	9	17	5
Class Diplopoda	4	6	6	8	2
Class Entognatha	1	2	2	2	1
Class Insecta	9	19	21	22	0
Class Malacostraca	3	5	6	13	8
Class Maxillopoda	1	1	4	7	1
Class Symphyla	1	1	1	1	0
Phylum Mollusca					
Class Gastropoda	2	6	9	12	0
Phylum Chordata					
Class Actinopterygii	2	2	4	4	0
Class Amphibia	1	1	6	9	0
Class Aves	1	2	2	2	0
Class Mammalia	4	5	8	12	0
Class Reptilia	1	4	5	5	0
Total	43	90	124	164	25

ligate taxa (*Phanetta subterranea*, *Porrhomma cavernicola,* and *Pseudosinella pecki*) have broad distributions that include several karst regions in the eastern United States. The other cave-obligate taxa are predominately endemic to North Carolina. No cave-obligate vertebrates have been documented from North Carolina to date.

Of the taxa included in the checklist above, at least 25 taxa are considered troglophiles/stygophiles, 50 taxa as trogloxenes/stygoxenes, and 22 as accidentals. All taxa classified as accidentals are considered common in surface habitats, except for *Mesodon andrewsae*. This terrestrial snail is considered Vulnerable (G3) globally and Imperiled-Vulnerable (S2S3) in North Carolina. All eight earthworms reported from North Carolina caves (Reynolds, 1994) are considered edaphic. The ecological classification of 44 taxa could not confidently be assigned to one specific category: seven taxa were classified as troglophiles or trogloxenes (stygophiles or stygoxenes) and 27 taxa as trogloxenes or accidentals.

Counties with the greatest number of caves with biological occurrences include Rutherford, McDowell, Swain, Henderson, Polk, and Avery counties (Table 3). Unsurprisingly, these counties also have the greatest subterranean species richness in North Carolina. However, most occurrences for each of these counties are for just a few cave systems. Twenty or more taxa have been documented at four caves (Table 4): Bat Cave complex in Rutherford County (34 taxa), Blowing Springs Cave in Swain County (27 taxa), Linville Caverns in McDowell County (27 taxa), and Rumbling Bald Cave in Rutherford County (22 taxa).

Caves with the greatest troglobitic species richness include the Bat Cave complex in Rutherford County, Blowing Springs Cave in Swain County, and Wind Cave in McDowell County, each with four troglobites documented. A particularly rich vertebrate fauna is known from Linville Caverns with 14 taxa, including four fishes, six salamanders, and four bat species. A diverse vertebrate fauna (>5 taxa) also has been documented to date at Rumbling Bald Cave in

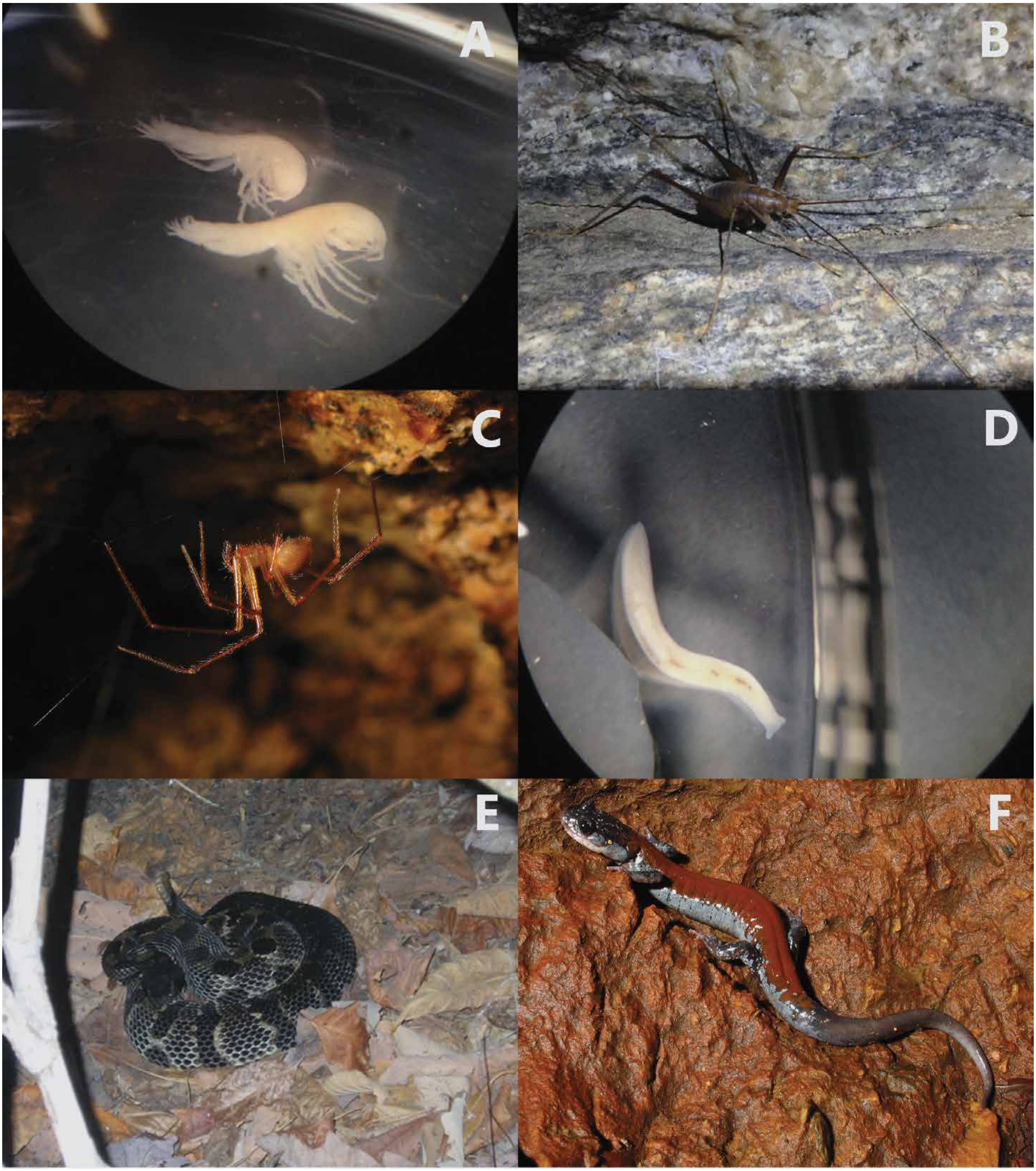

Figure 2. Representative cave life documented in North Carolina caves: A) *Stygobromus* sp. from Wind Cave, McDowell County (Photo by Cato Holler); B) *Euhadenoecus adelphus* from Bat Cave, Rutherford County (Photo by Cato Holler) C) *Nesticus* sp. from Wind Cave, McDowell County (Photo by Alan Cressler); D) *Phagocata* sp. from Wind Cave, McDowell County (Photo by Cato Holler); E) *Crotalus horridus* from Crotalus Shelter, McDowell County (Photo by Cato Holler); F) *Plethodon yonahlossee* from Wind Cave, McDowell County (Photo by Alan Cressler).

Table 2. Troglobitic and stygobitic taxa documented from caves and other subterranean habitats in North Carolina.

Species	Authority	No. of Occurrences	Counties
Caecidotea carolinensis	Lewis & Bowman, 1977	1	McDowell
Diacyclops jeanneli putei	(Yeatman, 1943)	1	Orange
Litocampa sp.		15	Henderson, Madison, McDowell, Polk, Rutherford, Swain
Nannopetalum vespertilio	Shear, 2003	1	Rutherford
Nesticus brimleyi	Gertsch, 1984	11	Polk, Rutherford
Phagocata carolinensis	Kenk, 1979	2	Burke, McDowell
Phagocata holleri	Kenk, 1979	1	Ashe
Phagocata procera	Kenk, 1984	7	Burke, Jackson, McDowell, Mitchell, Yancey
Phagocata pygmaea	Kenk, 1987	1	Stokes
Phagocata spuria	Kenk, 1987	2	McDowell
Phanetta subterranea	(Emerton, 1875)	4	McDowell, Rutherford, Yancey
Porrhomma cavernicola	(Keyserling, 1886)	2	Swain
Pseudosinella flatua	Christiansen & Bellinger, 1996	1	Swain
Pseudosinella gisini carolina	Christiansen & Bellinger, 1996	2	Rutherford
Pseudosinella orba	Christiansen, 1961	1	Polk
Pseudosinella pecki	Christiansen & Bellinger, 1980	1	Swain
Pseudosinella vespera	Christiansen & Bellinger, 1996	2	Rutherford, Swain
Pseudotremia shelleyi	Lewis, 2009	4	McDowell, Rutherford
Stygobromus araeus	(Holsinger, 1969)	1	Gates
Stygobromus carolinensis	Holsinger, 1978	2	Yancey
Stygobromus cf. *carolinensis*		1	Wilkes
Stygobromus indentatus	(Holsinger, 1967)	1	Nash
Stygobromus nov. sp. A		11	Avery, Burke, Henderson, McDowell, Mitchell
Stygobromus nov. sp. B		1	Jackson
Stygobromus sp.		4	Buncombe, McDowell, Polk

Rutherford County (8 taxa), Bat Cave complex in Rutherford County (6 taxa), Wind Cave in McDowell County (7 taxa), and Cooper's Cave in Yancey County (6 taxa) (Table 4).

Species of conservation concern

Several species documented from caves and other subterranean habitats in North Carolina are of conservation concern, including 14 species that are troglobites or stygobites (Table 5). Five species are on the IUCN Red List of Threatened Species. The bats *Myotis lucifugus* and *M. leibii* have been evaluated as Endangered and the Tri-colored Bat (*Perimyotis subflavus*) and groundwater amphipods *Stygobromus araeus* and *S. indentatus* have been evaluated as Vulnerable. Two additional bats, *M. septentrionalis* and *M. sodalis*, and the Hickory Nut Gorge Green Salamander (*Aneides caryaensis*) have been evaluated as Near Threatened. *Aneides caryaensis* likely will be placed on the IUCN Red List in the near future. The conservation status of four bat species on the IUCN Red List was recently increased in response to population declines associated with White-nose Syndrome (Solaris 2018a,b,c,d).

Based on NatureServe conservation rank criteria, 21 species are of conservation concern at the global level (G1–G3), many of which are troglobites or stygobites. *Nesticus cooperi*, *Stygobromus carolinensis*, *N. carolinensis*, and *Helicodiscus saludensis* are ranked as Critically Imperiled (G1). In addition, three species are ranked as Imperiled (G2), five species as Vulnerable (G3), six species as Critically Imperiled-Imperiled (G1G2), and three species as Imperiled-Vulnerable (G2G3). At the state level, 28 species of conservation concern, including two species ranked as Possibly Extinct (SH), seven species as Critically Imperiled (S1), four species as Imperiled (S2), five as Vulnerable (S3), seven species as Critically Imperiled-Imperiled (S1S2), and three species as Imperiled-Vulnerable (S2S3).

Nine species are listed as protected species in North Carolina by the North Carolina Wildlife Resources Commission (North Carolina Wildlife Resources Commission, 2017a; Table 5), including three species as Endangered, three species as Threatened, and three species as Special Concern. Three bats are also listed under the U.S. Endangered

Table 3. Subterranean occurrences and taxa by county and caves sampled in North Carolina. Percent is the number of caves with records divided by the total number of caves multiplied by 100 in a county. Some counties include sites that are not caves and are not included in this calculation.

County	No. of Records	No. of Taxa	No. of Sites with Records	Total No. of Caves	Percent (%)
Alexander	0	0	0	11	0.0
Alleghany	0	0	0	10	0.0
Anson	0	0	0	9	0.0
Ashe	3	2	2	32	6.3
Avery	27	14	12	136	8.1
Bladen	0	0	0	2	0.0
Buncombe	2	2	1	41	2.4
Burke	11	7	11	254	4.3
Caldwell	4	4	2	7	28.6
Chatham	7	4	6	1	0.0
Cherokee	0	0	0	8	0.0
Clay	0	0	0	17	0.0
Columbus	0	0	0	10	0.0
Craven	0	0	0	1	0.0
Dare	0	0	0	1	0.0
Davidson	3	3	2	8	25.0
Davie	0	0	0	1	0.0
Duplin	0	0	0	3	0.0
Forsyth	0	0	0	1	0.0
Gaston	0	0	0	20	0.0
Gates	1	1	1	0	···
Harnett	0	0	0	1	0.0
Haywood	0	0	0	38	0.0
Henderson	30	27	7	46	15.2
Hoke	0	0	0	4	0.0
Iredell	0	0	0	6	0.0
Jackson	9	9	4	23	13.0
Jones	1	1	1	4	0.0
Lincoln	0	0	0	1	0.0
Macon	12	10	3	24	12.5
Madison	15	12	5	28	17.9
Martin	0	0	0	2	0.0
Mecklenburg	0	0	0	2	0.0
McDowell	82	54	11	259	4.2
Mitchell	5	3	3	12	8.3
Montgomery	0	0	0	9	0.0
Nash	1	1	1	0	···
New Hanover	0	0	0	4	0.0
Northhampton	0	0	0	5	0.0
Onslow	4	4	2	5	20.0
Orange	2	2	1	11	0.0
Polk	30	25	8	17	47.1
Randolph	0	0	0	13	0.0
Rockingham	2	2	1	1	100.0
Rowan	0	0	0	2	0.0
Rutherford	137	76	20	186	10.8
Stokes	2	2	2	96	2.1
Surry	1	1	1	17	5.9
Swain	47	35	9	40	22.5
Transylvania	7	7	1	35	2.9
Union	0	0	0	2	0.0
Wake	1	1	1	12	8.3
Watauga	2	2	2	44	4.5
Wilkes	2	2	2	30	6.7
Yancey	25	17	5	31	9.7
Total	475	164	127	1,582	8.0

Table 4. List of the 14 most diverse cave systems in North Carolina.

Cave	County	No. of Taxa	No. of Troglobites	No. of Vertebrates	No. of Species of Conservation Concern
Bat Cave complex	Rutherford	34	4	6	8
Blowing Springs Cave	Swain	27	4	5	8
Linville Caverns	McDowell	27	1	14	4
Rumbling Bald Cave	Rutherford	22	3	8	7
Staircase Cave	McDowell	16	3	1	1
Wind Cave	McDowell	15	4	7	6
Breakdown Cave	Rutherford	13	2	4	4
Devil's Kitchen Cave	Henderson	12	0	0	0
Little Warrior Mountain Cave	Polk	11	3	1	2
Lost Nantahala Cave	Swain	11	2	2	4
Western Talus Tunnel	Rutherford	10	0	0	0
Sliding Rock Cave	Rutherford	10	1	3	4
Moonshiners Cave	Rutherford	9	1	4	4
Celo Cave	Yancey	9	0	4	4

Table 5. List of species of conservation concern and their conservation status documented from caves and other subterranean habitats in North Carolina. Conservation status includes IUCN Red List ranking, NatureServe global conservation rank, NatureServe state conservation rank, state designation by NCWRC, and federal status under the U.S. Endangered Species Act. Species considered troglobites or stygobites are in bold.

Scientific name	Common name	IUCN	NS Global	NS State	NC Status	US Status
Phagocata carolinensis	**Carolina Cave Planarian**		**G1G2**	**S1S2**		
Phagocata holleri	**Holler's Cave Planarian**		**G1G2**	**S1S2**		
Phagocata procera	**A Cave Planarian**		**G1G2**	**S1S2**		
Hypochilus coylei	A Lampshade Weaver		G3?	S3?		
Nesticus brimleyi	**A Cave Cobweb Spider**		**G1G2**	**S1?**		
Nesticus carolinensis	Linville Caverns Spider		G1?	S1		
Nesticus cooperi	Lost Nantahala Cave Spider		G1	S1		
Nesticus mimus	A Cobweb Spider		G2	S2?		
Pseudosinella flatua	**Blowing Springs Cave Springtail**		**G1G2**	**S1S2**		
Pseudosinella gisini carolina	**Carolina Cave Springtail**		**G3G4T1T2**	**S1S2**		
Pseudosinella pecki	**Peck's Cave Springtail**		**G2G3**	**SNR**		
Pseudosinella vespera	**Bat Cave Springtail**		**G2**	**S1S2**		
Stygobromus araeus	**Tidewater Interstitial Amphipod**	**VU**	**G3**	**SU**		
Stygobromus carolinensis	**Carolina Groundwater Amphipod**		**G1**	**S1**		
Stygobromus indentatus	**Tidewater Amphipod**	**VU**	**G3**	**SU**		
Stygobromus* nov. sp. *B	**A Cave Amphipod**		**GNR**	**S1?**		
Caecidotea carolinensis	**Bennett's Mill Cave Isopod**		**G2G3**	**SH**	LE	
Diacyclops jeanneli putei	**Carolina Well Copepod**		**G3G4**	**SH**	SC	
Helicodiscus saludensis	Corncob Snail		G1	S1?		
Mesodon andrewsae	Balsam Globe		G3	S2S3		
Mesomphix latior	Broad Button		G3G4	S2S3		
Aneides caryaensis	Hickory Nut Green Salamander	NT	G3G4	S2S3	LT	
Corynorhinus rafinesquii	Rafinesque's Big-eared Bat	LC	G3G4T2	S1	LE	LE
Corynorhinus townsendii virginianus	Virginia Big-eared Bat	LC	G3G4	S3	LT	
Myotis leibii	Eastern Small-footed Bat	EN	G4	S2	SC	
Myotis lucifugus	Little Brown Bat	EN	G3	S3		
Myotis septentrionalis	Northern Long-eared Bat	NT	G1G2	S2	LT	LT
Myotis sodalis	Indiana Bat	NT	G2	S1S2	LE	LE
Perimyotis subflavus	Tri-colored Bat	VU	G2G3	S3		
Crotalus horridus	Timber Rattlesnake	LC	G4	S3	SC	

Species Act, including *Myotis sodalis* and *Corynorhinus townsendii virginianus* as Endangered and *M. septentrionalis* as Threatened. Since White-nose Syndrome was first detected in North Carolina at a cave in Avery County in 2011, it has spread into 12 counties in western North Carolina (https://www.whitenosesyndrome.org/static-page/where-is-wns-now) resulting in significant population declines for several bat species. Most impacted have been populations of *M. septentrionalis*, *M. lucifugus*, *M. sodalis*, and *P. subflavus*, in which some populations have experienced over 90 percent declines (North Carolina Wildlife Resources Commission, 2017b). *Myotis septentrionalis* was recently listed as Threatened on the U.S. Endangered Species Act in 2015 due to population declines associated with White-nose Syndrome (USFWS, 2015). *Myotis lucifugus* and *P. subflavus* may be listed in the near future; their respective conservation statuses (both IUCN Red List and NatureServe) were elevated to higher risk of extinction ranks recently due to high mortality from White-nose Syndrome (e.g., Frick et al., 2010; Verant et al., 2012).

Conclusions and Future Directions

Although not nearly as extensive and common as in adjacent states, such as Georgia, Tennessee, and Virginia, caves and other subterranean habitats in North Carolina support a diverse community of invertebrates and vertebrates. Our review of the subterranean fauna of North Carolina includes at least 164 taxa representing five phyla and 90 families. While this review highlights the biodiversity found in subterranean habitats in the state, we aim for this assessment to serve as a base line for future biological surveys in caves and other subterranean habitats in the state. Moreover, we highlight the importance of caves and subterranean habitats for many species, not just bats and troglobites (obligate cave species).

A small percentage of caves in North Carolina have biological occurrence records (8.0%), and even a smaller fraction of sites has had comprehensive or repeated biological inventories conducted. Several counties, such as Buncombe, Burke, Haywood, Stokes, Transylvania, and Watauga counties, have >30 caves yet <4% of caves have even a single biological occurrence. This biodiversity knowledge shortfall is not limited to North Carolina, but even occurs in states with considerably greater karst and cave systems (e.g., Tennessee; Niemiller and Zigler, 2013). Thus, there is great potential to document new cave distributional records. With 20 described cave-obligate taxa, North Carolina is tied for 19th (with Pennsylvania) in cave-obligate species richness among U.S. states (Niemiller et al. 2019). Much of the cave-obligate diversity in North Carolina is endemic to the state. At least three undescribed taxa are noted in the literature and await formal taxonomic description. However, there is considerable potential to discover new taxa unknown to science in subterranean habitats of North Carolina, given the complex geological history, topography, and isolated nature of cave systems in much of the state.

Caves are important habitats for many species of vertebrates and invertebrates. Several caves and mines have been identified previously as important hibernacula for several threatened and endangered bat species in North Carolina (Boynton et al., 1992), and there is a growing body of literature that caves are critically important habitats for other vertebrates and invertebrates (e.g., Niemiller and Miller, 2009; Niemiller et al., 2016) for shelter, hibernation, food, and other aspects of life history.

Acknowledgements

We thank the following individuals for assistance with arranging access to caves and with bioinventories: the owners and management of Linville Caverns Incorporated, Chimney Rock, and Grandfather Mountain (the latter two are now state parks), along with the numerous private cave owners throughout the state. We also thank all past and present members of the Flittermouse Grotto. Peter Hertl did a considerable amount of collecting in the early days of the project. Bob Currie, Allen Boynton, Chris McGrath and other members of the U.S. Fish and Wildlife and N.C. Wildlife Resources Commission were kind to allow us to accompany them on many of their official bat inventories. Lead author (CH) is particularly grateful to his wife, Susan Holler, and three children, Oliver, Chris, and Nancy, for their encouragement and assistance in the field over many years. Thank you Alan Cressler for providing photographs. We especially thank the late Dr. John Cooper, former Director of Research and Collections at the North Carolina State Museum, who first inspired and encouraged cavers to initiate a cave fauna survey in the state. John was also instrumental in distributing many of the collected specimens to taxonomic specialists for identification. We also thank the following taxonomists for their assistance with this project: Tom Barr, Ken Christiansen, Fredrick Coyle, Lynn Ferguson, Marshal Hedin, John Holsinger, Roman Kenk, William Shear, Rowland Shelly, and Charles Stephen.

Literature Cited

Arroyo-Cabrales, J., and Ospina-Garces, S., 2016, *Myotis sodalis*: The IUCN Red List of Threatened Species 2016: e.T14136A22053184. http://dx.doi.org/10.2305/IUCN.UK.2016-1.RLTS.T14136A22053184.en. Downloaded on 04 January 2019.

Barr, T.C., Jr., 1968, Cave ecology and the evolution of troglobites: *in* Dobzhansky, T., Hecht, M.K., and Steere, W.C., eds., Evolutionary Biology, vol. 2: New York, Appleton-Century-crofts, p. 35–102.

Beane, J.C., Braswell, A.L., Mitchell, J.C., Palmer, W.M., and Harrison III, J.R., 2010, Amphibians and Reptiles of the Carolinas and Virginia, 2nd edition: Chapel Hill, The University of North Carolina Press, 274 p.

Boynton A., Holler, C., and Currie, B., 1992, Survey of important sites for listed and candidate bat species in western North Carolina: Final report to the U.S. Fish and Wildlife Service, 13 p.

Brandon, R.A., 1966, Systematics of the Salamander Genus *Gyrinophilus*: Urbana, University of Illinois Press, Illinois Biological Monographs 35, 82 p.

Brode, W.E., 1958, The occurrence of the pickerel frog, three salamanders and two snakes in Mississippi caves: Copeia, v. 1958, p. 47–48.

Bruno, M.C., Reid, J.W., and Perry, S.A., 2000, New records of copepods from Everglades National Park (Florida): description of two new species of *Elaphoidella* (Harpacticoida, Canthocamptidae), and supplementary description of *Diacyclops nearcticus* (Cyclopoida, Cyclopidae): Crustaceana, v. 73, p. 1171–1204.

Buhlmann, K.A., 2001, A biological inventory of eight caves in northwestern Georgia with conservation implications: Journal of Cave and Karst Studies, v. 63, p. 91–98.

Camp, C.D., and Jensen, J.B., 2007, Use of twilight zones of caves by plethodontid salamanders: Copeia, v. 2007, p. 594–604.

Christ, C.A., 2003, The invertebrate community of the Rumbling Bald Mountain Fissure Cave System: Unpublished report, Warren Wilson College, Ashville, North Carolina, 28 p.

Christiansen, K., and Bellinger, P., 1980, The Collembola of North America North of the Rio Grande: Grinnell College, Grinnell, Iowa, 1322 p.

Christiansen, K., and Bellinger, P., 1996, Cave *Pseudosinella* and *Oncopodura* new to science: Journal of Cave and Karst Studies, v. 58, p. 38–53.

Cliburn, J.W., and Middleton, Jr., A.L., 1983, The vertebrate fauna of Mississippi caves: Bulletin of the National Speleological Society, v. 45, p. 45–48.

Cooper, J.E., and Cooper, M.R., 1968, Cave-associated herpetozoa II: salamanders of the genus *Gyrinophilus* in Alabama caves: Bulletin of the National Speleological Society, v. 30, p. 19–24.

Culver, D. C., Christman, M. C., Elliott, W. R., Hobbs, H. H., and Reddell, J. R., 2003, The North American obligate cave fauna: regional patterns: Biodiversity & Conservation, v. 12, p. 441-468.

Culver, D.C., and Pipan, T., 2009, The Biology of Caves and Other Subterranean Habitats: Oxford University Press, 254 p.

Culver, D.C., Holsinger, J.R., and Feller, D.J., 2012, The fauna of seepage springs and other shallow subterranean habitats in the Mid-Atlantic Piedmont and Coastal Plain: Northeastern Naturalist, v. 19, p. 1–42.

Dearolf, K., 1956, Survey of North American cave vertebrates: Proceedings of the Pennsylvania Academy of Science, v. 30, p. 201–210.

Dodd, C.K. Jr., Griffey, M.L., and Corser, J.D., 2001, The cave associated amphibians of Great Smoky Mountains National Park: Review and monitoring: Journal of the Elisha Mitchell Scientific Society, v. 117, p. 139–149.

Dourson, D.C., 2013, Land Snails of the Great Smoky Mountains National Park and Southern Appalachians: Goatslug Publications, Bakersville, North Carolina, 336 p.

Ellison, L., O'Shea, T., Bogan, M., Everette, A., and Schneider, D., 2003, Existing data on colonies of bats in the United States: Summary and analysis of the US Geological Survey's bat population database: USGS/BRD/ITR-2003-0003, Fort Collins, Colorado, US Geological Survey.

Faber-Langendoen, D., Nichols, J., Master, L., Snow, K., Tomaino, A., Bittman, R., Hammerson, G., Heidel, B., Ramsay, L., Teucher, A., and Young, B., 2012, NatureServe Conservation Status Assessments: Methodology for Assigning Ranks, revised edition: Arlington, Virginia, NatureServe, 44 p.

Flock, B., 2013, 2013 Tennessee Bat Population Monitoring and White Nose Syndrome: Nashville, Tennessee Wildlife Resources Agency, TWRA Wildlife Technical Report 13-22, 17 p.

Flock, B., 2014, 2014 Bat Population Monitoring and White Nose Syndrome Surveillance: Nashville, Tennessee Wildlife Resources Agency, TWRA Wildlife Technical Report 14-07, 15 p.

Fong, D.W., Porter, M.L., and Slay, M.E., 2012, Cave Life of the Virginias: A Field Guide to Commonly Encountered Species: Huntsville, Alabama, Biology Section of the National Speleological Society. 41 p.

Frick, W.F., Pollock, J.F., Hicks, A.C., Langwig, K.E., Reynolds, D.S., Turner, G.G., Butchkoski, C.M., and Kunz, T.H., 2010, An emerging disease causes regional population collapse of a common North American bat species: Science, v. 329, p. 679–682. doi:10.1126/ science.1188594.

Gaddy, L.L., 1986a, Natural Area Reconnaissance: Little Warrior Mountain Cave: Unpublished report. 4 p.

Gaddy, L.L., 1986b, The spider fauna of three caves in the Southern Appalachians of North Carolina: Unpublished report. 7 p.

Garton, E.R., Grady, F., and Carey, S.D., 1993, The Vertebrate Fauna of West Virginia Caves: West Virginia Speleological Survey, Bulletin 11, 107 p.

Gertsch, W.J., 1984, The spider family Nesticidae (Araneae) in North America, Central America, and the West Indies: Texas Memorial Museum Bulletin, v. 31, p. 1–91.

Godwin, J.C., 2008, Biological Inventory of the Cave and Karst Systems of The Nature Conservancy's Sharp-Bingham Mountain Preserve: Auburn, Alabama, Alabama Natural Heritage Program, 40 p.

Hammerson, G., 2004, *Aneides aeneus*. The IUCN Red List of Threatened Species 2004: e.T1282A3385547. http://dx.doi.org/10.2305/IUCN.UK.2004.RLTS.T1282A3385547.en. Downloaded on 04 January 2019.

Hedin, M.C., 1997a, Molecular phylogenetics at the population/species interface in cave spiders of the southern Appalachians (Araneae: Nesticidae: *Nesticus*): Molecular Biology and Evolution, v. 14, p. 309–324.

Hedin, M.C., 1997b, Speciational history in a diverse clade of habitat-specialized spiders (Araneae: Nesticidae: *Nesticus*): inferences from geographic-based sampling: Evolution, v. 51, p. 1929–1945.

Hertl, P.T., 1981, A preliminary inventory survey and management plan for the Bat Cave complex, Bat Cave, North Carolina: Unpublished report for the North Carolina Nature Conservancy.

Highton, R., and Peabody, R.B., 2000, Geographic protein variation and speciation in salamanders of the *Plethodon jordani* and *Plethodon glutinosus* complexes in the southern Appalachian Mountains with the description of four new species: *in* Bruce, R.C., Jaeger, R.G., and Houck, L.D., eds. The Biology of Plethodontid Salamanders: New York, Kluwer Academic / Plenum Publishers, p. 31–93.

Hobbs III, H.H., 2012, Diversity patterns in the United States: *in* White, W.B., Culver, D.C., eds. Encyclopedia of Caves, 2nd edition: Amsterdam, Academic Press, p. 251–264.

Hoffman, R.L., 1981, On the taxonomic status, distribution, and subspecies of the milliped *Pseudotremia fracta* Chamberlin (Chordeumatida, Cleidogonidae): Brimleyana, v, 5, p. 135–144.

Holland, D.G., 1981, A survey of the reptiles and amphibians of Bat Cave: Report to the North Carolina Nature Conservancy. 17 p.

Holler, C.O., 1981, North Carolina's Bat caves: a significant region of tectonokarst: *in* Proceedings of the 8th International Congress of Speleology, Volume 1, Bowling Green, Kentucky, p. 190–191.

Holler, C.O., 2019, Pseudokarst: *in* White, W.B., Culver, D.C., and Pipan, T., eds. Encyclopedia of Caves, 3rd edition: Amsterdam, Academic Press, p. 836–849.

Holler, C.O., and Holler, S.G., 2009, Tectonic pseudokarst in western North Carolina: Field Trip Guidebook for the 15th International Congress of Speleology, National Speleological Society, Huntsville, Alabama, 36 p.

Holsinger, J.R., 1978, Systematics of the subterranean amphipod genus *Stygobromus* (Crangonyctidae), Part II: Species of the eastern United States: Smithsonian Contributions to Zoology, v. 266, p. 1–144.

Holsinger, J.R., and Peck, S.B., 1971, The invertebrate cave fauna of Georgia: National Speleological Society Bulletin, v. 33, p. 23–44.

Hubbell, T.H., and Norton, R.M., 1978, The systematics and biology of the cave-crickets of the North American tribe Hadenoecini (Orthoptera Saltatoria: Ensifera: Rhaphidophoridae: Dolichopodinae): Miscellaneous Publications Museum of Zoology, Univ. of Michigan, No. 156, 124 p.

Inland Water Crustacean Specialist Group, 1996a, *Stygobromus araeus*: The IUCN Red List of Threatened Species 1996: e.T20973A9240768. http://dx.doi.org/10.2305/IUCN.UK.1996.RLTS.T20973A9240768.en. Downloaded on 04 January 2019.

Inland Water Crustacean Specialist Group, 1996b, *Stygobromus identatus*: The IUCN Red List of Threatened Species 1996: e.T20993A9242203. http://dx.doi.org/10.2305/IUCN.UK.1996.RLTS.T20993A9242203.en. Downloaded on 04 January 2019.

International Union for the Conservation of Nature, 2012, IUCN Red List Categories and Criteria: Version 3.1. Second edition: Gland, Switzerland, and Cambridge, UK, IUCN, 32 p.

Kenk, R., 1979a, Freshwater triclads (Turbellaria) of North America. XI. *Phagocata holleri*, new species, from a cave in North Carolina: Proceedings of the Biological Society of Washington, v. 92, p. 389–393.

Kenk, R., 1979b, Freshwater triclads (Turbellaria) of North America. XII. Another new cave planarian from North Carolina: Brimleyana, v. 2, p. 91–96.

Kenk, R., 1984, Freshwater triclads (Turbellaria) of North America. XV. Two new subterranean species from the Appalachian region: Proceedings of the Biological Society of Washington, v. 97, p. 209–216.

Kenk, R., 1987, Freshwater triclads (Turbellaria) of North America. XVI. More on subterranean species of *Phagocata* of the eastern United States: Proceedings of the Biological Society of Washington, v. 100, p. 664–673.

Kofoid, C.A., 1900, The plankton of Echo River, Mammoth Cave: Transactions of the American Microscopical Society, v. 21, p. 113–126.

LeGrand, H.E., Ratcliffe, J.A., and Finnegan, J.T., 2014, Natural Heritage Program list of rare animal species of North Carolina: Raleigh, North Carolina, North Carolina Natural Heritage Program, Department of Environment and Natural Resources, 162 p.

Lewis, J.J., 2000, Five new species of *Pseudotremia* from caves in the Blue River area of southern Indiana (Diplopoda: Chordeumatida: Cleidogonidae): Myriapodologica, v. 6, p. 55–68.

Lewis, J.J., 2005a, Bioinventory of caves of the Cumberland escarpment area of Tennessee: Technical report. Tennessee Chapter of the Nature Conservancy, Nashville Tennessee, 158 p.

Lewis, J.J., 2005b, Six new species of *Pseudotremia* from caves of the Tennessee Cumberland Plateau: Zootaxa, v. 1080, p. 17–31.

Lewis, J.J., 2009, Eight new cavernicolous species of the milliped genus *Pseudotremia* (Diplopoda: Chordeumatida: Cleidogonidae): Virginia Museum of Natural History Special Publication, v. 16, p. 171–186.

Lewis, J.J., and Bowman, T.E., 1977, *Caecidotea carolinensis*, n. sp., the first subterranean water slater from North Carolina (Crustacea: Isopoda: Asellidae): Proceedings of the Biological Society of Washington, v. 90, p. 968–974.

Lewis, J.J., and Reid, J.W., 2007, Patterns and processes of groundwater invasion by copepods in the Interior Low Plateau of the United States: Acta Carsologica, v. 36, p. 279–289.

McAlpine, D.F., and Reynolds, J.W., 1977, Terrestrial Oligochaeta of some New Brunswick caves with remarks on their ecology: Canadian Field-Naturalist, v. 91, p. 360–366.

McAlpine, D.F., Reynolds, J.W., Fletcher, T.J., Trecartin, J.L., and Sabine, D.L., 2001, *Sparganophilus eiseni* (Oligochaeta, Sparganophilidae) in New Brunswick, an addition to the earthworm fauna of Maritime Canada: Megadrilogica, v. 8, p. 53–56.

Miller, B.T., and Niemiller, M.L., 2005, *Pseudotriton ruber*. Reproduction: Herpetological Review, v. 36, p. 429.

Miller, B.T., and Niemiller, M.L., 2008, Distribution and relative abundance of Tennessee Cave Salamanders (*Gyrinophilus palleucus* and *Gyrinophilus gulolineatus*) with an emphasis on Tennessee populations: Herpetological Conservation and Biology, v. 3, p. 1–20.

Miller, B.T., Niemiller, M.L., and Reynolds, R.G., 2008, Observations on egg-laying behavior and interactions among attending female Red Salamanders (*Pseudotriton ruber*) with comments on the use of caves by this species: Herpetological Conservation and Biology, v. 3, p. 203–210.

Niemiller, M.L., Glorioso, B.M., Gray, E., Miller, B.T., Jensen, J.B., and Keyes, T., 2006, *Pseudotriton ruber* ruber. Size and subterranean aggregation: Herpetological Review, v. 37, p. 438.

Niemiller, M.L., and Miller, B.T., 2009, A survey of the cave-associated herpetofauna of the eastern United States with an emphasis on salamanders: *in* Proceedings of the 15th International Congress of Speleology, Kerrville, Texas, p. 249–256.

Niemiller, M.L., Osbourn, M.S., Fenolio, D.B., Pauley, T.K., Miller, B.T., and Holsinger, J.R., 2010, Conservation status and habitat use of the West Virginia spring salamander (*Gyrinophilus subterraneus*) and spring salamander (*G. porphyriticus*) in General Davis Cave, Greenbrier co., West Virginia: Herpetological Conservation and Biology, v. 5, p. 32–43.

Niemiller, M.L., and Reeves, W.C., 2014, Predation by the stygophilic crayfish *Cambarus tenebrosus* on the salamander *Pseudotriton ruber* within a cave in Cannon County, Tennessee, USA: Speleobiology Notes, v. 6, p. 8–13. doi:10.5563/spbn.v6i0.62.

Niemiller, M.L., and Reynolds, R.G., eds, 2011, The Amphibians of Tennessee, Knoxville, Tennessee: University of Tennessee Press, 369 p.

Niemiller, M.L., Taylor, S.J., Slay, M.E., and Hobbs III, H.H., 2019, Diversity patterns in the United States and Canada: *in* Encyclopedia of Caves, 3rd edition (Culver, D.C., White, W.B., and Pipan, T., eds.). Elsevier, p. 163–176.

Niemiller, M.L., and Zigler, K.S., 2013, Patterns of cave biodiversity and endemism in the Appalachians and Interior Plateau of Tennessee, USA: PLOS ONE, v. 8, art. e64177, 17 p. doi:10.1371/journal. pone.0064177.

Niemiller, M.L., Zigler, K.S., and Fenolio, D.B., 2013, Cave Life of TAG: A Guide to Commonly Encountered Species in Tennessee, Alabama and Georgia: Huntsville, Alabama, Biology Section of the National Speleological Society, 45 p.

Niemiller, M.L., Zigler, K.S., Stephen, C.D.R., Carter, E.T., Paterson, A.T., Taylor, S.J., and Engel, A.S., 2016, Vertebrate fauna in caves of eastern Tennessee within the Appalachians karst region, USA: Journal of Cave and Karst Studies, v. 78, p. 1–24.

North Carolina Wildlife Resources Commission, 2015, North Carolina Wildlife Action Plan: Raleigh, North Carolina, North Carolina Wildlife Resources Commission, 1298 p.

North Carolina Wildlife Resources Commission, 2017a, Protected wildlife species of North Carolina: Raleigh, North Carolina, North Carolina Wildlife Resources Commission, 9 p.

North Carolina Wildlife Resources Commission, 2017b, Final performance report: White-nose syndrome surveillance and bat monitoring in North Carolina: White-nose syndrome grants to states: E-17-5.11.

O'Keefe, J.M., Pettit, J.L., Loeb, S.C., and Stiver, W.H., 2019, White-nose syndrome dramatically altered the summer bat assemblage in a temperate Southern Appalachian forest: Mammalian Biology, v. 98, p. 146–153.

Osbourn, M.S., 2005, The natural history, distribution, and phenotypic variation of cave-dwelling spring salamanders, *Gyrinophilus* spp. Cope (Plethodontidae), in West Virginia [Master's thesis]: Marshall University, 207 p.

Patton, A., Apodaca, J.J., Corser, J.D., Wilson, C.R., Williams, L.A., Cameron, A.D., and Wake, D.B., 2019, A new green salamander in the Southern Appalachians: Evolutionary implications of *Aneides aeneus* and implications for management and conservation with the description of a cryptic microendemic species: Copeia, v. 107, p. 748–763.

Pauley, T.K., 1993, Report of the Upland Vertebrates in the New River Gorge National River. Volumes 1-3 (1989-1992). US Department of the Interior, National Park Service.

Peck, S.B., and Lewis, J.J., 1978, Zoogeography and evolution of the subterranean invertebrate faunas of Illinois and southeastern Missouri: National Speleological Society Bulletin, v. 40, p. 39–63.

Petrie, J.S., 1942, Commercial caves: Bulletin of the National Speleological Society, v. 4, p. 58–63.

Poly, W.J., 2001, Nontroglobitic fishes in Bruffey-Hills Creek Cave, West Virginia, and other caves worldwide: Environmental Biology of Fishes, v. 62, p. 73–83.

Reeves, W.K., 2000a, *Caecidotea carolinensis* (Isopoda: Asellidae): first record of a stygobite from South Carolina: Journal of Cave and Karst Studies, v. 62, p. 18–19.

Reeves, W.K., 2000b, Invertebrate cavernicoles of the Great Smoky Mountains National Park, USA: Journal of the Elisha Mitchell Scientific Society, v. 116, n. 4, p. 334–343.

Reeves, W.K., 2001, Invertebrate and slime mold cavernicoles of Santee Cave, South Carolina, U.S.A.: Proceedings of the Academy of Natural Sciences of Philadelphia, v. 151, p. 81–85.

Reeves, W.K., Jensen, J.B., and Ozier, J.C., 2000, New faunal and fungal records from caves in Georgia, USA: Journal of Cave and Karst Studies, v. 62, p. 169–179.

Reeves, W.K., and Reynolds, J.W., 1999, New records of cave-dwelling earthworms (Oligochaeta: Lumbricidae, Megascolecidae, and Naididae) and other annelids (Aeolosomatida, Branchiobdellida, and Hirudinea) in the southeastern United States, with notes on their ecology: Megadrilogica, v. 7, p. 65–71.

Reid, J.W., 2004, New records and new species of the genus *Diacyclops* (Crustacea; Copepoda) from subterranean habitats in southern Indiana, USA: Jeffersoniana, v. 12, p. 1–65.

Reynolds, J.W., 1980, The earthworm family Sparganophilidae (Annelida, Oligochaeta) in North America: Megadrilogica, v. 3, p. 189–204.

Reynolds, J.W., 1994, Earthworms of North Carolina (Oligochaeta: Acanthodrilidae, Komarekionidae, Lumbricidae, Megascolecidae, Ocnerodrilidae and Sparganophilidae): Megadrilogica, v. 5, p. 53–72.

Schmidt, K., 1994, The Mystery Cave expeditions: American Currents, v. 20, p. 26–31.

Schultz, G.A., 1964, Two additional data on terrestrial isopod crustaceans: *Ligidium blueridgensis*, sp. nov., from Georgia and a North Carolina cave location for *Miktoniscus linearis* (Patience, 1908): Journal of the Elisha Mitchell Scientific Society, v. 80, p. 90–94.

Shear, W.A., 2003, The milliped family Trichopetalidae, Part 1: Introduction and genera *Trigenotyla* Causey, *Nannopetalum* n. gen., and *Causeyella* n. gen. (Diplopoda: Chordeumatida, Cleidogonoidea): Zootaxa, v. 321, p. 1–36.

Shear, W.A., 2008, Cave millipedes of the United States. VII. New species and records of the genus *Pseudotremia* Cope. I. Species from West Virginia, USA (Diplopoda, Chordeumatida, Cleidogonidae): Zootaxa, v. 1764, p. 53–65.

Shear, W.A., 2011, Cave millipedes of the United States. X. New species and records of the genus *Pseudotremia* Cope. 2. Species from Virginia, USA (Diplopoda, Chordeumatida, Cleidogonidae): Zootaxa, v. 3109, p. 1–38.

Shelley, R.M., 2000, Annotated checklist of the millipedes of North Carolina (Arthropoda: Diplopoda), with remarks on the genus *Sigmoria* Chamberlin (Polydesmida: Xystodesmidae): Journal of the Elisha Mitchell Scientific Society, v. 116, p. 177–205.

Sket, B., 2008, Can we agree on ecological classifications of subterranean animals? Journal of Natural History, v. 42, n. 21-22, p. 1549–1563. doi:10.1080/00222930801995762.

Slay, M.E., Niemiller, M.L., Taylor, S.J., and Sutton, M., 2016, Cave Life of the Ozarks: A Guide to Commonly Encountered Species in Arkansas, Missouri, and Oklahoma: Huntsville, Alabama: Biology Section of the National Speleological Society, 45 p.

Solari, S., 2018a, *Myotis leibii*. The IUCN Red List of Threatened Species 2018: e.T14172A22055716. http://dx.doi.org/10.2305/IUCN.UK.2018-2.RLTS.T14172A22055716.en. Downloaded on 04 January 2019.

Solari, S., 2018b, *Myotis lucifugus*. The IUCN Red List of Threatened Species 2018: e.T14176A22056344. http://dx.doi.org/10.2305/IUCN.UK.2018-2.RLTS.T14176A22056344.en. Downloaded on 04 January 2019.

Solari, S., 2018c, *Myotis septentrionalis*. The IUCN Red List of Threatened Species 2018: e.T14201A22064312. http://dx.doi.org/10.2305/IUCN.UK.2018-2.RLTS.T14201A22064312.en. Downloaded on 04 January 2019.

Solari, S., 2018d, *Perimyotis subflavus*. The IUCN Red List of Threatened Species 2018: e.T17366A22123514. http://dx.doi.org/10.2305/IUCN.UK.2018-2.RLTS.T17366A22123514.en. Downloaded on 04 January 2019.

Strayer, D.L., and Reid, J.W., 1999, Distribution of hyporheic cyclopoids (Crustacea: Copepoda) in the eastern United States: Archiv fur Hydrobiologie, v. 145, p. 79–92.

Taylor, N., and Mays, J., 2006, The salamanders *Eurycea longicauda* and *Plethodon glutinosus* in Gregorys Cave, TN: Monitoring and observations on ecology and natural history: Southeastern Naturalist, v. 5, p. 435–442. doi:10.1656/1528-7092(2006)5[435:TSELAP]2.0. CO;2.

Taylor, S.J., Krejca, J.K., Niemiller, M.L., Dreslik, M.J., and Phillips, C.A., 2015, Life history and demographic differences between cave and surface populations of the western slimy salamander, *Plethodon albagula* (Caudata: Plethodontidae), in central Texas: Herpetological Conservation and Biology, v. 10, p. 740–752.

Terwilliger, K., 1991, Virginia's endangered species: Proceedings of a symposium: McDonald and Woodward Publishing Co., Blacksburg, Virginia. 672 p.

Trauth, S.E., Robison, H.W., and Plummer, M.V., 2004, The Amphibians and Reptiles of Arkansas: Fayetteville, Arkansas: University of Arkansas Press. 421 p.

United States Fish and Wildlife Service, 2007, Indiana Bat (*Myotis sodalis*) draft recovery plan: first revision: United States Fish and Wildlife Service, 258 p.

United States Fish and Wildlife Service, 2015, Endangered and threatened wildlife and plants; threatened species status for the Northern Long-eared Bat with 4(d) rule: Federal Register, v. 80, p. 17974–18033.

Vandel, A., 1950, Biospeologica 71: Campagne speologique de C. Bolivar et R Jeannel dans l'Amerique du Nord (1928). Isopodes terrestres recueillis par C. Bolivar et R. Jeannel (1928) et le Dr. Henrot (1946): Arch. Zool. Exper. Gen., v. 87, p. 183–210.

Verant, M.L., Boyles, J.G., Waldrep, Jr., W., Wibbelt, G., and Blehert, D.S., 2012, Temperature-dependent growth of *Geomyces destructans*, the fungus that causes bat white-nose syndrome: PLOS One, v. 7, e46280.

Wallace, R.L., 1984, Biological survey report of the Great Smoky Mountains National Park caves: Unpublished report to Great Smoky Mountains National Park, 34 p.

Wallace, R.L., 2003, Biological survey report of the Great Smoky Mountains National Park caves: Tennessee Caver, v. 2, p. 3–23.

Yeatman, H.C., 1943, Rare cyclopoid copepods from wells in North Carolina: Journal of the Elisha Mitchell Scientific Society, v. 59, p. 27–36.

Ibrahem G. Wasti, Foo She Fui, Tan Qin Zhi, Cheh Wai Mun, Mohammad Hafiz Syukri Kassim, Mahadimenakbar Mohd Dawood, Noor Haliza Hasan, Vijay Kumar Subbiah, Faisal Ali Anwarali Khan, and Jaya Seelan Sathiya Seelan. Fungi from dead arthropods and bats of Gomantong Cave, northern Borneo, Sabah (Malaysia). *Journal of Cave and Karst Studies*, v. 82, no. 4, p. 261-275. DOI:10.4311/2019MB0146

FUNGI FROM DEAD ARTHROPODS AND BATS OF GOMANTONG CAVE, NORTHERN BORNEO, SABAH (MALAYSIA)

Ibrahem G. Wasti[1,2], Foo She Fui[1], Tan Qin Zhi[1], Cheh Wai Mun[1], Mohammad Hafiz Syukri Kassim[1], Mahadimenakbar Mohd Dawood[1], Noor Haliza Hasan[1], Vijay Kumar Subbiah[3], Faisal Ali Anwarali Khan[4], and Jaya Seelan Sathiya Seelan[1, C]

Abstract

Borneo is a biodiversity and ecotourism hotspot, yet one of its least-studied ecosystems is their limestone caves. Not many studies have been conducted on the role fungi play in tropical cave ecosystems, and no fungal surveys have been conducted in the caves of Sabah, Malaysia. Here, we assess the mycofloral diversity on bat and arthropod cadavers in one of the most popular ecotourism destinations of northern Borneo, Gomantong caves. Opportunistic sampling of cadavers within the Semud Hitam chamber of Gomantong cave yielded nine dead arthropods and four dead bats. Twenty-four culturable fungi were isolated, of which 14 morphological taxonomic units (MTU) were observed. Twelve of the 14 MTUs underwent molecular characterization of the ITS gene region to confirm identification. All fungi were Ascomycetes except for one Basidiomycete isolate. *Aspergillus* spp. had the highest occurrence (45.8%), followed by *Penicillium* spp. (25.0%), and *Fusarium* sp. (12.5%). *Ceratobasidium* sp., *Diaporthe* sp., *Pestalotiopsis* sp., and *Xylaria feejeensis* were isolated once each. No more than one fungal taxon was isolated from each arthropod cadaver, and not all arthropods yielded culturable fungi. Bat cadavers yielded 14 out of 24 isolates (58.3%), with the highest occurrence of the fungi sampled from their skin. Our results corroborate that bats and arthropods play a role in fungal dispersion and introduction in the cave because their exteriors are likely to harbor fungi they are exposed to in the environment. We also conclude that cadavers are important substrates for fungal growth and proliferation, perpetuating the role of fungi as important decomposers in caves. This study provides a baseline of information of the mycobiome of Bornean caves for future bioprospecting and potential biotechnological applications.

INTRODUCTION

The fungal diversity in caves and their influence on cave ecosystems have yet to be explored in Borneo. Many organisms cannot sustain themselves within the dark, cool, and nutrient-limited cave environment (Gunde-Cimerman et al., 1998). Despite this, fungi are one of the most dominant of all cave organisms with high rates of spore dissemination, colonization capability in various types of substrates, and tolerance to a wide range of pH values (Nováková, 2009; Bastian et al., 2010; Wang et al., 2010; Ogórek et al., 2013; Vanderwolf et al., 2013a). Over 1000 species of fungi stemming from over 500 genera have been found from caves throughout the world (Vanderwolf et al., 2013a). Most cave fungi are Ascomycetes, but Basidiomycetes and Zygomycetes are also found at lesser rates. Many of the fungi within caves are saprophytes that have been isolated from non-cave environments. While many cave-dwelling organisms are true troglobites, very few fungi are specialized in the cave ecosystem. It is the most likely scenario that cave fungi originated from environments external to caves (Zhang et al., 2017).

Fungi are known to interact with a wide array of organisms and play an important role in the greater ecosystems they are a part of, whether as symbionts, parasites, saprophytes, or a food source (Bastian et al., 2010; Arouja and Hughes, 2016). In wild animals, fungi found on ears, lungs, intestines, bladder, kidney, animal dung, brain, and skin may lead to fungal infections (Ainsworth and Austwick, 1955; Seelan et al., 2008, 2009). A few fungal species cause diseases in mammals because their high body temperatures promote fungal growth (Bergman and Casadevall, 2010; Garcia-Solache and Casadevall, 2010). For example, white-nose syndrome (WNS) is a disease that affects hibernating bats. It is caused by a visible white fungus, *Pseudogymnoascus destructans*, that grows on bats' muzzles and wings and has killed millions of bats in North America (Blehert et al., 2009; Lorch et al., 2011; Warnecke et al., 2012). Many non-pathogenic keratinophillic fungi can survive on animal fur, possibly due to less competition from soil fungi with higher saprophytic ability (Rees, 1967). Keratinophillic fungi have been isolated from animals such as cats, dogs, cows, rabbits, horses, rats, and donkeys (Aho, 1983; Bagy, 1986; Ali-Shtayeh et al., 1988). The most common fungi isolated from animals include *Aspergillus* spp., *Penicillium* spp., *Cladosporium* spp. and *Mucor* spp. (Aho, 1983; Ali-Shtayeh et al., 1988). Fungal dermatophytes have also been isolated from domestic animals, namely *Trichophyton* spp. and

[1] Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah, 88400 Jalan UMS, Kota Kinabalu, Sabah, Malaysia
[2] Faculty of Natural Science and Sustainability, University College Sabah Foundation, Jalan Sanzac, Sembulan, 88100 Kota Kinabalu, Sabah, Malaysia
[3] Biotechnology Research Institute, Universiti Malaysia Sabah, 88400 Jalan UMS, Kota Kinabalu, Sabah, Malaysia
[4] Faculty of Resource Science and Technology, Universiti Malaysia Sarawak, 94300 Kota Samarahan, Sarawak, Malaysia
[C]Corresponding author: seelan80@ums.edu.my

Microsporum spp., which shows that they are important in the transmission of disease-causing fungi (Ali-Shtayeh et al., 1988). Some keratinophilic fungal species isolated from domestic animals are pathogenic to humans and animals, namely *A. fumigatus*, *Stachybotrys chartarum*, *Scopulariopsis brevicaulis*, and *Cephalosporium acremonium* (Bagy, 1986). Isolation of fungi from animals, especially those from biodiversity-rich ecosystems like the tropics, may lead to the important discovery of novel bioactive compounds (Higginbotham et al., 2014). They showed that fungi isolated from sloth hair have anti-malarial, anti-bacterial, and anti-cancer bioactivity.

Although previous studies on the fungal biomes of bats in Borneo have been conducted, they were not done on bats that were captured in or near a cave environment (Seelan et al., 2008, 2009). Around the world, a plethora of studies on cave fungi from a variety of different substrates have been conducted, including bats, bat guano, invertebrates, soil, rocks, walls, water, and air (Vanderwolf et al., 2013a). In another study, thirty bats from four caves and one mine in the United States yielded 182 fungal isolates (Johnson et al., 2013). These fungi were mainly from the division Ascomycota, while Basidiomycota only made up 14% of the isolates. The most common genera isolated from the bat wings were *Cladosporium*, *Fusarium*, *Mortierella*, and *Penicillium*. A study on cave walls, ceilings, and sediment from six caves led to the discovery of 675 fungal isolates composed mainly of Ascomycota, suggesting that common cosmopolitan ascomycetes will likely dominate studies that utilize culture-dependent methods (Zhang et al., 2014). While different hosts and substrates in caves result in varying assemblages of fungi, it has been suggested that the specific environmental characteristics of the cave itself plays a significant role on the type of fungi isolated (Johnson et al., 2013; Vanderwolf et al., 2016a).

Arthropod-associated fungi and entomopathogenic fungi have been isolated from caves from many regions of the world (Gunde-Cimerman et al., 1998; Santamaria and Faille, 2007; Jurado et al., 2008; Yoder et al., 2009; Polovinko et al., 2010; Bastian et al., 2010; Porca et al., 2011; Vanderwolf et al., 2016a). Cave invertebrates can thrive in the nutrient-limited cave environment because they are able to utilize a broad range of food sources for sustenance (Smrž et al., 2015). Fungal conidia can act as a food source for cave insects, as demonstrated with *Folsomia candida* (Smrž et al., 2015). Cave isopods were shown to prefer saprophytic fungi growing on bat guano as one of their food sources because it is a source of polyunsaturated fatty acids, an essential nutrient. A world review of cave fungi showed that 201 species of fungi from 89 genera had been isolated from arthropods (Vanderwolf et al., 2013a). Most of these were Ascomycetes and Zygomycetes. Many entomopathogenic fungi in caves are specialized to infect specific hosts. For example, *Rhachomyces* spp. are infectious to carabid beetles that are highly specialized to the cave environment (Santamaria and Faille, 2007). Cave entomopathogenic fungi may also be generalists by nature. For example, the known generalist insect pathogen, *Beauveria bassiana,* was isolated from multiple dead insects in the caves of West Siberia and made up 68% of all isolates (Polovinko et al. 2010). Furthermore, arthropods may carry fungal spores that are pathogenic to other cave fauna (Vanderwolf et al., 2016b). Despite the importance of cave fungal studies that have been conducted on all the major continents of the world, none have been conducted in Malaysian Borneo (Vanderwolf et al., 2013a).

The Gomantong cave system (5°31'30"N 118°04'15"E) is located in the 3,297 hectare Gomantong Forest Reserve, Kinabatangan, Sabah (North Borneo), and it is part of the largest limestone outcrop in the area, Gomantong Hill (Lundberg and McFarlane, 2012). Kinabatangan is known for its rich biodiversity, where at least 51 mammal species, including 10 primate species, have been recorded in the area (Boonratana and Sharma, 1997). The area surrounding the forest reserves are almost exclusively utilized for the monocrop production of palm oil. The cave itself is famous for its swiftlet nest farming and birds nest harvesting (Ismail, 1999; Hobbs, 2004; Lundberg and McFarlane, 2012). Since 2012, Gomantong caves get around 13,000 to 15,000 thousand visitors annually, mostly composed of foreigners, according to the Sabah Wildlife Department. At least 13 species of bats have been recorded from this cave, including some of the common *Hipposideros* spp., *Rhinolopus* spp., and *Myotis gomantongensis* (Abdullah et al., 2007). So far, there has not been any entomological or mycological survey studies conducted in Gomantong cave.

With the development of species barcodes in ecological studies, there are now large amounts of phylogenetic data on species isolated in caves (Woese et al., 1990; Barton et al., 2004; Lahaye et al., 2008). However, no such studies on cave mycota have been published on the caves of Sabah. With the help of DNA barcoding, establishing baseline ecological data for fungal species in areas where these types of studies are scant increases the possibility of discovering novel fungi.

MATERIALS AND METHODS

Site Description

Semud Hitam, Gomantong cave was visited twice, on October 6, 2017 and January 23, 2018 (Fig. 1). The cave is composed of two main sections, Semud Hitam (Black Cave) and Semud Putih (White Cave). Birds nest harvesting apparatus are visible throughout the cave, and the harvesting itself is done by employees of the municipality (Lundberg

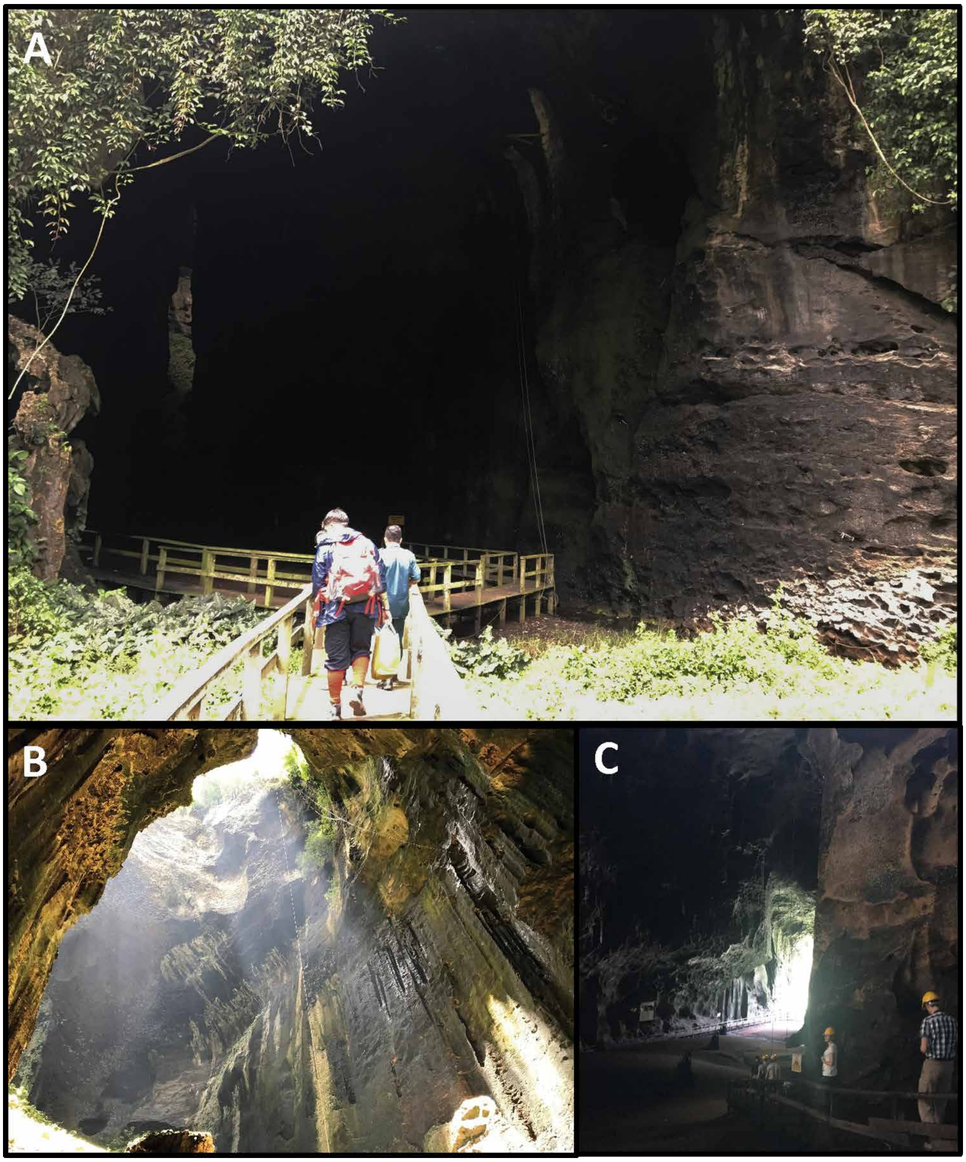

Figure 1. A. Cave entrance of Semud Hitam, Gomantong Cave. B. Ceiling opening at the back end of Semud Hitam. C. Foreign tourists on boardwalk passage in the cave. Note that the boardwalk was built for ease of access for people as piles of bat guano and running water streams cover the cave floor.

and McFarlane, 2012). Because Semud Hitam is easily accessible by tourists due to its boardwalk, it is by far the most visited part of the cave (Fig. 2). Some sections of the cave have their walls written on or vandalized by visitors. Hundreds of bats are visible in the ceiling of the cave. A strong pungent smell of ammonia is always present due to the floor of the cave being covered in heaps of bat guano. A diverse array of invertebrates is found throughout the cave on guano piles, walls, ceilings, and even the walkway itself (Lundberg and McFarlane, 2012). At the end of the boardwalk, a large portion of the cave is exposed to the canopy and allows sunlight into the whole back end of the cave. In this back section of the cave, past the boardwalk, there are patches of grassy areas with a plethora of organic litter. Cave temperature and relative humidity were measured during both visits in the cave light zone, twilight zone, and dark zone (Table 1).

Sampling

Opportunistic sampling of bat and arthropod cadavers was undertaken and their positions within the cave were recorded (Fig. 3). All of the insect cadavers were collected aseptically with sterilized tweezers and sealed within sterile centrifuge tubes. The distance from the cave entrance and light zone of each sample were also recorded during sampling (Table 2). The arthropod cadavers were stored in a cooler filled with ice until transportation back to the laboratory where samples were stored at 4 °C. Bat cadavers were in the early stages of decomposition and had no obvious signs of fungal growth. They were identified on site and subsequently swabbed using sterile cotton swabs on five different body parts (i.e. anus, ear, skin, mouth, and hair). The swabs were inoculated into sterile centrifuge tubes containing 900 µL PBS buffer until further processing.

Fungal Isolation

In the laboratory, arthropod cadavers were identified to at least the genus level by using dichotomous keys (Imes, 1992; Chinery, 2005). Any hyphae visibly growing from the cadaver were inoculated onto Potato Dextrose Agar (PDA) incorporated with the antibiotic streptomycin sulfate (40 µg/mL). Isolations were performed in triplicate using the three-point method until pure isolates were produced. Bat cadaver swab samples were serially diluted 10-fold up to 10^{-4}. Of the 10^{-2} to 10^{-4} dilutions, 0.1 mL aliquots were spread on the PDA plates. Dilutions were performed in triplicate using sterile distilled water. All inoculated plates were incubated for 3–7 days at room temperature (25 °C) and in the dark. Isolates were grouped into morphological taxonomic units (MTU) based on the colony morphology and micro-morphological characteristics, i.e. colony color, texture, growth patterns, hyphae, conidia size and shape, and conidiophores.

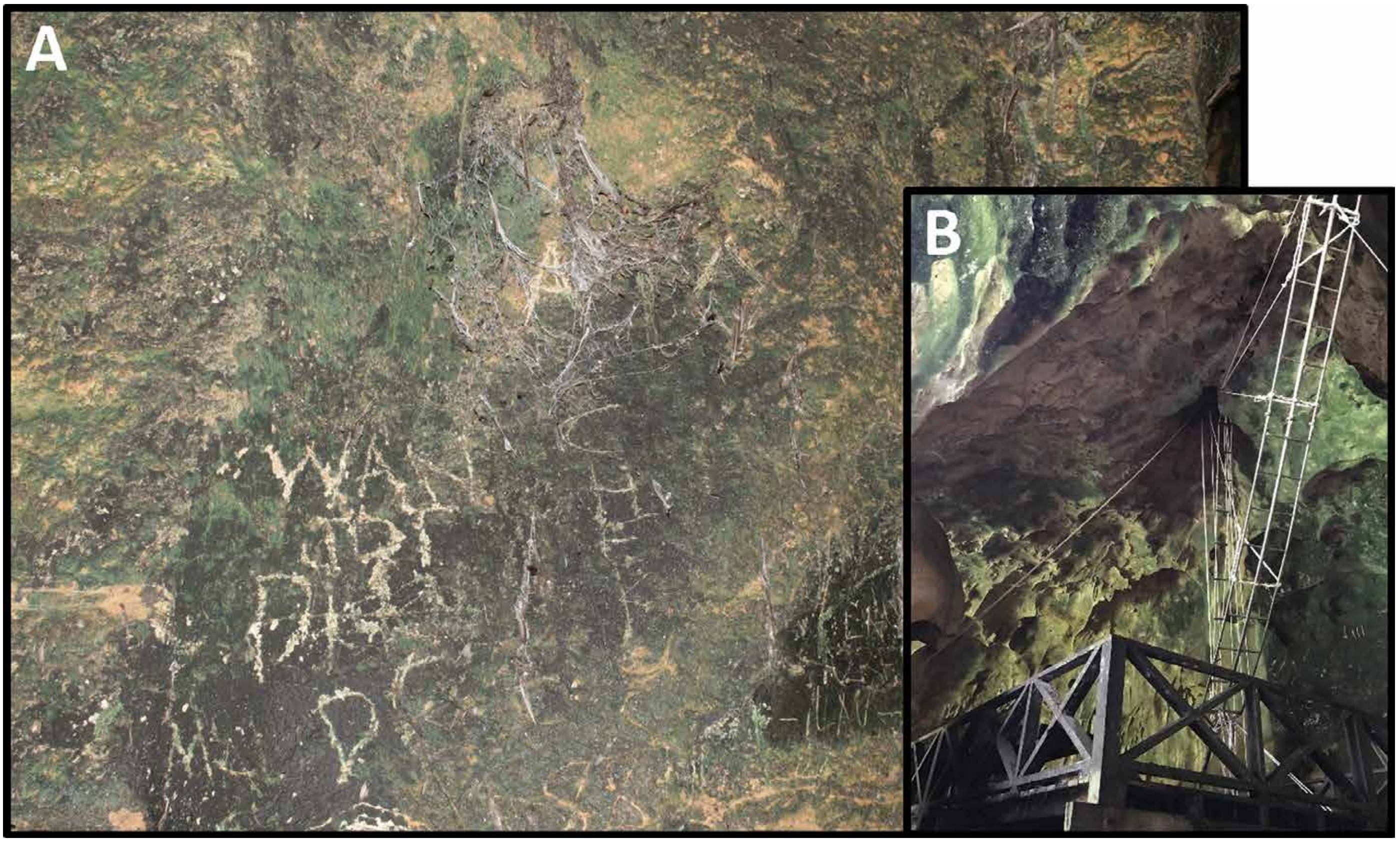

Figure 2. A. Vandalism on the cave wall by tourists. B. One of the swiftlet nest harvesting apparatus. Care takers of the swiftlet nest farms reside in shacks immediately outside the cave.

Table 1. Temperature and relative humidity in Gomantong cave. The approximate distance from cave entrance and light zone were also recorded.

Date	Light Zone			Twilight Zone			Dark Zone		
	Temp, °C	RH, %	Dist., m	Temp, °C	RH, %	Dist., m	Temp, °C	RH, %	Dist., m
Oct 6, 2017	29	93	5	26	100	35	26	100	70
Jan 23, 2018	30	92	5	29	92	35	27	100	70

Identifications were carried out by comparing the morphological characteristics of the fungi to universal identification keys described by Raper and Fennell (1965), Klich (2002), and Domsch et al. (2007).

PCR Amplification and Sequencing

Molecular identification was performed on all arthropod cadaver fungi isolates and on six bat cadaver fungi isolates. The E.Z.N.A DNA Fungal Kit (Omega Bio-Tek, USA) was used to extract DNA from pure cultures of isolates according to the manufacturer's instruction. The 5.8S sequences were amplified with primers ITS1 (5'-TCC GTA GGT GAA CCT GCG G-3') and ITS4 (5'-TCC TCC GCT TAT TGA TAT TGA TAT GC-3') (White et al.; 1990) (Vilgalys Mycology Lab). PCR amplification was carried out in a total volume of 50 µL with the following reagents from Promega (USA): 2 µL of each primer (10 pmol/**µ**L), 0.25 µL *Taq* DNA polymerase (5 units/µL**)**, 10 µL PCR Buffer (5X), 4 µL $MgCl_2$ (25mM), 1 µL dNTPmix (10mM), 2 µL DNA template (~25 ng/mL). PCR cycles were performed in a Bio Rad T100 Thermal Cycler. For amplification, the conditions were 95 °C for 3 min of the initial denaturation, followed by 35 cycles of 94 °C for 30 s of denaturation, 53 °C for 30 s of annealing, and 72 °C for 1 min of extension. A final extension of 72 °C for 10 min was added to complete the process. The PCR products were then electrophoresed in a 1 % agarose gel for 30 mins and subsequently stained with gel red for visualization. Next, the PCR products were purified using Column-Pure PCR Clean-Up Kit (Applied Biological Materials, Inc., Richmond, BC) according to the protocol of the manufacturer. DNA sequencing was performed using the BigDye Terminator v3.1 on a ABI3500 sequencer (Applied Biosystems). Sequencing service was provided by MyTACG (Taiwan). The ITS forward and reverse primers were used in the cycle sequencing. The resulting reads were aligned to obtain the full-length amplicon sequence (BioEdit version 7.0.5.3) and submitted to GenBank. Once the sequencing was completed, the ITS barcode sequences generated from all isolates were queried against NCBI nucleotide sequence using basic local alignment search tool (BLASTn) to ascertain their closest relationships (Zhang et al., 2000).

RESULTS

Fungi are prevalent in the cave environment, and cave fauna, wind, water, and humans all play important roles in spore dispersion and translocation in and out of the cave. In the current study, a total of 24 axenic fungal isolates were obtained from six out of nine arthropod cadavers and all four bat cadavers sampled (Table 2). Ten pure cultures were isolated from arthropod cadavers and 14 from bat cadavers (Table 3). Isolated fungi were separated

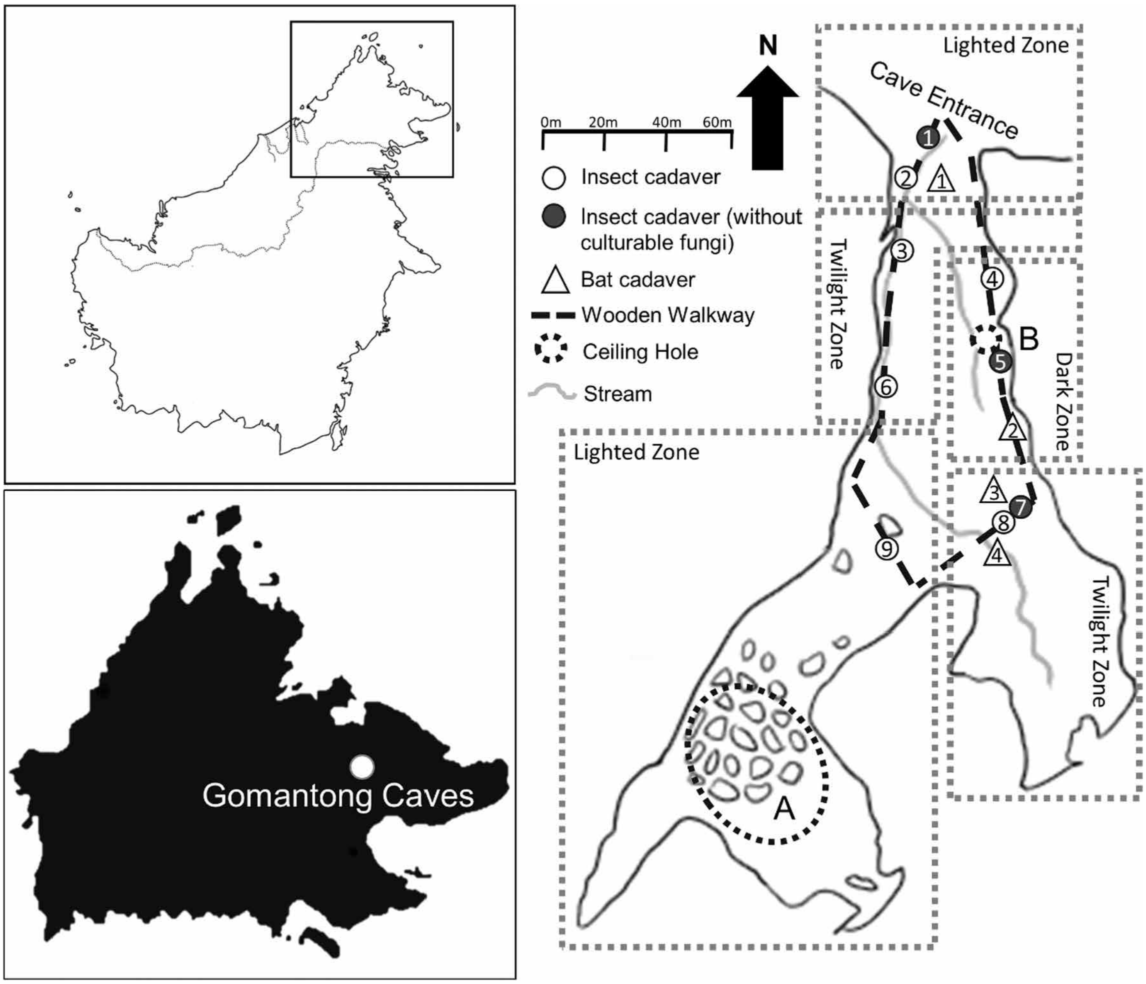

Figure 3. Layout of Semud Hitam, Gomantong Cave, Sukau, Sabah Malaysia. The cave entrance is located in the north end of the cave. The cave is accessible via a wooden walkway that was built for ease of access for tourists and swiftlet nest farmers. The tall mouth of the cave entrance and the presence of two large ceiling apertures (A and B) make the cave relatively well-lit during the afternoon hours. There are areas that do not receive direct sunlight and other areas that are completely dark hidden between walls, nooks, and crevices. All 14 cadavers were collected and their relative distances from the cave entrance measured and labelled by light zone.

Table 2. Distance of arthropod and bat cadaver samples from the entrance and number of fungal isolates obtained.

Light Zone	Sample ID	Sample species	Distance from Entrance, m	No. of Fungal Isolates
Lighted	A01	*Periplaneta americana*	8	0
	A02	*Thereuopoda* sp.	20	2
	A09	*Periplaneta americana*	108	1
	B01	*Cynopterus brachyotis*	19	8
Twilight	A03	*Periplaneta americana*	28	2
	A06	*Periplaneta americana*	63	1
	A07	*Periplaneta americana*	91	0
	A08	*Trigonilus corallinus*	93	1
	B03	*Balionycteris maculata*	85	2
	B04	*Chaerephon plicatus*	94	1
Dark	A04	*Trigonilus corallinus*	33	3
	A05	*Thereuopoda* sp.	45	0
	B02	*Hipposideros diadema*	76	3

Table 3. Fungi isolated per insect and bat cadaver species. Body parts sources are listed for bat cadaver hosts.

Host Species	Fungal Species	Source	No. of isolates
Arthropod			
Periplaneta americana	*Aspergillus flavus*	Wings	1
	Aspergillus luteovirescens	Abdomen	1
	Ceratobasidium sp.	Thorax	1
	Fusarium solani	Thorax	1
Thereuopoda sp.	*Fusarium solani*	Abdomen	1
	Penicillium sclerotiorum	Abdomen	1
Trigonilus corallinus	*Aspergillus sclerotiorum*	Body	1
	Diaporthe sp.	Body	1
	Fusarium solani	Body	1
	Penicillium shearii	Head	1
Bat			
Balionycteris maculata	*Aspergillus restrictus*	Skin, Hair	2
Cynopterus brachyotis	*Aspergillus flavus*	Skin	1
	Aspergillus ochraceus	Ear	1
	Aspergillus restrictus	Anal, Skin, Ear	3
	Penicillium citrinum	Skin, Oral	2
	Pestalotiopsis sp.	Oral	1
Hipposideros diadema	*Aspergillus restrictus*	Hair	1
	Penicillium paxilli	Skin, Hair	2
Chaerephon plicatus	*Xylaria feejeensis*	Oral	1

Table 4. Molecular characterization based on ITS barcode similarities to the NCBI database.

Isolate	NCBI Identification	E value	Identification, %	Host Species
GMT01	*Fusarium solani*	0.0	97.4	*Thereupoda* sp. (A02)
GMT02	*Penicillum sclerotiorum*	0.0	98.2	*Thereupoda* sp. (A02)
GMT03	*Fusarium solani*	0.0	99.8	*Periplaneta americana* (A03)
GMT04	*Ceratobasidium* sp.	0.0	96.2	*Periplaneta americana* (A03)
GMT05	*Diaporthe* sp.	0.0	98.8	*Trigonilus corallinus* (A04)
GMT06	*Aspergillus sclerotiorum*	0.0	100	*Trigonilus corallinus* (A04)
GMT07	*Fusarium solani*	0.0	99.0	*Trigonilus corallinus* (A04)
GMT08	*Aspergillus luteovirescens*	0.0	99.8	*Periplaneta americana* (A06)
GMT09	*Penicillium shearii*	0.0	99.7	*Trigonilus corallinus* (A08)
GMT10	*Aspergillus flavus*	0.0	100	*Periplaneta americana* (A09)
GMC05	*Penicillium paxilli*	0.0	100	*Hipposideros diadema* (B02)
GMC06	*Penicillium paxilli*	0.0	100	*Hipposideros diadema* (B02)
GMC09	*Xylaria feejeensis*	0.0	99.7	*Chaerephon plicatus* (B04)
GMC10	*Penicillium citrinum*	0.0	99.5	*Cynopterus brachyotis* (B01)
GMC14	*Aspergillus flavus*	0.0	100	*Cynopterus brachyotis* (B01)
GMC15	*Penicillium citrinum*	0.0	100	*Cynopterus brachyotis* (B01)

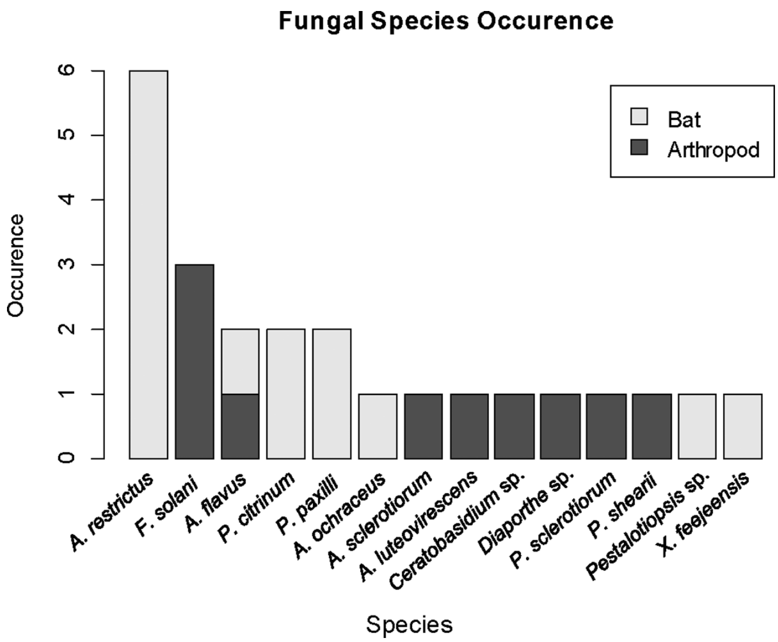

Figure 4. Fungal species occurrence from bat and arthropod cadavers in Gomantong Cave. *A. restrictus* was the mode (n=6), followed by *F. solani* (n=3).

into 14 MTUs based on their macro- and micromorphology. Sixteen out of the 24 pure isolates underwent molecular characterization. Molecular analysis was prioritized for isolates that could not be identified solely on morphology and to corroborate identification of cryptic taxa at the species level. The ITS barcode PCR amplicons were about 600 bp in size for all of the 16 isolates. After DNA sequencing, the BLASTn data from the 16 isolates resulted in 11 operational taxonomic units (OTU) (Table 4). The results indicated that all OTUs belonged to the division Ascomycota except for *Ceratobasidium* sp., from the division Basidiomycota. The majority of the isolated strains belonged to the order Eurotiales and the family Aspergillaceae. For the *Ceratobasidium* sp. and *Diaporthe* sp. isolates reported in this study, ITS gene sequences were not enough to differentiate the isolates up to the species level. The fungal genera isolated were *Aspergillus* (45.8%), *Penicillium* (25.0%), *Fusarium* (12.5%), *Ceratobasidium* (4.2%), *Diaporthe* (4.2%), *Pestalotiopsis* (4.2%), and *Xylaria* (4.2%). *Aspergillus restrictus* was isolated a total of six times, accounting for 25% of all isolates (Fig, 4).

We observed that different species of fungi were present on different species of arthropods (Figs. 5 and 6). Four species of fungi were isolated from *Periplaneta americana* (Cockroach) and *Trigonilus corallinus* (Asian/Rusty millipede) cadavers. *Thereuopoda* sp. (Gantcave/Cave centipede) recorded only two species of fungal isolates.

Four different species of bat cadavers were collected during sampling for this study. *Cynopterus brachyotis* (Lesser short-nosed fruit bat) yielded five species of fungi, which was the most for any one sample in this study. Two species of fungi were isolated from *Hipposideros diadema* (Diadem leaf-nosed bat). Only one species of fungi was isolated from each *Balionycteris maculata* (Spotted-winged fruit bat) and *Chaerephon plicatus* (Wrinkle-lipped free-tailed bat) cadaver. The bats' skin had the highest di-

Figure 5. Dead arthropod samples in order of distance from entrance. A. A01, *P. americana*. B. A02, *Thereuopoda* sp.. C. A03, *P. americana*. D. A04, *T. corallinus*. E. A05, *Thereuopoda* sp.. F. A06, *P. americana*. G. A07, *P. americana*. H. A08, *T. corallinus*. I. A09, *P. americana*.

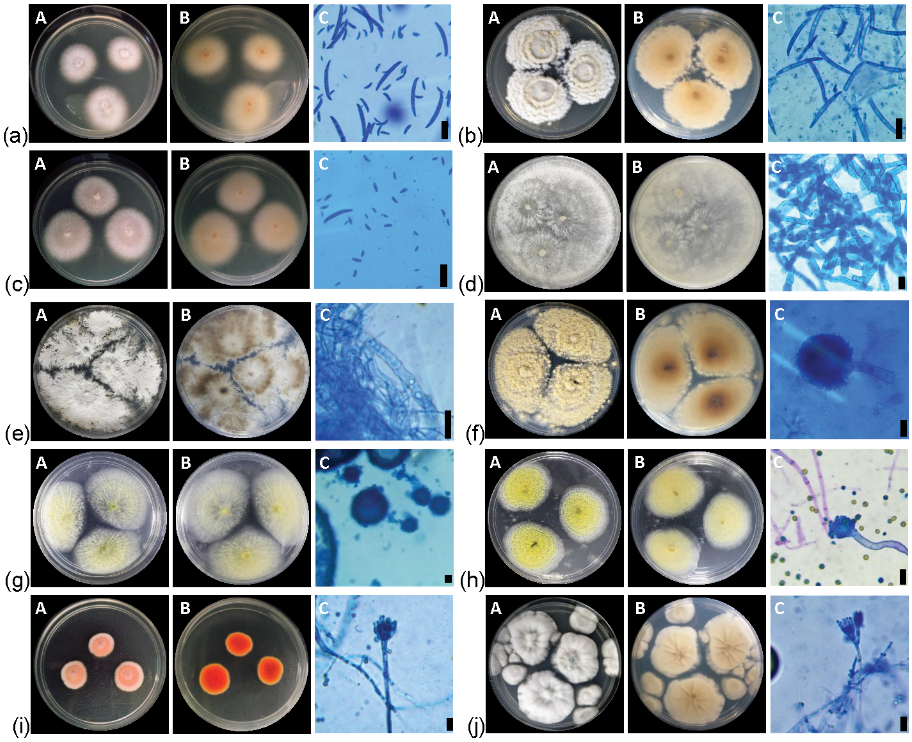

Figure 6. (a) *Fusarium solani* GMT01; (b) GMT03; (c) GMT07; (d) *Ceratobasidium* sp. GMT04; (e) *Diaporthe* sp. GMT05; (f) *Aspergillus sclerotiorum* GMT06; (g) *Aspergillus terricola* GMT08; (h) *Aspergillus flavus* GMT10; (i) *Penicillium sclerotiorum* GMT02; (j) *Penicillium shearii* GMT09. A. Colony surface on PDA. B. Colony reverse on PDA. C. Micromorphological characteristics (*i.e.* conidia, conidiophores, hyphae). Scale = 10 µm.

versity and occurrence for any of the body parts with four species of fungi from five isolates (Fig. 7). The different fungal isolates from all bat cadavers are shown (Fig. 8).

DISCUSSION

Ours is the first study that describes the occurrence of fungi in a cave from Sabah, Malaysia. Fourteen fungal isolates were found on bat cadavers and ten fungal isolates from arthropod cadavers in this study. Both the bat and arthropod cadavers had similar fungal diversity, each yielding eight different species of fungi. Twenty-three of the 24 fungal isolates found in the study were ascomycetes, the remaining one being *Ceratobasidium* sp., a basidiomycete. The most frequently isolated fungal genera in this study were *Aspergillus*, *Penicillium*, and *Fusarium*, which are ubiquitous in non-cave environments (Domsch et al., 2007). Findings from this study are congruent with previous reports where the Ascomycota, especially fungi from genera *Aspergillus*, *Fusarium*, and *Penicillium*, are the dominant division of fungi isolated from cave environment (Nováková, 2009; Voyron et al., 2011, Vanderwolf et al., 2013a). A study conducted previously on cadavers and skeletons of various fauna from cave and mine environments found that 12 out of 39 fungal isolates were identified as Mucoromycota (Nováková et al., 2018). *Mucor* is considered as one of the dominant genera of fungi isolated from cadavers throughout the various stages of decomposition along with *Aspergillus*, *Penicillium*, and *Candida* (Sidrim et al., 2009). The lack of Mucoromycota in this study may be explained by difference in media type used in our study compared to theirs. Using exclusively PDA and MEA during incubation and isolation in this study likely gave preference for rapidly growing ubiquitous fungi, especially *Aspergillus* and *Penicillium*.

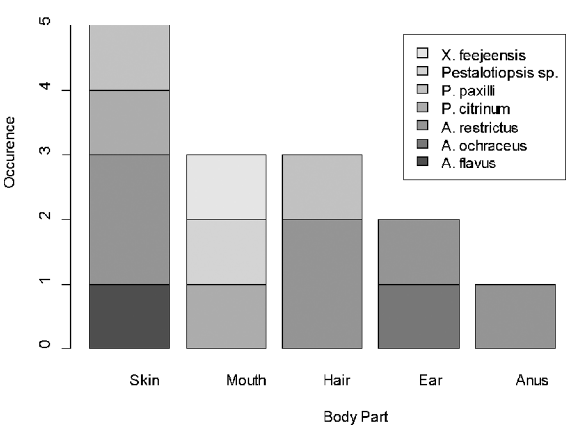

Figure 7. Number of fungal isolates per body part. The skin of bats had the highest fungal isolates (n=5, 4 species), and anus had the lowest (n=1, 1 species).

Most studies on cave fungi utilize culture-dependent methods of isolation before proceeding to morphological or molecular characterization (Vanderwolf et al., 2013a; Man et al., 2015; Zhang et al., 2017; Nováková et al., 2018; Visagie et al., 2019). Because of limited finances, manpower, and time, this initial study exclusively utilized culture-based methods of isolation. Culture-independent community-based studies on cave fungi are scant, but they have been conducted in recent years (Zhang et al., 2014; Zhang and Cai, 2019). Culture-based methods tend to produce results that over-represent rapidly growing cosmopolitan fungi. Whether fastidious fungi will grow and be observed in culture is heavily influenced by temperature, length of incubation, type of medium used, and aerobic conditions (Bills, 1995; Collado et al., 2007; Unterseher and Schnittler, 2009; Tristan et al., 2012). Culture-dependent methods are known to only reveal around 0.6−8.0% of total the total fungal species in a sample (Hibbett et al., 2009; Hawksworth and Lucking, 2017) so culture-dependent methods are greatly limiting and hinder our understanding of the overall role fungi play in the ecosystems they inhabit. Community based culture-independent methods can generate millions of raw sequences at a time, yielding in the hundreds to thousands of fungal OTUs per sample (Winter et al., 2017; Zhang and Cai, 2019). On the other hand, Zhang and Cai (2019) reported that culture-dependent and culture-independent methods tend to show similar fungal diversity, but culture-independent methods are more likely to find uncommon fungi in their re-

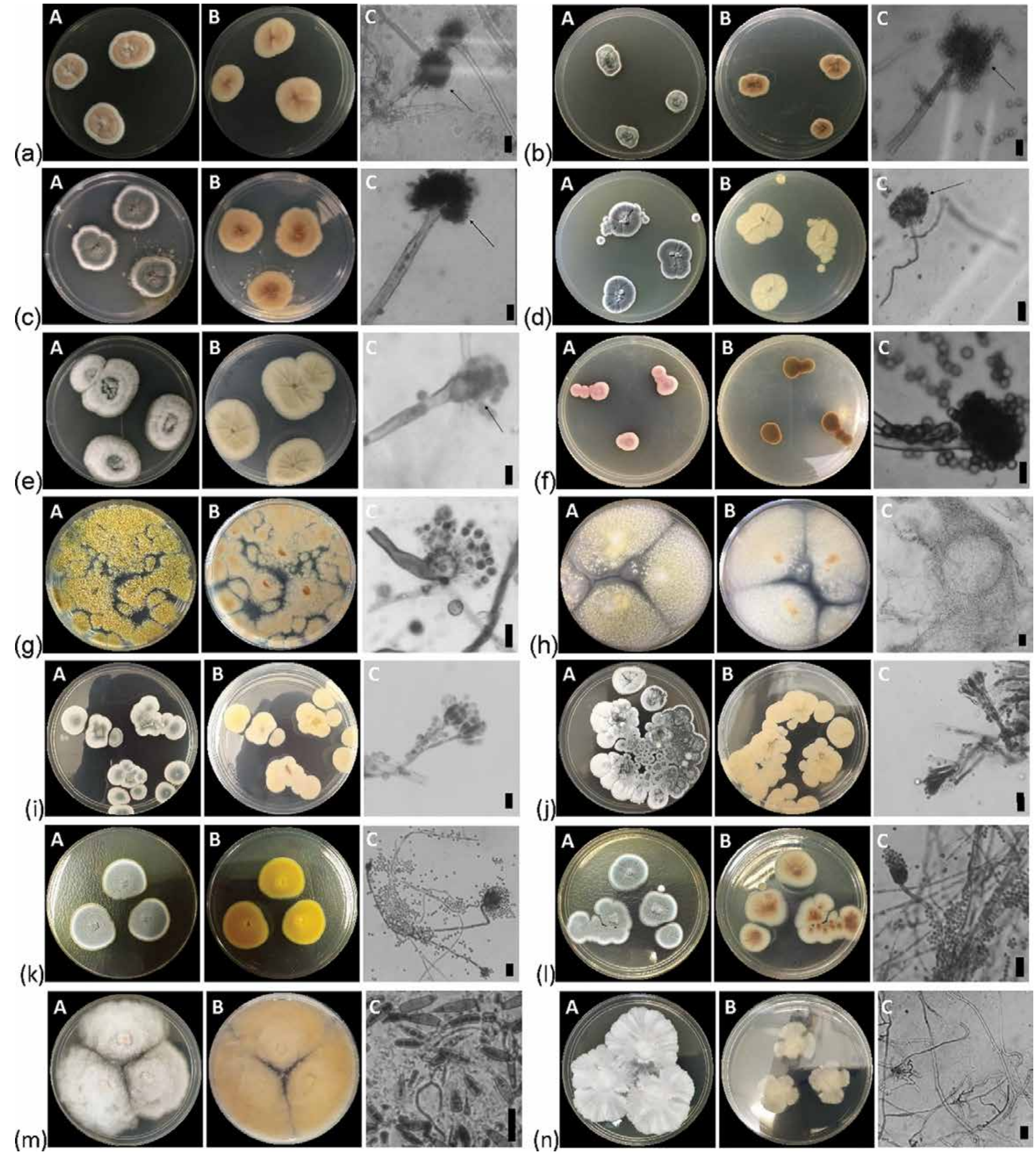

Figure 8. (a) *Aspergillus restrictus* GMC01; (b) GMC02; (c) GMC03; (d) GMC06; (e) GMC07; (f) GMC11; (g) *Aspergillus flavus* GMc13; (h) *Aspergillus ochraceus* GMC12; (i) *Penicillium citrinum* GMC09; (j) GMC14; (k) *Penicillium paxilli* GMC04; (l) GMC05; (m) *Pestalotiopsis* sp. GMC10; (n) *Xylaria feejeensis* GMC08. A. Colony surface on PDA. B. Colony reverse on PDA. C. Micromorphological characteristics (*i.e.* conidia, conidiophores, hyphae). Scale = 10 µm.

spective substrates. They also found that around 3.6−12.0% of the total OTUs in each respective cave were exclusive to that cave only, although most of these OTUs were unidentified to the genus level. Thus, fungal studies utilizing culture-based methods are still primary and will remain necessary as long as they are required to describe new strains and species, in addition to being cost-effective and more readily available.

Bats can travel faster and traverse larger distances than arthropods, which may account for the higher number of fungal isolates as seen in this study. In this study, six out of the nine fungal species isolated from bats (71.4% of isolates) were isolated from the two frugivorous bat hosts compared to the two insectivorous ones. Although our sample size is not large enough to statistically conclude which group of bats are more efficient as fungal carriers and dispersers, our current data shows that frugivorous bats carried a higher fungal load than insectivorous bats. In a previous study in Sarawak-Borneo, 17 *Aspergillus* spp. from six species of bats, namely *Aspergillus restrictus*, *A. sydowii*, *A. niger*, *A. clavatus*, and *A. japonicus* were recorded (Seelan et al., 2008). They reported that the anus and ear yielded the largest number of fungal isolates and showed high fungal diversity. It was noted that various substrate types in wild animal populations resulted in various types of mycoflora, and that the mycoflora found on bats are highly correlated to the food consumed (higher in fruit-eating bats than insect-eating bats) and their roosting site (Seelan et al., 2008). Although fungi and fungal spores exist throughout the cave environment, bats are likely key regulators for the mycoflora of caves, as they are the key transporters in and out of the caves and contribute to guano and carcass deposition (Vanderwolf et al., 2013a, 2013b).

Most studies on bat mycoflora have focused on bat fur (Larcher et al., 2003; Beguin et al., 2005) and *P. destructans* related surveys (Blehert et al., 2008; Gargas et al., 2009; Puechmaille et al., 2011; Johnson et al., 2013; Zukal et al., 2014). Although there have been bat cadavers sampled for fungal isolation in these studies, none have specifically studied fungal diversity exclusively on cadavers until Nováková et al. (2018). Prior to our study, 67 species of fungi have been reported from bat cadavers found in caves throughout the world, with *Crysosporium merdarium* bring the most frequently isolated (Nováková et al., 2018). Only five other studies had reported fungi from bat cadavers in caves (Zeller, 1996; Wibbelt et al., 2010; Voyron et al., 2011; Vanderwolf et al., 2013b, 2016a, Nováková, 2018). None of the eight fungal species identified from bat cadavers in our study were reported in prior studies on cadavers in caves, and thus are reported for the first time here. None of them were in the genus *Chrysosporium*, although this is not surprising as fungi from this genus were previously isolated from long-dead decomposed bats.

The bat cadavers in our study were in the early stages of decomposition and were still identifiable morphologically *in situ*. Nováková et al. (2018) sampled cadavers from both early and late stages of decomposition, even carcasses of only fur and bone, which may have also contributed to the difference in mycobiome from bat cadavers in both studies. Because fungal growth rates are faster on dead bats than live bats, the increased number of bat cadavers in a cave will likely affect fungal diversity found on live bats in the same cave especially after a mass-mortality event (Vanderwolf et al., 2016a).

Along with bats, studies on the insect diversity of Gomantong cave remain scant, with no published reports on arthropod-associated fungi or entomopathogenic fungi in Gomantong cave. The presence of fungi is expected on cave arthropods because many fungi are entomophilous, entomogenous, or entomopathogenic (Ogórek et al., 2013). The arthropods collected in our study were not commonly sampled in previous cave studies, and all three different species of arthropods yielded culturable fungi. Various cave butterflies, crickets, diplopods, harvestmen, moths, mites, and spiders have been previously cultured for fungi, none of which were sampled in our study (Kubátová and Dvorák, 2005, Vanderwolf et al., 2016b; Nováková et al, 2018). The genera *Aspergillus* and *Fusarium* tied for having the highest number of isolates for arthropod hosts (n = 3). But all three *Fusarium* isolates were identified as *F. solani*, whereas the three *Aspergillus* isolates were identified as the three different species, i.e. *A. flavus*, *A. luteovirescens*, and *A. sclerotiorum*. *Penicillium* was the only other genus isolated multiple times (n = 2). *Aspergillus*, *Fusarium*, and *Penicillium* are known to have entomogenous species recorded from previous surveys, and it is not surprising to find these taxa in our study (Jurado et al., 2008; Bastian et al., 2010; Vanderwolf et al., 2016b). Whenever *Aspergillus*, *Fusarium*, and *Penicillium* fungi are isolated from cave environmental samples, arthropods should always be considered as major vectors, dispersers, and hosts. One of the most frequent entomopathogenic fungi to be identified in prior cave studies, *Isaria furinosa*, was not isolated from any arthropod cadavers in this study (Kubátová and Dvorák, 2005). A reduction in the arthropod population in caves, naturally or artificially, could be a way to reduce fungal abundance in the cave or control fungal contamination to other areas within a cave system.

The different species of fungi documented on different arthropod cadavers may be due to differences in movement patterns, feeding location, diet, aggregation and interaction with other individuals, and other external factors. Insects are known to feed on fungi (Šustr et al., 2005; Jacobs et al., 2017). Guano, which is a known substrate of cave fungi, are also feeding grounds for mites that eat guano inhabiting bacteria and fungi (Smrž et al., 2015). Similar arthropods in Gomantong cave, where most of the cave floor is covered in heaps of guano, could be dispersing fungal spores throughout the cave unintentionally. Cockroaches are well known omnivorous scavengers and are likely feeders on sundry organic matter in guano heaps. These same cockroaches would be unintentionally inoculating fungi on bat guano within the cave, as well as dispersing fungal spores already proliferating on the guano to other areas of the cave that can act as substrates, i.e. dead wood (Marcot, 2017), sediment (Taylor et al., 2014), and cave walls (Bastian et al., 2009).

All 14 taxa of fungi isolated from this study have saprophytic properties. Eight of them have been isolated from cave environments prior to this study (Vanderwolf et al., 2013a). *A. luteovirescens*, *A. ochraceus*, *Diaporthe* sp., *P. sclerotiorum*, *P. shearii*, and *Xylaria feejeensis*, have not been isolated from the cave environment prior to this study. *Ceratobasidium* sp. and *Diaporthe* sp. were not able to be identified at the species level based solely on their ITS barcode. It is unlikely to get a good representation of the overall mycobiome of any cave by evaluating a limited type of substrate or host, i.e. only sampling cadavers. Microbial distribution in caves is heavily determined by the susceptibility of the host, bio-receptivity of the substrate, and environmental conditions (Cuezva et al., 2009; Jurado et al., 2010).

Aspergillus and *Penicillium* accounted for the highest proportion of fungal diversity in this study, constituting five and four different species out of a total of 14, respectively. They also constituted 17 of the 24 total isolates (70.8%). *Aspergillus restrictus* was isolated six times (25%) from three different species of bats. *Aspergillus flavus* was the only species isolated from a bat and an arthropod cadaver, *C. brachyotis* and *P. Americana*, respectively. *Aspergillus* spp. are commonly known saprophytes from soil and plant debris (Domsch et al., 2007). *Aspergillus* and *Penicillium* are the first and second most reported genera in cave mycological studies aside from the genera *Geomyces* and *Histoplasma*, which have a high occurrence due to the many studies focusing specifically on WNS and histoplasmosis, respectively (Vanderwolf et al, 2013a), which is consistent with the finding that *Aspergillus* spp. and *Penicillium* spp. are some of the most frequently-isolated fungi from cave soil, rocks, and bat guano (Lorch et al., 2012; Man et al., 2015). *Aspergillus* spp. and *Penicillium* spp. were some of the most commonly isolated fungi recovered from bat wings, fur, and skin in North American caves (Johnson et al., 2013; Vanderwolf et al., 2013b). In fact, a variety of *Aspergillus* and *Penicillium* species have been isolated from dead bats and arthropods in caves as well (Voyron et al., 2011; Nováková, 2018). The fungi identified from cadavers in this study are likely to be found growing on other environmental substrates within the cave, making it easy for the fungi to utilize the cadavers as a carbon source as soon as death occurs.

Fusarium solani (12.5%), *Ceratobasidium* sp. (4.2%), and *Diaporthe* sp. (4.2%), were isolated from arthropod cadavers and *Pestalotiopsis* sp. (4.2%) and *Xylaria feejeensis* (4.2%) were isolated from bat cadavers in this study. These taxa of fungi are known plant pathogens and plant endophytes, which suggests a strong influence of the surrounding plant diversity on the fungal communities in Gomantong cave. *Ceratobsidium* sp. is the only basidiomycete of all the isolates in this study. Basidiomycetes are less common in the cave environment, but they are the second most frequent division to be isolated (Vanderwolf et al., 2013a). All of these isolates have been reported in the cave environment prior to this study, except *Diaporthe* sp. and *X. feejeensis*, and thus, they are reported for the first time here (Vanderwolf et al., 2013a). *Xylaria* spp. are fast-growing fungi usually found in healthy plant tissue (Petrini and Petrini, 1985; Davis et al., 2003), and fungi from this genus have been isolated in the cave from guano, soil, and wood prior to this study (Vanderwolf et al., 2013a). *Fusarium solani* was the second most isolated taxa in this study, which is congruent with prior findings because this species has been widely reported in caves and is considered a natural part of the cave ecosystem (Bastian et al., 2009). *Pestalotiopsis* sp. has garnered increased attention in recent years because they produce many important secondary metabolites (Strobel et al., 1996, 2002; Aly et al., 2010; Xu et al., 2010; Maharachchikumbra et al., 2011).

Since these fungi are plant pathogens and endophytes, it may seem unlikely that these fungi would be isolated from bat and arthropods cadavers. However, it is conceivable that the bats and arthropods interacted with these fungi or their spores while traversing the cave or the surrounding forest environment. Bats exit the cave on a regular basis due to their feeding habits and likely interact with *Pestalotiopsis* sp. and *X. feejeensis* while foraging food in the forest. The arthropods could have picked up *Ceratobasidium* sp., *Diaporthe* sp., and *F. solani* mycelia or spores while feeding on decaying organic material, either near the cave entrance or the rear cave floor where plant life is abundant. Cave invertebrates also tend to reside in heaps of guano that are known reservoirs for fungi (Šustr et al., 2005; Nieves-Rivera et al., 2009; Nováková, 2009). Another possibility is that the fungi colonized the cadavers after death, which could happen due to spore dispersal by environmental means, other motile fauna, or human influence.

The geological features of Semud Hitam may have a direct effect on its mycota. The cave has a large cave opening that is about 80 m high and about 30 m wide (Lundberg and McFarlane, 2012). Also, in the back end of Semud Hitam, there is a large opening in the ceiling. The sunlight allows for there to be grassy patches exclusively in this part of the cave area (B, Fig, 1; ceiling hole A, Fig. 3). The presence of autotrophs on the cave floor itself is expected to affect the overall fungal diversity of Gomantong cave, and greater fungal diversity is expected near both the cave entrance and back opening (Shapiro and Pringle, 2010; Kuzmina et al., 2012; Mulec et al., 2012). These openings expose a good portion of the cave to precipitation and sunlight and serves as another point of entry for ambient spores, water, organic content, and airflow. Recent studies have suggested that the fungal communities outside the cave play a major role on the fungal diversity within the cave (Zhang and Cai, 2019). Another source of water, organic matter, and spores stems from rainwater being vertically filtered through the soil and rock above the cave (Ikner et al., 2007). During both visits, temperature and relative humidity data did not vary by much (Table 1). But, there was a discernible decrease in temperature and increase in humidity going from the lighted zones to the dark zones. Air temperature and humidity play a significant role in the microbial diversity in the environment (Ogórek et al., 2013). The dark zone of the cave had the lowest temperatures and relative humidity of 100% during both visits. Gomantong cave showed high relative humidity similar to caves in temperate regions (Nováková et al., 2018).

Gomantong cave, especially Semud Hitam, is a popular ecotourism destination for foreigners and locals. Human visitation may affect cave fungal diversity in a number of ways. There is significant evidence showing that increased human traffic into a cave system will cause contamination of indigenous fungal species by non-indigenous microorganisms (Porca et al., 2011; Griffin et al., 2014). Humans are also responsible for introducing nutrients into a cave (Ikner et al., 2007; Chelius et al., 2009, Shapiro and Pringle, 2010; Pusz et al., 2015). Previous reports have correlated human visitation to lower levels of fungal diversity, but interestingly caves with no human visitations show extremely low fungal abundance (Shapiro and Pringle, 2010). Vandalism in Gomantong cave can be seen on cave walls (Fig. 2A), and previous studies have shown that it affects microbial diversity, although localized to those specific areas (Shapiro and Pringle, 2010). We cannot say how human visitation has changed the mycobiome of Gomantong cave prior to human visitation as the cave has been utilized for swiftlet nest farming for multiple generations. Nonetheless, the increased levels of ecotourism in recent decades have likely affected the microbial diversity in the cave either through spore translocations or nutrient introductions, especially along the boardwalk. All of the samples in this study were collected near or on the boardwalk itself. Comparing the mycobiome of caves with high visitation versus those of low visitation in Sabah has never been done before, and future studies of this nature are highly recommended to better understand tropical cave ecosystems.

Aspergillus flavus and *F. solani* are known opportunistic human pathogens. Although *Diaporthe* sp. was not identified to the species level, it is known that *Diaporthe phoenicicola* causes scleral keratitis in humans (Gajjar et al., 2011). Recent visitors of caves should always be aware of opportunistic fungal pathogens on the rare chance that they present medical symptoms. More surveys need to be conducted to truly evaluate the potential of emerging infectious diseases of fauna residing in the tropical caves of Borneo.

Currently, we are unaware of any deleterious fungal diseases that affect bats and insects in Sabah's limestone caves. No *P. destructans* or *Geomyces* spp. were isolated in this study, likely due to the warmer tropical climate. As the keystone species in many ecosystems, particularly their roles as plant pollinators and insect population control (Mickleburg, et al., 2002; Kunz and Fenton, 2003; Lobova et al., 2009), it is pivotal to monitor the general health of bat populations and to identify any risks that pose a threat to their general wellness. A decline in the population of bats may lead to a cascade of ecological changes that could pose a significant loss to diversity of Bornean bats. Additionally, no obligate entomopathogenic fungi were isolated in this study, but the limited number of sampling days and limited access to all areas of the cave may have played a significant factor. We expect that with more extensive sampling in the future similar entomopathogenic strains that exist in the tropical forests of Borneo would overlap into their caves as well.

Living bats and arthropods are not a food source for most fungi, but their cadavers can be reservoirs for both live fungi and fungal spores. Environmental factors, anthropogenic disturbance, and the natural movement of cave fauna will disperse fungi proliferating on these cadavers into the surrounding environment. Dispersed fungi and their spores will find other suitable substrates to thrive on in the cave, perpetuating further growth. Some saprobic fungi, including ones isolated in this study, can act as opportunistic pathogens to cave fauna and humans (Bastian et al., 2009; Voyron et al., 2011). However, considering their low pathogenic potential to bats and insects, it is likely that the fungi did not play a role in the death of these animals. It is plausible that these animals came in contact with the spores or hyphae from the surface, while traversing the cave environment, or the saprophytic fungi colonized the host cadavers after their deaths. This work corroborates the idea that arthropods and bats contribute to the translocation of fungal spores in and out of the cave and dispersion within the cave itself. There was no evidence of any fungus that required cadavers as their obligatory substrate.

CONCLUSIONS

Our study is the first to report on the mycobiome of a cave in Borneo and serves as a baseline study to propel future interest and develop skills of researchers. Cave fauna, specifically bats and insects, harbor a multitude of fungi on or in their bodies that represent the mycobiome profile of the surrounding environment. The 14 species of fungi reported from 24 isolates in this study very likely account for an extremely small percentage of the total assemblage of fungi residing within Gomantong caves due to the multitude of potential substrates suitable for fungal growth. None of the fungi isolated are obligate cave dwellers since all taxa have been reported from outside the cave environment. Ongoing studies cultivating fungi from various environmental samples from Gomantong cave is currently in progress. We urge that more studies on cave fungi in Borneo be conducted for their enormous biological and industrial potential, and that future studies use both culture-dependent and culture-independent methods.

ACKNOWLEDGEMENTS

The authors acknowledge support from Ministry of Education Malaysia (MOE) and Universiti Malaysia Sabah (UMS) who supported this study under the grants FRGS/1/2017/WAB13/UMS/02/2 and Graduate Research Fellowship to IGW GUG0143-1/2017, respectively. We are also grateful to Sabah Forestry Department (Permit No.: JPHTN/TKKH(P-SH)100-14/18/2JILID36(47)) and Sabah Wildlife Department for providing access to the sampling site. We also thank the Institute of Tropical Biology and Conservation, Universiti Malaysia Sabah for providing transportation and facilities. We also thank the two anonymous reviewers for improving the manuscript.

REFERENCES

Abdullah, M.T., Hall, L.S., Tissen, O.B., Tuuga, A., and Cranbrook, S., 2007, The large bat caves of Malaysian Borneo: Bat Research News, v. 48, p. 99–100.

Aho, R., 1983, Saprophytic fungi isolated from the hair of domestic and laboratory animals with suspected dermatophytosis: Mycopathologia, v. 83, p. 65–73. https://doi.org/10.1007/BF00436886

Ainsworth, G.C., and Austwick, P.K.C., 1955, A survey of animal mycoses in Britain: mycological aspects: Transactions of the British Mycological Society, v. 38, p. 369–386. https://doi.org/10.1016/S0007-1536(55)80040-X

Ali-Shtayeh, M.S., Arda, H.M., Hassouna, M., and Shaheen, S.F., 1988, Keratinophilic fungi on the hair of cows, donkeys, rabbits, cats, and dogs from the West Bank of Jordan: Mycopathologia, v. 104, p. 109–121.

Aly, A.H., Debbab, A., Kjer, J., and Proksch, P., 2010, Fungal endophytes from higher plants: a prolific source of phytochemicals and other bioactive natural products: Fungal Diversity, v. 41, p. 1–16. https://doi.org/10.1007/s13225-010-0034-4

Arouja, J.P.M., and Hughes, D.P., 2016, Diversity of entomopathogenic fungi: Which groups conquered the insects body?: Advances in Genetics, v. 94, p. 1–39. https://doi.org/10.1016/bs.adgen.2016.01.001

Bagy, M.M.K., 1986, Fungi on the hair of large mammals in Egypt: Mycopathologia, v. 93, p. 73–75. https://doi.org/10.1007/BF00437737

Barton, H.A., Taylor, M.R., and Pace, N.R., 2004, Molecular phylogenetic analysis of a bacterial community in an oligotrophic cave environment: Geomicrobiology Journal, v. 21, p. 11–20. https://doi.org/10.1080/01490450490253428

Bastian, F., Alabouvette, C., and Saiz□Jimenez, C., 2009, The impact of arthropods on fungal community structure in Lascaux Cave: Journal of Applied Microbiology, v. 106, p. 1456–1462. https://doi.org/10.1111/j.1365-2672.2008.04121.x

Bastian, F., Jurado, V., Nováková, A., Alabouvette, C., and Saiz-Jimenez, C., 2010, The microbiology of Lascaux cave: Microbiology, v. 156, p. 644–652. https://doi.org/10.1099/mic.0.036160-0

Beguin, H., Larcher, G., Nolard, N., and Chabasse, D., 2005, *Chrysosporium chiropterorum* sp. nov., isolated in France, resembling *Chrysosporium* state of *Ajellomyces capsulatus* (*Histoplasma capsulatum*): Medical Mycology, v. 43, p. 161–169. https://doi.org/10.1080/13693780400006096

Bergman, A., and Casadevall, A., 2010, Mammalian endothermy optimally restricts fungi and metabolic costs: MBio, v. 1, e00212-10. https://doi.org/10.1128/mBio.00212-10

Bills, G.F., 1995, Analyses of microfungal diversity from a user's perspective: Canadian Journal of Botany, v. 73, p. 33–41. https://doi.org/10.1139/b95-222

Blehert, D.S., Hicks, A.C., Behr, M., Meteyer, C.U., Berlowski-Zier, B.M., Buckles, E.L., Coleman, J.T.H., Darling, S.R., Gargas, A., Niver, R., Okoniewski, J.C., Rudd, R.J., and Stone, W.B., 2009, Bat white-nose syndrome: an emerging fungal pathogen?: Science, v. 323, p. 227. https://doi.org/10.1126/science.1163874

Boonratana, R. and Sharma, D.S., 1997, Checklist of wildlife species recorded in the Lower Kinabatangan, Sabah: Journal of Wildlife Management and Research Sabah, v. 1, p. 47–60.

Chelius, M.K., Beresford, G., Horton, H., Quirk, M., Selby, G., Simpson, R.T., and Moore, J.C., 2009, Impacts of alterations of organic inputs on the bacterial community within the sediments of Wind Cave, South Dakota, USA: International Journal of Speleology, v. 38, no.1. https://doi.org/10.5038/1827-806X.38.1.1Chinery, M., 2005. Collins Complete Guide to British Insects. HarperCollins.

Collado, J., Platas, G., Paulus, B., and Bills, G. F., 2007, High-throughput culturing of fungi from plant litter by a dilution-to-extinction technique: FEMS Microbiology Ecology, v. 60, p. 521–533. https://doi.org/10.1111/j.1574-6941.2007.00294.x

Cuezva, S., Sanchez-Moral, S., Saiz-Jimenez, S., and Cañaveras, J.C., 2009, Microbial communities and associated mineral fabrics in Altamira Cave, Spain: International Journal of Speleology, v. 38, p. 83–92. https://doi.org/10.5038/1827-806X.38.1.9

Davis, E.C., Franklin, J.B., Shaw, A.J., and Vilgalys, R., 2003, Endophytic *Xylaria* (Xylariaceae) among liverworts and angiosperms: phylogenetics, distribution, and symbiosis: American Journal of Botany, v. 90, p. 1661–1667. https://doi.org/10.3732/ajb.90.11.1661

Domsch, K.H., Gams, W., and Anderson, T.H., 2007, Compendium of Soil Fungi: Eching, Germany, 672 p. https://doi.org/10.1111/j.1365-2389.2008.01052_1.x

Gajjar, D.U., Pal, A.K., Parmar, T.J., Arora, A.I., Ganatra, D.A., Ghodadra, B.K., and Vasavada, A. R., 2011, Fungal scleral keratitis caused by *Phomopsis phoenicicol*:. Journal of Clinical Microbiology, v. 49, p. 2365–2368. https://doi.org/10.1128/JCM.02449-10

Garcia-Solache, M.A., and Casadevall, A., 2010, Global warming will bring new fungal diseases for mammals: mBio, v. 1, p. 1–3. https://doi.org/10.1128/mBio.00061-10

Gargas, A., Trest, M. T., Christensen, M., Volk, T. J., and Blehert, D. S., 2009, *Geomyces destructans* sp. nov. associated with bat white-nose syndrome: Mycotaxon, v. 108, p. 147–154. https://doi.org/10.5248/108.147

Griffin, D. W., Gray, M. A., Lyles, M. B., and Northup, D. E., 2014, The transport of nonindigenous microorganisms into caves by human visitation: A case study at Carlsbad Caverns National Park: Geomicrobiology Journal, v. 31, p. 175–185. https://doi.org/10.1080/01490451.2013.815294

Gunde-Cimerman, N., Zalar, P., and Jeram, S., 1998, Mycoflora of cave cricket *Troglophilus neglectus* cadavers. Mycopathologia, v. 141, p. 111–114. https://doi.org/10.1023/A:1006947524503

Hawksworth, D.L., and **Lücking**, R., 2017, Fungal diversity revisited: 2.2 to 3.8 million species: Microbiology Spectrum, v. 5, p. 1–17. https://doi.org/10.1128/microbiolspec.FUNK-0052-2016

Hibbett, D.S., Ohman, A., and Kirk, P.M., 2009, Fungal ecology catches fire: New Phytologist: v. 184, p. 279–282. https://doi.org/10.1111/j.1469-8137.2009.03042.x

Higginbotham, S., Wong, W.R., Linington, R.G., Spadafora, C., Iturrado, L., and Arnold, A.E., 2014, Sloth hair as a novel source of fungi with potent anti-parasitic, anti-cancer and anti-bacterial bioactivity: PloS One, v. 9, p. 1–10. https://doi.org/10.1371/annotation/10c47668-8c49-48d2-ba65-60420af463b6

Hobbs, J.J., 2004, Problems in the harvest of edible birds' nests in Sarawak and Sabah, Malaysian Borneo: Biodiversity & Conservation, v. 13, p. 2209–2226. https://doi.org/10.1023/B:BIOC.0000047905.79709.7f

Ikner, L. A., Toomey, R. S., Nolan, G., Neilson, J. W., Pryor, B. M., and Maier, R. M., 2007, Culturable microbial diversity and the impact of tourism in Kartchner Caverns, Arizona: Microbial Ecology, v. 53, p. 30–42. https://doi.org/10.1007/s00248-006-9135-8

Imes, R., 1992, Practical Entomologist: Simon and Schuster.

Ismail, M.Y., 1999, Social control and bird's nest harvesting among the Idahan: Japanese Journal of Southeast Asian Studies, v. 37, p. 3–17.

Jacobs, A., Msimang, D., and Venter, E., 2017, First survey of the fungi from Bakwena Cave in South Africa suggests low human disturbance: Journal of Cave and Karst Studies, v. 79, p. 89–94. https://doi.org/10.4311/2016MB0146

Johnson, L.J., Miller, A.N., Mccleery, R.A., Mcclanahan, R., Kath, J.A., Lueschow, S., and Porras-Alfaro, A., 2013, Psychrophilic and psychrotolerant fungi on bats and the presence of *Geomyces* spp. on bat wings prior to the arrival of white nose syndrome: Applied and Environmental Microbiology, v. 79, p. 5465–5471. https://doi.org/10.1128/AEM.01429-13

Jurado, V., Laiz, L., Rodriguez-Nava, V., Boiron, P., Hermosin, B., Sanchez-Moral, S., and Saiz-Jimenez, C., 2010, Pathogenic and opportunistic microorganisms in caves: International Journal of Speleology, v. 39, p. 15–24. https://doi.org/10.5038/1827-806X.39.1.2

Jurado, V., Sanchez-Moral, S., and Saiz-Jimenez, C., 2008, Entomogenous fungi and the conservation of the cultural heritage: A review: International Biodeterioration & Biodegradation, v. 62, p. 325–330. https://doi.org/10.1016/j.ibiod.2008.05.002

Klich, M.A., 2002, Identification of common Aspergillus species: Centraalbureau voor schimmelcultures, Utrecht, The Netherlands, 116 p. https://doi.org/10.1017/S0269915X03243123

Kubátová, A., and Dvorák, L., 2005, Entomopathogenic fungi associated with insect hibernating in underground shelters: Czech Mycology, v. 57, p. 221. https://doi.org/10.33585/cmy.57303

Kunz, T.H, Fenton, M.B., eds., 2003, Bat Ecology: University of Chicago Press, Chicago, Illinois, p. 779.

Kuzmina, L.Y., Galimzianova, N.F., Abdullin, S.R., and Ryabova, A.S., 2012, Microbiota of the Kinderlinskaya cave (South Urals, Russia): Microbiology, v. 81, p. 251–258. https://doi.org/10.1134/S0026261712010109

Lahaye, R., van der Bank, M., Bogarin, D., Warner, J., Puplin, F., Gigot, G., Maurin, O., Duthoit, S., Barraclough, T.G., and Savolainen, V., 2008, DNA barcoding the floras of biodiversity hotspots: Proceedings of the National Academy of Science, USA, v. 105, no. 8, p. 2923–2928. https://doi.org/10.1073/pnas.0709936105

Larcher, G., Bouchara, J.P., Pailley, P., Montfort, D., Beguin, H., De Bièvre, C., and Chabasse, D., 2003, Fungal biota associated with bats in Western France: Journal de Mycologie Medicale, v. 13, p. 29–34.

Lobova, T.A., Geiselman, C.K., and Mori, S.A., 2009, Seed dispersal by bats in the Neotropics: Memoirs of the New York Botanical Garden, v. 101, p. 471.

Lorch, J.M., Lindner, D.L., Gargas, A., Muller, L.K., Minnis, A.M., and Blehert, D.S., 2013, A culture-based survey of fungi in soil from bat hibernacula in the eastern United States and its implications for detection of *Geomyces destructans*, the causal agent of bat white-nose syndrome: Mycologia, v. 105, p. 237–252. https://doi.org/10.3852/12-207

Lundberg, J., and Mcfarlane, D.A., 2012, Post-speleogenetic biogenic modification of Gomantong caves, Sabah, Borneo. Geomorphology, v. 157, p. 153–168. https://doi.org/10.1016/j.geomorph.2011.04.043

Maharachchikumbura, S.S., Guo, L.D., Chukeatirote, E., Bahkali, A.H., and Hyde, K.D., 2011, Pestalotiopsis—morphology, phylogeny, biochemistry and diversity: Fungal Diversity, v. 50, p. 167–187. https://doi.org/10.1007/s13225-011-0125-x

Malloch, D., and Blackwell, M., 1993, Dispersal biology of ophiostomatoid fungi, *in* Wingfield, M.J., Seifert, K.A., Webber, J.F., eds., Ceratocystis and Ophiostoma. Taxonomy, Ecology and Pathologenicity: Saint Paul, MN, USA, APS Press, p. 195–206.

Man, B., Wang, H., Xiang, X., Wang, R., Yun, Y., and Gong, L., 2015, Phylogenetic diversity of culturable fungi in the Heshang Cave, central China: Frontiers in Microbiology, v. 6, 1158. https://doi.org/10.3389/fmicb.2015.01158

Marcot, B.G., 2017, A review of the role of fungi in wood decay of forest ecosystems: Portland, OR: US Department of Agriculture, Forest Service, Pacific Northwest Research Station, Research Note PNW-RN-575 p. 1–31.

Mickleburgh, S.P., Hutson, A.M., and Racey, P.A., 2002, A review of the global conservation status of bats: Oryx, v. 36, p. 18–34. https://doi.org/10.1017/S0030605302000054

Mulec, J., Vaupotiˇc, J., and Walochnik, J., 2012, Prokaryotic and eukaryotic airborne micoorganisms as tracers of microclimatic changes in the underground (Postojna Cave, Slovenia): Microbial Ecology, v. 64, p. 654–667. https://doi.org/10.1007/s00248-012-0059-1

Nieves-Rivera, Á.M., Santos-Flores, C.J., Dugan, F.M., and Miller, T.E., 2009, Guanophilic fungi in three caves of southwestern Puerto Rico: International Journal of Speleology, v. 38, p. 61–70. https://doi.org/10.5038/1827-806X.38.1.7

Nováková, A., 2009, Microscopic fungi isolated from the Domica Cave system (Slovak Karst National Park, Slovakia): a review: International Journal of Speleology, v. 38, no. 1, p. 71–82. https://doi.org/10.5038/1827-806X.38.1.8

Nováková, A., Kubátová, A., Sklenár,, F., and Hubka, V., 2018, Microscopic fungi on cadavers and skeletons from cave and mine environments: Czech Mycology, v. 70, p. 101–121. https://doi.org/10.33585/cmy.70201

O'Donnell, K., Sutton, D.A., Fothergill, A., McCarthy, D., Rinaldi, M.G., Brandt, M.E., Zhang, N., and Geiser, D.M., 2008, Molecular phylogenetic diversity, multilocus haplotype nomenclature, and in vitro antifungal resistance within the *Fusarium solani* species complex: Journal of Clinical Microbiology, v. 46, p. 2477–2490. https://doi.org/10.1128/JCM.02371-07

Ogórek, R., Lejman, A., and Matkowski, K., 2013, Fungi isolated from Niedźwiedzia cave in Kletno (lower Silesia, Poland): International Journal of Speleology, v. 42, p. 161–166. https://doi.org/10.5038/1827-806X.42.2.9

Otero, J.T., Ackerman, J.D., and Bayman, P., 2004, Differences in mycorrhizal preferences between two tropical orchids: Molecular Ecology, v. 13, p. 2393–2404. https://doi.org/10.1111/j.1365-294X.2004.02223.x

Pflitsch, A., and Piasecki, J., 2003, Detection of an airflow system in Niedzwiedzia (Bear) cave, Kletno, Poland: Journal of Cave and Karst Studies, v. 65, p. 160–173.

Petrini, L., and Petrini, O, 1985, Xylariaceous fungi as endophytes. Sydowia, v. 38, p. 216–234.

Polovinko, G.P., Yaroslavtseva, O.N., Teshebaeva, Z.A., and Kryukov, V.Y., 2010, Dominating species of entomophilous ascomycetes anamorphs in West Siberia, Primorsky krai, and Kyrgyzstan: Contemporary Problems of Ecology, v. 3, p. 515–521. https://doi.org/10.1134/S1995425510050024

Porca, E., Jurado, V., Martin-Sanchez, P.M., Hermosín, B., Bastian, F., Alabouvette, C., and Sáiz-Jiménez, C., 2011, Aerobiology: an ecological indicator for early detection and control of fungal outbreaks in caves: Ecological Indicators, v. 11, p. 1594–1598. https://doi.org/10.1016/j.ecolind.2011.04.003

Poulson T.L., 1972, Bat guano ecosystems: Bulletin of the National Speleological Society, v. 72, p. 55–59.

Puechmaille, S.J., Wibbelt, G., Korn, V., Fuller, H., Forget, F., Mühldorfer, K., Kurth, A., Bogdanowicz, W., Borel, C., Bosch, T., Cherezy, T., Drebet, M., G**örföl**, T., Haarsma, A., Herhaus, F., Hallart, G., Hammer, M., Jungmann, C., Bris, Y.L., Lutsar, L., Masing, M., Mulkens, B., Passior, K., Starrach, M., Wojtaszewski, A., Z**ö**phel, U., and Teeling, E., 2011, Pan-European distribution of white-nose syndrome fungus (*Geomyces destructans*) not associated with mass mortality: PloS One, v. 6, e19167. https://doi.org/10.1371/journal.pone.0019167

Pusz, W., Ogórek, R., Knapik, R., Kozak, B., and Bujak, H., 2015, The occurrence of fungi in the recently discovered Jarkowicka cave in the Karkonosze Mts. (Poland): Geomicrobiology Journal, v. 32, p. 59–67. https://doi.org/10.1080/01490451.2014.925010

Raper, K.B., and Fennell, D.I., 1965, The Genus *Aspergillus*: Baltimore, Williams and Wilkins, 686 p.

Rees, R.G. (1967). Keratinophilic fungi from Queensland—I. Isolations from animal hair and scales: Sabouraudia: Journal of Medical and Veterinary Mycology, v. 5, p. 165–172. https://doi.org/10.1080/00362176785190351

Santamaria, S., and Faille, A., 2007, Rachomyces (Ascomycota, Laboulbeniales) parasites on cave-inhabiting Carabid beetles from the Pyrenees: Nova Hedwigia, v. 85, p. 159–186. https://doi.org/10.1127/0029-5035/2007/0085-0159

Seelan, S.S.J., and Anwarali, F., 2009, Case report of a new pathogenic variant of *Aspergillus fumigatus* isolated from *Hipposideros cervinus* (Chiroptera: Hipposideridae) in Sarawak, Malaysia: Journal of Threatened Taxa, v. 1, p. 190–191. https://doi.org/10.11609/JoTT.o2084.190-91

Seelan, S.S.J., Anwarali, F.A., Sepiah, M., and Abdullah, M.T., 2008, Bats (chiropteran) reported with *Aspergillus* species from Kubah National Park, Sarawak, Malaysia. Journal of Tropical Biology and Conservation, v. 4, p. 81–97.

Shapiro, J., and Pringle, A., 2010, Anthropogenic influences on the diversity of fungi isolated from caves in Kentucky and Tennessee: The American Midland Naturalist, v. 163, p. 76–86. https://doi.org/10.1674/0003-0031-163.1.76

Sidrim, J.J.C., Moreira Filho, R.E., Cordeiro, R.A., Rocha, M.F.G., Caetano, E.P., Monteiro, A.J., and Brilhante, R.S.N., 2010, Fungal microbiota dynamics as a postmortem investigation tool: Focus on *Aspergillus*, *Penicillium* and *Candida* species: Journal of Applied Microbiology, v. 108, p. 1751–1756. https://doi.org/10.1111/j.1365-2672.2009.04573.x

Smrž, J., Kováč, Ľ., Mikeš, J., **Šustr**, V., Lukešová, A., Tajovsky, K., Nováková, A., and Režňáková, P., 2015, Food sources of selected terrestrial cave arthropods: Subterranean Biology, v. 16, p. 37–46. https://doi.org/10.3897/subtbiol.16.8609

Strobel, G., Yang, X., Sears, J., Kramer, R., Sidhu, R.S., and Hess, W.M., 1996, Taxol from *Pestalotiopsis microspora*, an endophytic fungus of *Taxus wallachiana*: Microbiology, v. 142, p. 435–440. https://doi.org/10.1099/13500872-142-2-435

Strobel, G., Ford, E., Worapong, J., Harper, J.K., Arif, A.M., Grant, D.M., Fung, P.C.W., and Chau, R.M.W., 2002, Isopestacin, an isobenzofuranone from *Pestalotiopsis microspora*, possessing antifungal and antioxidant activities: Phytochemistry, v. 60, p. 179–183. https://doi.org/10.1016/S0031-9422(02)00062-6

Šustr, V., Elhottová, D., Krištůfek, V., Lukešová, A., Nováková, A., Tajovský, K., and Tříska, J., 2005, Ecophysiology of the cave isopod *Mesoniscus graniger* (Frivaldszky, 1865) (Crustacea: Isopoda): European Journal of Soil Biology, v. 41, p. 69–75. doi:10.1016/j.ejsobi.2005.09.008. https://doi.org/10.1016/j.ejsobi.2005.09.008

Taylor, E.L.S., Ferreira, R.L., Cardoso, P.G., and Stoianoff, M.A.R., 2014, Cave entrance dependent spore dispersion of filamentous fungi isolated from various sediments of iron ore cave in Brazil: a colloquy on human threats while caving: Ambient Science, v. 1, p.16––28. https://doi.org/10.21276/ambi.2014.01.1.ra02

Tristan, C., Cécile, R., Xavier, C., Desprez-Loustaua, M.L., Corinne, V., 2012, Spatial variability of phyllosphere fungal assemblages: genetic distance predominates over geographic distance in a European beech stand (*Fagus sylvatica*): Fungal Ecology, v. 5, p. 509–520. https://doi.org/10.1016/j.funeco.2011.12.004

Unterseher, M., and Schnittler, M., 2009, Dilution-to-extinction cultivation of leaf-inhabiting endophytic fungi in beech (*Fagus sylvatica* L.)–different cultivation techniques influence fungal biodiversity assessment: Mycological Research, v. 113, p. 645–654. https://doi.org/10.1016/j.mycres.2009.02.002

Vanderwolf, K.J., Malloch, D., McAlpine, D.F., and Forbes, G.J., 2013a, A world review of fungi, yeasts and slime molds in caves: International Journal of Speleology, v. 42, no. 1, p. 77–96. https://doi.org/10.5038/1827-806X.42.1.9

Vanderwolf, K.J., McAlpine, D.F., Malloch, D., and Forbes, G.J., 2013b, Ectomycota associated with hibernating bats in eastern Canadian caves prior to the emergence of white-nose syndrome: Northeastern Naturalist, v. 20, p. 115–131. https://doi.org/10.1656/045.020.0109

Vanderwolf, K.J., Malloch, D., and McAlpine, D.F., 2016a, Fungi on white-nose infected bats (*Myotis* spp.) in Eastern Canada show no decline in diversity associated with *Pseudogymnoascus destructans* (Ascomycota: Pseudeurotiaceae): International Journal of Speleology, v. 45, p. 43–50. https://doi.org/10.5038/1827-806X.45.1.1946

Vanderwolf, K., Malloch, D., and McAlpine, D., 2016b, Ectomycota associated with arthropods from bat hibernacula in eastern Canada, with particular reference to *Pseudogymnoascus destructans*: Insects, v. 7, p. 16. https://doi.org/10.3390/insects7020016

Visagie, C.M., Yilmaz, N., Vanderwolf, K., Renaud, J.B., Sumarah, M.W., Houbraken, J., Assebgui, R., Seifert, K.A., and Malloch, D., 2019, Penicillium diversity in Canadian bat caves, including a new species, *P. speluncae*: Fungal Systematics and Evolution, v. 5, p. 1–15. https://doi.org/10.3114/fuse.2020.05.01

Voyron, S., Lazzari, A., Riccucci, M., Calvini, M., and Varese, G., 2011, First mycological investigation on Italian bats: Hystrix, the Italian Journal of Mammalogy, v. 22, p. 189–197.

Wang, W., Ma, X., Ma, Y., Mao, L., Wu, F., Ma, X., and Feng, H., 2010, Seasonal dynamics of airborne fungi in different caves of the Mogao Grottoes, Dunhuang, China: International Biodeterioration & Biodegradation, v. 64, p. 461–466. https://doi.org/10.1016/j.ibiod.2010.05.005

White, T.J., Burns, T., Lee, S. and Taylor, J., 1990, Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics, *in* Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J., eds., PCR protocols: a guide to methods and applications: San Diego, Academic Press, p. 315–322. https://doi.org/10.1016/B978-0-12-372180-8.50042-1

Wibbelt, G., Kurth, A., Hellmann, D., Weishaar, M., Barlow, A., Veith, M., Prüger, J., G**örföl***, T., Grosche, L., Bontadina, F., Zöphel, U., Seidl, H.,* ***Cryan, P.M., Blehert, D.S.***, 2010, White-nose syndrome fungus (*Geomyces destructans*) in bats, Europe: Emerging Infectious Diseases, v. 16, p. 1237. https://doi.org/10.3201/eid1608.100002

Winter, A.S., Hathaway, J.J., Kimble, J.C., Buecher, D.C., Valdez, E.W., Porras-Alfaro, A., Young, J.M, Read, K.J.H., and Northup, D.E., 2017, Skin and fur bacterial diversity and community structure on American southwestern bats: effects of habitat, geography and bat traits: PeerJ, v. 5, e3944.

Woese, C.R., Kandler, O., and Wheelis, M.L., 1990, Towards a natural system of organisms: proposal for the domains Archaea, Bacteria, and Eucarya: Proceedings of the National Academy of Science, USA, v. 87, no. 12, p. 4576–4579. https://doi.org/10.1073/pnas.87.12.4576

Xu, J., Ebada, S.S., and Proksch, P., 2010, *Pestalotiopsis* a highly creative genus: chemistry and bioactivity of secondary metabolites: Fungal Diversity, v. 44, p. 15–31. https://doi.org/10.1007/s13225-010-0055-z

Yoder, J.A., Benoit, J.B., Christensen, B.S., Croxall, T.J., and Hobbs, H.H., 2009, Entomopathogenic fungi carried by the cave orb weaver spider, *Meta ovalis* (Araneae, Tetragnathidae), with implications for mycoflora transfer to cave crickets: Journal of Cave and Karst Studies, v. 71, p. 116–120.

Zeller, L., 1966, Keratinophilic fungi from the “Baradla” cave in Aggtelek: Annals of the University of Sciences Budapest, Section Biology, v. 8, p. 375–388.

Zhang, Z., Schwartz, S., Wagner, L., and Miller, W., 2000, A greedy algorithm for aligning DNA sequences. Journal of Computational Biology, v. 7, p. 203–214. https://doi.org/10.1089/10665270050081478

Zhang, T., Victor, T.R., Rajkumar, S.S., Li, X., Okoniewski, J.C., Hicks, A.C., Davis, A.D., Broussard, K., LaDeau, S.L., Chaturvedi, S., and Chaturvedi, V., 2014, Mycobiome of the bat white nose syndrome affected caves and mines reveals diversity of fungi and local adaptation by the fungal pathogen *Pseudogymnoascus* (*Geomyces*) *destructans*: PloS One, v. 9, e108714. https://doi.org/10.1371/journal.pone.0108714

Zhang, Z.F., and Cai, L., 2019, Substrate and spatial variables are major determinants of fungal community in karst caves in Southwest China: Journal of Biogeography, v. 46, p. 1504–1518. https://doi.org/10.1111/jbi.13594

Zhang, Z.F., Liu, F., Zhou, X., Liu, X.Z., Liu, S.J., and Cai, L., 2017, Culturable mycobiota from Karst caves in China, with descriptions of 20 new species. Persoonia: Molecular Phylogeny and Evolution of Fungi, v. 39, p. 1–31. https://doi.org/10.3767/persoonia.2017.39.01

Zukal, J., Bandouchova, H., Brichta, J., Cmokova, A., Jaron, K.S., Kolarik, M., Kovacova, V., Kubátová, A., Nováková, A., Orlov, O., Pikula, J., Presetnik, P., Šuba, J., Zahradníková Jr., A., and Martínková, N., 2014, White-nose syndrome fungus: a generalist pathogen of hibernating bats: PLoS One, v. 9, e97224. https://doi.org/10.1371/journal.pone.0097224.

Julian J. Lewis, Marc A. Milne, Charles D. R. Stephen, and Daniel C. Dourson. Karst sinkholes as foci of biodiversity in the Hoosier National Forest. *Journal of Cave and Karst Studies*, v. 82, no. 4, p. 276-292. DOI:10.4311/2020LSC0103

KARST SINKHOLES AS FOCI OF BIODIVERSITY IN THE HOOSIER NATIONAL FOREST

Julian J. Lewis[1, C], Marc A. Milne[2], Charles D. R. Stephen[3], and Daniel C. Dourson[4]

Abstract

Sinkholes are a well-known, but poorly studied, aspect of karst environments. In 2015, the Hoosier National Forest in southern Indiana, USA, commissioned a study of sinkhole habitats to assess their ecological role. The ecosystems of 26 sinkholes were evaluated to determine if sinkhole floor biological communities and species richness were a function of the surrounding plant community. Each sinkhole was sampled four times for five target groups of invertebrates at intervals of approximately three months, for a total of 104 visits. The sampling resulted in finding 140 taxa, including 31 land snails, 14 millipedes, 3 terrestrial isopods, 83 spiders and 9 pseudoscorpions. Of exceptional note were at least 12 new state records and a probable new species of pseudoscorpion. Several of these species appear to be endemic to sinkhole habitats. A link was confirmed between species richness and the surrounding plant community, specifically that the highest biodiversity was found in sinkholes surrounded by native deciduous forest, followed by native glades. Sinkholes in fields from which deciduous forest had been removed possessed markedly decreased species diversity, as did non-native plantings of pines. Sinkhole habitats had a significantly higher species richness than adjacent non-sinkhole control sites. Moreover, the arthropod communities that were found in each sinkhole within each plant community type were different from each other and the surrounding non-sinkhole areas. These data suggest that sinkholes are more than just depressions in epigean landscapes, but possess unique invertebrate communities linked to the surrounding plant community.

INTRODUCTION

Between 2015 and 2017 a project was initiated by the Hoosier National Forest (HNF) to evaluate the biological communities of sinkholes and their relationship with the surrounding forest environment. This study was motivated in part by a desire to better manage the forest through an improved understanding of the effects of modifying forest plant communities on sensitive karst sinkhole ecosystems.

The HNF spans approximately 800 km^2 and is spread across nine counties in southcentral Indiana (Fig. 1). The geologic setting (Powell, 1961; Frushour, 2012; Lewis, 2012; Lewis and Lewis, 2012b) is comprised of the sandstone-capped ridges of the Crawford Upland, with limestone-floored valleys where caves and springs are common. In ridges penetrated by these caves, sinkholes tend to occur in places where the sandstone cap has collapsed into underlying limestone caves. Frequently this resulted in sandstone-walled sinks where little if any limestone is available for calciphilic animals, such as terrestrial snails. In contrast, the eastern edge of the HNF lies in the Mitchell Sinkhole Plain, a region characterized by copious numbers of sinkholes formed entirely in limestone.

Extensive literature exists on forest (e.g., Homoya et al., 1985) and cave ecology (e.g., Culver and Pipan, 2009), but little has been written on that of the sinkhole ecotone that connects these surface and subterranean environments. As a frame of reference from which to start in the evaluation of the sinkhole fauna, the HNF cave fauna has been well-characterized after two decades of bioinventory projects (Lewis, 1998a, 1998b, 2011; Lewis et al., 2002, 2004; Lewis and Lewis, 2008, 2009, 2012a). Beyond that, the literature concerning sinkholes mostly addresses three topics: (1) engineering nuisances that swallow homes and complicate construction projects; (2) the wetlands and associated communities that are created when sinkholes become plugged (Scott, 1910; White, 1930); and (3) fossil remains (primarily Tertiary in age), mostly animals that fell into pit-like sinkholes and were unable to escape (Farlow et al., 2001).

The goals of this project were to relate the fauna of sinkholes with four kinds of surrounding plant communities and to test whether native plant communities harbored greater sinkhole fauna species richness. The two hypotheses for the project were: (1) that sinkhole floor fauna communities were a function of the surrounding plant community type: and (2) species richness was a function of the presence of native deciduous forest in which the communities had evolved since the end of the last Pleistocene glacial advance.

METHODS

The methods for the project were chosen to facilitate identification and characterization of the chosen sinkholes, to provide data necessary to quantify the amount of leaf litter present in each sink, the extent of the invertebrate com-

[1] Lewis and Associates LLC, Cave, Karst and Groundwater Biological Consulting, 17903 State Road 60, Borden, IN 47106
[2] Department of Biology, University of Indianapolis, 1400 E. Hanna Avenue, Indianapolis, IN 46227
[3] Department of Biological Sciences, Auburn University, 101 Rouse Life Sciences, Auburn, AL 36849
[4] Dourson Environmental Consulting, 1128 Gritter Ridge Road, Stanton, KY 40380
[C] Corresponding Author: lewisbioconsult@gmail.com

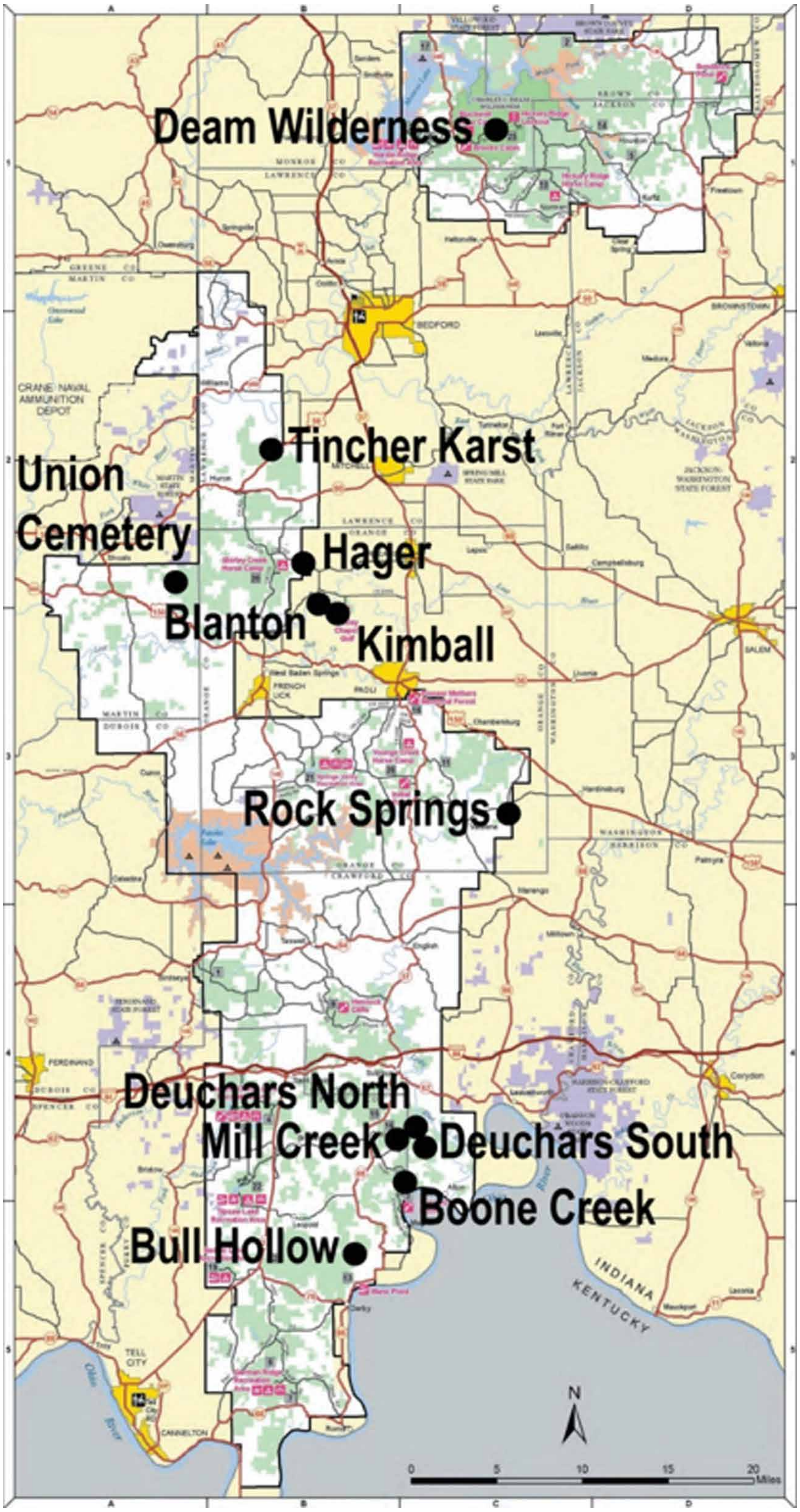

Figure 1. Map of the purchase boundary of the Hoosier National Forest in southern Indiana, with tracts labelled in which sinkholes were investigated.

munity, and the identification of some of the species present. Particular constraints were time, limited funding, and personnel with taxonomic expertise. Some of the methods expressed here were relevant for aspects of the project that were tangential to the faunistic portion of the project and will be further discussed in a future paper focusing on the environment of HNF sinkholes.

Twenty-five sinkholes were evaluated between September 2015 and June 2016. The Forest Service suggested that sinkholes with cave entrances be eliminated from consideration to ascertain a clear distinction between this project and several others that focused on caves. The Forest Service made no stipulations as to which sinkholes were selected, although it was requested that a variety of shapes and sizes be chosen, including some that were only subtly different from the surrounding landscape. Control sites were selected in adjacent non-sinkhole habitats for comparison with deciduous forest, glade grasslands, and pine plantings (at Tincher Special Karst, Bull Hollow, and Deuchars North, respectively).

Each sink and control site were visited four times, at approximately three-month intervals such that there was sampling coverage during all seasons. Additionally, a single large sink was studied with the same protocol between July 2016 and May 2107. A total of 104 visits were conducted. Site names were chosen based on Forest Service designation, if available (e.g., Kimball Tract, Blanton Tract), or a nearby landmark (e.g., Union Cemetery). Location coordinates were obtained with a Garman Oregon 450 GPS. Dimensions (length, width, and depth) were measured with a laser measuring device or a tape measure (Table 1). Extensive data concerning other environmental parameters (e.g., temperature, humidity, soil pH) were gathered and will be discussed in a separate paper.

Sinkholes surrounded by four plant communities were studied: (1) deciduous forest – comprised of native tree species that in the HNF are predominantly oaks (*Quercus* spp.), sugar maple (*Acer saccharum*), and hickories (*Carya* spp.) (Woodall et al., 2007); (2) glades – natural openings in deciduous forests in rocky areas with shallow soil, with sparse trees and meadows inhabited by warm-season grasses and other prairie plants; (3) pine plantations – row plantings of pines (*Pinus* spp.) that are otherwise rare or absent in Indiana forests, comprising monocultures that are sporadically invaded by maple trees that contribute some deciduous forest leaves

Table 1. Site designations, location, and physical dimensions of the HNF sinkholes surveyed. Length is the greatest dimension of the sinkhole. The last two columns report results of the quantity of leaf litter measured in a floor sample and the number of target species found.

Site (with abbreviation)	County	Elevation (ft)	Latitude	Longitude	Length (m)	Width (m)	Depth (m)	Litter (g/0.25m^2)	Target Species
Bull Hollow 1 (BH1)	Perry	492	38.06787	86.52930	6.6	4.9	2.7	291	14
Bull Hollow 2 (BH2)	Perry	498	38.06712	86.52917	5.3	4.6	1.7	538	25
Boone Creek 1 (BC1)	Perry	550	38.13249	86.47109	5.8	4.8	2.6	322	21
Boone Creek 2 (BC2)	Perry	572	38.13223	86.46971	2.6	1.6	2.1	141	16
Mill Creek 1 (MC1)	Perry	539	38.16869	86.46843	5.4	3.6	2.2	127	10
Mill Creek 2 (MC2)	Perry	531	38.16872	86.46838	3.1	2.2	1.1	31	8
Deuchars North 1 (DN1)	Crawford	587	38.17293	86.46063	5.8	5.7	1.4	856	13
Deuchars South 1 (DS1)	Crawford	590	38.17120	86.46040	7.7	4.5	1.6	368	9
Deuchars South 2 (DS2)	Crawford	624	38.17153	86.46053	8.0	6	1.6	85	10
Deuchars South 3 (DS3)	Crawford	628	38.17180	86.46048	3.2	2.4	0.7	719	3
Rock Springs 1 (RS1)	Orange	792	38.44100	86.36134	6.6	4.4	4.0	1010	25
Rock Springs 2 (RS2)	Orange	778	38.44122	86.36031	5.3	4.8	1.8	323	25
Rock Springs 3 (RS3)	Orange	782	38.43805	86.35993	22.6	20.1	7.1	1469	38
Blanton 1 (B1)	Orange	546	38.62447	86.52769	3.4	1.8	1.0	47	12
Blanton 2 (B2)	Orange	559	38.62348	86.52850	4.7	1.6	0.8	1933	25
Kimball 1 (K1)	Orange	596	38.6261	86.54997	5.5	4.9	1.7	1360	36
Kimball 2 (K2)	Orange	575	38.62622	86.54868	11.9	9.8	2.2	1845	31
Kimball 3 (K3)	Orange	575	38.62777	86.54655	7.3	6.4	1.2	409	24
Kimball 4 (K4)	Orange	568	38.62781	86.54818	10.7	9.5	2.0	341	14
Hager 1 (H1)	Orange	567	38.65608	86.56608	7.0	4.7	4.9	82	5
Hager 2 (H2)	Orange	630	38.65606	86.56811	4.7	1.7	1.1	419	15
Tincher 1 (T2)	Lawrence	625	38.75628	86.61287	15.3	12.9	8.7	317	20
Tincher 2 (T2)	Lawrence	610	38.75681	86.61308	3.2	2.6	0.4	1970	31
Union Cemetery 1 (UC1)	Martin	812	38.64027	86.70113	5.4	4.2	1.4	2367	19
Union Cemetery 2 (UC2)	Martin	812	38.64036	86.70095	2.8	2.6	0.34	550	12
Deam Wilderness 1 (DW1)	Monroe	771	39.01291	86.37473	7.2	3.9	1.3	396	9
Tincher control	Lawrence	677	38.75552	86.61522	n/a	n/a	n/a	187	2
Bull Hollow control	Perry	499	38.06783	86.52935	n/a	n/a	n/a	98	2
Deuchars North control	Crawford	585	38.17180	86.46062	n/a	n/a	n/a	209	2

to the forest leaf litter; and (4) fields – former crop land or pasture abandoned from agricultural use and dominated by weedy plants, like goldenrod (*Solidago* spp.) and ironweed (*Vernonia* spp.). The plant community in which each sinkhole occurred was characterized by field identifications at each site into one of the four types.

During the initial visit of each sink a 0.25 m^2 quadrat was placed in the central drain area of the sink floor. The depth of the leaf litter from the surface to the top of the underlying soil was measured. All organic material present was removed from the quadrat and weighed. The litter sample was reduced by sifting through a 1.0 cm^2 mesh screen, bagged for transport, and placed in a Berlese funnel at the laboratory to extract invertebrates. During subsequent visits to the sinkholes, qualitative collecting was done by thoroughly searching a transect 0.3 m wide and extending from the center of the sinkhole to the rim. A different transect was set for each collection due to the disruptive nature of sampling. Leaf litter from the transect was screened onto a white surface and target invertebrates placed into 70% ethanol for transport to the laboratory.

Figure 2. Example of a typical quadrat placement in Bull Hollow sinkhole 1.

Five invertebrate groups were chosen as targets for collection and identification based on the availability of taxonomic expertise. The target groups and specialists were: terrestrial isopods and millipedes (J. Lewis), spiders (M. Milne), pseudoscorpions (C. Stephen) and terrestrial snails (D. Dourson). Specimens were shipped to the institutions of the authors responsible for identification. Vouchers for pseudoscorpions are deposited at the Auburn University Museum of Natural History; terrestrial isopods are deposited in the collection of the Smithsonian Institution. Other vouchers remain in the collections of the authors.

To determine if there was a significant difference in species richness between the controls and the sinkhole habitats, a *t*-test was completed. To compare species richness among habitat types, an ANOVA was employed. Additionally, a Tukey HSD test was done to determine which specific habitats were significantly different than others. To compare the taxonomic makeup of the communities (composed of all target species) at various sites in different habitat types, including controls, a non-metric multidimensional scaling analysis (NMDS) was completed in R (R Development Core Team, 2019) version 3.4.3 using the associated vegan package (Oksanen et al., 2019). Data were log normal transformed prior to the NMDS. Between-habitat comparisons were analyzed in R with a pairwise multilevel comparison analysis, using the pairwise adonis function in vegan. Plots were created in R with the package ggplot2 (Wickham et al., 2019).

TRACT DESCRIPTIONS

Locations of the 26 sinkholes are shown in Figure 1, with coordinates and dimensions in Table 1. The sinkholes ranged from 2.6 m to 22.6 m long in the greatest dimension (mean 6.8), 1.6 m to 20.1 m wide (mean 5.2) and 0.3 m to 8.7 m deep (mean 2.2). Brief descriptions follow for the sinkholes chosen among 12 Forest Service tracts in the HNF.

Bull Hollow Tract

A remote area accessed by all-terrain vehicle, located on the ridge above the confluence of Bull Hollow and Oil Creek, about 3 km north of the confluence with the Ohio River. An area of extensive glades, sink 1 is steep-walled in a glade with sparse leaf litter on bare clay with an open drain (Fig. 3). The native grass, River Oats (*Chasmanthium latifolium*), occurred on the margins of the sink. Sink 2 was in sparse deciduous forest on the edge of the forest-glade interface, with more leaf litter present in the floor than observed in sink 1.

Boone Creek Tract

This tract is characterized by extensive glades and occurs on the north side of a ridge one km northwest of the Ohio River. Sinkhole plant communities were characterized by sparse forest and River Oats (*C. latifolium*) on the sinkhole margins. Sink 1 had a slightly over-hanging wall with limestone exposed on the floor. Litter was sparse with slabs of rock scattered on the floor under which was a drain hole that always had an air temperature approximating local cave temperatures (ca. 12.5 °C). Sink 2 was small, steep-walled, bordered by limestone and leading into an open drain. At this sinkhole little organic litter was present; the floor and walls were mostly bare soil and limestone.

Figure 3. Bull Hollow sinkhole 1.

Mill Creek Tract

This site is an old field, currently being converted by HNF personnel to native grasses. Trash and other remnants of old buildings indicate the presence of a former homesite. Sink 1 had vertical sandstone walls that overhung the floor slightly on one side. This sink was judged as significantly disturbed, with the floor containing trash, road gravel, and wood ashes. A mostly dead, exotic Tree-of-heaven (*Ailanthus altissima*) overhung one end of the sink. A floor drain had air temperature consistently near local cave temperature. The adjacent sink 2 was shallower and formed completely in soil with no visible rock.

Deuchars North and South Tracts

Just northeast of the Mill Creek Tract, the four sinks in the two tracts designated Deuchars North and South are in mature monoculture pine plantings on opposite sides of a county road. The North Deuchars sink 1 was an open bowl-shaped depression with a thick layer of forest litter, primarily pine needles with some maple leaves. Three sinks were selected in South Deuchars. Sinks 1 and 3 were floored with pine mulch (bark and needles). Sink 2 was filled to the rim with trash, including quantities of broken glass and rusty metal that may have been a source of chemical pollution negatively affecting the sinkhole fauna.

Rock Springs Tract

This tract is named after the Rock Springs Church located about 1 km to the northwest; no springs are present on this upland area that drains into the Patoka River. Three sinks were chosen for evaluation, two studied the first year and the third singled out for attention the following year because of its large size. All were in established deciduous forest and occurred just above the sandstone and limestone contact, with limestone exposed in sinks 1 and 3. Sink 1 was steep-walled with an active drain. Sink 2 was bowl-shaped with no apparent drain. Sink 3 was steep-walled and a hand line was used for entry due to its depth of over 7 meters. Sink 3 had an overhanging rock shelter with exposed limestone under the sandstone, and a hole that did not function as a drain but breathed air that was consistently near cave temperature. The overhang in sink 3 was the only site inhabited by the cave cricket (*Ceuthophilus stygius*) found in dense clusters and the Cave Salamander (*Eurycea lucifuga*).

Blanton Tract

This tract is on the western margin of the Mitchell Sinkhole Plain and contains two entrances to the 35+ km Lost River Cave System. Both of the sinkholes selected in this tract had limestone exposed in the walls. Sink 1 occurred in an old field and was narrow and elongate. Sink 2 was in deciduous forest, formed along an enlarged joint and appeared like a rectangular stone box. An abandoned wooden shed was a short distance away and one end of the sink was covered by a large bale of fence wire, obvious evidence of past human disturbance.

Figure 4. Kimball Tract sinkhole 1.

Kimball Tract

Prior to purchase by the HNF, this tract had been held by a company maintaining it for a timber harvest. The tract forms a peninsula surrounded by the dry bed of Lost River, comprised of dense sinkhole formation under a moderately mature deciduous forest that was not recently harvested. The sinkholes occur at the sandstone and limestone contact. Sink 1 was a somewhat elongate bowl-shaped depression (Fig. 4). Sinks 2 and 4 were similar, but broader and more open. Sink 3 was broad and shallow, with many chunks of limestone on the floor that were suspected to have been the result of human placement there. An old stone-lined cistern was a few meters away from the sink.

Hager Tract

The two sinks chosen in this tract were in different habitats. Sink 1 was in a large, flat, lowland field overgrown with weeds that would have been almost impenetrable without the trail bush-hogged by Forest Service personnel to allow access during this project. The sink had one scraggly tree growing above the steep wall of the sink above what looked like a mostly buried metal tank. This sink was the only one that changed markedly over the course of the year it was visited, with an open drain that breathed out water vapor that created a dense hoarfrost on the wall during mid-winter. In the spring the wall above the drain collapsed and completely sealed the drain with loose soil. Sink 2 was near the top of a hill adjacent to a wide swath of mowed gas pipeline right-of-way. The sink was rectangular with sandstone walls.

Tincher Karst Special Area

This area is one of the most rugged parts of the HNF. Two sinks were selected on the karst valley floor between two large sandstone ridges. Sink 1 was the deepest of any selected and the second largest overall. A spring emerging from the ridge side some distance from the sink created a waterfall that plummeted down the vertical limestone wall of the sink and disappeared in breakdown. The floor of sink 1 was consistently wet and much of the leaf litter disappeared into the drain, leaving bare clay substrate. Sink 2, in contrast, was a broad, shallow depression that trapped leaves. Despite its small size, sink 2 was the third most biodiverse sink studied.

Union Cemetery Tract

Two sinks were selected in this remote area that lies near the top of a ridge. The first sinkhole was a bowl-shaped depression lacking any drain. A small spring occurred at the base of the small slope beyond the rim of the sink. Sink 2 was the smallest of any evaluated, a shallow depression only a third of a meter deep that trapped little leaf litter.

Deam Wilderness

This karst area is comprised of a narrow sliver of limestone thrust to the surface adjacent to the Mt. Carmel Fault Zone. Only one sink was located that did not have an open vertical fissure or pit. This sink, in a mature pine planting, was an elongate bowl-shaped depression that sloped toward a drain hole. The sparse litter on the floor was a combination of pine needles and maple leaves.

RESULTS AND DISCUSSION

A total of 140 taxa from the target groups of fauna were found in the 26 sinkholes (Tables 2−6). These included 31 land snails, 14 millipedes, 3 terrestrial isopods, 83 spiders, and 9 pseudoscorpions (Tables 2−6). Of the 140 taxa, 113 were found in 5 or fewer sites, and another 17 in 10 or fewer sinks, accounting for about 93% of the target fauna collected. Of the species that made recurrent use of sinkholes, eight taxa occurred in 11−15 sites, and only the spiders *Leucauge venusta* and *Pirata* spp. occurred in more than 15 sinks. These are common epigean spiders of the Midwest with no particular affinity to sinkholes that has been reported (Sierwald et al., 2005). *Leucauge venusta* was found in 18 sinks, where its webs were spun on taller plants during the summer and autumn months. In contrast, *Pirata* spp., including three specific taxa as well as seemingly ubiquitous juveniles, were found in moist litter in 24 sinks.

Spiders were the most diverse faunal group (Tables 4−6). Eleven species of spiders were new state records (Milne et al., 2017) at the time of their discovery in HNF sinks: *Cicurina itasca, Ceratinops latus, Ceratinopsidis formosa, Mermessus maculatus, Walckenaeria communis, Gladicosa pulchra, Phrurolithus singulus, Scotinella redempta, Pisaurina dubia, Neon nelli*, and *Robertus frontatus.*

Several rare species were encountered in certain sinkholes. The pseudoscorpion *Kleptochthonius griseomanus* was found only in Kimball sink 1 (Fig. 5). It was previously known only from the entrance zones of four caves in Crawford and Perry counties, Indiana (Muchmore, 2000; Lewis and Lewis, 2012a), with one or two pseudoscorpions found in each cave. It was thus surprising to find over 30 specimens in a single litter sample taken from Kimball sink 1, which suggests that the preferred habitat of this species may be sinkhole floors and not caves. At each sampling event the sinkhole floor, rim, and mid-wall areas were sampled, but *K. griseomanus* was only collected from the floor. The finding of *Chitrella* sp. (family Syarinidae) in Rock Springs sink 1 is significant. This specimen likely represents an undescribed species and is a range expansion for the genus, which in eastern North America was previously only known from one epigean locality in Tennessee and caves in Tennessee, Virginia, and West Virginia (Malcolm and Chamberlin, 1960; Muchmore, 1963, 1973).

Among the millipedes, *Conotyla bollmani* was reported by Hoffman and Lewis (1997) from caves in the northern half of the Mitchell Plain and Crawford Upland. Shear (1971) reported an unspecified record outside of a cave. In caves, the species is troglomorphic, with reduced eyes and depigmented. *Conotyla bollmani* was found in 15 HNF sinkholes,

Table 2. Terrestrial snails occurring in HNF sinkholes.

Site	*Anguispira alternata*	*Anguispira kochi*	*Carychium exile*	*Cochlicopa morseana*	*Deroceras* sp.	*Discus patulus*	*Euchemotrema fraternum*	*Euconolus* sp.	*Gastrocopta pentodon*	*Glyphalinia indentata*	*Glyphalinia wheatleyi*	*Haplotrema concavum*	*Inflectarius inflectus*	*Inflectarius rugeli*	*Megapallifera mutabilis*	*Mesodon thyroidus*	*Mesodon* sp.	*Mesomphix cupreus*	*Mesomphix vulgatus*	*Neohelix albolabris*	*Pallifera fosteri*	*Patera laevior*	*Punctum minutissimum*	*Punctum* sp.	*Stenotrema hirsutum*	*Strobilops labyrinthicus*	*Triodopsis tridentata*	*Triodopsis vulgata*	*Ventridens* sp. *(ligera)*	*Zonitoides limatulus*
Bull Hollow sink 1												X					X													
Bull Hollow sink 2												X											X							
Boone Creek sink 1			X									X															X	X		
Boone Creek sink 2			X									X										X					X	X		
Mill Creek sink 1																														
Mill Creek sink 2																														
Deuchars North sink 1																										X				
Deuchars South sink 1																														
Deuchars South sink 2																														
Deuchars South sink 3			X																											
Rock Springs sink 1																				X				X						
Rock Springs sink 2											X	X								X							X			
Rock Springs sink 3	X		X							X		X																		
Blanton sink 1												X																X		
Blanton sink 2	X		X			X		X	X	X				X									X			X	X			
Kimball sink 1											X	X						X									X			
Kimball sink 2		X								X		X	X														X			
Kimball sink 3	X	X	X									X				X			X		X				X		X			
Kimball sink 4	X																										X		X	
Hager sink 1															X															
Hager sink 2					X																									
Tincher sink 1			X				X				X		X														X			
Tincher sink 2			X	X					X		X		X			X						X								X
Union Cemetery sink 1							X					X	X							X										
Union Cemetery sink 2			X																	X										
Deam Wilderness sink 1													X					X												
Bull Hollow control																														
Tincher control																														
Deuchars North control																														

although adult males were encountered only from sinks in the Rock Springs tract, where they were darkly pigmented with normal eyes. The finding of the millipede *Cleidogona unita* is a new record for Indiana and represents a range expansion for the species. This millipede was reported by Shear (1971) from habitats along the sandstone escarpment in southern Illinois and Mammoth Cave National Park, Kentucky. It was found in sinks in the Bull Hollow, Deuchars and Tincher tracts, where it co-occurred with *Conotyla bollmani.*

Terrestrial snails were relatively scarce given the presence of limestone in karst topography. Without question, calcium carbonate is an essential mineral to land snails for regulation of bodily processes, reproduction, and most importantly, shell-building. Land snails obtain calcium in several ways including consuming soil particles from calcareous substrates, eating decaying leaf matter, almost certainly by ingesting Physarales slime molds which precipitate amor-

Table 3. Millipedes and terrestrial isopods occurring in HNF sinkholes.

Site	*Abacion* sp.	*Apheloria virginiensis*	*Cambala* sp.	*Chaetaspis albus*	*Cleidogona unita*	*Conotyla bollmani*	*Euryurus leachi*	*Pseudopolydesmus serratus*	*Ptyoiulus impressus*	*Oxidus gracilis*	*Ophyiulus pilosus*	*Scytonotus granulatus*	*Uroblaniulus* sp.	*Miktoniscus medcofi*	*Trachelipus rathkei*	*Ligidium elrodii*
Bull Hollow sink 1			X			X										
Bull Hollow sink 2	X				X	X							X		X	X
Boone Creek sink 1						X		X							X	X
Boone Creek sink 2						X		X				X		X	X	X
Mill Creek sink 1								X							X	X
Mill Creek sink 2						X										X
Deuchars North sink 1					X	X									X	
Deuchars South sink 1																X
Deuchars South sink 2							X	X				X				
Deuchars South sink 3								X								
Rock Springs sink 1						X		X		X	X	X				
Rock Springs sink 2		X				X	X			X	X					
Rock Springs sink 3				X		X	X	X		X	X	X				X
Blanton sink 1										X	X				X	
Blanton sink 2	X						X	X		X	X				X	
Kimball sink 1	X			X		X		X	X	X	X			X		
Kimball sink 2						X				X	X		X			
Kimball sink 3								X		X	X				X	X
Kimball sink 4						X				X	X					
Hager sink 1											X					
Hager sink 2						X		X		X	X	X			X	X
Tincher sink 1					X	X		X			X					X
Tincher sink 2	X					X	X	X		X	X					
Union Cemetery sink 1											X	X				X
Union Cemetery sink 2																
Deam Wilderness sink 1								X			X					
Bull Hollow control																
Tincher control																
Deuchars North control																

phous calcium carbonate, and gleaning calcium from the shells and bones of deceased animals. Terrestrial gastropods living around carbonate cliffs can exhibit large and diverse populations but show significant declines in abundance in as little as 50 m from a calcareous source or limestone cliff (Dourson et al., 2013).

The most unusual terrestrial snail encountered was originally described by Pilsbry (1948) as the ecological morph *Anguispira kochi* form *aperta,* from an unspecified habitat in Spring Mill State Park. The form was synonymized with *A. kochi* by Hubricht (1985). Specimens with the morphology of this ecological morph were found in Kimball Tract sinks 2 and 3. Although the location at Spring Mill was unspecified, like the Kimball Tract, the entire state park lies in the Mitchell Sinkhole Plain.

The most common land snail encountered during the study was the carnivorous *Haplotrema concavum*, documented at 11 sites, followed by *Triodopsis tridentata*, and *Carychium exile*: both species were found at 9 sites. All three

Table 4. Spiders occurring in HNF sinkholes.

Site	*Agelenopsis pennsylvanica*	*Agyneta unimaculata*	*Agyneta parva*	*Allocosa noctuabunda*	*Anahita punctulata*	*Anyphaena celer*	*Anyphaena* sp.	*Araneus marmoreus*	*Ariadna bicolor*	*Bathyphantes pallidus*	*Calositticus cutleri*	*Centromerus cornupalpis*	*Centromerus latidens*	*Ceratinops latus*	*Ceratinopsidis formosa*	*Ceratinopsis interpres*	*Ceraticelus fissiceps*	*Cicurina arcuata*	*Cicurina brevis*	*Cicurina itasca*	*Cicurina* sp.	*Colonus puerperus*	*Cyclosa* sp.	*Dipoena* sp.	*Drassyllus fallens*	*Eurypois* sp.	*Faiditus* sp.	*Frontinella communis*	*Ghelna canadensis*	*Gladicosa pulchra*	*Hyptiotes cavaticus*	*Leucauge venusta*	*Lycosidae* sp.
Bull Hollow sink 1																					X						X					X	
Bull Hollow sink 2					X								X		X																	X	
Boone Creek sink 1					X		X						X																X			X	
Boone Creek sink 2			X																													X	
Mill Creek sink 1										X		X						X															
Mill Creek sink 2										X													X										
Deuchars North sink 1					X																X							X		X		X	
Deuchars South sink 1											X																					X	
Deuchars South sink 2					X													X														X	
Deuchars South sink 3																																	
Rock Springs sink 1					X								X				X	X			X											X	
Rock Springs sink 2										X			X								X	X										X	
Rock Springs sink 3		X								X		X		X				X	X		X										X	X	
Blanton sink 1							X			X												X											
Blanton sink 2							X			X		X														X						X	
Kimball sink 1	X		X										X								X							X				X	
Kimball sink 2								X																X								X	
Kimball sink 3													X																			X	
Kimball sink 4																		X														X	
Hager sink 1										X																							
Hager sink 2										X		X														X							
Tincher sink 1												X																				X	
Tincher sink 2				X	X		X													X				X	X							X	
Union Cemetery sink 1					X	X						X				X								X									
Union Cemetery sink 2													X																			X	
Deam Wilderness sink 1																		X															
Bull Hollow control																																	
Tincher control			X						X																								
Deuchars North control																		X															X

species can be found in both calcareous and acidic environments. Of the 31 species documented in the study area, 87% are found in both substrates.

The highest number of target species was found in sinkholes within native deciduous forest, followed by glades, then pine plantings and fields, and the non-sinkhole control areas (Fig. 7). We infer species richness to be a function of the presence of organic litter, which accumulates in sinkholes surrounded by forest, but is almost absent in the field habitats. Statistical correlation of quadrat litter weight versus site target species was skewed by the presence of dense mats of pine needles in sinks surrounded by pines that tended to be more mulch-like and inhospitable the heavier the layer became. Some sinks in deciduous forest, e.g., Union Cemetery Sink 1, also had the litter weight skewed by the presence of a chunk of dense, heavy wood occurring in the quadrat.

Table 5. Spiders occurring in HNF sinkholes (continued).

Site	*Maevia inclemens*	*Mangora placida*	*Mangora* sp.	*Mermessus maculatus*	*Mermessus tridentatus*	*Metepeira* sp.	*Neoantistea agilis*	*Neoantistea magna*	*Neoantistea* sp.	*Neon nelli*	*Neriene clathrata*	*Neriene radiata*	*Neriene* sp.	*Origanates rostratus*	*Oxyopes* sp.	*Pholcomma hirsutum*	*Pholcus* sp.	*Phrurolithus singulus*	*Phrurolithus* sp.	*Phrurotimpus alarius*	*Phrurotimpus borealis*	*Phrurotimpus* sp.	*Pirata alachuus*	*Pirata apalacheus*	*Pirata insularis*	*Pirata* sp.	*Pisaurina dubia*	*Pisaurina* sp.	*Robertus frontatus*
Bull Hollow sink 1										X		X								X						X			
Bull Hollow sink 2		X										X										X	X			X		X	
Boone Creek sink 1				X								X														X			
Boone Creek sink 2	X																									X			
Mill Creek sink 1						X												X								X			
Mill Creek sink 2															X											X		X	
Deuchars North sink 1																				X									
Deuchars South sink 1		X										X											X			X		X	
Deuchars South sink 2			X																							X	X		
Deuchars South sink 3																										X			
Rock Springs sink 1				X								X										X				X		X	
Rock Springs sink 2				X			X	X															X			X		X	
Rock Springs sink 3		X		X			X		X	X		X					X		X	X				X		X			X
Blanton sink 1				X																						X		X	
Blanton sink 2															X											X			
Kimball sink 1		X		X	X							X										X				X		X	
Kimball sink 2																							X			X			
Kimball sink 3				X																	X	X	X			X			
Kimball sink 4																				X						X		X	
Hager sink 1											X	X																	
Hager sink 2				X																			X			X			
Tincher sink 1					X									X									X		X				
Tincher sink 2								X					X			X				X		X				X			
Union Cemetery sink 1																						X				X			
Union Cemetery sink 2							X					X								X						X			
Deam Wilderness sink 1				X										X												X			
Bull Hollow control																						X							
Tincher control																													
Deuchars North control																													

Sinkhole habitats had a significantly higher species richness than control sites (t_7 = 3.122, p < 0.05; Fig. 8). Species richness differed significantly among habitats (F = 9.125, p < 0.0005; Fig. 9). Specifically, deciduous forests were significantly more species-rich than fields (p < 0.005) and pine habitats (p < 0.005). The community of target species varied significantly by habitat (F = 33.24, p < 0.0001; Fig. 10). Specifically, the community of target species within the control habitats was significantly different from within the deciduous forest (p < 0.01), fields (p < 0.01), and pine habitats (p < 0.05). Moreover, the community of target species within the deciduous forest habitat was significantly different from within the field (p < 0.01) and the pine habitats (p < 0.01). All other comparisons between habitats were not significantly different in the pairwise multilevel comparison analysis.

The relationship of sinkhole dimensions to biodiversity was analyzed in several ways, but none returned conclusive results. The presence of the greatest number of target species in the deepest sinkhole (38 species at Rock Springs sinkhole 3) intuitively suggests a correlation, but 31 species were collected from Tincher Karst sinkhole 2, which was

Table 6. Spiders (continued) and pseudoscorpions occurring in HNF sinkholes.

Site	Salticidae sp.	*Schizocosa saltatrix*	*Scotinella redempta*	*Scotinella* sp.	*Synema parvulum*	*Talanites exlineae*	*Tapinocyba emertoni*	*Tenuiphantes sabulosus*	*Theridion* sp.	*Theridiosoma gemmosum*	*Theridula* sp.	*Tmarus* sp.	*Trabeops aurantiacus*	*Uloborus* sp.	*Verrucosa arenata*	*Wadotes calcaratus*	*Wadotes hybridus*	*Wadotes* sp.	*Walckenaeria communis*	*Xysticus fraternus*	*Xysticus* sp.	*Apochthonius moestus*	*Apochthonius* sp.	*Chitrella* sp.	Chthoniidae sp.	*Chthonius tetrachelatus*	*Kleptochthonius griseomanus*	*Microbisium parvulum*	*Microbisium* sp.	Neobisiidae sp.
Bull Hollow sink 1			X			X					X											X								
Bull Hollow sink 2			X	X		X										X				X			X						X	
Boone Creek sink 1		X	X											X		X						X								
Boone Creek sink 2								X																						
Mill Creek sink 1																					X									
Mill Creek sink 2																										X				
Deuchars North sink 1		X				X																								X
Deuchars South sink 1								X																						
Deuchars South sink 2								X																						
Deuchars South sink 3																														
Rock Springs sink 1			X					X								X						X	X	X						
Rock Springs sink 2		X					X	X														X								X
Rock Springs sink 3			X				X	X	X	X					X	X		X				X						X		
Blanton sink 1											X																			
Blanton sink 2			X																X											
Kimball sink 1			X					X		X	X	X	X					X				X					X			X
Kimball sink 2								X		X		X										X								
Kimball sink 3					X													X												
Kimball sink 4																		X				X			X					
Hager sink 1													X																	
Hager sink 2								X																						
Tincher sink 1				X				X		X																				X
Tincher sink 2			X														X					X	X							
Union Cemetery sink 1			X					X				X				X		X												
Union Cemetery sink 2								X														X		X					X	
Deam Wilderness sink 1																						X								
Bull Hollow control	X																													
Tincher control																														
Deuchars North control																														

one of the shallowest sampled at 0.4 m depth. The correlation coefficient of species diversity as a function of sinkhole depth was weak at 0.23. Several other approaches to model sinkholes as cone or bowl-shaped depressions were analyzed to search for correlations with species diversity with similar results, until plant community subsets of the data were used. The strongest correlation was for sinks associated with glades, where a strong 0.98 correlation was found between modeled sink size and species richness, but a larger sample size is necessary to pursue this avenue of analysis further.

Figure 5. The pseudoscorpion *Kleptochthonius griseoanus* from Kimball Tract sinkhole 1.

Figure 6. The landsnail *Anguispira kochi* from Kimball Tract sinkhole 3.

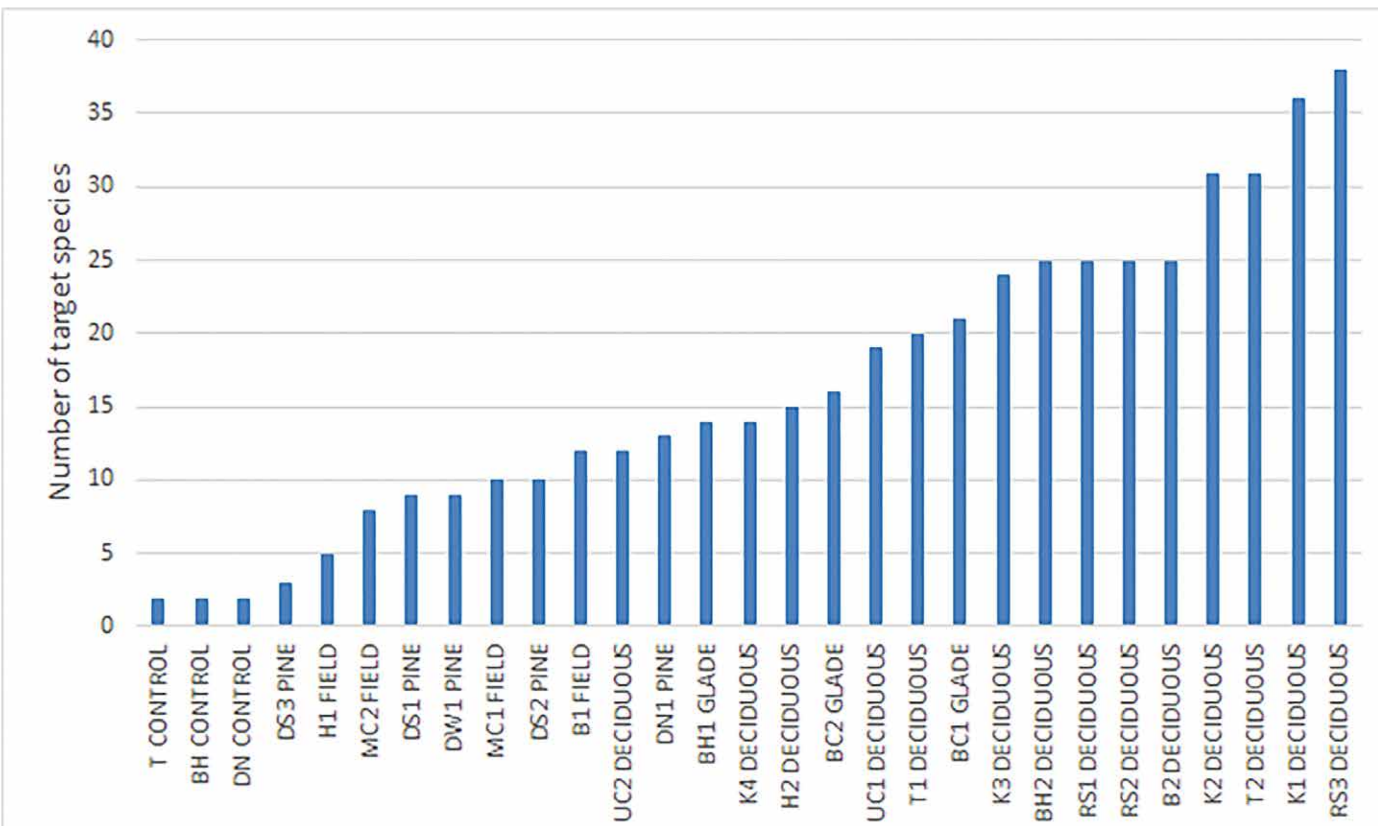

Figure 7. Histogram showing the number of target species sorted by surrounding plant community (sink designation abbreviations in Table 1).

The highest biodiversity in sinkholes resulted from the confluence of fauna from different contributing pools:

1. Widespread Eastern North America fauna – opportunistic species with wide ranges that are likely to occur in a variety of suitable habitats. These included terrestrial snails *Carychium exile, Anguispira alternata*, or Orchard Spider *Leucauge venusta*.
2. Southeastern fauna – warm latitude species on the northern edge of their ranges, using environmentally buffered habitats moderated against cold winter temperatures. These included the terrestrial snails *Inflectarius ru-*

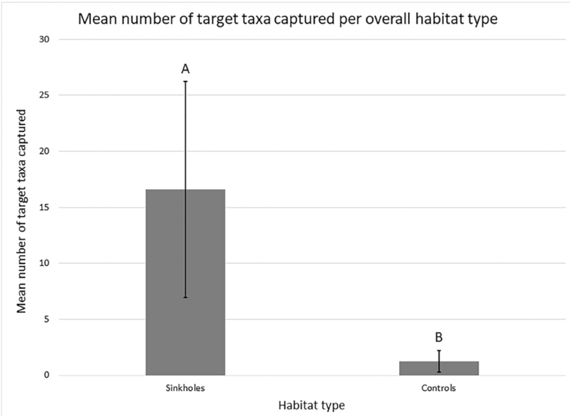

Figure 8. Mean species richness comparison between sinkhole habitats and controls, with different letters indicating significant differences at $p < 0.05$.

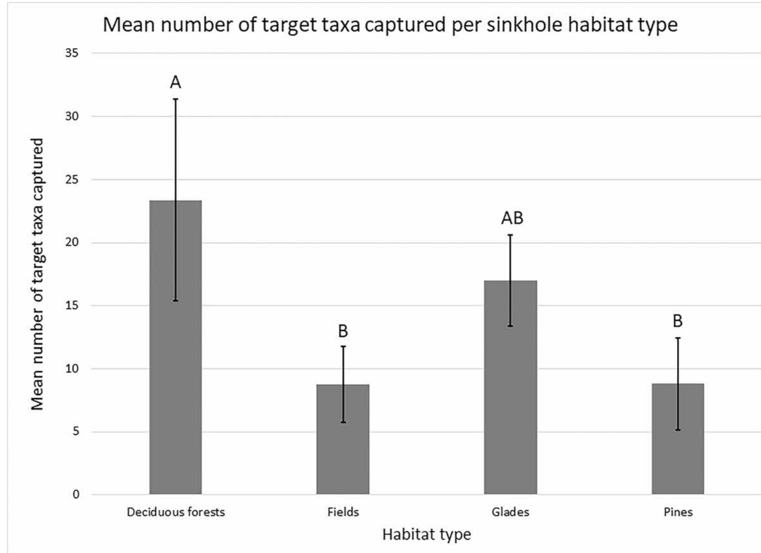

Figure 9. Mean species richness per sinkhole habitat type, with different letters indicating significant differences at $p < 0.0005$.

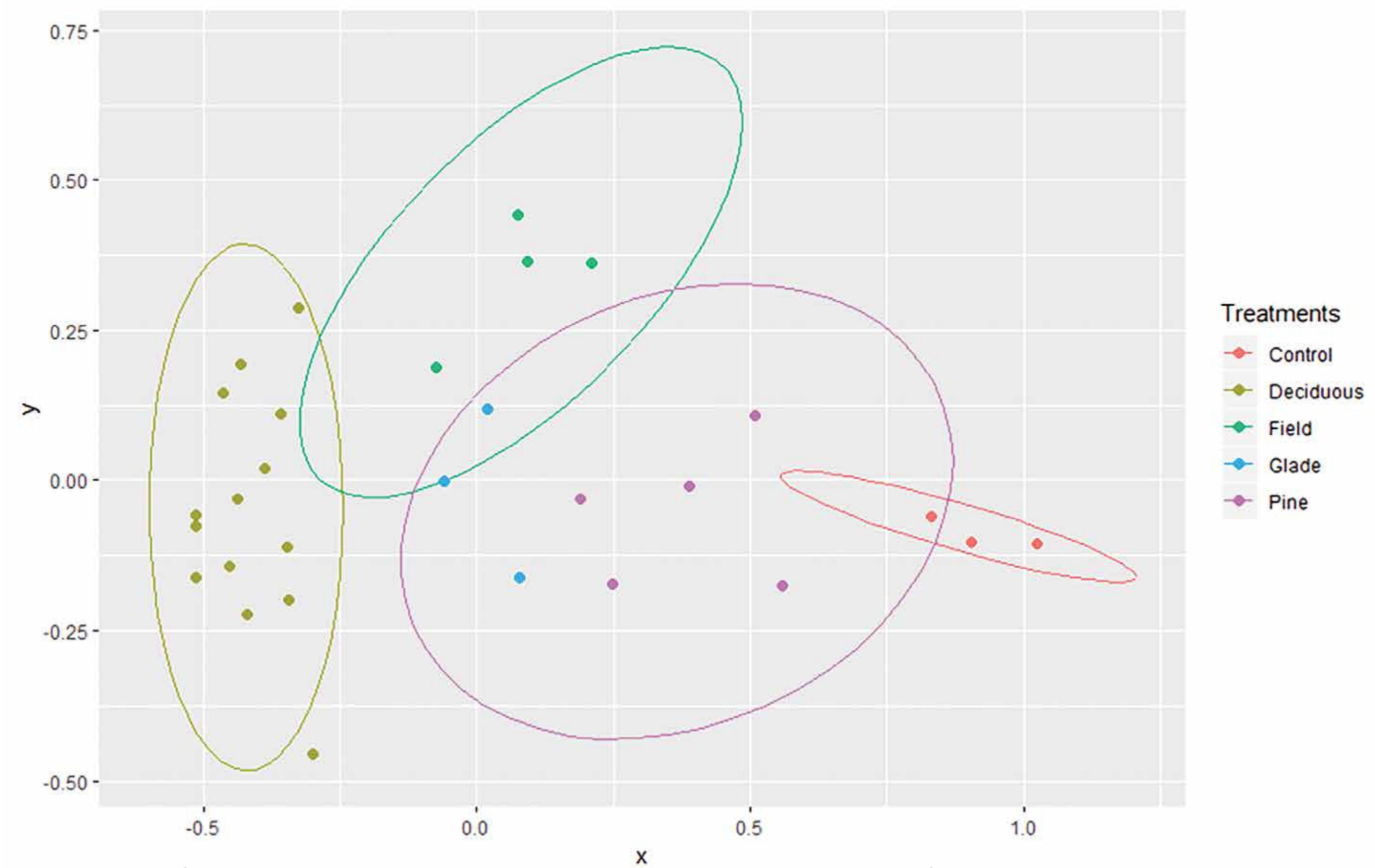

Figure 10. Plot of non-metric multidimensional scaling analysis comparing communities of target species within various habitats. Each dot represents such a community at a site. Ellipses represent 95% confidence intervals. The glade habitat lacked enough replicate sites to accurately predict a 95% confidence interval on this plot. This statistic produces an ordination based on a dissimilarity matrix without labelled axes.

geli, Mesomphix vulgatus, terrestrial isopods *Ligidium elrodii*, *Miktoniscus medcofi*, and spiders *Anahita punctulata, Talanites exlineae.*

3. Northern species – species adapted to inhabit cooler, northerly temperate latitudes, living in sinkholes and caves along the southern edge of their ranges, using environmentally-buffered habitats moderated against hot

summer temperatures. Examples included the terrestrial snail *Zonitoides limatulus*, and spiders *Cicurina arcuata, Cicurina itasca, Centromerus cornupalpis*, and the millipede *Scytonotus granulatus*.

4. Endemic fauna – habitat-restricted species known only from southern Indiana or the somewhat broader karst region of the northern Interior Low Plateaus physiographic province (i.e., parts of Kentucky, southern Indiana, and adjacent southern Illinois). Southern Indiana species included the pseudoscorpion *Kleptochthonius griseomanus*, and millipede *Conotyla bollmani;* northern Interior Low Plateaus karst region species included the millipede *Cleidogona unita.* The pseudoscorpion *Chitrella* sp. falls into the latter category and is likely an undescribed species of particular interest; the two described species in this genus are obligate stygobionts.
5. Exotic species – opportunistic, cosmopolitan species introduced from Eurasia, e.g., terrestrial isopod *Trachelipus rathkei*, and the millipedes *Oxidus gracilis* and *Ophyiulus pilosus*.

CONCLUSIONS

The importance of biological studies of sinkholes was highlighted by the range expansion of eleven species of spiders, the range expansion of the millipede *C. unita* into Indiana, the finding of the pseudoscorpion *K. griseomanus* outside of a cave habitat, and the range expansion and potentially new species of the pseudoscorpion genus *Chitrella*. Evidence is provided for sinkhole floor communities being influenced by the plant communities present at their rims: unique sinkhole fauna assemblages were observed when sinkholes were clustered by their surrounding plant community (Fig. 5). The presence of deciduous forest had a positive impact on sinkhole fauna species richness and abundance (Figs. 2 and 4). Correspondingly, target fauna species richness and abundance declined in sinkholes where deciduous trees had been removed and replaced with fields or pine monocultures.

Of interest for conservation management of sinkholes, it is pertinent to point out that other research suggests a greater role of dissolved organic carbon percolating into caves from sinkholes lacking discrete openings, or via other portals into the epikarst (Gibert, 1986; Graening and Brown, 2003; Simon et al., 2007), with a de-emphasis on the importance of organic debris entering open sinkhole drains or cave entrances. The results of the HNF sinkhole project, along with that of other cited research, suggest that sinkholes are as important as cave entrances in terms of both ecological function and biological diversity in karst ecosystems.

ACKNOWLEDGEMENTS

This project was funded by the U.S. Forest Service, Hoosier National Forest, Bedford, Indiana. Our greatest thanks go to Steve Harriss, the Karst Coordinator for the HNF, who conceived of the project, facilitated its funding, suggested sites, and arranged for logistical support in a forest where some of the project sinks were many miles from the nearest road. Likewise, Ron Scott, the Lands and Minerals Program Manager for the HNF, provided field and logistical support. Charles Boswell kindly provided transportation to the remote Bull Creek sinkholes on Mogan Ridge. As always, the support of the HNF Forest Supervisor, Mike Chaveas, is appreciated in our work on the national forest. Field support was also provided by Keith Dunlap (Indiana Karst Conservancy), Salisa Lewis (Lewis and Associates), and Dr. Gavin Bradley (University of Louisville).

REFERENCES

Culver, D.C., and Pipan, T., 2009, The biology of caves and other subterranean habitats: Oxford, England, U.K., Oxford University Press, 256 p.

Dourson, D.C., Langdon, K., and Dourson, J., 2013, Land snails of the Great Smoky Mountains National Park and southern Appalachians, Tennessee & North Carolina: Bakersville, NC, USA, Goatslug Publications, 336 p.

Farlow, J.O. et al., 2001, The Pipe Creek sinkhole biota, a diverse late Tertiary continental fossil assemblage from Grant County, Indiana: The American Midland Naturalist, v. 145, p. 367–378. https://doi.org/10.1674/0003-0031(2001)145[0367:TPCSBA]2.0.CO;2.

Frushour, S.S., 2012, A guide to caves and karst of Indiana: Bloomington, IN, USA, Indiana University Press, 142 p.

Gibert, J., 1986, Ecologie d'un système karstique jurassien. Hydrogéologie, dérive animale, transits de matières, dynamique de population de *Niphargus* (Crustacé Amphipode): Mémoires de biospéologie, v. 13, p. 1–379.

Graening, G.O., and Brown, A.V., 2003, Ecosystem dynamics and pollution effects and pollution effects in an Ozark cave stream: Journal of the American Water Resources Association, v. 39, p. 1497–1505. https://doi.org/10.1111/j.1752-1688.2003.tb04434.x.

Hoffman, R.L., and Lewis, J.J., 1997, *Pseudotremia conservata*, a new cleidogonid milliped (Diplopoda: Chordeumatida), with a synopsis of the cavernicolous millipeds of Indiana: Myriapodologica, v. 4, p. 107–119.

Homoya, M.A., Abrell, D.B., Aldrich, J.R., and Post, T.W., 1985, The natural regions of Indiana: Proceedings of the Indiana Academy of Science, v. 94, p. 245–268.

Hubricht, L., 1985, The distributions of the native land mollusks of the Eastern United States: Fieldiana Zoology, p. 1–191. https://doi.org/10.5962/bhl.title.3329.

Lewis, J.J., 2011, Conservation assessment of subterranean communities of the Hoosier National Forest: U.S. Forest Service, U.S. Department of Agriculture, 60 p.

Lewis, J.J., 2012, Subterranean ecosystems, *in* Whitaker, J.O., Jr. and Amlaner, C.J., Jr. eds., Habitats and Ecological Communities of Indiana: Presettlement to Present, Bloomington, IN, USA, Indiana University Press, p. 156–176.

Lewis, J.J., 1998a, The subterranean fauna of the Blue River area. Final Report. Volume 1.: Species at Risk Program, U.S. Geological Survey; The Nature Conservancy; Natural Heritage Program, Indiana DNR, Division of Nature Preserves, 241 p.

Lewis, J.J., 1998b, The subterranean fauna of the Blue River area. Final Report. Volume 2.: Species at Risk Program, U.S. Geological Survey; The Nature Conservancy; Natural Heritage Program, Indiana DNR, Division of Nature Preserves, 60 p.

Lewis, J.J., Burns, R., and Lewis, S., 2004, The subterranean fauna of the Hoosier National Forest: U.S. Forest Service, U.S. Department of Agriculture, 190 p.

Lewis, J.J., Burns, R., and Rafail, S., 2002, The subterranean fauna of the Hoosier National Forest: U.S. Forest Service, U.S. Department of Agriculture, 115 p.

Lewis, J.J., and Lewis, S.L., 2012a, Subterranean fauna of the Hoosier National Forest: Patoka Lake and Lost River tracts: U.S. Forest Service, U.S. Department of Agriculture, 136 p.

Lewis, J.J., and Lewis, S.L., 2012b, The cave fauna of Indiana, *in* A guide to caves and karst of Indiana, Bloomington, IN, USA, Indiana University Press, p. 15–23.

Lewis, J.J., and Lewis, S.L., 2008, The subterranean fauna of the Hoosier National Forest. Final Report: U.S. Forest Service, U.S. Department of Agriculture, 186 p.

Lewis, J.J., and Lewis, S.L., 2009, The subterranean fauna of the Hoosier National Forest. Final Report: U.S. Forest Service, U.S. Department of Agriculture, 127 p.

Malcolm, D.R., and Chamberlin, J.C., 1960, The pseudoscorpion genus *Chitrella* (Chelonethida, Syarinidae): American Museum Novitates, p. 1–19.

Milne, M.A., Foster, B., Lewis, J.J., Bishop, L., Hoffman, A., Ploss, T., and Deno, B., 2017, Spiders in Indiana: Seventy-one new and updated distribution records: Proceedings of the Indiana Academy of Science, v. 125, p. 75–85.

Muchmore, W.B., 2000, New species and records of *Kleptochthonius* from Indiana (Pseudoscorpionida, Chthoniidae): Journal of Arachnology, v. 28, p. 293–299. https://doi.org/10.1636/0161-8202(2000)028%5b0293:NSAROK%5d2.0.CO;2.

Muchmore, W.B., 1963, Redescription of some cavernicolous pseudoscorpions (Arachnida, Chelonethida) in the collection of the Museum of Comparative Zoology: Breviora, v. 188, p. 1–16.

Muchmore, W.B., 1973, The genus *Chitrella* in America (Pseudoscorpionida, Syarinidae): Journal of the New York Entomological Society, v. 81, p. 183–192.

Oksanen, J. et al., 2019, vegan: Community Ecology Package:, https://cran.r-project.org/package=vegan.

Pilsbry, H.A., 1948, Land mollusca of North America (north of Mexico). Volume II. Part 2: The Academy of Natural Sciences of Philadelphia Monographs, v. 3.

Powell, R.L., 1961, Caves of Indiana: Indiana Department of Conservation No. 8, 127 p.

Scott, W., 1910, The fauna of a solution pond: Proceedings of the Indiana Academy of Science, v. 20, p. 395–442.

Shear, W.A., 1971, The millipede family Conotylidae in North America with a description of the new family Adritylidae (Diplopoda: Chordeumida): Bulletin of the Museum of Comparative Zoology, v. 141, p. 55–98.

Sierwald, P., Draney, M.L., Prentice, T., Pascoe, F., Sandlin, N., Lehman, E.M., Medland, V., and Louderman, J., 2005, The spider species of the Great Lakes states: Proceedings of the Indiana Academy of Science, v. 114, p. 111–206.

Simon, K.S., Pipan, T., and Culver, D.C., 2007, A conceptual model of the flow and distribution of organic carbon in caves: Journal of Cave and Karst Studies, v. 69, p. 279–284.

White, H.W., 1930, The phytoplankton of a solution pond, with special reference to the periodicity of certain algae: Proceedings of the Indiana Academy of Science, v. 30, p. 123–140.

Wickham, H., Chang, W., Henry, L., Pedersen, T.L., Takahashi, K., Wilke, C., Woo, K., Yutani, H., and RStudio, 2019, ggplot2: Create elegant data visualisations using the grammar of graphics:, https://cran.r-project.org/package=ggplot2.

Woodall, C.W., Perez, J.A., and Thake, T.R., 2007, Forest resources of the Hoosier National Forest. Resource Bulletin NRS-18 2007: U.S. Forest Service, U.S. Department of Agriculture, 551 p. https://doi.org/10.2737/NRS-RB-18.

„Explore, understand and protect"

is the main goal of the International Year of Caves and Karst.
With your help, we seek to:

- **improve** public understanding of how caves and karst touch the daily lives of billions of people
- **promote** the importance of caves and karst through sustainable development, particularly in water quality and quantity, agriculture, geotourism/ecotourism, and natural/cultural heritage
- **demonstrate** how the study and proper management of caves and karst is critical to global economic and environmental health
- **build** worldwide educational capacity through activities targeted on cave and karst science
- **promote** awareness of the interdisciplinary nature of cave and karst science and management, and emphasize how interactions between different areas of science and management will be needed increasingly in future research, education, and environmental protection
- **establish** durable partnerships to ensure that these activities, goals and achievements continue in the future beyond the International Year of Caves and Karst.

International Year 2021 of Caves and Karst

Union Internationale de Spéléologie
www.uis-speleo.org

GUIDE TO AUTHORS

The *Journal of Cave and Karst Studies* is a multidisciplinary journal devoted to cave and karst research. The *Journal* is seeking original, unpublished manuscripts concerning the scientific study of caves or other karst features. Authors do not need to be members of the National Speleological Society, but preference is given to manuscripts of importance to North American speleology.

LANGUAGES: The *Journal of Cave and Karst Studies* uses American-style English as its standard language and spelling style, with the exception of allowing a second abstract in another language when room allows. In the case of proper names, the *Journal* tries to accommodate other spellings and punctuation styles. In cases where the Editor-in-Chief finds it appropriate to use non-English words outside of proper names (generally where no equivalent English word exist), the *Journal* italicizes them. However, the common abbreviations i.e., e.g., et al., and etc. should appear in roman text. Authors are encouraged to write for our combined professional and amateur readerships

CONTENT: Each paper will contain a title with the authors' names and addresses, an abstract, and the text of the paper, including a summary or conclusions section. Acknowledgments and references follow the text. Manuscripts should be limited to 6,000 words and no more than 10 figures and 5 tables. Larger manuscripts may be considered, but the *Journal* reserves the right to charge processing fees for larger submissions.

ABSTRACTS: An abstract stating the essential points and results must accompany all articles. An abstract is a summary, not a promise of what topics are covered in the paper.

STYLE: The *Journal* consults The Chicago Manual of Style on most general style issues.

REFERENCES: In the text, references to previously published work should be followed by the relevant author's name and date (and page number, when appropriate) in brackets. All cited references are alphabetical at the end of the manuscript with senior author's last name first, followed by date of publication, title, publisher, volume, and page numbers. Geological Society of America format should be used (see http://www.geosociety.org/documents/gsa/pubs/GSA_RefGuide_Examples.pdf). Please do not abbreviate periodical titles. Web references are acceptable when deemed appropriate. The references should follow the style of: Author (or publisher), year, Webpage title: Publisher (if a specific author is available), full URL (e.g., http://www.usgs.gov/citguide.html), and the date the website was accessed in brackets. If there are specific authors given, use their name and list the responsible organization as publisher. Because of the ephemeral nature of websites, please provide the specific date. Citations within the text should read: (Author, Year).

SUBMISSION: Manuscripts are to be submitted via the PeerTrack submission system at http://www.edmgr.com/jcks/. Instructions are provided at that address. At your first visit, you will be prompted to establish a login and password, after which you will enter information about your manuscript and upload your manuscript, tables, and figure files. Manuscript files can be uploaded as DOC, WPD, RTF, TXT, or LaTeX. Note: LaTeX files should not use any unusual style files; a LaTeX template and BiBTeX file may be obtained from the Editor-in-Chief. Table files can be uploaded as DOC, WPD, RTF, TXT, or LaTeX files and figure files can be uploaded as TIFF, AI, EPS, or CDR files. Extensive supporting data may be placed on the Journal's website as supplemental material at the discretion of the Editor-in-Chief. The data that are used within a paper must be made available upon request. Authors may be required to provide supporting data in a fundamental format, such as ASCII for text data or comma-delimited ASCII for tabular data.

DISCUSSIONS: Critical discussions of papers previously published in the *Journal* are welcome. Authors will be given an opportunity to reply. Discussions and replies must be limited to a maximum of 1000 words and discussions will be subject to review before publication. Discussions must be within 6 months after the original article appears.

MEASUREMENTS: All measurements will be in Systeme Internationale (metric) except when quoting historical references. Other units will be allowed where necessary if placed in parentheses and following the SI units.

FIGURES: Figures and lettering must be neat and legible. Figure captions should be on a separate sheet of paper and not within the figure. Figures should be numbered in sequence and referred to in the text by inserting (Fig. x). Most figures will be reduced, hence the lettering should be large. Photographs must be sharp and high contrast. Figures must have a minimum resolution of 300 dpi for acceptance. Please do not submit JPEG images.

TABLES: See http://caves.org/pub/journal/PDF/Tables.pdf to get guidelines for table layout.

COPYRIGHT AND AUTHOR'S RESPONSIBILITIES: It is the author's responsibility to clear any copyright or acknowledgement matters concerning text, tables, or figures used. Authors should also ensure adequate attention to sensitive or legal issues such as land owner and land manager concerns or policies and cave location disclosures.

PROCESS: All submitted manuscripts are sent out to at least two experts in the field. Reviewed manuscripts are then returned to the author for consideration of the referees' remarks and revision, where appropriate. Revised manuscripts are returned to the appropriate Associate Editor who then recommends acceptance or rejection. The Editor-in-Chief makes final decisions regarding publication. Upon acceptance, the senior author will be sent one set of PDF proofs for review. Examine the current issue for more information about the format used.

Journal of Cave and Karst Studies

Volume 82 Number 4 December 2020

CONTENTS

Visit us at www.caves.org/pub/journal