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Front cover: A Berry Cave Salamander (*Gyrinophilus gulolineatus*) from the type-locality, Roane Co., Tennessee. Photo by Matthew Niemiller.

M.L. Niemiller, K.S. Zigler, C.D.R. Stephen, E.T. Carter, A.T. Paterson, S.J. Taylor, and A.S. Engel – Vertebrate fauna in caves of eastern Tennessee within the Appalachians karst region, USA. *Journal of Cave and Karst Studies,* v. 78, no. 1, p. 1–24. DOI: 10.4311/2015LSC0109

VERTEBRATE FAUNA IN CAVES OF EASTERN TENNESSEE WITHIN THE APPALACHIANS KARST REGION, USA

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Abstract: More than one-fifth of the documented caves in the United States occur in Tennessee. The obligate subterranean biota of Tennessee is rich and diverse, with 200 troglobionts reported from over 660 caves. Fifty troglobionts are known from just 75 of the 1,469 caves in the Appalachian Valley and Ridge physiographic province of eastern Tennessee. Tennessee's Valley and Ridge has been under-sampled relative to other karst areas in the state, limiting our knowledge of cave and karst species diversity and distributions and compromising our ability to identify habitats and species potentially at risk from anthropogenic threats, such as urban sprawl near the metropolitan area of Knoxville. Knowledge of nontroglobiontic species inhabiting caves, including vertebrates, is particularly sparse in this region. Although caves have long been recognized as critical habitats for several bat species, the importance of caves for other vertebrate taxa has received less attention. Caves are important habitats for many other nontroglobiontic vertebrates and should be considered in the management and conservation of these species. Our decade-long study bioinventoried 56 caves in 15 counties and begins to address knowledge gaps in distributions and cave use by vertebrates in the Valley and Ridge and adjacent Blue Ridge Mountains of eastern Tennessee within the Appalachians karst region. In addition, we conducted a thorough review of the literature and museum databases for additional species-occurrence records in those provinces of eastern Tennessee. From these sources, we present an annotated list of 54 vertebrate taxa, including 8 fishes, 19 amphibians (8 anurans and 11 salamanders), 6 reptiles, 3 birds, and 18 mammals. Three species are included on the IUCN Red List of Threatened Species, while six species are at risk of extinction based on NatureServe conservation rank criteria. Ten bat species are known from 109 caves in 24 eastern Tennessee counties. Our bioinventories documented five bat species in 39 caves, including new records of the federally endangered Gray Bat (*Myotis grisescens*). We observed visible evidence of white-nose syndrome caused by the fungal pathogen *Pseudogymnoascus destructans* at four caves in Blount, Roane, and Union counties. We documented two new localities of the only troglobiontic vertebrate in the Valley and Ridge, the Berry Cave Salamander (*Gyrinophilus gulolineatus*). Despite these efforts, significant sampling gaps remain—only 7.7% of known caves in the Valley and Ridge and Blue Ridge Mountains of eastern Tennessee have records of vertebrate-species occurrence. Moreover, few caves in eastern Tennessee have experienced repeated, comprehensive bioinventories, with the exception of periodic surveys of hibernating bats at selected caves. Future bioinventory efforts should incorporate multiple visits to individual caves, if possible, and more efforts should focus on these understudied areas of eastern Tennessee.

Introduction

Caves and karst habitats are important in varying degrees to many vertebrate species, most of which are not troglobionts. Many species of amphibians and mammals use caves for foraging, reproduction, or refuges from harsh conditions on the surface during periods of drought or extreme temperatures. Several species of bats, such as *Myotis grisescens*, roost in caves during the summer in large colonies or use caves for hibernation during the winter. Caves are the sites of reproduction and nesting for several species of salamanders. Some fish and salamander species that occur in surface habitats, such as *Cottus carolinae* and *Gyrinophilus porphyriticus*, can also live their entire lives in caves. Understanding the distribution and extent of cave utilization is important for proper conservation and management of these vertebrate species, but few

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comprehensive, annotated lists of extant vertebrate cave fauna have been published for the eastern United States. Cliburn and Middleton (1983) discussed the vertebrate fauna of Mississippi caves, and Garton et al. (1993) reviewed the vertebrate cave fauna of West Virginia. Faunal lists at varying geographic scales have been published for taxa such as fishes (Poly, 2001; Poly and Boucher, 1996) and amphibians and reptiles (Himes et al., 2004; Niemiller and Miller, 2009).

More caves are known in Tennessee than in any other state in the United States, with over 10,000 documented caves as of 2015 occurring in two major karst regions, the Interior Low Plateau and the Appalachians (Niemiller and Zigler, 2013). The Appalachians karst region extends from southeastern New York to northwestern Georgia and northeastern Alabama. In Tennessee, the Appalachians karst region includes karst within the Valley and Ridge province and Blue Ridge Mountains. The Valley and Ridge is flanked to the west by the Cumberland Plateau and to the east by the Blue Ridge Mountains, which contain limited exposures of karst. The Appalachians karst region is the second largest karst area in Tennessee, after the Highland Rim of the Interior Low Plateau.

Bioinventories of the subterranean fauna of Tennessee have been conducted for more than a century (e.g., Cope and Packard, 1881; Hay, 1902; Lewis, 2004, 2005; Lewis et al., 2010; Dixon and Zigler, 2011; Wakefield and Zigler, 2012). Most records are included in a report by Holsinger and Culver (1988) summarizing invertebrate cave fauna from Valley and Ridge bioinventories conducted between 1961 and 1980 in seven northeastern Tennessee counties and from approximately five hundred caves in Virginia. In the Blue Ridge Mountains of eastern Tennessee, studies of cave fauna are limited to those in and immediately adjacent to Great Smoky Mountains National Park (Wallace, 1984, 2003; Reeves, 2000; Dodd et al., 2001; Mays, 2002; Taylor and Mays, 2006). Niemiller and Zigler (2013) recently compiled over twenty-two hundred records of obligate subterranean species in Tennessee, and listed 200 invertebrate and vertebrate troglobiontic species identified from 661 caves in the state. As of 2013, troglobiontic faunal records exist for just 5.1% of known caves (75 of 1,469) in the Valley and Ridge province of eastern Tennessee and 7.6% of known caves (13 of 171) in the Blue Ridge Mountains (Niemiller and Zigler, 2013). From these karst regions, only 50 and 9 troglobiontic species have been documented, respectively.

Vertebrate cave fauna records of the Appalachians karst region in eastern Tennessee are largely limited to distributional records of cave-roosting bat species documented in state biological databases, such as those of Tennessee Natural Heritage Inventory Program and other government reports, and to studies of salamanders, mainly from caves in Great Smoky Mountains National Park (Dodd et al., 2001; Taylor and Mays, 2006) and particularly the Berry Cave Salamander *Gyrinophilus gulolineatus* (e.g., Miller and Niemiller, 2007, 2008; Niemiller et al., 2010). Considerable cave biodiversity remains to be discovered and documented. The Appalachians karst region has been under-sampled compared to many other regions of the state (Niemiller and Zigler, 2013). To begin filling in sampling gaps in Valley and Ridge and adjacent karst in the Blue Ridge Mountains of eastern Tennessee, we inventoried the fauna of 56 caves in 15 counties between October 23, 2004 and August 3, 2015, with the intention of creating annotated lists of vertebrates and invertebrates that will supplement faunal surveys that have already been published or that might be underway. The invertebrate fauna of the Appalachians karst region of eastern Tennessee will be addressed separately. For the vertebrate fauna lists, we compiled cave occurrence records for these areas of eastern Tennessee from a variety of published and unpublished literature sources, databases, and museum collections. We include these data to produce the first comprehensive faunal list of vertebrates from caves in this region.

Materials and Methods

We conducted bioinventories of 56 caves representing 107 cave visits within the Appalachian Valley and Ridge and adjacent Blue Ridge Mountains of eastern Tennessee (Table 1, Fig. 1). Many of these caves had never been bioinventoried. Survey sites included Blount (1 cave), Campbell (1), Carter (2), Claiborne (3), Grainger (1), Jefferson (2), Knox (21), Loudon (3), McMinn (2), Meigs (1), Monroe (6), Rhea (1), Roane (5), Sevier (1), and Union (6) counties. Cave descriptions, maps, and locations are maintained by the Tennessee Cave Survey (http://www.subworks.com/tcs/), and we report the TCS cave inventory number with the cave name. We emphasized Knox County, where *Gyrinophilus gulolineatus* may potentially exist and could be at risk from anthropogenic impacts.

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. For fishes, amphibians, and reptiles, we made a concerted effort to capture each individual observed either by hand or with dip nets to confirm identification and obtain voucher photographs. Depending on the extent of the cave system, each survey typically involved 2 to 4 surveyors (maximum 12), with a search effort of 2 to 36 person-hours per cave visit.

All statistical analyses were conducted in the R statistical computing environment (v.3.1.2; R Core Team, 2014). We explored possible relationships between species richness and horizontal length of caves, a proxy for available habitat because the caves sampled were predominantly horizontal in development, and between species richness and the number of bioinventories conducted at a cave using

Pearson correlation analyses. A weighted Pearson correlation analysis in the R package weights (v.0.80; Pasek, 2015) was employed to explore the relationship between species richness and cave length weighted by the number of bioinventories conducted at each cave. We also employed a Wilcoxon rank sum test to assess if wet caves differed from dry caves with respect to species diversity. Caves bioinventoried were classified as either wet or dry, where each cave was classified based on the dominant habitat type present, aquatic or terrestrial. Of the 56 caves bioinventoried, 36 caves were classified as wet and 20 caves as dry. When applicable, we report mean values ± standard deviation.

We searched for additional distributional records of vertebrates in eastern Tennessee 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. Database sources included biodiversity databases maintained by the Tennessee Natural Heritage Inventory Program (TNNH) and the Bat Population Database. We also queried the VertNet database (http://www.vertnet.org), a web portal to search accessions of over 170 vertebrate museum collections from 12 countries. Institutions for which accessions included specimens collected from eastern Tennessee include Carnegie Museum of Natural History (CM), Kansas University Biodiversity Institute (KU), Museum of Vertebrate Zoology at University of California-Berkeley (MVZ), North Carolina Museum of Natural Sciences (NCSM), Sam Noble Oklahoma Museum of Natural History (OMNH), Santa Barbara Museum of Natural History (SBMNH), Museum of Texas Tech University (TTU), University of Michigan Museum of Zoology (UMMZ), Smithsonian Institution National Museum of Natural History (USNM), and Western Foundation of Vertebrate Zoology (WFVZ). We attempted to georeference each distributional record using the TCS database of caves.

The annotated list of 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., Etnier and Starnes, 1993; Niemiller and Reynolds, 2011; Niemiller et al., 2013). 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 troglobiont (synonym: troglobite) or stygobiont (synonym: 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). Troglobionts and stygobionts 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 are capable of completing 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.

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 June 29, 2015]) and NatureServe (http://www.natureserve.org/ [accessed June 29, 2015]), is included to provide a better understanding of the distribution and biogeography of subterranean organisms in eastern Tennessee, 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 animals in Tennessee (Withers, 2009). 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 make a determination on 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; and (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).

Table 1. List of caves bioinventoried in the Appalachian Valley and Ridge and adjacent Blue Ridge Mountains of eastern Tennessee, including survey dates, Tennessee Cave Survey (TCS) cave number, and number of species documented for five classes of vertebrates observed: F – fishes; A – amphibians; R – reptiles; B – birds; and mammals (separated into bats and NM, non-bat mammals).

County	Cave	TCS No.	Date	F	A	R	B	NM	Bats	Total
Blount	Tuckaleechee Caverns	BA11	2014: 20 Mar		1				2	3
Campbell	Panther Cave No. 1	CM8	2015: 23 Mar		4		1	2	2	9
Carter	Carter Saltpeter Cave	CR1	2014: 14 May		1	1			1	3
Carter	Rockhouse Cave	CR3	2014: 14 May							0
Claiborne	Sour Kraut Cave	CB46	2015: 1 Jun		1		1	1		3
Claiborne	Buis Saltpeter Cave	CB48	2015: 1 Jun		1		1	1		3
Claiborne	Kings Saltpeter Cave	CB52	2015: 30 May		2		1	1		4
Grainger	Indian Cave	GA4	2014: 22 Feb; 29 Jun	1	3				3	7
Jefferson	Tater Cave	JF8	2015: 3 Aug		2			1	1	4
Jefferson	Silo Pit Cave	JF71	2015: 3 Aug		3	1				4
Knox	Campbell Cave	KN1	2014: 23 Dec				1	2	2	5
Knox	Cherokee Bluff Cave	KN4	2015: 7 Mar						1	1
Knox	Mudflats Cave	KN9	2004: 20 Nov 2005: 6 Jan; 30 Dec 2006: 12 Nov 2007: 7 Jun 2008: 2 Oct 2014: 5 Apr; 20 Oct	1	4			1	2	8
Knox	Carter Cave	KN14	2008: 21 May		3			1	1	5
Knox	Keller Bend Cave	KN16	2013: 16 May		1			1	1	3
Knox	Blowing Hole Cave	KN19	2013: 16 May		3			1	1	5
Knox	Cherokee Caverns	KN22	2014: 5 Apr		1				1	2
Knox	Cruze Cave	KN24	2004: 31 Oct 2005: 6 Jan; 6 Mar; 31 Dec 2006: 18 Jul; 10 Sep; 19 Nov 2008: 19 May; 7 Jul 2013: 13 May; 15 Jun 2014: 10 Apr; 11 May; 19 Jun; 14 Aug; 13 Oct		4			1	2	7
Knox	Meads Quarry Cave	KN28	2004: 23 Oct 2006: 4 Nov 2007: 22 Apr; 9 Sep; 8, 24 Nov 2008: 24, 31 Jan; 1, 6, 30 Mar; 10, 30 Apr; 15 May; 4, 27 Jun; 30 Jul; 10 Sep 2013: 5 Oct		8	1		2	3	14
Knox	Christian Cave	KN49	2005: 17 Sep	2	3				2	7
Knox	Kirkpatrick Cave	KN62	2014: 9 Feb; 6 Jul		1				2	3
Knox	Unreported Cave	KN90	2014: 5 Apr		2			1	1	4
Knox	Brents Cave	KN112	2012: 8 May		1				1	2
Knox	Burnett Cave	KN125	2008: 21 May		2					2
Knox	Chriscroft Cave	KN127	2014: 20 Oct		2			1	1	4
Knox	The Lost Puddle	KN145	2012: 8 May		2				1	3
Knox	Ebenezer Rising Cave	KN150	2004: 20 Nov	2	1		1	2		6
Knox	Meads River Cave	KN151	2004: 23 Oct 2007: 22 Apr; 8, 24 Nov; 2 Dec		8	1			1	10
Knox	Fifth Entrance Cave	KN167	2004: 23 Oct 2007: 8 Nov		4					4
Knox	Aycock Spring Cave	KN172	2005: 17 Sep	2	5					7

Table 1. Continued.

County	Cave	TCS No.	Date	F	A	R	B	NM	Bats	Total
Knox	Steamboat Crawl	KN173	2007: 5 Apr		3					3
Loudon	Blankenship Cave	LN1	2014: 25 Jan		1				1	2
Loudon	Benjos Cave	LN11	2014: 30 Aug		2					2
Loudon	Phantom Insurgence Cave	LN22	2014: 30 Aug	2	5			1	1	9
McMinn	Small Cave	MM5	2014: 10 May		6		1	2	2	11
McMinn	Too Small Cave	MM6	2014: 10 May		2		1	1		4
Meigs	Sensabaugh Cave	ME3	2014: 31 Aug		3			1	1	5
Monroe	The Lost Sea	MO1	2014: 9 Sep							0
Monroe	Gay Cave	MO3	2013: 16 Nov		2			2	1	5
Monroe	Morgan Cave	MO5	2013: 26 Oct		3			2	1	6
Monroe	Nobletts Cave	MO6	2014: 26 Nov		2			1	1	4
Monroe	Lick Creek Cave	MO8	2013: 16 Nov	3			1	1	1	6
Monroe	Alans Hideway Cave	MO9	2013: 16 Nov		2		1		1	4
Rhea	Grassy Creek Cave	RH2	2014: 22 Dec		3			1	2	6
Roane	Berry Cave	RN3	2004: 17 Dec 2005: 5 Mar 2014: 28 Jun	3	5		1	1	2	12
Roane	Cave Creek Cave	RN5	2007: 7 Jun 2014: 28 Jun	2	4		1	1	1	9
Roane	Eblen Cave	RN6	2005: 30 Dec 2013: 15 May	1	3			2	2	8
Roane	Big Cave	RN13	2005: 5 Mar		2				2	4
Roane	Chimney Cave	RN14	2005: 5 Mar						1	1
Sevier	Two County Cave	SV36	2014: 5 Jul	1	1			1		3
Union	Oaks Cave	UN5	2015: 23 Mar		1		1	2	3	7
Union	Wright Cave	UN9	2015: 21 Mar		2		1	1	1	5
Union	Big Cave	UN10	2015: 22 Mar		3		1	2		6
Union	Rogers Hollow Cave	UN23	2015: 22 Mar		1		1	1	1	4
Union	Mossy Spring Cave	UN25	2015: 22 Mar		3			2		5
Union	Ellison Hollow Cave	UN46	2015: 22 Mar		1		1			2

Two additional ranks associated with extinction exist: GH (Possibly Extinct) and GX (Presumed Extinct). At the global scale, a Questionable rank qualifier (Q) can be used to denote uncertainty in the conservation status rank (e.g., G2Q). 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.

Results and Discussion

Annotated List of Vertebrate Fauna of the Appalachians Karst Region in Eastern Tennessee

Phylum Chordata

Subphylum Vertebrata

Class Actinopterygii

Order Cypriniformes

Family Cyprinidae

Genus *Luxilus*

***Luxilus chrysocephalus* Rafinesque, 1820 (AC) Striped Shiner**

Localities: Rhea Co.: Grassy Creek Cave (RH2)*.

Conservation status: IUCN: Least Concern; NatureServe: G5 (S5 in Tennessee).

Comments: An adult was observed in the main stream near the upstream quarry entrance of Grassy Creek Cave. This represents the first report of this species from a cave. We hypothesize that fish enter caves either from being washed in during flooding events or following streams upstream and entering caves through springs, which may be the situation for Grassy Creek Cave.

References: * present study.

Genus *Nocomis*

***Nocomis micropogon* (Cope, 1865) (AC) River Chub**

Localities: Monroe Co.: Lick Creek Cave (MO8)*, karst window NNE of Lick Creek Cave*.

Conservation status: IUCN: Least Concern; NatureServe: G5 (S5 in Tennessee).

Comments: A single fish was observed in the sump pool of Lick Creek Cave. Three fish were observed in a shallow pool at the base of a karst window ca. 250 m NNE of the

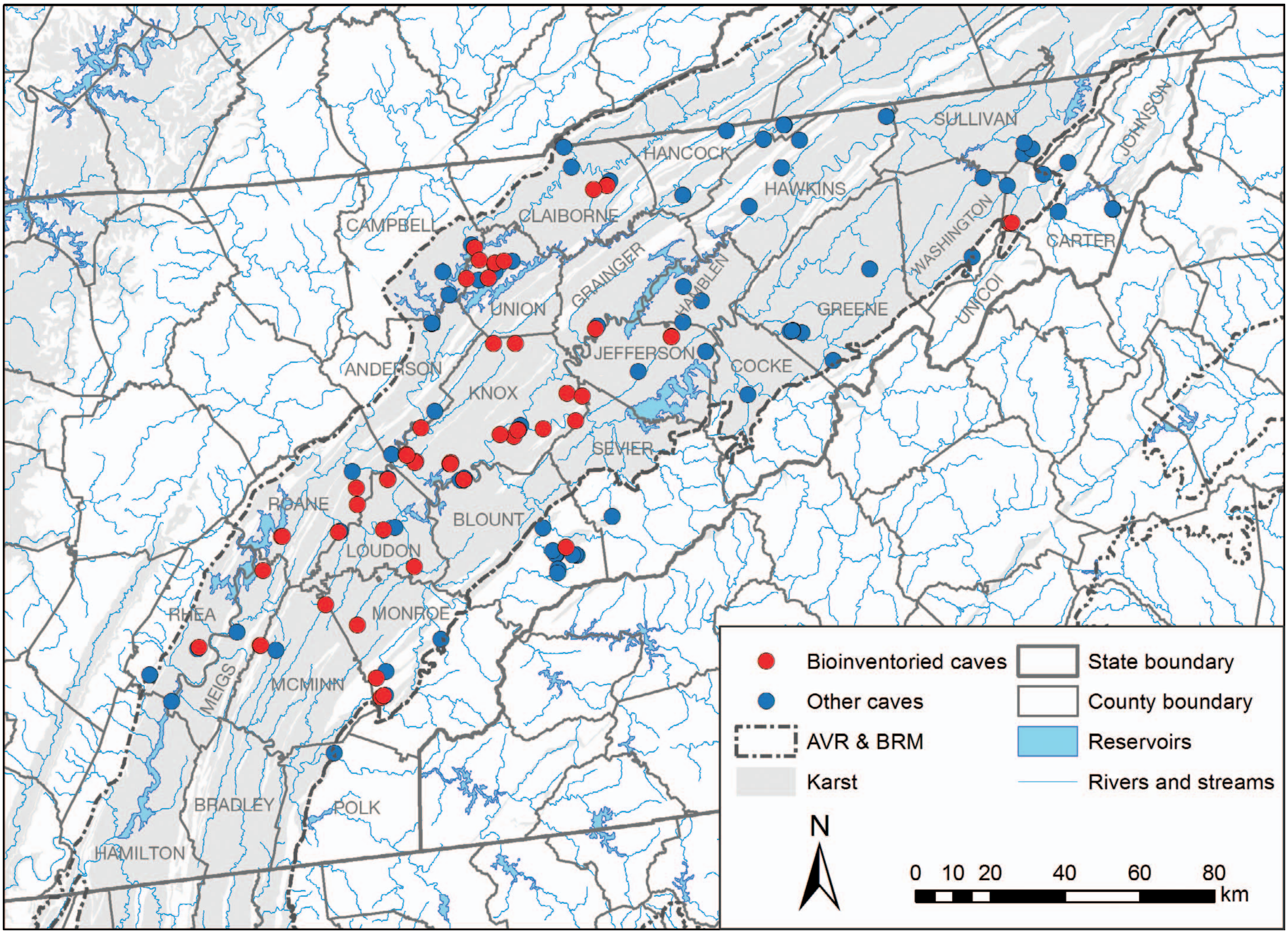

Figure 1. Map of 56 caves bioinventoried and 76 additional caves with vertebrate records in the Appalachian Valley and Ridge and adjacent Blue Ridge Mountains of eastern Tennessee from 24 counties. Karst carbonate rock are depicted in gray based on the U.S. Karst Map (Weary and Doctor, 2014).

spring entrance to Lick Creek Cave. This species was previously reported from caves in West Virginia (Poly and Boucher, 1996).

References: * present study.

Genus *Notropis*

***Notropis* spp. (AC) Unidentified shiner**

Localities: Knox Co.: Christian Cave (KN49)*, Ebenezer Rising Cave (KN150)*, Aycock Spring Cave (KN172)*; Loudon Co.: Phantom Insurgence Cave (LN22)*; Rhea Co.: Grassy Creek Cave (RH2)*; Roane Co.: Berry Cave (RN3)*, Cave Creek Cave (RN5)*.

Conservation status: Not applicable.

Comments: Shiners were observed but could not be captured to confirm species identity. Several *Notropis* species have been reported from caves in the eastern United States (Bailey, 1933; Kuehne, 1966; Armstrong and Williams, 1971; Relyea and Sutton, 1973; Pearson and Boston, 1995; Poly and Boucher, 1996; Poly, 2001; Ruhl, 2005) and may wash into caves during flooding events and become trapped after waters recede. Fifteen *Notropis* species are known from the Appalachian Valley and Ridge of eastern Tennessee (Etnier and Starnes, 1993), and several genera of Cyprinidae have been reported from caves in nearby regions of northern Alabama (Rheams et al., 1992), including *Notropis* minnows. It is possible that cave systems may allow some aquatic surface species to disperse across hydrological drainage divides (Ray et al., 2014).

References: * present study.

Genus *Rhinichthys*

***Rhinichthys atratulus* (Hermann, 1804) (AC) Eastern Blacknose Dace**

Localities: Claiborne Co.: a cave near New Tazewell[1].

Conservation status: IUCN: Least Concern; NatureServe: G5 (S5 in Tennessee).

Comments: Evermann and Hildebrand (1914) collected eight specimens from a cave near New Tazewell in October

1893. This species has also been reported from caves in West Virginia (Reese, 1934; Dearolf, 1956; Poly and Boucher, 1996; Poly, 2001).

References: [1] Evermann and Hildebrand (1914).

Order Perciformes
Family Centrarchidae
Genus *Lepomis*

***Lepomis cyanellus* Rafinesque, 1819 (AC) Green Sunfish**

Localities: Loudon Co.: Phantom Insurgence Cave (LN22)*; Roane Co.: Berry Cave (RN3)*.

Conservation status: IUCN: Least Concern; NatureServe: G5 (S5 in Tennessee).

Comments: This sunfish has been reported from several caves in the eastern United States (Jones and Hettler, 1959; McDaniel and Gardner, 1977; Pearson and Boston, 1995; Poly and Boucher, 1996; Poly, 2001; Ruhl, 2005).

References: * present study.

***Lepomis macrochirus* Rafinesque, 1819 (AC) Bluegill**

Localities: Knox Co.: Ebenezer Rising Cave (KN150)*; Monroe Co.: Lick Creek Cave (MO8)*; Rhea Co.: Grassy Creek Cave (RH2)*; Roane Co.: Berry Cave (RN3)*.

Conservation status: IUCN: Least Concern; NatureServe: G5 (S5 in Tennessee).

Comments: This species has been reported from caves in several states in the eastern United States (Franz et al., 1994; Pearson and Boston, 1995; Poly and Boucher, 1996; Lewis, 1998; Poly, 2001; Ruhl, 2005).

References: * present study.

Order Scorpaeniformes
Family Cottidae
Genus *Cottus*

***Cottus carolinae* (Gill, 1861) (SP) Banded Sculpin**

Localities: Grainger Co.: Indian Cave (GA4)*; Knox Co.: Christian Cave (KN49)*, Aycock Spring Cave (KN172)*; Monroe Co.: Lick Creek Cave (MO8)*; Roane Co.: Cave Creek Cave (RN5)*, Eblen Cave (RN6)*; Sevier Co.: Two County Cave (SV36)*.

Conservation status: IUCN: Least Concern; NatureServe: G5 (S5 in Tennessee).

Comments: *Cottus carolinae* is the most commonly observed fish in caves of the Interior Low Plateau and Appalachian Valley (Cope and Packard, 1881; Bailey, 1933; Dearolf, 1956; Pearson and Boston, 1995; Poly and Boucher, 1996; Ruhl, 2005; Niemiller et al., 2006). Many populations likely live year-round in caves and some populations are noted as possessing troglomorphic traits (Espinasa and Jeffery, 2003; Espinasa et al., 2013). Although common in several eastern Tennessee caves, no populations in this region are known to be troglomorphic.

References: * present study.

Order Siluriformes
Family Ictaluridae
Genus *Ameiurus*

***Ameiurus natalis* (Lesueur, 1819) (SX/AC) Yellow Bullhead**

Localities: Knox Co.: Mudflats Cave (KN9)*.

Conservation status: IUCN: Least Concern; NatureServe: G5 (S5 in Tennessee).

Comments: This species is known from caves in Alabama, Florida, Kentucky, and Mississippi (Armstrong and Williams, 1971; Relyea and Sutton, 1973; Cliburn and Middleton, 1983; Franz et al., 1994; Pearson and Boston, 1995; Poly, 2001). This is the first report from a cave in eastern Tennessee.

References: * present study.

Class Amphibia
Order Anura
Family Bufonidae
Genus *Anaxyrus*

***Anaxyrus americanus* (Holbrook, 1836) (AC) American Toad**

Localities: Blount Co.: Gregory Cave (BA4)[1].

Conservation status: IUCN: Least Concern; NatureServe: G5 (S5 in Tennessee).

Comments: Dodd et al. (2001) report this toad from the entrance of Gregory Cave in Cades Cove, Great Smoky Mountains National Park.

References: [1] Dodd et al. (2001).

***Anaxyrus fowleri* (Hinckley, 1882) (AC) Fowler's Toad**

Localities: Blount Co.: Gregory Cave (BA4)[1].

Conservation status: IUCN: Least Concern; NatureServe: G5 (S5 in Tennessee).

Comments: Dodd et al. (2001) report this toad from the entrance of Gregory Cave in Cades Cove, Great Smoky Mountains National Park.

References: [1] Dodd et al. (2001).

Family Hylidae
Genus *Pseudacris*

***Pseudacris crucifer* (Wied-Neuwied, 1838) (AC) Spring Peeper**

Localities: Knox Co.: Meads River Cave (KN151)*.

Conservation status: IUCN: Least Concern; NatureServe: G5 (S5 in Tennessee).

Comments: This treefrog has been infrequently reported from caves (Black, 1971; Prather and Briggler, 2001; Godwin, 2008; Niemiller and Miller, 2009). Most of these records are thought to represent accidental occurrences, but P. crucifer may seek refuge in small caves during drought conditions (Prather and Briggler, 2001).

References: * present study.

***Pseudacris feriarum* (Baird, 1854) (AC) Upland Chorus Frog**

Localities: Knox Co.: Steamboat Crawl (KN173)*.

Conservation status: IUCN: Least Concern; NatureServe: G5 (S5 in Tennessee).

Comments: This treefrog has been infrequently reported from caves (Black, 1971; Osbourn, 2005; Godwin, 2008; Niemiller and Miller, 2009).

References: * present study.

Table 2. Ecological classification, conservation status, and number of eastern Tennessee caves and counties from which species are documented by bioinventories of 56 caves (Current) and from the literature, museum, and database sources for other caves (Other).

Species	Ecological Classification	IUCN Red List	NatureServe Status	Gov Status	Caves Current	Caves Other	Caves Total	Counties
Fishes								
Luxilus chrysocephalus	AC	LC	G5, S5		1	0	1	1
Nocomis micropogon	AC	LC	G5, S5		1	0	1	1
Notropis spp.	AC				7	0	7	4
Rhinichthys atratulus	AC	LC	G5, S5		0	1	1	1
Lepomis cyanellus	AC	LC	G5, S5		2	0	2	2
Lepomis macrochirus	AC	LC	G5, S5		4	0	4	4
Cottus carolinae	SP	LC	G5, S5		7	0	7	5
Ameiurus natalis	SX/AC	LC	G5, S5		1	0	1	1
Amphibians (Frogs and Toads)								
Anaxyrus americanus	AC	LC	G5, S5		0	1	1	1
Anaxyrus fowleri	AC	LC	G5, S5		0	1	1	1
Pseudacris crucifer	AC	LC	G5, S5		1	0	1	1
Pseudacris feriarum	AC	LC	G5, S5		1	0	1	1
Lithobates catesbeianus	TX	LC	G5, S5		4	0	4	2
Lithobates clamitans	TX	LC	G5, S5		9	1	10	7
Lithobates palustris	TX	LC	G5, S5		11	2	13	7
Lithobates sylvaticus	TX/AC	LC	G5, S5		0	1	1	1
Amphibians (Salamanders)								
Desmognathus conanti	AC	LC	G5, S5		1	1	2	2
Desmognathus quadramaculatus	AC	LC	G5, S4		0	1	1	1
Eurycea cirrigera	TX	LC	G5, S5		1	0	1	1
Eurycea longicauda	TP/TX	LC	G5, S5		4	3	7	6
Euryea lucifuga	TP	LC	G5, S5		38	14	48	19
Eurycea wilderae	AC	LC	G5, S5		2	2	4	2
Gyrinophilus gulolineatus	SB	EN	G1Q, S1	FC, ST	9	9	11	4
Gyrinophilus porphyriticus	TP	LC	G5, S5		18	12	25	11
Plethodon dorsalis	TP/TX	LC	G5, S4		9	2	10	3
Plethodon glutinosus	TP	LC	G5, S5		19	13	31	11
Pseudotriton ruber	TP	LC	G5, S5		3	1	4	3
Reptiles (Snakes)								
Diadophis punctatus	AC	LC	G5, S5		1	0	1	1
Lampropeltis nigra	AC	LC	G5, S5		0	1	1	1
Pantherophis spiloides	AC	LC	G5, S5		0	1	1	1
Thamnophis sirtalis	AC	LC	G5, S5		1	0	1	1
Agkistrodon contortrix	AC	LC	G5, S5		0	1	1	1
Reptiles (Turtles)								
Terrapene carolina	AC	VU	G5, S4		2	0	2	2
Birds								
Cathartes aura	TX/AC	LC	G5, S5		0	1	1	1
Coragyp satratus	TX/AC	LC	G5, S4		0	4	4	2
Sayornis phoebe	TX	LC	G5, S5		17	1	18	8
Mammals (Bats)								
Corynorhinus rafinesquii	TX	LC	G3G4, S3		0	11	11	6
Eptesicus fuscus	TX	LC	G5, S5		10	34	41	16
Lasiurus borealis	AC	LC	G4, S5		0	2	2	1
Lasiurus cinereus	TX/AC	LC	G4, S5		0	2	2	2
Myotis grisescens	TX	NT	G3, S2	FE, SE	6	35	37	17
Myotis leibii	TX	LC	G3G4, S2S3	SD	0	6	6	4
Myotis lucifugus	TX	LC	G3, S5		3	26	29	12

Table 2. Continued.

Species	Ecological Classification	IUCN Red List	NatureServe Status	Gov Status	Caves Current	Caves Other	Caves Total	Counties
Myotis septentrionalis	TX	LC	G1G2, S4	FT	1	21	21	10
Myotis sodalis	TX	EN	G2, S1	FE, SE	0	13	13	6
Perimyotis subflavus	TX	LC	G3, S5		37	63	89	22
Mammals (non-Bats)								
Canis latrans	TX/AC	LC	G5, S5		3	0	3	2
Procyon lotor	TX	LC	G5, S5		27	2	29	12
Castor canadensis	TX	LC	G5, S5		2	0	2	2
Neotoma magister	TX	NT	G3G4, S3	SD	7	6	13	7
Peromyscus gossypinus	TX	LC	G5, S5		0	1	1	1
Peromyscus leucopus	TX	LC	G5, S5		2	0	2	2
Peromyscus sp.	TX/AC				2	0	2	2
Blarina brevicauda	AC	LC	G5, S5		1	0	1	1

Note: FE = Federally Endangered, FT = Federally Threatened, FC = Federal Candidate Species, SE = State Endangered, ST = State Threatened, and SD = State Deemed in Need of Management.

Family Ranidae
Genus *Lithobates*
***Lithobates catesbeianus* (Shaw, 1802) (TX)**
American Bullfrog

Localities: Knox Co.: Cruze Cave (KN24)*, Meads Quarry Cave (KN28)*, Meads River Cave (KN151)*; McMinn Co.: Small Cave (MM5)*.

Conservation status: IUCN: Least Concern; NatureServe: G5 (S5 in Tennessee).

Comments: This species and *L. clamitans* are frequently found in caves with aquatic habitat, particularly near entrances. It has been reported from several caves in Tennessee (Barr, 1953; Lewis, 2005; Niemiller and Miller, 2009).

References: * present study.

***Lithobates clamitans* (Rafinesque, 1820) (TX) Green Frog**

Localities: Blount Co.: Gregory Cave (BA4)[1]; Grainger Co.: Indian Cave (GA4)*; Knox Co.: Meads Quarry Cave (KN28)*, Meads River Cave (KN151)*, Fifth Entrance Cave (KN167)*, Aycock Spring Cave (KN172)*; Loudon Co.: Phantom Insurgence Cave (LN22)*; Monroe Co.: Alans Hideaway Cave (MO9)*; Roane Co.: Berry Cave (RN3)*; Sevier Co.: Two County Cave (SV36)*.

Conservation status: IUCN: Least Concern; NatureServe: G5 (S5 in Tennessee).

Comments: *Lithobates clamitans* has been reported from several caves in Tennessee (Barr, 1953; Dodd et al., 2001; Lewis, 2005; Niemiller and Miller, 2005, 2009).

References: [1] Dodd et al. (2001); * present study.

***Lithobates palustris* (LeConte, 1825) (TX) Pickerel Frog**

Localities: Blount Co.: Gregory Cave (BA4)[2]; Campbell Co.: Panther Cave No. 1 (CM8)*; Knox Co: Christian Cave (KN49)*, Ebenezer Rising Cave (KN150)*, Meads River Cave (KN151)*, Fifth Entrance Cave (KN167)*, Aycock Spring Cave (KN172)*; Loudon Co.: Phantom Insurgence Cave (LN22)*; McMinn Co.: Small Cave (MM5)[1]; Meigs Co.: Sensabaugh Cave (ME3)*; Roane Co.: Berry Cave (RN3)*, Cave Creek Cave (RN5)*, Eblen Cave (RN6)*.

Conservation status: IUCN: Least Concern; NatureServe: G5 (S5 in Tennessee).

Comments: This species is commonly found in caves with streams in entrance and twilight areas (Cliburn and Middleton, 1983; Garton et al., 1993; Buhlmann, 2001; Camp and Jensen, 2007; Niemiller and Miller, 2009).

References: VertNet: [1] NCSM; [2] Dodd et al. (2001); * present study.

***Lithobates sylvaticus* (LeConte, 1825) (TX/AC) Wood Frog**

Localities: Blount Co.: Gregory Cave (BA4)[1].

Conservation status: IUCN: Least Concern; NatureServe: G5 (S5 in Tennessee).

Comments: Dodd et al. (2001) report this frog from the entrance of Gregory Cave in Cades Cove, Great Smoky Mountains National Park.

References: [1] Dodd et al. (2001).

Order Caudata
Family Plethodontidae
Genus *Desmognathus*
***Desmognathus conanti* Rossman, 1958 (TX) Spotted Dusky Salamander**

Localities: Blount Co.: Whiteoak Blowhole Cave (BA2)[1,2]; Knox Co.: Burnett Cave (KN125)*.

Conservation status: IUCN: Least Concern; NatureServe: G5 (S5 in Tennessee).

Comments: This species is found in a variety of aquatic habitats in forest areas, including springs, seeps, spring runs, and small- to medium-sized streams (Wyckoff and Niemiller, 2011). Although it occasionally can be found in and around the entrances of caves that issue at the surface as springs in Tennessee, it is seldom observed in the dark zone (Niemiller and Miller, 2009).

References: [1,2] Wallace (1984, 2003); * present study.

Desmognathus quadramaculatus (Holbrook, 1840) (AC) Black-Bellied Salamander

Localities: Blount Co.: Rainbow Cave (BA26)[1,2].

Conservation status: IUCN: Least Concern; NatureServe: G5 (S4 in Tennessee).

Comments: Wallace (1984, 2003) observed one individual at the entrance to Rainbow Cave in Great Smoky Mountain National Park.

References: [1,2] Wallace (1984, 2003).

Genus _Eurycea_

Eurycea cirrigera (Green, 1818) (TX) Southern Two-Lined Salamander

Localities: Loudon Co.: Phantom Insurgence Cave (LN22)*.

Conservation status: IUCN: Least Concern; NatureServe: G5 (S5 in Tennessee).

Comments: This salamander has been infrequently reported from caves in Tennessee (Lewis, 2005; Niemiller and Miller, 2007, 2009), as well as northwestern Georgia (Camp and Jensen, 2007). This is the first report from the Valley and Ridge of eastern Tennessee.

References: * present study.

Eurycea longicauda (Green, 1818) (TP/TX) Long-Tailed Salamander

Localities: Blount Co.: Gregory Cave (BA4)[1–4], Tory Shields Bluff Cave (BA56)[1,2]; Campbell Co.: Panther Cave No. 1 (CM8)*; Knox Co.: Meads Quarry Cave (KN28)*; McMinn Co.: Small Cave (MM5)*; Monroe Co.: Morgan Cave (MO5)*; Sevier Co.: Stupkas Cave (SV42)[2].

Conservation status: IUCN: Least Concern; NatureServe: G5 (S5 in Tennessee).

Comments: This species is a common inhabitant of caves in the Interior Low Plateau and Appalachian Valley (Garton et al., 1993; Buhlmann, 2001; Dodd et al., 2001; Lewis, 2005; Osbourn, 2005; Camp and Jensen, 2007; Niemiller and Miller, 2009). It is known to reproduce in caves in Tennessee (Dodd et al., 2001; Taylor and Mays, 2006).

References: [1,2] Wallace (1984, 2003); [3] Dodd et al. (2001); [4] Taylor and Mays (2006); * present study.

Eurycea lucifuga Rafinesque, 1822 (TP) Cave Salamander

Localities: Anderson Co.: Norris Dam Cave No. 2 (AN32)[2]; Blount Co.: Tuckaleechee Caverns (BA11)[5,7,*], Calf Cave No. 2 (BA20)[5,7]; Campbell Co.: Panther Cave No. 1 (CM8)*; Carter Co.: Carter Saltpeter Cave (CR1)*; Claiborne Co.: Sour Kraut Cave (CB46)*, Kings Saltpeter Cave (CB52)*; Grainger Co.: Indian Cave (GA4)[2,*]; Hancock Co.: Goodmans Cave (HN5)[6]; Jefferson Co.: Mutton Hollow Cave (JF41)[4], Silo Pit Cave (JF71)*; Knox Co.: Mudflats Cave (KN9)*, Carter Cave (KN14)*, Keller Bend Cave (KN16)*, Blowing Hole Cave (KN19)*, Cruze Cave (KN24)*, Meads Quarry Cave (KN28)[2,10,*], Christian Cave (KN49)*, Keller Bluff Cave No. 1 (KN61)[3], Unreported Cave (KN90)*, Brents Cave (KN112)*, Chriscroft Cave (KN127)*, The Lost Puddle (KN145)*, Meads River Cave (KN151)*, Fifth Entrance Cave (KN167)*, Steamboat Crawl (KN173)*; Loudon Co.: Blankenship Cave (LN1)*, Benjos Cave (LN11)*, Phantom Insurgence Cave (LN22)*; McMinn Co.: Small Cave (MM5)*, Too Small Cave (MM6)*; Meigs Co.: Sensabaugh Cave (ME3)*; Monroe Co.: Morgan Cave (MO5)*, Nobletts Cave (MO6)[6,*]; Rhea Co.: Grassy Creek Cave (RH2)[6], Marler Cave (RH4)[9]; Roane Co.: Berry Cave (RN3)*, Cave Creek Cave (RN5)*, Eblen Cave (RN6)*, Big Cave (RN13)*; Sevier Co.: Stupkas Cave (SV42)[5,7,8]; Sullivan Co.: Morrell Cave (SL6)[1]; Union Co.: Wright Cave (UN9)*, Big Cave (UN10)*, Rogers Hollow Cave (UN23)*, Mossy Spring Cave (UN25)*, Ellison Hollow Cave (UN46)*; Washington Co.: Keplinger Cave (WS3)[3].

Conservation status: IUCN: Least Concern; NatureServe: G5 (S5 in Tennessee).

Comments: *Eurycea lucifuga* is the most common salamander found in caves of Tennessee (Niemiller and Miller, 2009). Niemiller et al. (2009a) report on reproduction of this species from Meads Quarry Cave. A female and eight hatchlings with yolk sacs were found in a rimstone pool in Blankenship Cave (LN1) on 25 January 2014.

References: VertNet: [1] CM, [2] USNM, [3] TNNH; [4] Ives (1951); [5,6,7] Wallace (1984, 1989, 2003); [8] Dodd et al. (2001); [9] Lewis (2005); [10] Niemiller et al. (2009a); * present study.

Eurycea wilderae Dunn, 1920 (AC) Blue Ridge Two-Lined Salamander

Localities: Blount Co.: Whiteoak Blowhole Cave (BA2)[1,2], Gregory Cave (BA4)[3]; Monroe Co.: Gay Cave (MO3)*, Alans Hideaway Cave (MO9)*.

Conservation status: IUCN: Least Concern; NatureServe: G5 (S5 in Tennessee).

Comments: This species has been found infrequently in caves, typically near entrances.

References: [1,2] Wallace (1984, 2003); [3] Dodd et al. (2001); * present study.

Genus _Gyrinophilus_

Gyrinophilus gulolineatus Brandon, 1965 (SB) Berry Cave Salamander

Localities: Knox Co.: Mudflats Cave (KN9)[4–9,11,12,*], Meads Quarry Cave (KN28)[1,4,7–9,11,12,*], Christian Cave (KN49)[8,9,11,12,*], The Lost Puddle (KN145)*, Meads River Cave (KN151)[8,12,*], Fifth Entrance Cave (KN167)[8,12,*], Aycock Spring Cave (KN172)[8,9,11,12,*]; McMinn Co.: Small Cave (MM5)*, roadside ditch near Oostanaula Creek south of Athens[2–4,7–10,12]; Meigs Co.: Blythe Ferry Cave (ME1)[9,12]; Roane Co.: Berry Cave (RN3, type-locality)[2–4,7–9,11,12,*].

Conservation status: IUCN: Endangered (B1ab(iii)+2ab(iii)); NatureServe: G1Q (S1 in Tennessee); Candidate for listing under the U.S. Endangered Species Act; listed as Threatened in Tennessee (Withers, 2009).

Comments: The records from The Lost Puddle and Small Cave represent new localities for this rare salamander.

References: VertNet: [1] NCSM; [2,3] Brandon (1965, 1966); [4,5] Simmons (1975, 1976); [6] Caldwell and Copeland (1992); [7,8] Miller and Niemiller (2007, 2008); [9,10] Niemiller and Miller (2010, 2011); [11,12] Niemiller et al. (2008, 2010); * present study.

***Gyrinophilus porphyriticus* (Green, 1827) (TP) Spring Salamander**

Localities: Blount Co.: Whiteoak Blowhole Cave (BA2)[6,7]; Rainbow Cave (BA26)[8]; Claiborne Co.: Buis Saltpeter Cave (CB48)*, Kings Saltpeter Cave (CB52)*; Grainger Co.: Indian Cave (GA4)[2,3,*]; Hawkins Co.: Pearson Cave (HW12)[1], Sensabaugh Saltpeter Cave (HW13)[2]; Knox Co.: Mudflats Cave (KN9)[4,5], Carter Cave (KN14)*, Cruze Cave (KN24)[9–11,*], Meads Quarry Cave (KN28)[4,*], Kirkpatrick Cave (KN62)*, Burnett Cave (KN125)*, Meads River Cave (KN151)[9,10,*]; Loudon Co.: Phantom Insurgence Cave (LN22)*; McMinn Co.: Small Cave (MM5)*, Wattenbarger Cave (MM9)[2]; Meigs Co.: Sensabaugh Cave (ME3)*; Monroe Co.: Nobletts Cave (MO6)*; Roane Co.: Cave Creek Cave (RN5)[9,10,*], Eblen Cave (RN6)*; Union Co.: Oaks Cave (UN5)*, Wolf Cave (UN8)[2,3], Big Cave (UN10)*, Mossy Spring Cave (UN25)*.

Conservation status: IUCN: Least Concern; NatureServe: G5 (S5 in Tennessee).

Comments: *Gyrinophilus porphyriticus* likely can be found in caves throughout the Appalachian Valley and Ridge province and Blue Ridge Mountains of eastern Tennessee. This species occurs syntopically and occasionally hybridizes with *G. gulolineatus* at Mudflats Cave, Meads Quarry Cave, and Meads River Cave in Knox County (Niemiller et al. 2008, 2009b). Both *G. porphyriticus* and *G. gulolineatus* were found at Small Cave in McMinn County.

References: VertNet: [1] KU, [2] USNM; [3] Brandon (1966); [4,5] Simmons (1975, 1976); [6,7] Wallace (1984, 2003); [8] Dodd et al. (2001); [9,10] Miller and Niemiller (2007, 2008); [11] Niemiller et al. (2008); * present study.

Genus *Plethodon*

***Plethodon dorsalis* Cope, 1889 (TP/TX) Northern Zigzag Salamander**

Localities: Jefferson Co.: Tater Cave (JF8)*, Silo Pit Cave (JF71)*; Knox Co.: Mudflats Cave (KN9)[1,*], Carter Cave (KN14)*, Blowing Hole Cave (KN19)*, Meads Quarry Cave (KN28)*, Unreported Cave (KN90)*, Meads River Cave (KN151)*, Steamboat Crawl (KN173)*; Rhea Co.: Marler Cave (RH4)[2].

Conservation status: IUCN: Least Concern; NatureServe: G5 (S4 in Tennessee).

Comments: This species is closely related to the Southern Zigzag Salamander (*P. ventralis*); they are difficult to distinguish morphologically. Both species occur in caves in Tennessee (Lewis, 2005; Niemiller and Miller, 2009). Females are known to nest in small cavities in cave walls or crevices in clay floors (Miller et al., 1998; Niemiller and Miller, 2008).

References: [1] Simmons (1975); [2] Lewis (2005); * present study.

***Plethodon glutinosus* (Green, 1818) (TP) Northern Slimy Salamander**

Localities: Blount Co.: Whiteoak Blowhole Cave (BA2)[3,5], Bull Cave (BA3)[5], Gregory Cave (BA4)[2,3,5,6,8], Calf Cave No. 1 (BA19)[3,5,6], Calf Cave No. 2 (BA20)[3,5], Rainbow Cave (BA26)[6], Tory Shields Bluff Cave (BA56)[3,5]; Campbell Co.: Panther Cave No. 1 (CM8)*; Jefferson Co.: Tater Cave (JF8)*, Silo Pit Cave (JF71)*; Knox Co.: Mudflats Cave (KN9)*, Blowing Hole Cave (KN19)*, Cherokee Caverns (KN22)*, Cruze Cave (KN24)[2,*], Meads Quarry Cave (KN28)*, Chriscroft Cave (KN127)*, Aycock Spring Cave (KN172)*; Loudon Co.: Benjos Cave (LN11)*; McMinn Co.: Small Cave (MM5)*, Too Small Cave (MM6)*, Wattenbarger Cave (MM9)[2]; Monroe Co.: Gay Cave (MO3)*, Morgan Cave (MO5)*, Nobletts Cave (MO6)[4], Double Sump Cave (MO13)[1]; Rhea Co.: Marler Cave (RH4)[7]; Roane Co.: Berry Cave (RN3)*, Cave Creek Cave (RN5)*, Big Cave (RN13)*; Sevier Co.: Stupkas Cave (SV42)[3,5]; Union Co.: Big Cave (UN10)*.

Conservation status: IUCN: Least Concern; NatureServe: G5 (S5 in Tennessee).

Comments: This salamander is commonly encountered in caves throughout its range (Garton et al., 1993; Buhlmann, 2001; Dodd et al., 2001; Lewis, 2005; Taylor and Mays, 2006; Camp and Jensen, 2007; Godwin, 2008; Niemiller and Miller, 2009; Niemiller and Reeves, 2014).

References: VertNet: [1] CM, [2] USNM; [3,4,5] Wallace (1984, 1989, 2003); [6] Dodd et al. (2001); [7] Lewis (2005); [8] Taylor and Mays (2006); * present study.

Genus *Pseudotriton*

***Pseudotriton ruber* (Latreille, 1801) (TP) Red Salamander**

Localities: Blount Co.: Whiteoak Blowhole Cave (BA2)[1,2]; Knox Co.: Aycock Spring Cave (KN172)*; Union Co.: Wright Cave (UN9)*, Mossy Spring Cave (UN25)*.

Conservation status: IUCN: Least Concern; NatureServe: G5 (S5 in Tennessee).

Comments: This species is frequently found in the twilight zone of caves with streams throughout the Interior Low Plateau and Appalachian Valley (Buhlmann, 2001; Osbourn, 2005; Camp and Jensen, 2007; Godwin, 2008; Miller et al., 2008; Niemiller and Miller, 2009; Niemiller and Reeves, 2014). *Pseudotriton ruber* utilizes cave streams and associated aquatic habitats for reproduction (Miller and Niemiller, 2005; Niemiller et al., 2006; Miller et al., 2008).

References: [1,2] Wallace (1984, 2003); * present study.

Class Reptilia
Order Squamata
Suborder Serpentes
Family Colubridae
Genus *Diadophis*
***Diadophis punctatus* (Linneaus, 1766) (AC) Ring-Necked Snake**

Localities: Knox Co.: Meads River Cave (KN151)*.

Conservation status: IUCN: Least Concern; NatureServe: G5 (S5 in Tennessee).

Comments: An adult was found crawling on a mud bank on 2 December 2007 in Meads River Cave (KN151) a few days after heavy rainfall. The snake was likely washed into the cave during flash flooding.

References: * present study.

Genus *Lampropeltis*
***Lampropeltis nigra* (Yarrow, 1882) (AC) Black Kingsnake**

Localities: Sullivan Co.: Reagans Grapevine Cave (SL138)[1].

Conservation status: IUCN: Least Concern; NatureServe: G5 (S5 in Tennessee).

Comments: Bailey (1988) reported seeing this species in February 1987.

References: [1] Bailey (1988).

Genus *Pantherophis*
***Pantherophis spiloides* (Dumeril, Bibron, & Dumeril, 1854) (AC) Gray Ratsnake**

Localities: Blount Co.: Gregory Cave (BA4)[1].

Conservation status: IUCN: Least Concern; NatureServe: G5 (S5 in Tennessee).

Comments: Dodd et al. (2001) report this species from the entrance of Gregory Cave in Cades Cove, Great Smoky Mountains National Park.

References: [1] Dodd et al. (2001).

Family Natricidae
Genus *Thamnophis*
***Thamnophis sirtalis* (Linnaeus, 1758) (AC) Common Gartersnake**

Localities: Carter Co.: Carter Saltpeter Cave (CR1)*.

Conservation status: IUCN: Least Concern; NatureServe: G5 (S5 in Tennessee).

Comments: An adult was observed at the bottom of the large entrance sink in the dark zone on 14 May 2014, likely as a consequence of accidentally falling into the cave.

References: * present study.

Family Viperidae
Genus *Agkistrodon*
***Agkistrodon contortrix* (Linnaeus, 1766) (AC) Copperhead**

Localities: Blount Co.: Gregory Cave (BA4)[1].

Conservation status: IUCN: Least Concern; NatureServe: G5 (S5 in Tennessee).

Comments: Dodd et al. (2001) report this species from the entrance of Gregory Cave in Cades Cove, Great Smoky Mountains National Park.

References: [1] Dodd et al. (2001).

Order Testudines
Family Emydidae
Genus *Terrapene*
***Terrapene carolina* (Linnaeus, 1758) (AC) Eastern Box Turtle**

Localities: Jefferson Co.: Silo Pit Cave (JF71)*; Knox Co.: Meads Quarry Cave (KN28)*.

Conservation status: IUCN: Vulnerable; NatureServe: G5 (S4 in Tennessee).

Comments: An adult male was observed just inside the main entrance of Meads Quarry Cave on 4 November 2006. An adult male with a cracked carapace also was observed in the twilight zone at Silo Pit Cave on 3 Aug 2015. This species commonly falls into pits or gets washed into caves during flooding.

References: * present study.

Class Aves
Order Cathartiformes
Family Cathartidae
Genus *Cathartes*
***Cathartes aura* (Linnaeus, 1958) (TX/AC) Turkey Vulture**

Localities: Washington Co.: unknown cave[1].

Conservation status: IUCN: Least Concern; NatureServe: G5 (S5 in Tennessee).

Comments: This species is known to nest at the entrances and within the twilight zone of caves, particularly those occurring in bluffs (Coles, 1944; Lewis, 2005).

References: VertNet: [1] SBMNH.

Genus *Coragyps*
***Coragyps atratus* (Bechstein, 1793) (TX/AC) Black Vulture**

Localities: Sullivan Co.: cave in Slaughter Bluff on Holston River[1], cave on south fork of Holston River[2]; Washington Co.: cave near Johnson City[1]; cave on Watauga River NW of Johnson City[3].

Conservation status: IUCN: Least Concern; NatureServe: G5 (S4 in Tennessee).

Comments: Like *Cathartes aura,* this species is known to nest at the entrances and within the twilight zone of caves (Lyle, 1931).

References: VertNet: [1] SBMNH, [2] WFVZ; [3] Lyle (1931).

Order Passeriformes
Family Tyrannidae
Genus *Sayornis*
***Sayornis phoebe* (Latham, 1790) (TX) Eastern Phoebe**

Localities: Campbell Co.: Panther Cave No. 1 (CM8)*; Claiborne Co.: Sour Kraut Cave (CB46)*, Buis Saltpeter Cave (CB48)*, Kings Saltpeter Cave (CB52)*; Knox Co.:

Campbell Cave (KN1)*, Ebenezer Rising Cave (KN150)*; McMinn Co.: Small Cave (MM5)*, Too Small Cave (MM6)*; Monroe Co.: Lick Creek Cave (MO8)*, Alans Hideaway Cave (MO9)*; Rhea Co.: Marler Cave (RH4)[1]; Roane Co.: Berry Cave (RN3)*, Cave Creek Cave (RN5)*; Union Co.: Oaks Cave (UN5)*, Wright Cave (UN9)*, Big Cave (UN10)*, Rogers Hollow Cave (UN23)*, Ellison Hollow Cave (UN46)*.

Conservation status: IUCN: Least Concern; NatureServe: G5 (S5 in Tennessee).

Comments: This flycatcher is common throughout the eastern United States and is frequently encountered in entrances and the twilight zone of caves, where it nests on rocky ledges or in crevices.

References: [1] Lewis (2005); * present study.

Class Mammalia
Order Carnivora
Family Canidae
Genus *Canis*
***Canis latrans* Say, 1823 (TX/AC) Coyote**

Localities: McMinn Co.: Small Cave (MM5)*; Union Co.: Oaks Cave (UN5)*, Big Cave (UN10)*.

Conservation status: IUCN: Least Concern; NatureServe: G5 (S5 in Tennessee).

Comments: Fresh scat and tracks were observed just inside the entrance to Small Cave, and fresh scat was observed in the two Union County caves.

References: * present study.

Family Procyonidae
Genus *Procyon*
***Procyon lotor* (Linnaeus, 1758) (TX) Raccoon**

Localities: Blount Co.: Tory Shields Bluff Cave (BA56)[1,2]; Campbell Co.: Panther Cave No. 1 (CM8)*; Claiborne Co.: Sour Kraut Cave (CB46)*, Buis Saltpeter Cave (CB48)*, Kings Saltpeter Cave (CB52)*; Jefferson Co.: Tater Cave (JF8)*; Knox Co.: Campbell Cave (KN1)*, Mudflats Cave (KN9)*, Cruze Cave (KN24)*, Meads Quarry Cave (KN28)*, Unreported Cave (KN90)*, Chriscroft Cave (KN127)*; Loudon Co.: Phantom Insurgence Cave (LN22)*; McMinn Co.: Small Cave (MM5)*, Too Small Cave (MM6)*; Monroe Co.: Gay Cave (MO3)*, Morgan Cave (MO5)*, Nobletts Cave (MO6)*, Lick Creek Cave (MO8)*; Rhea Co.: Grassy Creek Cave (RH2)*, Marler Cave (RH4)[3]; Roane Co.: Berry Cave (RN3)*, Cave Creek Cave (RN5)*, Eblen Cave (RN6)*; Sevier Co.: Two County Cave (SV36)*; Union Co.: Oaks Cave (UN5)*, Wright Cave (UN9)*, Rogers Hollow Cave (UN23)*, Mossy Spring Cave (UN25)*.

Conservation status: IUCN: Least Concern; NatureServe: G5 (S5 in Tennessee).

Comments: Dead adults were found in Cruze Cave, Kings Saltpeter Cave, and Nobletts Cave, while tracks and/or scat were observed in the other caves. Raccoons often enter caves in search of crayfish, frogs, salamanders, and other prey.

References: [1,2] Wallace (1984, 2003); [3] Lewis (2005); * present study.

Order Chiroptera
Family Vespertilionidae
Genus *Corynorhinus*
***Corynorhinus rafinesquii* (Lesson, 1827) (TX) Rafinesque's Big-Eared Bat**

Localities: Blount Co.: Whiteoak Blowhole Cave (BA2)[11], Bull Cave (BA3)[1,8], Gregory Cave (BA4)[1,6,7], Kelly Ridge Cave (BA6)[1,8,10,11], Scott Gap Cave (BA7)[6,7,11], Calf Cave No. 2 (BA20)[5]; Greene Co.: Double Mouth Cave (GN6)[1,9]; Hancock Co.: unknown cave[4]; Hawkins Co.: Pearson Cave (HW12)[2]; Monroe Co.: Ballplay Cave (MO10)[3]; Sevier Co.: Stupkas Cave (SV42)[6,7].

Conservation status: IUCN: Least Concern; NatureServe: G3G4 (S3 in Tennessee).

Comments: Although we did not observe Rafinesque's Big-Eared Bats during our bioinventories, this species has been reported from several eastern Tennessee caves, primarily during winter hibernaculum surveys.

References: [1] Bat Population Database; VertNet: [2] KU, [3]MVZ, [4] TNNH; [5] Rabinowitz and Nottingham (1979); [6,7] Wallace (1984, 2003); [8] Samoray (2011); [9] Holliday (2012); [10,11] Flock (2013, 2014).

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

Localities: Anderson Co.: Springhill Saltpeter Cave (AN3)[9,10]; Blount Co.: Whiteoak Blowhole Cave (BA2)[4,5], Bull Cave (BA3)[1,7], Gregory Cave (BA4)[4,5], Kelly Ridge Cave (BA6)[1,9,10], Whiteoak Saltpeter Cave (BA27)[1,7,9,10]; Campbell Co.: Meredith Cave (CM5)[1,7], Norris Dam Cave (CM7)[1,8,9], Panther Cave No. 1 (CM8)*; Carter Co.: Carter Saltpeter Cave (CR1)[1,6], Grindstaff Cave (CR2)[1,6,7,10], Renfro Cave (CR6)[10], Poga Road Cave (CR31)[1,6], Elk Mills Cave (CR34)[1,6]; Claiborne Co.: Sour Kraut Cave (CB46)[9], Buis Saltpeter Cave (CB48)[9], cave near Harrogate[3]; Grainger Co.: Indian Cave (GA4)[9,10,*]; Greene Co.: Cochran Cave (GN32)[1,8]; Hamblen Co.: Soard Cave (HB3)[9], Corner Store Cave (HB22)[10]; Jefferson Co.: Tater Cave (JF8)[9], Rouse Cave (JF26)[9]; Knox Co.: Campbell Cave (KN1)*, Mudflats Cave (KN9)*, Blowing Hole Cave (KN19)[9], Meads Quarry Cave (KN28)[1,8,*], Cave Spring Cave No. 2 (KN47)[1,8], Kirkpatrick Cave (KN62)*; McMinn Co.: Small Cave (MM5)*; Meigs Co.: Eves Cave (ME2)[10]; Monroe Co.: Ballplay Cave (MO10)[2]; Roane Co.: Berry Cave (RN3)*, Cave Creek Cave (RN5)[10], Eblen Cave (RN6)*, Big Cave (RN13)*, Marble Bluff Cave (RN19)[1,10]; Sullivan Co.: Morrell Cave (SL6)[1,6,7,9,10], Kaylor Cave (SL46)[1,6]; Union Co.: Oaks Cave (UN5)[1,6–8], Herd O Coons Cave (UN32)[9].

Conservation status: IUCN: Least Concern; NatureServe: G5 (S5 in Tennessee).

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

References: [1] Bat Population Database; VertNet: [2] MVZ, [3] OMNH; [4,5] Wallace (1984, 2003); [6] Lamb and Wyckoff (2010); [7] Samoray (2011); [8] Holliday (2012); [9,10] Flock (2013, 2014); * present study.

Genus *Lasiurus*

Lasiurus borealis (Muller, 1776) (AC) Eastern Red Bat

Localities: Rhea Co.: Grassy Creek Cave (RH2)[1], Starve Rock Cave (RH7)[1].

Conservation status: IUCN: Least Concern; NatureServe: G4 (S5 in Tennessee).

Comments: This bat is typically associated with forests but has been found in caves on occasion (Mohr, 1952; Quay and Miller, 1955; Myers, 1960). This species will swarm around cave entrances, particular in autumn; however, bats that enter caves typically die, presumably because they become disoriented and are unable to find their way back to an entrance (Best and Dusi, 2014).

References: [1] VertNet: KU.

Lasiurus cinereus (Beauvois, 1796) (TX/AC) Hoary Bat

Localities: Campbell Co.: Meredith Cave (CM5)[1]; Rhea Co.: Starve Rock Cave (RH7)[1].

Conservation status: IUCN: Least Concern; NatureServe: G4 (S5 in Tennessee).

Comments: This bat is typically associated with forests but has been documented roosting in caves (Myers, 1960; Best and Dusi, 2014).

References: [1] VertNet: KU.

Genus *Myotis*

Myotis grisescens Howell, 1909 (TX) Gray Bat

Localities: Anderson Co.: Little Turtle Cave (AN38)[5]; Campbell Co.: Meredith Cave (CM5)[9], Norris Dam Cave (CM7)[1,9], Panther Cave No. 1 (CM8)[5]; Claiborne Co.: English Cave (CB9)[1,12], Station Creek Cave (CB17)[9], Upper Coonsies Creek Cave (CB47)[1,2,8,9]; Cocke Co.: Rattling Cave (CO2)[1,8,9]; Grainger Co.: Indian Cave (GA4)[1,3,4,8,9,13,14,*], Coon Cave (GA12)[9]; Greene Co.: Arch Cave (GN1)[9], Cedar Creek Cave (GN3)[9], Stillhouse Cave (GN7)[1,12], Cochran Cave (GN32)[1,9,13]; Hancock Co.: Rockhouse Cave (HN27)[9], Upstream Cave (HN77)[1,12]; Hawkins Co.: Hasson Cave (HW6)[1], Horner Cave (HW9)[1,9], Pearson Cave (HW12)[1,2,9,10,12–14]; Jefferson Co.: Rouse Cave (JF26)[13]; Knox Co.: Mudflats Cave (KN9)[9], Baloney Cave (KN18)[9], Blowing Hole Cave (KN19)[9], Christian Cave (KN49)*; Loudon Co.: Ghost Cave (LN3)[5], Browder Bluff Cave No. 3 (LN7)[5], Phantom Insurgence Cave (LN22)*; Meigs Co.: Blythe Ferry Cave (ME1)[1,2,9], Eves Cave (ME2)[1,9,13], Sensabaugh Cave (ME3)[1,2,9,*]; Rhea Co.: Grassy Creek Cave (RH2)[1,2,6–9,*], Starve Rock Cave (RH7)[2,9]; Roane Co.: Marble Bluff Cave (RN19)[1]; Sullivan Co.: Morrell Cave (SL6)[1,7–9,12–14]; Union Co.: Oaks Cave (UN5)[1,2,8–12,*]; Wright Cave (UN9)[1,12], Lost Creek Cave (UN19)[9].

Conservation status: IUCN: Near Threatened; NatureServe: G3 (S2 in Tennessee); listed as Endangered under the U.S. Endangered Species Act; listed as Endangered in Tennessee (Withers, 2009).

Comments: *Myotis grisescens* is one of three bats federally listed as endangered that can be found in Tennessee, the others being the Indiana Bat (*M. sodalis*) and the Northern Long-Eared Bat (*M. septentrionalis*). Populations throughout Tennessee are well-monitored because large colonies are only found in approximately eight caves nationally (Martin 2007). Indian Cave, Grassy Creek Cave, and Oaks Cave are sites for summer maternity colonies, while Sensabaugh Cave is a summer bachelor colony (Martin, 2007). Martin (2007) included Mudflats Cave and Blowing Hole Cave as Priority 4 sites, but the species has not been observed at these caves in recent years (this study). Gray Bats have not been reported previously from Christian Cave or Phantom Insurgence Cave. An estimated 250 bats and fresh guano piles were observed at Christian Cave on 17 September 2005. At Phantom Insurgence Cave on 30 August 2014, an estimated 2,000 bats were observed roosting above a pool in the large entrance chamber. Although no roosting bats were observed in Grassy Creek Cave at the time of the bioinventory, large and extensive guano piles from a recent summer colony and several skeletons of dead bats were present throughout the cave during our visit on 22 December 2014.

References: [1] Bat Population Database; VertNet: [2] KU, [3] UMMZ, [4] USNM; [5] TNNH; [6] Wallace (1989); [7] Harvey (1994); [8] Harvey and Britzke (2002); [9] Martin (2007); [10] Lamb and Wyckoff (2010); [11] Samoray (2011); [12] Holliday (2012); [13,14] Flock (2013, 2014); * present study.

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

Localities: Anderson Co.: Springhill Saltpeter Cave (AN3)[4]; Blount Co.: Kelly Ridge Cave (BA6)[1]; Carter Co.: Poga Road Cave (CR31)[1,3], Elk Mills Cave (CR34)[1,3]; Union Co.: Lost Creek Cave (UN19)[2]; Washington Co.: Hampton Cave[2].

Conservation status: IUCN: Least Concern; NatureServe: G3G4 (S2S3 in Tennessee); listed as Deemed in Need of Management in Tennessee (Withers 2009).

Comments: The Eastern Small-Footed Bat is the smallest bat species in the eastern United States. It is rare throughout its range in Tennessee, where it is found primarily in the eastern two-thirds of the state. Many records of this species are from winter surveys, when it can be found hibernating in caves and mines. Hampton Cave is not known in the TCS database and could not be georeferenced.

References: [1] Bat Population Database; VertNet: [2] OMNH; [3] Lamb and Wyckoff (2010); [4] Flock (2013).

***Myotis lucifugus* (LeConte, 1831) (TX) Little Brown Bat**

Localities: Anderson Co.: Springhill Saltpeter Cave (AN3)[10,11], Toilet Bowl Cave (AN14)[10]; Blount Co.: Whiteoak Blowhole Cave (BA2)[1,5–11], Bull Cave (BA3)[1,3,8], Gregory Cave (BA4)[1,9–11], Kelly Ridge Cave (BA6)[1,8,10,11], Scott Gap Cave (BA7)[1,8–11], Tuckaleechee Caverns (BA11)*, Rainbow Cave (BA26)[1,8], Whiteoak Saltpeter Cave (BA27)[1,8–10], Tory Shields Bluff Cave (BA56)[5,6]; Campbell Co.: Norris Dam Cave (CM7)[10]; Carter Co.: Grindstaff Cave (CR2)[1,8]; Claiborne Co.: English Cave (CB9)[1,9], Sour Kraut Cave (CB46)[10], Buis Saltpeter Cave (CB48)[10]; Grainger Co.: Indian Cave (GA4)[4]; Hamblen Co.: Corner Store Cave (HB22)[11]; Knox Co.: Cruze Cave (KN24)*, Meads Quarry Cave (KN28)*; Meigs Co.: Eves Cave (ME2)[10,11]; Rhea Co.: Grassy Creek Cave (RH2)[2]; Roane Co.: Marble Bluff Cave (RN19)[10]; Sullivan Co.: Morrell Cave (SL6)[1,8,11]; Union Co.: Oaks Cave (UN5)[2], Wright Cave (UN9)[1,9], Jolley Saltpeter Cave (UN12)[11], Lost Creek Cave (UN19)[1,2,9], Herd O Coons Cave (UN32)[10].

Conservation status: IUCN: Least Concern; NatureServe: G3 (S5 in Tennessee).

Comments: The range of this species includes all of eastern Tennessee; however, only a few individuals were noted during this study. The individual observed in Tuckaleechee Caverns on 20 March 2014 had characteristic white fungal growth of white-nose syndrome on the muzzle. *Myotis lucifugus* populations throughout the eastern United States have sustained significant losses due to WNS, and Ingersoll et al. (2013) have suggested that its IUCN Red List status be increased from Least Concern to Endangered in some areas. Declines have also been noted for some eastern Tennessee populations (Flock 2013, 2014).

References: [1] Bat Population Database; VertNet: [2] KU,[3] OMNH,[4] UMMZ; [5,6] Wallace (1984, 2003); [7] Lamb and Wyckoff (2010); [8] Samoray (2011); [9] Holliday (2012); [10,11] Flock (2013, 2014); * present study.

***Myotis septentrionalis* (Trovessart, 1897) (TX) Northern Long-Eared Bat**

Localities: Blount Co.: Whiteoak Blowhole Cave (BA2)[1,5–10], Bull Cave (BA3)[1,3,8], Gregory Cave (BA4)[1,9–11], Kelly Ridge Cave (BA6)[10], Scott Gap Cave (BA7)[1,8], Whiteoak Saltpeter Cave (BA27)[1,9,10]; Carter Co.: Carter Saltpeter Cave (CR1)[1,7], Grindstaff Cave (CR2)[1,7]; Claiborne Co.: Sour Kraut Cave (CB46)[10], Buis Saltpeter Cave (CB48)[10]; Grainger Co.: Indian Cave (GA4)[4]; Greene Co.: Cedar Creek Cave (GN3)[4]; Meigs Co.: Eves Cave (ME2)[11]; Rhea Co.: Grassy Creek Cave (RH2)[2]; Roane Co.: Cave Creek Cave (RN5)[11], Marble Bluff Cave (RN19)[1,8,9,11]; Sullivan Co.: Morrell Cave (SL6)[1,7]; Union Co.: Oaks Cave (UN5)[1,2,7,*], Wright Cave (UN9)[1,9], Jolley Saltpeter Cave (UN12)[11], Herd O Coons Cave (UN32)[10].

Conservation status: IUCN: Least Concern; NatureServe: G1G2 (S4 in Tennessee); listed as Threatened under the U.S. Endangered Species Act.

Comments: We observed a single individual at Oaks Cave in March 2015. United States Fish and Wildlife Service (2013) found that listing this species is warranted, as it is one of the bat species most impacted by white-nose syndrome, and proposed to list *M. septentrionalis* as an endangered species throughout its range. The species was listed as Threatened under the Endangered Species Act, effective 4 May 2015 (United States Fish and Wildlife Service, 2015).

References: [1] Bat Population Database; VertNet: [2] KU, [3] OMNH, [4] TNNH; [5,6] Wallace (1984, 2003); [7] Lamb and Wyckoff (2010); [8] Samoray (2011); [9] Holliday (2012); [10,11] Flock (2013, 2014); * present study.

***Myotis sodalis* Miller & Allen, 1928 (TX) Indiana Bat**

Localities: Blount Co.: Whiteoak Blowhole Cave (BA2)[1,4–12], Bull Cave (BA3)[1,2,4,5,7,9], Kelly Ridge Cave (BA6)[1,7,9,11,12], Scott Gap Cave (BA7)[1,4,5,7,9–12], Rainbow Cave (BA26)[1,9], Whiteoak Saltpeter Cave (BA27)[1,9,11,12]; Campbell Co.: Meredith Cave (CM5)[7], Norris Dam Cave (CM7)[7]; Claiborne Co.: English Cave (CB9)[3]; Grainger Co.: Indian Cave (GA4)[7], Coon Cave (GA12)[3]; Hawkins Co.: Pearson Cave (HW12)[1,7]; Union Co.: Jolley Saltpeter Cave (UN12)[3].

Conservation status: IUCN: Endangered A2ac; NatureServe: G2 (S1 in Tennessee); listed as Endangered under the U.S. Endangered Species Act; listed as Endangered in Tennessee (Withers, 2009).

Comments: We did not observe any Indiana Bats during our bioinventories. However, several priority sites exist in eastern Tennessee for this endangered species (United States Fish and Wildlife Service, 2007).

References: [1] Bat Population Database; VertNet: [2] OMNH, [3] TNNH; [4,5] Wallace (1984, 2003); [6] Harvey and Britzke (2002); [7] United States Fish and Wildlife Service (2007); [8] Lamb and Wyckoff (2010); [9] Samoray (2011); [10] Holliday (2012); [11,12] Flock (2013, 2014).

Genus *Perimyotis*

***Perimyotis subflavus* (Cuvier, 1832) (TX) Tri-Colored Bat**

Localities: Anderson Co.: Springhill Saltpeter Cave (AN3)[13,14], Toilet Bowel Cave (AN14)[13]; Blount Co.: Whiteoak Blowhole Cave (BA2)[1,6–8,10–14], Bull Cave (BA3)[1,4,6–8,11], Gregory Cave (BA4)[1,6–8,12–14], Kelly Ridge Cave (BA6)[1,11,13,14], Scott Gap Cave (BA7)[1,6–8,11–14], Tuckaleechee Caverns (BA11)*, Hatcher Cave (BA12)[6], Calf Cave No. 1 (BA19)[7], Calf Cave No. 2 (BA20)[7,8], Rainbow Cave (BA26)[1,7,8,11], Whiteoak Saltpeter Cave (BA27)[1,6,11–14], Tory Shields Bluff Cave (BA56)[6–8]; Campbell Co.: Meredith Cave (CM5)[1,11], Norris Dam Cave (CM7)[1,10–13], Panther Cave No. 1 (CM8)*; Carter Co.: Carter Saltpeter Cave (CR1)[1,10,*], Grindstaff Cave (CR2)[1,10–12,14], Renfro Cave (CR6)[14], Poga Road Cave (CR31)[1,10], Elk Mills Cave (CR34)[1,10]; Sculpture Cave

(CR63)[1,10], Conway Cave (CR66)[1,10]; Claiborne Co.: English Cave (CB9)[1,12], Sour Kraut Cave (CB46)[13], Buis Saltpeter Cave (CB48)[1,11,13]; Grainger Co.: Indian Cave (GA4)[13,14,*]; Greene Co.: Poplar Cave (GN5)[1,12], Still House Cave (GN7)[1,12], Cochran Cave (GN32)[1,12,13], Afton Cave (GN69)[13]; Hamblen Co.: Soard Cave (HB3)[13], Saltpeter Cave (HB4)[14], Corner Store Cave (HB22)[14]; Hancock Co.: Cantwell Valley Cave (HN2)[1,12], Upstream Cave (HN77)[1,12]; Jefferson Co.: Tater Cave (JF8)[13,*], Rouse Cave (JF26)[13]; Knox Co.: Campbell Cave (KN1)*, Cherokee Bluff Cave (KN4)*, Mudflats Cave (KN9)*, Carter Cave (KN14)*, Keller Bend Cave (KN16)*, Blowing Hole Cave (KN19)[13,*], Cherokee Caverns (KN22)*, Cruze Cave (KN24)*, Meads Quarry Cave (KN28) [1,12,*], Cave Spring Cave No. 2 (KN47)[1,12], Christian Cave (KN49)[1,12,*], Keller Bluff Cave No. 1 (KN61)[5], Kirkpatrick Cave (KN62)*, Unreported Cave (KN90)*, Brents Cave (KN112)*, Chriscroft Cave (KN127)*, The Lost Puddle (KN145)*, Meads River Cave (KN151)*; Loudon Co.: Blankenship Cave (LN1)[14,*]; McMinn Co.: Small Cave (MM5)*; Meigs Co.: Blythe Ferry Cave (ME1)[14], Eves Cave (ME2)[13,14]; Monroe Co.: Gay Cave (MO3)*, Morgan Cave (MO5)*, Nobletts Cave (MO6)*, Lick Creek Cave (MO8)*, Alans Hideaway Cave (MO9)*, Ballplay Cave (MO10)[3], Luther Cave (MO11)[13]; Polk Co.: Gee Cave (PO1)[1,12–14]; Rhea Co.: Grassy Creek Cave (RH2)[2,*], Marler Cave (RH4)[9]; Roane Co.: Berry Cave (RN3)*, Cave Creek Cave (RN5)[14,*], Eblen Cave (RN6)*, Big Cave (RN13)*, Chimney Cave (RN14)*, Marble Bluff Cave (RN19)[1,10–14], Smith Cave (RN37)[1,12]; Sevier Co.: Stupkas Cave (SV42)[7,8]; Sullivan Co.: Morrell Cave (SL6)[1,10–14], Kaylor Cave (SL46)[1,10]; Union Co.: Oaks Cave (UN5)[1,10–12,*], Wright Cave (UN9)[1,12,*], Jolley Saltpeter Cave (UN12)[14], Lost Creek Cave (UN19)[1,12], Rogers Hollow Cave (UN23)*, Herd O Coons Cave (UN32)[13]; Washington Co.: The Man Cave (WS8)[14], Hampton Cave[4].

Conservation status: IUCN: Least Concern; NatureServe: G3 (S5 in Tennessee).

Comments: *Perimyotis subflavus* is among the most commonly encountered bats in caves of eastern Tennessee, and this bat was the most prevalent in our study. Seven dead and three live *P. subflavus* with characteristic white-nose syndrome symptoms were observed at Eblen Cave in May 2013 (Fig. 2). Three Tri-Colored Bats with characteristic WNS symptoms were observed at both Wright Cave and Oaks Cave in Union County in March 2015. Tri-Colored Bat populations are suffering significant losses due to WNS throughout its range (Turner et al., 2011; Ingersoll et al., 2013). The strongest effects of the disease have been on populations in localities with more severe winter climate, where hibernation time is longer and the fungal pathogen has greater opportunity to infect hibernating bats. Reexamination of the IUCN Red List status for this species in the coming years may be needed (Ingersoll et al., 2013). Hampton Cave is not known in the TCS database and could not be georeferenced.

References: [1] Bat Population Database; VertNet: [2] KU, [3] MVZ, [4] OMNH, [5] TNNH; [6] Rabinowitz (1981); [7,8] Wallace (1984, 2003); [9] Lewis (2005); [10] Lamb and Wyckoff (2010); [11] Samoray (2011); [12] Holliday (2012); [13,14] Flock (2013, 2014); * present study.

Order Rodentia
Family Castoridae
Genus *Castor*
***Castor canadensis* Kuhl, 1820 (TX) American Beaver**

Localities: Knox Co.: Ebenezer Rising Cave (KN150)*; Roane Co.: Eblen Cave (RN6)*.

Conservation status: IUCN: Least Concern; NatureServe: G5 (S5 in Tennessee).

Comments: A beaver and associated lodge were observed just inside the entrance of Ebenezer Rising Cave. Two adults were observed near the cave stream within Eblen Cave on 30 December 2005.

References: * present study.

Family Cricetidae
Genus *Neotoma*
***Neotoma magister* Baird, 1858 (TX) Allegheny Woodrat**

Localities: Carter Co.: Grindstaff Cave (CR2)[2]; Hancock Co.: Little Rockhouse Cave (HN19)[1], unknown cave[1]; Hawkins Co.: Barretts Cave (HW26)[1]; Knox Co.: Carter Cave (KN14)*, Keller Bend Cave (KN16)*, Blowing Hole Cave (KN19)*, Ebenezer Rising Cave (KN150)*; Monroe Co.: Gay Cave (MO3)*, Morgan Cave (MO5)*; Rhea Co.: Marler Cave (RH4)[3]; Union Co.: Mossy Spring Cave (UN25)*; Washington Co.: Keplinger Cave (WS3)[1].

Conservation status: IUCN: Near Threatened; NatureServe: G3G4 (S3 in Tennessee); listed as Deemed in Need of Management in Tennessee (Withers 2009).

Comments: Although this species was not observed directly, evidence for its occurrence (i.e., latrines, caches, and nests) was noted in several caves. Kennedy et al. (2012) state that *N. magister* occurs only in central Tennessee, with *N. floridana* replacing this species west of the Tennessee River in western Tennessee and also in the Appalachian Valley and Ridge of eastern Tennessee. But Best and Dusi (2014) show the range of *N. magister* includes eastern Tennessee. These two species are identical in general appearance and can be distinguished by the shape of the palate and by genetic examination.

References: VertNet: [1] TNNH; [2] Conaway and Howell (1953); [3] Lewis (2005); * present study.

Genus *Peromyscus*
***Peromyscus gossypinus* (LeConte, 1853) (TX) Cotton Deermouse**

Localities: Grainger Co.: Indian Cave (GA4)[1].

Conservation status: IUCN: Least Concern; NatureServe: G5 (S5 in Tennessee).

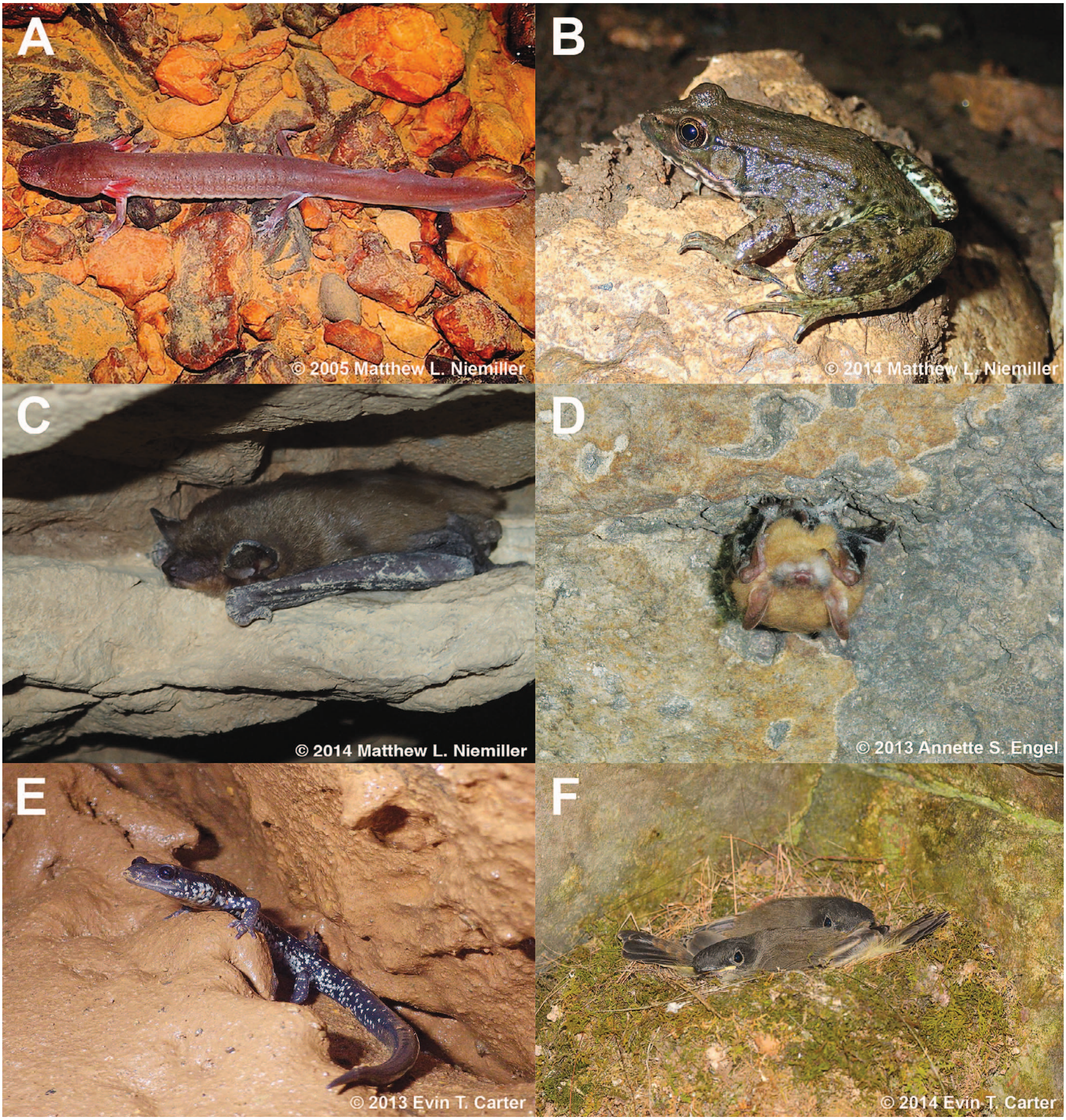

Figure 2. Vertebrate cave life documented in Appalachian Valley and Ridge and Blue Ridge Mountains caves of eastern Tennessee: A) an adult Berry Cave Salamander *Gyrinophilus gulolineatus* from Roane County; B) Green Frog *Lithobates clamitans* from Loudon County; C) Big Brown Bat *Eptesicus fuscus* from Knox County; D) Tri-Colored Bat *Perimyotis subflavus* from Roane County exhibiting fungal growth characteristic of white-nose syndrome; E) Northern Slimy Salamander *Plethodon glutinosus* from Monroe County; and F) fledgling Eastern Phoebes *Sayornis phoebe* from McMinn County.

Comments: Two specimens were collected from Indian Cave in December 1911.

References: VertNet: [1] UMMZ.

Peromyscus leucopus (Rafinesque, 1818) (TX) White-Footed Deermouse

Localities: Campbell Co.: Panther Cave No. 1 (CM8)*; Meigs Co.: Sensabaugh Cave (ME3)*.

Conservation status: IUCN: Least Concern; NatureServe: G5 (S5 in Tennessee).

Comments: Two adults and a nest were observed just inside the entrance to Panther Cave No. 1. Three adults were observed in the dark zone of the main upper-level passage at Sensabaugh Cave on 31 August 2014.

References: * present study.

Peromyscus sp. (TX/AC) Unidentified mouse

Localities: Knox Co.: Campbell Cave (KN1)*; Union Co.: Big Cave (UN10)*.

Conservation status: Not applicable.

Comments: Mouse scat was found in the dark zone on a mud bank at Big Cave.

References: * present study.

Order Soricomorpha
Family Soricidae
Genus _Blarina_
Blarina brevicauda (Say, 1823) (AC)
Northern Short-Tailed Shrew

Localities: Knox Co.: Meads Quarry Cave (KN28)*.

Conservation status: IUCN: Least Concern; NatureServe: G5 (S5 in Tennessee).

Comments: A recently deceased *B. brevicauda* was found in a crawl passage in Meads Quarry Cave between the downstream and main entrances.

References: * present study.

Summary of Bioinventory and Literature Records

For this study, we bioinventoried 56 caves during 107 individual surveys (Table 1) and documented 38 vertebrate taxa that represented 275 total species occurrence records. A mean of 4.9 ± 2.9 taxa were observed per cave. Ten or more species were documented from four caves: Meads Quarry Cave (KN28; 14 species), Berry Cave (RN3; 12 species), Small Cave (MM5; 11 species), and Meads River Cave (KN151; 10 species). We were able to verify 36 vertebrate species: 6 fishes, 15 amphibians (5 anurans and 10 salamanders), 3 reptiles, 1 bird, and 11 mammals. Amphibians accounted for most vertebrate occurrences (47.3% of the total records), with salamanders comprising the most prevalent order of vertebrates (37.8% of the total records). Bats (20.7% of the total records) and non-bat mammals (16.0% of the total records) also were frequently documented during bioinventories. No stygobiont fishes were documented during bioinventories, although three cyprinid, two centrarchid, one cottid, and one ictalurid species were recorded. The Cave Salamander *Eurycea lucifuga*, Tri-Colored Bat *Perimyotis subflavus*, Raccoon *Procyon lotor*, Northern Slimy Salamander *Plethodon glutinosus*, and Spring Salamander *Gyrinophilus porphyriticus* were the five most commonly encountered species, found in 38, 37, 27, 19, and 18 caves, respectively (Table 2). Thirteen observed taxa were considered accidentals in cave habitats, 14 were trogloxenes, 5 were troglophiles, and 1 was a stygobite (Table 2). All taxa classified as accidentals are considered common in surface habitats. The ecological classification of five taxa (*Ameiurus natalis*, *Eurycea longicauda*, *Plethodon dorsalis*, *Canis latrans*, and *Peromyscus* sp.) could not be reliably assigned to one category, and therefore three taxa were considered accidentals or either trogloxenes or stygoxenes and two were considered troglophiles or trogloxenes.

The amount of available habitat may be positively related to species richness of troglobionts in caves at the regional scale, where areas of higher cave density (as a proxy for available habitat) harbor greater species richness (Christman and Culver, 2001; Culver et al., 2003, 2006), or at the scale of individual caves, where larger caves support greater species richness (Schneider and Culver, 2004). From our data, there was a positive correlation between vertebrate species richness and cave length as a proxy for available habitat after accounting for sampling effort, according to weighted Pearson correlation analysis (correlation coefficient $r = 0.340$, $P\text{-}value = 0.01$). However, interpreting such a trend is likely confounded by variation in cave length and complexity, availability of habitat types such as wet or dry, surface land cover above caves and around entrances, and seasonal timing of cave bioinventories. In particular, seasonality might be especially important, as certain species, such as hibernating bats, are much more likely to be observed in caves during particular seasons. A positive correlation existed between species richness and the number of bioinventories conducted at a cave ($r = 0.498$, $P\text{-}value < 0.001$). Consequently, multiple bioinventories spanning different seasons are recommended to adequately document the vertebrate fauna of individual caves. The presence of substantial aquatic habitat also appears important. Wet caves (5.8 ± 3.2 taxa) exhibited greater species richness than dry caves (3.3 ± 1.3 taxa), according to Wilcoxon rank sum test value $W = 3008$, $P\text{-}value < 0.001$. Unsurprisingly, predominantly aquatic caves contained species most often associated with aquatic habitats, and mammals were most often observed in dry caves.

Our review of available literature, biodiversity databases, and museum collections yielded an additional 298 species occurrences, 258 of which were unique from species occurrences recorded during our bioinventories, and 40 that were the same as species occurrences generated through our bioinventories. Bats accounted for the vast majority of additional occurrences (65.1%), with many of these occurrences documented during winter white-nose syndrome surveys in the last five years (e.g., Lamb and

Wyckoff, 2010; Samoray, 2011; Holliday, 2012; Flock, 2013, 2014). From the literature, 10 or more vertebrate species were documented from 11 caves: Gregory Cave (BA4; 15 species), Meads Quarry Cave (KN28; 14 species), Small Cave (MM5; 12 species), Berry Cave (RN3; 12 species), Whiteoak Blowhole Cave (BA2; 11 species), Indian Cave (GA4; 11 species), Cave Creek Cave (RN5; 11 species), Panther Cave No. 1 (CM8; 10 species), Mudflats Cave (KN9; 10 species), Meads River Cave (KN151; 10 species), and Grassy Creek Cave (RH2; 10 species).

Of the 533 unique species occurrence records, 522 could be georeferenced to 132 caves, including the 56 caves bioinventoried in this study. In total, 54 vertebrate taxa have been documented in eastern Tennessee caves (Table 2), including 8 fishes, 19 amphibians (8 anurans and 11 salamanders), 6 reptiles (5 snakes and 1 turtle), 3 birds, and 18 mammals (10 bats and 8 non-bat mammals). The Tri-Colored Bat (*Perimyotis subflavus*), Cave Salamander (*Eurycea lucifuga*), Big Brown Bat (*Eptesicus fuscus*), Gray Bat (*Myotis grisescens*), and Northern Slimy Salamander (*Plethodon glutinosus*) were the five most commonly encountered species, with 89, 48, 41, 37, and 31 total locations, respectively (Table 2). Twenty-one taxa were considered accidentals in cave habitats, 18 were trogloxenes, 5 were troglophiles, and 1 was a stygobite (Table 2). All taxa classified as accidentals are considered common in surface habitats. The ecological classification of nine taxa (*Ameiurus natalis*, *Lithobates sylvaticus*, *Eurycea longicauda*, *Plethodon dorsalis*, *Cathartes aura*, *Coragyps atratus*, *Lasiurus cinereus*, *Canis latrans*, and *Peromyscus* sp.) could not reliably be assigned to one category, and therefore seven taxa were considered accidentals or either trogloxenes or stygoxenes, and two were considered troglophiles or trogloxenes.

Species of Conservation Concern

Thirty-two of the vertebrate taxa in the Appalachian Valley and Ridge and The Blue Ridge Mountains karst areas of eastern Tennessee rank as Least Concern under IUCN Red List criteria (Table 2). However, two taxa rank as Near Threatened, one as Vulnerable, and one as Endangered. Based on NatureServe conservation rank criteria, thirty of the species are globally ranked as Secure (G5), one as Apparently Secure or Vulnerable (G3G4), three as Vulnerable (G3), one as Imperiled or Vulnerable (G2G3), and one as Critically Imperiled (G1). At the state level, NatureServe conservation ranks are similar to the IUCN listings, with thirty species Secure (S5), three Apparently Secure (S4), one Vulnerable (S3), one Imperiled (S2), and one Critically Imperiled (S1). Three of the observed species are tracked by the state of Tennessee (Withers, 2009). *Myotis grisescens* (Gray Bat) is listed as Endangered, *Gyrinophilus gulolineatus* (Berry Cave Salamander) as Threatened, and *Neotoma magister* (Allegheny Woodrat) is Deemed in Need of Management. All of the accidental species observed in bioinventoried caves have a NatureServe global rank of G5 (Secure), with the exception of *Lasiurus borealis*, which has a global rank of G4 (Apparently Secure).

The Berry Cave Salamander (*Gyrinophilus gulolineatus*) is the only stygobiontic vertebrate known from the Valley and Ridge in Tennessee (Brandon, 1965, 1966; Miller and Niemiller, 2007, 2008; Niemiller et al., 2010). This species is a rare neotenic salamander previously known from just nine localities in Knox, McMinn, Meigs, and Roane counties (Miller and Niemiller, 2008; Niemiller and Miller, 2010, 2011). We increased the known localities of this rare salamander within that area to 11 caves. Because of few occurrences, low population densities, and threats associated with urban development and quarrying activities, *G. gulolineatus* is a candidate species for listing on the U.S. Endangered Species List (U.S. Fish and Wildlife Service Species Assessment; http://ecos.fws.gov/docs/candidate/assessments/2014/r4/D03B_V01.pdf).

We documented five bat species from 39 caves. Four species are considered Vulnerable by NatureServe, and two species, *M. grisescens* (Gray Bat) and *M. septentrionalis* (Northern Long-Eared Bat), are listed on the U.S. Endangered Species List as Endangered and Threatened, respectively. Five caves housed populations of *M. grisescens* that were either directly observed or hypothesized to occur on the basis of recent evidence of inhabitation such as fresh guano piles. All of these *M. grisescens* occurrences, except Loudon County, had been previously reported; see the range map compiled by Tennessee Bat Working Group. *Myotis septentrionalis* was recently listed as Threatened under the Endangered Species Act in May 2015 (United States Fish and Wildlife Service, 2015) because of population declines associated with the emergent infectious disease white-nose syndrome, which is caused by the fungus *Pseudogymnoascus destructans* (Pseudeurotiaceae). Although we only observed a single individual, at Oaks Cave in Union County in March 2015, *M. septentrionalis* is known from 20 additional caves in eastern Tennessee (Table 2). The distribution of *M. sodalis* (Indiana Bat) extends into eastern Tennessee with both hibernacula and summer non-maternity roosting colonies; it is also a federally endangered species. This bat was not detected in any of the bioinventoried caves, but is known from 13 caves in eastern Tennessee. The state of Tennessee tracks *M. leibii* (Eastern Small-Footed Bat), because it is Deemed in Need of Management. We did not observe this species, but it has been reported from six caves in eastern Tennessee.

The bats *M. lucifugus* (Little Brown Bat) and *Perimyotis subflavus* (Tri-Colored Bat) were previously considered Secure (G5), but their respective NatureServe conservation ranks were downgraded in 2012 to at best Vulnerable (G3) because of severe population declines. These declines were

Table 3. Number of georeferenced vertebrate records and taxa from the bioinventory reported in this paper and from the literature for counties in the kast area of the Appalachian Valley and Ridge and adjacent Blue Ridge Mountains. The number of caves with records is compared to the number of caves known to the Tennessee Cave Survey.

County	Number Records	Number Taxa	Sampled Caves	Total AVR-BRM Caves[a]	Percent
Anderson	8	6	4	66	6.1
Blount	71	23	12	87	13.8
Bradley	0	0	0	3	0.0
Campbell	20	13	3	38	7.9
Carter	20	8	8	70	11.4
Claiborne	24	10	6	154	3.9
Cocke	1	1	1	63	1.6
Grainger	13	11	2	106	1.9
Greene	11	5	7	112	6.3
Hamblen	6	3	3	81	3.7
Hamilton	0	0	0	35	0.0
Hancock	6	4	5	81	6.2
Hawkins	8	5	5	69	7.2
Jefferson	13	8	4	104	3.8
Johnson	0	0	0	25	0.0
Knox	114	29	24	165	14.5
Loudon	15	11	5	22	22.7
McMinn	18	12	3	12	25.0
Meigs	13	10	3	4	75.0
Monroe	31	15	9	13	69.2
Polk	1	1	1	6	16.7
Rhea	20	15	3	8	37.5
Roane	42	18	7	50	14.0
Sevier	8	8	2	66	3.0
Sullivan	9	7	3	175	1.7
Unicoi	0	0	0	9	0.0
Union	47	16	10	56	17.9
Washington	3	3	2	42	4.8
Total	522	54[b]	132	1722	7.7

[a] AVR = Appalachian Valley and Ridge; BRM = Blue Ridge Mountains.
[b] Number of distinct taxa.

attributed to high mortality from white-nose syndrome (Frick et al., 2010; Maher et al., 2012; Verant et al., 2012; Minnis and Lindner, 2013). The first confirmed cases of bats infected with white-nose syndrome in Tennessee were in winter 2009–2010 (Lamb and Wyckoff, 2010). Although thought to be widely distributed across eastern Tennessee, *M. lucifugus* was observed in just three caves during this study. At Tuckaleechee Caverns in Blount County, one specimen had the characteristic white fungal growth on the muzzle on 20 March 2014. The most frequently encountered bat species was *P. subflavus*, which we observed in 37 of 56 caves (66.1%) surveyed. Evidence of white-nose syndrome infecting *P. subflavus* was observed at Eblen Cave in Roane County on 15 May 2013. In this cave, a single bat was discovered with white fungal growth on the muzzle, and several dead bats were observed throughout the cave. Evidence of white-nose syndrome infecting *P. subflavus* was also observed in March 2015 at Oaks Cave and Wright Cave in Union County. The disease was first detected in Blount County in 2012 (Holliday, 2012), Roane County in 2014 (Flock, 2014), and Union County in 2013 (Flock, 2013).

Conclusions and Future Directions

Caves and associated subterranean habitats are important for many vertebrate species for reproduction, hibernation, refuge, or other aspects of their life histories. Although caves have long been recognized as critical habitats for several species of bats, the importance of caves for other vertebrate taxa has received less attention, with the exception of some salamanders (e.g., Niemiller and Miller, 2009; Goricki et al., 2012). This study advances our understanding of cave use by vertebrates by providing insights into the richness and distribution of vertebrate taxa in the Appalachian Valley and Ridge and the Blue

Ridge Mountains of eastern Tennessee. Prior to this study, 298 vertebrate occurrence records representing 35 taxa existed from at least 100 caves that could be georeferenced. Our efforts nearly doubled the number of records by generating an additional 235 unique species occurrence records for 36 taxa from 56 caves. Thirty-two of the 56 caves previously lacked any vertebrate records. In combination with literature data, 54 vertebrate taxa in total have been documented from 132 caves that could be georeferenced in 24 counties in eastern Tennessee. Significant numbers of fishes, amphibians, reptiles, birds, and mammals use caves in eastern Tennessee, and several species regularly use caves. However, overall only 7.7% of known caves in those parts of eastern Tennessee now have vertebrate occurrence records. Few caves in eastern Tennessee have had comprehensive bioinventories, and only a small fraction of those have had repeated bioinventories for all vertebrates, with the exception of periodic surveys of winter hibernating bats at select caves (e.g., Lamb and Wyckoff, 2010; Samoray, 2011; Holliday, 2012; Flock, 2013, 2014). Moreover, several karst areas still remain poorly investigated, particularly in Bradley, Claiborne, Cocke, Grainger, Hamblen, Hamilton, Jefferson, Johnson, Sevier, Sullivan, and Unicoi counties, where less than 5% of known caves in each county have a vertebrate occurrence record (Table 3). A growing body of literature suggests that caves provide important habitat for non-troglobiontic vertebrates. Caves should continue to be considered as resources to be protected in the management and conservation of vertebrate fauna.

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SURFACE AND SUBSURFACE KARSTIFICATION OF AQUIFERS IN ARID REGIONS: THE CASE STUDY OF CHESHME-ALI SPRING, NE IRAN

Mohammad Shokri[1]*, Javad Ashjari[2], and Gholamhossain Karami[1]

Abstract: Objective karstification assessment is a key component of hydrogeological studies of aquifers. In this research, surface and subsurface karst development have been assessed, based on different methods, to get insights into karstification processes in the area of the Cheshme-Ali karstic aquifer, located in northeast Iran. GIS information, remote sensing, and field measurements of fracture density and frequency have been used to determine surface karst development. The monthly spring discharge rate and physicochemical parameters of the Cheshmeh-Ali Spring in 2003–2004 were used to determine subsurface karstification rate. Additional monthly measurements of discharge rate and chemical parameters were carried out in 2010–2011. The evaluation of surface karstification (S_k) illustrates moderate karstification of 51.47 percent. The response of the aquifer to precipitation shows the impact of one or more conduits in the water level fluctuation zone that generate a rapid response of the spring to large precipitation events. The spring hydrograph analysis indicates a pseudo-diffuse flow system in the region. The hydrograph and chemograph analyses demonstrate average subsurface karstification index of 1.7 m. Finally, it is concluded that although both methods demonstrate karstification processes in an aquifer, these kind of reservoirs still require more intelligible approaches, as well as comparable methods for their description.

Introduction

Karstic aquifers are the most attractive reservoirs for water exploitation. These aquifers are very important resources of water supply in arid and semi-arid regions. While demand for water is high in dry regions, access to surface water is very limited. Aquifers store groundwater during the wet season, then naturally release it gradually to surface springs or rivers.

It is crucial to identify surface and subsurface karstification processes. Typical karst topography is related to subterranean drainage, and therefore, geomorphology and hydrology are closely interrelated (De Waele et al., 2009, 2011). Aerial photograph and satellite image interpretation may be of considerable help in the identification of the karst surface landforms. Techniques are currently supported by automatic tools with a high potential for graphical representation of karst development, such as GIS with incorporation of remotely sensed data. These types of data have significant advantages over traditional methods because they can cover broad areas relatively quickly, have better accuracy and precision, are accessible in a digital format, and can be easily updated (Melelli et al., 2012).

However, field mapping and acquisition of geological data remain the best way of obtaining accurate information to check the validity of the interpretation of the GIS data. Deducing the principles of karstification from geological structures were highlighted in the works of White (2002) and Ford and Williams (2007). According to Jameson (2006), in order to study karst evolution, structural features should be identified and analyzed. Features most commonly develop along preexisting fractures, joints, and bedding planes, which represent the initial flow path of the water through the rock. Over time, a variety of larger features can develop into cave systems, with sinkholes and deep valleys as surface expressions that control surface development of karst landscapes (Šebela et al., 2005; Parise, 2008; Pepe and Parise, 2014; Gutiérrez et al., 2014). Fractures play an important role in transmitting water from diffuse recharge on the land surface to the conduits, as well as to wells drilled in karst aquifers (Kiraly, 2003). Water flow will enhance the dissolution, particularly in fractures with large apertures.

Karst-spring hydrograph and chemograph analyses are the signature of subsurface karst development. Panagopoulos and Lambrakis (2006) demonstrated that by studying time-series analysis of rainfall as input and spring discharges as output along with hydrodynamic and recession curves analysis we can evaluate the whole of karst aquifers. The response times of karst aquifers depend on various factors, such as the contribution of allogenic recharge and internal runoff, the carrying capacity and internal structure of conduit system, and the area of the groundwater basin (White, 2002). The combined analysis of karst spring hydrographs and chemographs allows a more refined characterization of the karst drainage systems (Drake and Harmon,

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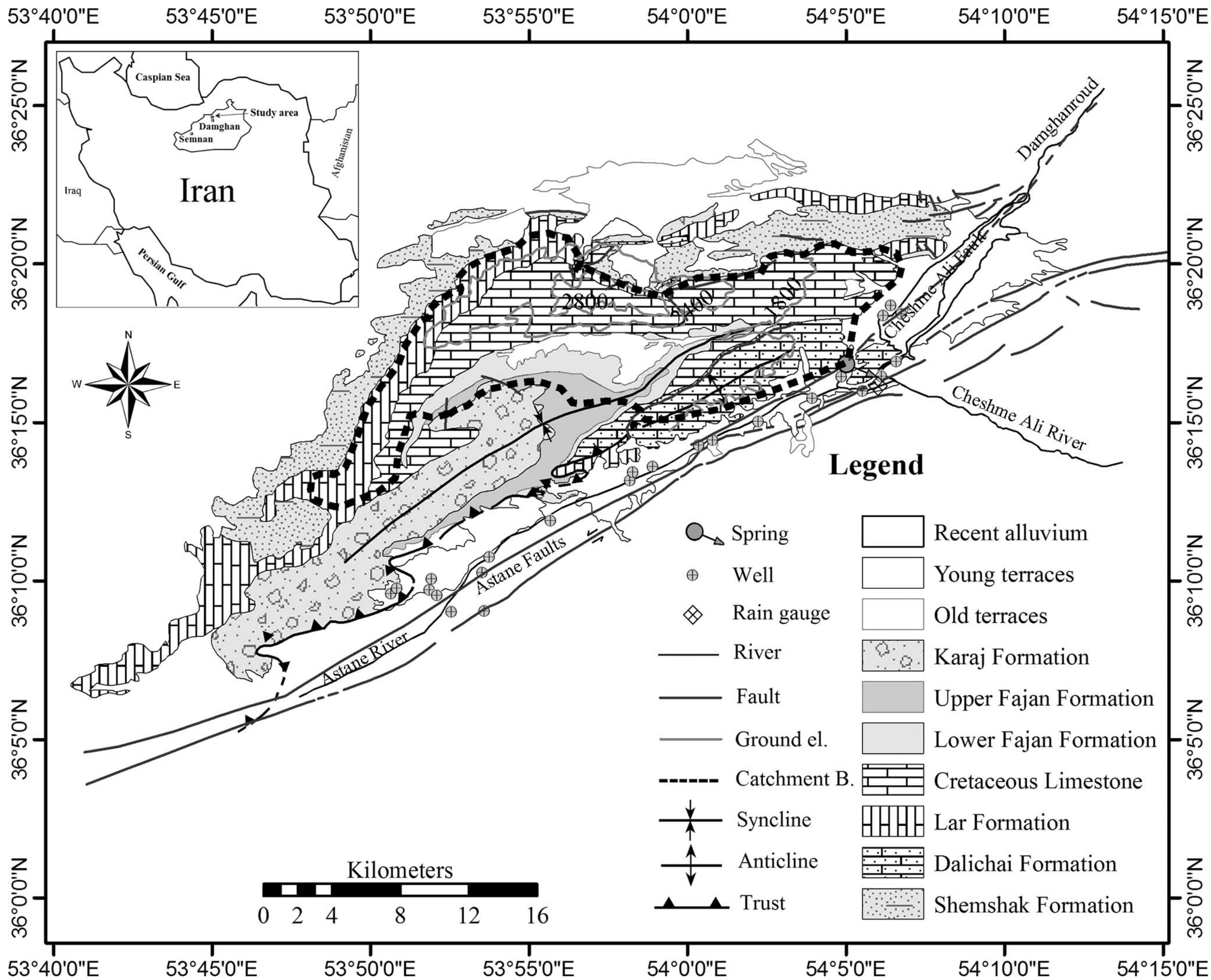

Figure 1. The location and geological map of the study area.

1973; Sauter, 1992; Jeannin and Sauter, 1998; Dewandel et al., 2003; Parise et al., 2015).

The object of this study is to evaluate surface and subsurface karstification in a catchment area of a permanent and high flow karstic spring located in an arid region of Iran. The study area is the basin of Cheshme-Ali Spring, with average discharge rate of about 500 L s^{-1} and mean annual rainfall of about 257 mm.

Identification and Geological Setting of Study Area

The study area is situated in the province containing the city of Damghan in northeastern Iran. The geological map and the stratigraphical units of this area are shown in Figure 1. The region lies in the Alborz Ridge Mountains of Iran. The Cheshme-Ali Basin consists of two series of tight anticlines, with the northern limb of the northern anticline eroded (Shokri et al., 2011). The Shemshak Formation, Early Triassic to Middle Jurassic, consists of fine sandstone, siltstone, and shale, and is over 1020 m thick (Aghanabati, 2006). Hydrogeologically, the formation is the bedrock of the Cheshme-Ali Basin's aquifer. The Dalichai Formation is a Middle Jurassic marly limestone with a thickness of more than 100 meters that crops out at the southern limb of the southern anticline. The limestone of the Lar Formation, Late Jurassic, and the Cretaceous Formation are the main karstic aquifers exposed in the area. The karstic formations are underlain and overlain by impermeable sandy marl of the Shemshak and Fajan Formations, respectively. The Fajan Formation (Paleocene–Eocene) consists of conglomerates, red sandstones, and sandy marl. The Karaj Formation (Middle Eocene) is composed of green tuff with frequent sandstone fragments.

Geomorphologically, several micro-karst landforms have been observed in abundance on the exposed limestone, but the field investigations showed no solution features such as sinkholes, caves, dolines, shafts, or closed depressions and highlighted the important role played by structural elements such as faults, joints, folds, and bedding planes. Two major faults controlled the basin development. The Astane Fault, a reverse left-lateral displacement fault, crosses south of it and is 75 km in length, and the Cheshme-Ali Fault shows a trend of N50E. Several minor normal, trust, or strike-slip faults cross the basin (Shokri et al., 2011; Shokri, 2012).

The highest, mean, and lowest elevations of the catchment basin are 3200, 2100, and 1510 m.a.s.l., respectively. No rain gauge station is located in the catchment area of the spring. According to the recorded data at the nearest rain gauge, about 2.5 km south from the spring and at 1450 m elevation, the long term mean annual rainfall is 129 mm. Several rivers cross the area, but none of them cross the anticlines. The rivers are ephemeral, largely fed by the upstream surface watershed, and are dry for much of the year (Fig. 1). A qanat is a man-made horizontal gallery to collect groundwater by gravity flow (Wulff, 1968). Neither wells nor qanats were built to exploit water from the karst layers. Twenty-four wells were identified that extract groundwater from the alluvial aquifer adjacent to the karst aquifer.

Methods

The following data were used in this research: 1:25000 topographic maps, 1:100000 geologic maps, 2001 Landsat images, 2007 panchromatic IRS images, long-term records of 14 rain gauges, and temperature data of the region's weather stations, as well as discharge, specific conductance, water temperature, pH, and major ions of the spring water. The data were obtained from a variety of sources, including the Geology Survey of Iran, Regional Water Board of Semnan, Space Organization of Iran, Karami (2004), and Survey Organizing of Iran. The present research is based on GIS and remote sensing systems, field studies, and physicochemical interpretations of the Cheshmeh-Ali Spring.

GIS techniques and remote sensing were used to determine the surface karstification factors in the study area. Lithology, topography, vegetation, lineament density and spacing, fault density and spacing, drainage network, temperature, and precipitation were used as interpretation elements to extract the thematic layers. The overlay of these map layers in GIS produces a composite map of the karstification components. To determine which factors affect surface karstification and to what degree, experts were consulted to provide judgments on importance of criteria. By using the analytic hierarchy process (AHP), a score for each criterion (X_i) on each layer was determined. These considerations were then converted to criteria weights (W_i). The AHP technique is based on a pair-wise criteria comparison that has been widely used in geosciences and other fields as well (e.g., Ho, 2008; Sinha et al., 2008; Mondal and Maiti, 2013; Daman Afshar and Majlesi, 2013).

The weighted linear combination of W_i and X_i results in a karstification index for each layer. By the above process, the surface karstification map was produced. Several field checks have been completed to assess and validate the procedure. The field studies also included measurements of orientation, spacing, and aperture of fracture elements.

The Cheshmeh-Ali Spring's physicochemical parameters were used to determine the subsurface karstification in the basin. As an index of karstification, the recession coefficient of the spring hydrograph was determined using Mangin's method (1975). The Grasso et al. (2003) method of comparison of the variation of spring water chemistry to discharge was also used to study subsurface karstification.

Results and Discussions

Catchment Area of Spring

The catchment area of Cheshme-Ali's Spring was determined using the method of Ashjari and Raeisi (2006). This method assumes that there is no allogenic stream input and that the variation of storage over the measured hydrological year is insignificant. In $A = V/(10^3 PI)$, A is the catchment area of the spring (km^2), V the total discharge of the spring during one hydrological year (m^3), P the average annual precipitation (mm y^{-1}), and I is a recharge coefficient (dimensionless) that varies from 0 to 1. The calculated catchment area should be compared to the probable boundary of the spring watershed area.

The mean discharge of the spring was 411 L s^{-1} during the hydrological year 2003–2004. The comparison of the spring discharge at the beginning and end of the year demonstrated negligible variation in the aquifer storage. Using a rainfall elevation equation of the region, average precipitation P was estimated to be 257 mm. Field verifications of morphology and the existence of valleys that are normally dry except for temporary water runoff due to heavy rains indicate low permeability of the rock mass. The recharge coefficient I was estimated to be 0.38 according to Ashjari and Raeisi (2006). The geological setting and the water budget calculation have shown that the catchment area of the Cheshme-Ali Spring is about 132 km^2 and is restricted to the northern and southern limbs of the calcareous and marly limestone anticlines (Fig. 1).

The exposed cores of the anticlines are dominantly made of the calcareous Lar and Cretaceous Formations and of the marly limestone of Dalichai Formation. There is no hydraulic connectivity between the northern and southern limbs in most parts of the anticlines, except in the plunge apexes, because the elevation of the Shemshak Formation under the crest of the anticline is higher than the adjacent alluvial aquifers in these areas. Karst water originates from the calcareous formations and discharges at the spring. Therefore, karst water from the limbs is expected to flow along the foot of the anticlines and finally

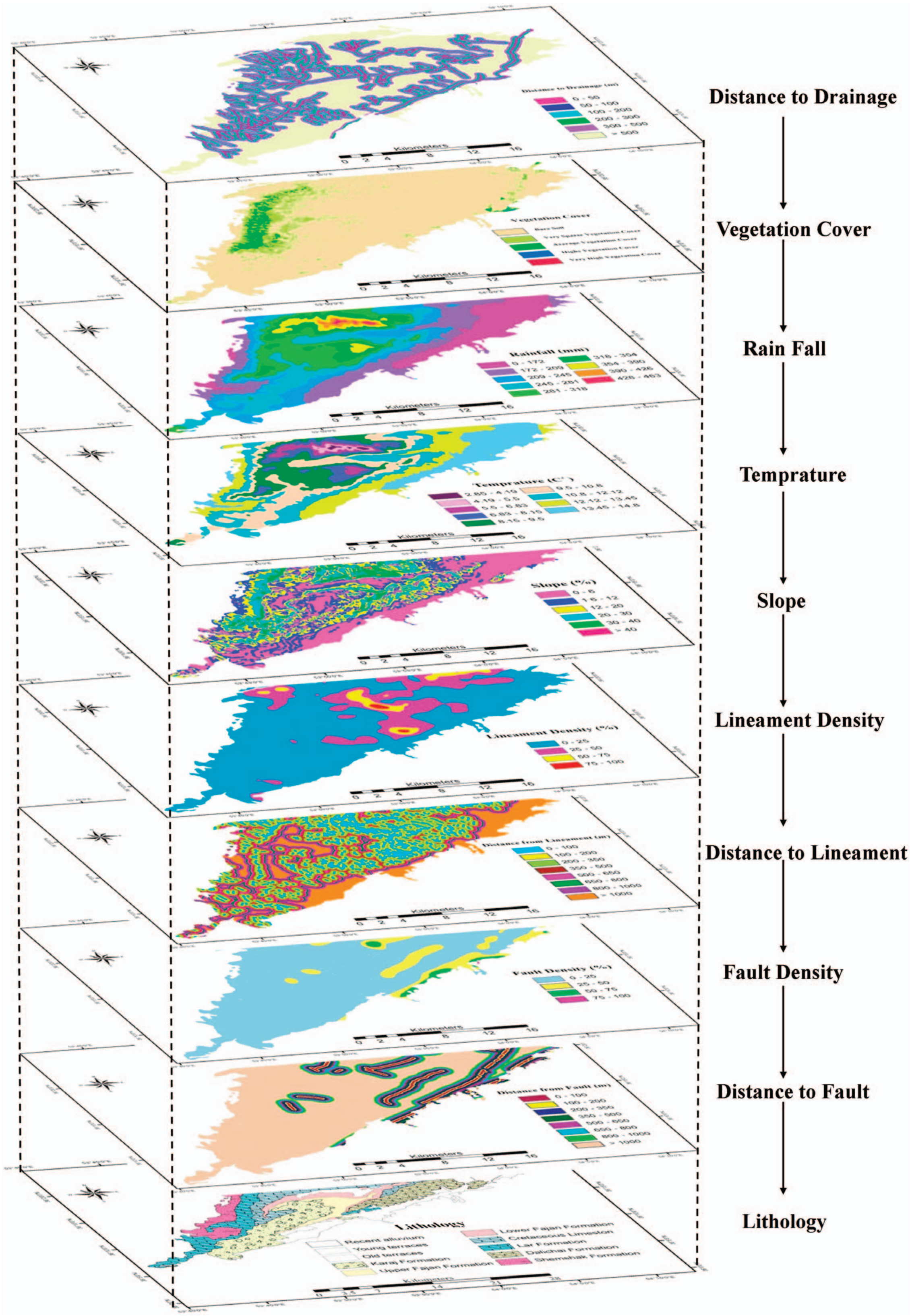

Figure 2. The ten database layers used in the GIS analysis, prepared as described in the text.

emerge from the Cheshme-Ali Spring, which acts as the local base level of the aquifer.

GIS and Remote-Sensing Method

Remote-sensing and GIS techniques were used to create a surface karstification map in the region. The GIS technique contains several layers of information, combining the data collected from the various sources and integrated into the system. To facilitate access to specific categories of information, these were combined within 10 main layers: lithology, distance to faults, fault density, distance to lineament, lineament density, slope, temperature, rainfall, vegetation cover, and distance to drainage (Fig. 2). Since nearly the whole catchment is bare and without soil cover, we did not consider a soil cover layer in this research. The lithology layer was based on published geological maps, satellite images, and field checks. Carbonate successions of the area were considered as karstifiable layers, whereas alluvial and sandy marly formations are unkarstified rocks. The Dalichai, Lar, and Cretaceous Limestone Formations consist of carbonate rocks with different level of purity. According to Stöcklin and Setudehnia (1971), the Dalichai Formation is less pure than the other carbonate layers.

Faults can guide water to penetrate into the ground; therefore, karstic processes can developed along them and result in a variety of karst morphologies (Jafarbeyglou et al., 2012). Large faults are rarely represented by a single surface fracture. Minor faults usually feather off at acute angles as a consequence of the wrenching of the rock. Shear fractures are often oriented parallel to or close to the associated structures (Ford and Williams, 2007). Opportunities for karst development are most likely in the center of the fault zone. A distance map to faults was prepared in GIS. To score fault effects on karstification, the inverse distance weighting to center method was used. The highest score was given to the center zone of the faults, and the lowest score to distances farther than one kilometer from middle line. We used nine distance zones: 0–100 m, 100–200m, 200–350m, 350–500m, 500–650m, 650–800m, 800–1000 m, and >1000 m. The margin of the Astane Fault achieved the highest score.

The fault-density map was prepared by the density function of ArcGIS and classified into four density zones, 0–25%, 25–50%, 50–75%, and 75–100%.

The zones with the highest lineament density are often those with the most intense karst development (Goldscheider and Drew, 2007). Lineaments have a narrow, linear trend and are detectable on high-altitude and satellite images (Ford and Williams, 2007). In this study, lineaments were detected by high-pass directional filters and edge enhancement filter on IRS panchromatic images in four directions by ILWIS 3 software. Maps of lineament are important tools that may help to designate karstifiable sites. Two sets of thematic maps were prepared to include lineament effect on karst development: lineament density and inverse distance weighting to lineament. A distance map of lineaments was prepared, subdividing the area into nine zones: 0–100m, 100–200m, 200–350m, 350–500m, 500–650m, 650–800m, 800–1000m and >1000m. The trends of lineaments are N60E to N90E, and N60W to N90W. The lineament density map was prepared by the density function of ArcGIS, and classified into four groups: 0–25%, 25–50%, 50–75% and 75–100%. The highest densities are distributed in the crests and at the north plunge of the anticlines.

The slope map was derived from a digital elevation model. The slopes were classified into six classes. The slope class of 0–6 degrees dominated the map, followed by 6–12 degrees. The lower average slopes were observed at the limbs, footslopes and crest line of folds. There is an inverse relationship between slope and karstification; gentler slopes promote karst development (White, 1988). Therefore, the lowest score was assigned to the steepest slopes.

The main source of recharge is autogenic, from direct precipitation over the karstic aquifer of the area. The temperature has an inverse effect on the karstification rate. Precipitation, rather than temperature, is the principal meteorological control on solution (Ford and Williams, 2007). Thematic maps data of precipitation and temperature were derived by applying rainfall-elevation and temperature-elevation relations to the DEM data. On their map layers, precipitation and temperature are classified into nine groups. The peaks of anticlines had the highest precipitations and lowest temperatures, which received the highest scores.

Vegetation is found to have an important role in surface karst development through absorption of calcium and magnesium by roots and carbon dioxide production (Ford and Williams, 2007). The normalized-difference vegetation index (NDVI) was used to prepare the layer for vegetation cover. The NDVI is directly related to photosynthetic capacity and, therefore, to the karstification capacity. The vegetation map shows five types of vegetation cover in the area: bare, very weak, weak, average, and intense, based on the NDVI. The intense-vegetation spots were distributed in the northwest of the catchment area. The basin is mostly classified as having an average vegetation cover.

The drainage pattern is distinguished from other types of lineaments to highlight its role on karstification. The drainage information was derived from the topographic maps at 1:25000 scale and the panchromatic IRS images of the area. Then a map was prepared in GIS of the distance to drainage grouped into six stages, <50 m, 50–100 m, 200–300 m, 300–500 m, and >500 m. In the study area, the northern anticline has a tighter drainage pattern than the southern anticline.

To assign a weight to each layer, previous research and personal judgment have been used. It is extremely difficult to assign weights for relative karstification to the

Table 1. The weights (%) given to the GIS data layers in Figure 2 to generate final surface karstification map.

Data Layer	Lithology	Precipitation	Fault Density	Lineament Density	Distance to Faults	Distance to Lineaments	Vegetation Cover	Distance to Drainage	Slope	Temperature
Weight	24	13	18	15	11	9	5	2	2	1

different layers involved in basin mapping for relative karstification. The final weights given to the categories in the ten data layers, which were determined by using the analytic hierarchy process, are listed in Table 1.

The final relative surface karstification map is presented in Figure 3. The accuracy of this model has been verified by field checks. The karstified rocks of the catchment are classified into four groups (Table 2). Non-karstified sections coincide with non-carbonate layers, and highly karstified zones are in the northern anticline of the spring-water catchment.

The average surface karstification (S_k) as a percentage was calculated by

$$S_K = \frac{\sum_{i=1}^{n} (A_i K_{C_i})}{A} \times 100 \quad (1)$$

where A_i represents the area of each karstification class, K_{C_i} represents the karstification coefficient of that class and A represents the catchment area of aquifer. In each class, overall distributive pixelated size of potential

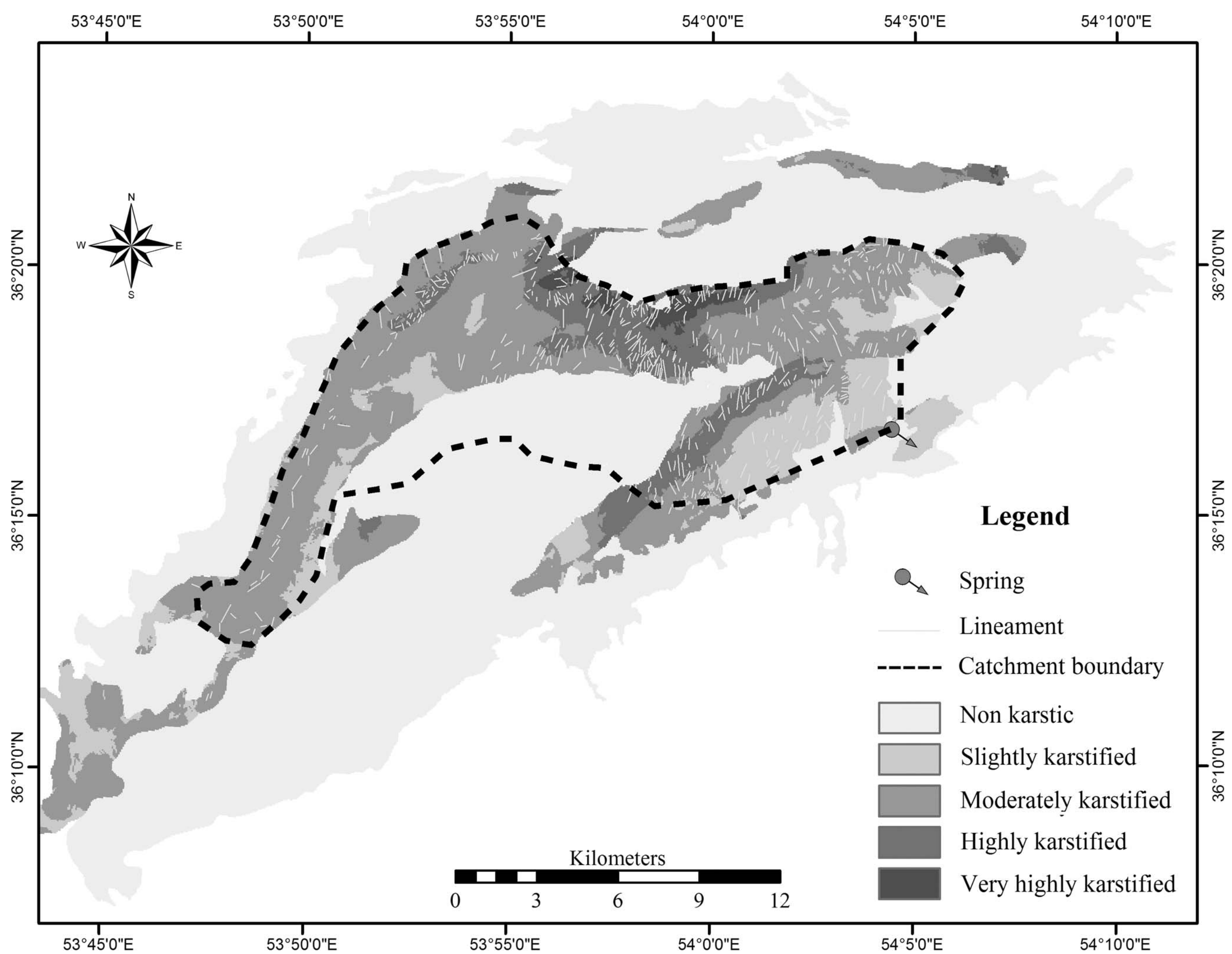

Figure 3. The relative surface karstification in the study area determined by weighting the data in the layers of Figure 2 according to the percentages in Table 1.

Table 2. Determination of the average surface karstification (%) in the Cheshme-Ali basin based on area and karstification coefficient at each level of karstification intensity.

Karst Development Classification	Area, km^2	Karstification Coefficient, %
Slightly karstified	24.3	25
Moderately karstified	81.0	50
Highly karstified	25.1	75
Very highly karstified	3.58	100
Surface Karstification = 51.47 %		

krastification according to actual surficial elements should be taken into account when estimating karstification coefficient. In fact, the coefficients represent relative potential of the surface of terrains regarding their potential of permeability and dissolution. If a class has a general low potential of karstification, it gets a low weight and vice versa. Although the proposed indices are based on logical numerical values, they are inherently qualitative criteria. The mean surface karstification of the Cheshme-Ali catchment is 51.47%, which suggests a moderate karstification rate.

Structural Analysis

Cheshme-Ali aquifer is recharged only by direct precipitation over bare limestones, through joints, fractures and discontinuous elements. Aloui and Chaabani (2006) presented a statistical method for evaluation of karstification in fractures at Jebel Feriana in Tunisia that is used here as well. Based on this method, karstification of bedrock I_k is proportional to openness and fracture intensity and can be estimated $I_k = O \times I_f$, where O represents the average openness and I_f describes the characteristic fracturing of the site (Aloui and Chaabani, 2006). The fracture characteristic of a site is defined by $I_f = (n \times n_c)/(s \times e)$, where s represents surface, n is the number of fractures, n_c is the number of beds crossed by fractures (i.e., fracture depths) and e is the mean fracture spacing (Aloui and Chaabani, 2006). In this study, fracture characteristics have been measured at different stations for evaluating the surface karst development (Fig. 4). The limestone layer is very thick in the study area; therefore, the number of beds n_c has been considered as single unit. The numerical result of karstification in fractures at several stations of the Cheshme-Ali Basin is shown in Table 3. The mean openness of fractures in the Cretaceous and Lar Formations is higher than the Dalichai Formation. Therefore, the karstification index I_k is expected to have higher values in Lar and Cretaceous Formations.

Hydrograph and Chemograph Analysis

Response of an aquifer to external precipitation pulses can be reflected in springs' hydrographs and chemographs, which are mainly controlled by internal flow paths and karstification of the aquifer. The Cheshme-Ali Spring is the only resurgence of the studied aquifer. The discharge rates and the main physicochemical parameters of the spring were measured monthly for two periods during 2003–2004 (first year) and 2010–2011 (second year) by Karami (2004) and Shokri (2012), respectively. The only input to the aquifer is the direct diffuse recharge of precipitation over anticlines.

The maximum (minimum) measured discharge rates are 435 (391) and 755 (425) L s^{-1} in the first and second year, respectively. Although the ratio of maximum to minimum discharge is relatively low in both measured periods, the flow rate differences are notably high between the two periods. Daily rainfall data were plotted to compare spring discharge fluctuation (Fig. 5). The total annual rainfall, according to the Astane station, was 99 and 114 mm in the first and second years, respectively. Such a small difference

Figure 4. Examples of fractures in different stations of the Cheshme-Ali basin.

Table 3. Fracture characteristics in several stations of the three karst formations of the Cheshme-Ali basin. The components of the table are described in the text.

		Location							
No	Formation	Latitude, °N	Longitude, ° E	n	e, cm	S, m^2	O, cm	I_f	I_k
1	Dalichai	36°17′03″	54°03′13″	6	64	23	1.2	4.1×10^5	4920
2	Dalichai	36°17′12″	54°03′35″	10	30.5	28	0.8	1.2×10^6	9600
3	Dalichai	36°15′28″	54°00′51″	14	82.3	33	1.63	5.2×10^5	8476
4	Dalichai	36°14′21″	53°58′48″	8	52	25	1.35	6.1×10^5	8235
5	Dalichai	36°17′50″	54°03′03″	8	115	35	1.1	2.0×10^5	2200
6	Cretaceous	36°18′10″	54°02′14″	6	90	15	1.4	4.4×10^5	6160
7	Cretaceous	36°17′57″	54°01′44″	5	130	41	2.6	9.4×10^4	2444
8	Cretaceous	36°17′35″	54°01′19″	7	70	26	3.5	4.0×10^5	14000
9	Lar	36°17′47″	53°51′10″	6	37	13	1.6	1.2×10^6	19200
10	Lar	36°17′47″	53°51′31″	11	115	20	2.95	4.7×10^5	14108
11	Lar	36°17′34″	53°51′57″	18	55	30	4.2	1.1×10^6	46200
12	Lar	36°17′47″	53°52′10″	14	120	38	3.6	3.1×10^5	11160

in annual rainfall cannot be the cause of the large discharge difference in the spring. The time distributions of rainfalls were compared to the discharge curve of the spring in both periods. In the rainy season, the rainfall distribution was more uniform over time in the first period relative to the second period. The most important rainfall event was an intense precipitation of 53 mm during three consecutive days, March 11–14, 2011, which is equal to about half of the annual rainfall in the second year. The peak discharge was detected after two months lag time, whereas it was estimated to be five months in the earlier year (Fig. 5).

A recession curve is the portion of the hydrograph that extends from a discharge peak to the base of the next rise. To determine flow regime of the spring, Mangin's approach (1975) was used. The exponential coefficients α represent inherent aquifer characteristics such as material porosity and internal karstification (Amit et al., 2002; Kovács et al., 2005; Fiorillo, 2011). The results of analysis are presented in Table 4. Only one recession coefficient, α, which is a rare situation in practice, was obtained in the two periods of measurements. The recession coefficient of the spring in the second period is seven times that of the first period. There is no quick flow in the first year, but a small amount of water (0.3%) emerges as a quick-flow component in the second period. Thus, the aquifer flow is diffuse. Also, the presence of well-interconnected karst fissures, along with the absence of large caves in the phreatic zone, can regulate water discharge and smooth variation in a hydrograph. According to Malik (2007), the inner karstification of the spring's catchment area is categorized as low degree.

Geological investigation demonstrated that the Lar Formation has more potential for karstification than the Dalichai Formation due to its high purity. The Lar and

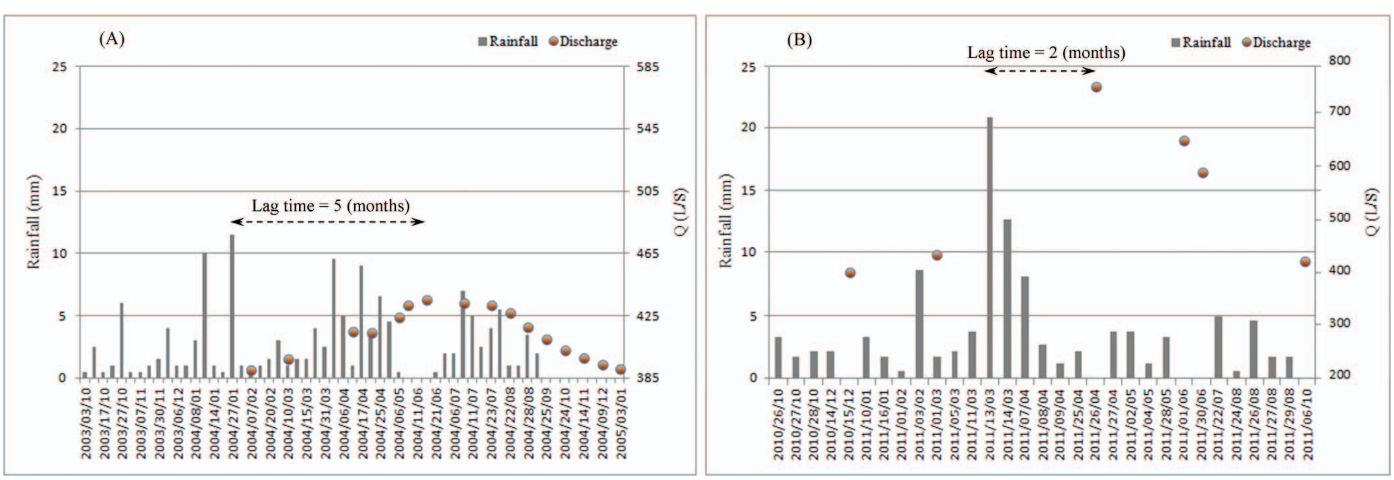

Figure 5. The records of daily rainfall and discharge of the Cheshme-Ali Spring during the 2003–2004 study year (A) and the 2010–2011 study year (B). Note that while the rainfall scales are the same in the two parts, the discharge scales are not.

Table 4. Summary of the main characteristics of the recession curve analysis of the Cheshme-Ali Spring in different hydrological years. Sufficient details regarding the components of the table are presented below.

Year	Q_{max}, m^3s^{-1}	Q_{min}, m^3s^{-1}	$q_0{}^b$, m^3s^{-1}	α, d^{-1}	$q_0{}^*$, m^3s^{-1}	t_i, d	μ, d^{-1}	ε, d^{-1}	$(V_0{}^b/V_0)$, %	$(V_0{}^*/V_0)$, %
2003−2004	0.435	0.391	0.435	5.20×10^{-4}	…	…	…	…	100	…
2010−2011	0.755	0.425	0.720	3.53×10^{-3}	0.035	36	0.027	0.0335	99.7	0.3

Notes: Q_{max} and Q_{min} are, respectively, maximum and minimum discharge of the spring; $q_0{}^b$, discharge at the beginning of the recession for the base flow; α, baseflow coefficient; $q_0{}^*$, discharge at the beginning of recession for the quickflow, t_i, duration of quickflow; μ and ε, parameters adapted for the curves of the quickflow; V_0, total dynamic aquifer volume; $V_0{}^b$ and $V_0{}^*$ are initial volumes that will be drained during baseflow and quickflow.

Cretaceous Formations are exposed in more than 90% of the spring's catchment area, while the Dalichai Formation crops out in about 10% of the catchment, near the spring. The aquifer must collect groundwater from the Lar and Cretaceous Formations and deliver it to the Dalichai Formation. Since Dalichai is not a heavily karsted water-bearing layer, it acts as regulator of the groundwater flow. Although the majority of the groundwater discharging at the spring originates from the Lar and Cretaceous Formations, hydraulic responses to external pulses are controlled by the Dalichai Formation.

The water table fluctuates up and down as a result of seasonal and annual change in precipitation and drought and wet conditions that create pathways near the water table and provide a very fast way to transport water in an aquifer. During high recharge or wet cycles, the water table, especially proximal to a spring, may be higher than the typical elevation of the water table. This hypothesis can explain the short lag time during the second year, 2010–2011, as due to the high rate of precipitation in those three days and the consequent recharge to the aquifer.

Grasso et al. (2003) stated that the concentration of dissolved materials in a spring is a function of discharge rate. This relationship allows for the prediction of aquifer geometry (volume/surface ratio and mean flow-path length). The relationship of concentration and discharge allows us to estimate two parameters, α and A, where A is a function of calcite saturation and α depends on the spatial dimension of the karstic network based on $\ln(C_t) = \ln(A) - \alpha \ln(Q_t)$. By depicting ln($C$) versus ln($Q$) on a graph, the α and A parameters can be determined. Graphically, α is equal to the slope of the line and A will be recognized by extrapolating the line to the vertical axis. Concentration is, however, dependent on two geometric parameters of conduit networks, the area-to-volume ratio (AVR) and the average flow path (AFP). The AVR is dependent on the average void aperture of the submerged karstic networks which can be considered as a karstification index and used for comparison of different karst systems (Grasso et al., 2003). The α parameter shows an intrinsic characteristic of each karstic system that varies directly with the conduit networks AVRs and inversely with their AFPs according to $\alpha = 1900\,AVR/AFP$ (Grasso et al., 2003).

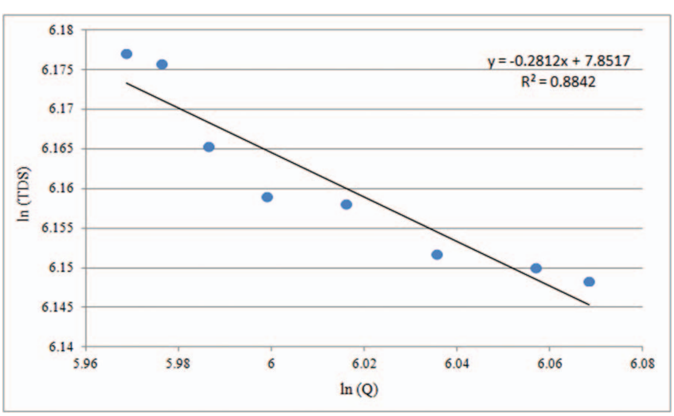

Figure 6. The relationship between discharge of the Cheshme-Ali Spring and its total dissolved solids concentration in a log-log plot. The parameters of the line are used to estimate the index of subsurface karstification of the aquifer.

The AFP depends on the hydrogeological catchment area of the spring and is estimated from the square root of its area. The result of plotting ln(C_t) versus ln(Q_t), where C_t is total dissolved solids, for Cheshme-Ali Spring is shown in Figure 6. α is equal to 0.2812, and A is equal to 2565 (ln(A) is 7.85). The average flow path AFP, which has been estimated as the square root of the catchment area, is 11.48 km. From these calculations, the area-to-volume ratio AVR, which is also referred to as the karstification index, is estimated to be 1.7 m based on the calculation of α. The estimated value shows a low to average extent of karstification.

Conclusions

The surface and subsurface indexes are signatures of karst development that can reflect hydraulic characteristics and the physicochemical response of karst aquifers. GIS and remote-sensing studies revealed that the karstification rate is moderate in the catchment area of Cheshme-Ali Spring. It is also possible to determine spatial zonation of karstification at the regional scale. The physicochemical characteristics of Cheshme-Ali Spring indicated that the subsurface karstification is moderate, and it is unlikely to determine its variation in the aquifer by the spring hydrochemical analysis.

Lithologically, the spring catchment area consists of distal limestone and proximal marly limestone relative to the spring's location. The latter is less karstified than the limestone portion and provides a groundwater flow regulator, limiting the discharge of the spring. The presence of this heterogeneity and the elongated shape of the aquifer cause the aquifer to store water for long periods and discharge water continuously and smoothly during the arid season. Consequently, in spite of a clear definition of karstification degree, we need to explore a comprehensive quantitive method to evaluate the karstification degree in geomorphologic, hydraulic, and engineering aspects. The main limit of this work has been the absence of a long period of discharge and physicochemical records of the spring with high temporal resolution. Ideally, these data would be available at least at a weekly interval for a minimum of two years. These data could provide a link between precipitation variability and fluctuations, as well as flow paths and spring response to external pulses.

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K.B. Kunt, R.S. Özkütük, M. Elverici, Y.M. Marusik, and G. Karakaş – *Harpactea karaschkhan* sp. n., a new cave-dwelling blind spider species from the Mediterranean Region of Turkey. *Journal of Cave and Karst Studies,* v. 78, no. 1, p. 36–40. DOI: 10.4311/2015LSC0106

HARPACTEA KARASCHKHAN SP. N., A NEW CAVE-DWELLING BLIND SPIDER SPECIES FROM THE MEDITERRANEAN REGION OF TURKEY

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Abstract: A new troglobitic species, *Harpactea karaschkhan* sp. n. (females only), inhabiting Yalandünya Mağarası of Gazipaşa (Antalya Province, Turkey) is described. Detailed morphological description and illustrations of the new species are provided. The relationships of the new species are discussed.

Introduction

Obligatory cave-dwelling organisms (troglobionts) have attracted attention of the scientific community for almost two centuries. Evolution of troglomorphic traits, such as loss of eyes and pigment, or gains, such as elongated appendages and increases in number or functionality of nonvisual sense organs, often obscures taxonomic relationships due to convergence. On the other hand, narrow distribution ranges and high endemism make troglobionts important subjects of biogeographic studies (Porter, 2007).

In spiders, troglomorphy usually results in loss of eyes and depigmentation. Such species are rather common, although their number varies within family or genus. One of the richest groups in troglobionts is the subfamily Harpacteinae in family Dysderidae.

Among Harpacteinae, reduction of eyes is common in the genera *Folkia* Kratochvíl, 1970 and *Stalagtia* Kratochvíl, 1970; eyes are absent in the monotypic genus *Sardostalita* Gasparo, 1999; and eyes are reduced in *Minotauria attemsi* Kulczyński, 1903 and completely absent in *M. fagei* (Kratochvíl, 1970). With only a few exceptions, species in the genus *Harpactea* Bristowe, 1939 possess fully developed eyes. Eyes are strongly reduced in *H. persephone* Gasparo, 2011 (Kournas Cave, Chania Prefecture, Crete, Greece), and posterior median eyes are absent in *H. sanctidomini* Gasparo, 1997 (Tremiti Islands, Italy). Eyes are completely lost in *Harpactea* only in two known cases, *H. stalitoides* Ribera, 1993 (Iberian Peninsula) and *H. strinatii* Brignoli, 1979 (Diros Caves, Peloponnese, Greece).

The third known case of a completely blind *Harpactea* is the new species *Harpactea karaschkhan* sp. n., described here from Turkey. Our description is based on females; the male is unknown. Illustrations of taxonomically important body parts and female reproductive organs are provided.

Material and Methods

Studied material was collected from the type locality, on the walls of the cave by using a hand aspirator. The specimens were preserved in 70% ethanol and deposited in the Anadolu University Zoology Museum. Digital images of copulatory organs were taken with a Leica DFC295 digital camera attached to a Leica S8AP0 stereomicroscope; five to fifteen photographs were taken in different focal planes and combined using Automontage software. All measurements are in mm, with methods following Chatzaki and Arnedo (2006). Terminology for the copulatory organs is adapted from Deeleman-Reinhold (1993).

The following abbreviations are used in the description: Dimensions of carapace and abdomen, AL, abdominal length; CL, carapace length, CWmax, maximum carapace width; CWmin, minimum carapace width. Chelicera: ChF, length of cheliceral fang; ChG, length of cheliceral groove; ChL, total length of chelicera (lateral external view). Depository: AUZM, Anadolu University Zoology Museum, Eskişehir, Turkey.

Taxonomy

Harpactea Bristowe, 1939

Harpactea karaschkhan sp, n.

Material examined. Holotype ♀ (AUZM), Turkey, Antalya Province, Gazipaşa District, Beyrebucak Village, cave Yalandünya Mağarası (36°13′9.77″N; 32°24′16.64″E), 05 January 2013, K.B. Kunt leg. Paratypes 1 ♀, 1 juvenile (AUZM), same locality and date as holotype, Y.M. Marusik leg.

Diagnosis: *Harpactea karaschkhan* sp. n. can be easily distinguished from other species of *Harpactea* found in Turkey and elsewhere by its troglomorphic characters and presence of retrolateral spines on anterior femora, normally absent in this genus. While the new species is similar to the Cretan endemic *Harpactea persephone* in the

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Table 1. Leg measurements of *Harpactea karaschkhan* sp. n. (Holotype ♀ / Paratype ♀).

Leg	Fe	Pa	Ti	Me	Ta	Total
I	1.60 / 1.64	1.00 / 1.08	1.20 / 1.48	1.12 / 1.36	0.40 / 0.50	5.32 / 6.06
II	1.50 / 1.60	0.83 / 1.04	1.25 / 1.32	1.00 / 1.16	0.38 / 0.38	4.96 / 5.50
III	1.20 / 1.40	0.63 / 0.72	0.95 / 1.20	1.15 / 1.28	0.42 / 0.44	4.35 / 5.04
IV	1.60 / 1.80	0.80 / 0.92	1.36 / 1.60	1.78 / 1.96	0.43 / 0.55	5.97 / 6.83

Fe = femur, Pa = patella, Ti = tibia, Me = metatarsus, and Ta = tarsus.

mentioned diagnostic characters, the vulva of *H. karaschkhan* sp. n. is unique.

Derivatio nominis: Karaş Han is God of Darkness in Turkish and Altaic mythology.

Measurements [Holotype ♀ / Paratype ♀]: AL 2.00 / 2.40; CL 1.83 / 2.04; CWmax 1.30 / 1.40; CWmin 0.80 / 0.88; ChF 0.44 / 0.51; ChG 0.33 / 0.35; ChL 0.81 / 0.90. Leg measurements are given in Table 1.

Description of holotype female: Small-sized spider. Carapace yellow-light brown, darker in the cephalic region, smooth, covered with sparsely distributed brownish hairs, longer and directed forward in the cephalic region. Fovea indistinct. Eyes absent (Figs. 1a,b). Labium, gnathocoxae, and chelicerae reddish-brown. Sternum dirty yellow, brownish at edges. Sternum, labium, gnathocoxae, and chelicerae covered with brownish hairs of similar type and density (Fig. 1c). Cheliceral groove with four teeth, retromarginal teeth smaller than promarginal teeth. Among retromarginal teeth, the proximal tooth is conical and located a bit distally compared to the interspace between the promarginal teeth; the distal tooth is triangular, larger than the other and located at the middle of the cheliceral groove (Fig. 1d). Abdomen yellowish, cylindrical, covered with fine brown hairs.

Legs same color as abdomen. All legs with brownish discoloration at articular points of trochanter with coxa and femur. Tarsus with three tarsal claws. Numbers of denticles on tarsal claws: over 10 on legs I and II; 2 on leg III; absent on leg IV. Scopulae weakly developed, almost absent on tarsus and metatarsus of legs III and IV. Anterior femora with numerous prolateral and dorsal spines (Figs. 1e,f) and one to three prominent retrolateral spines; posterior femora with many spines; one prolateral spine on patella II; four spines on the dorsal side of patellae III and IV; three or four ventral and prolateral spines on tibia II; posterior tibiae and metetarsi with many spines; one dorsal spine on coxa III. Leg formula 4123. Details of leg spination are given in Table 2.

Vulva: Vulva almost equally sclerotized on the entire surface. Distal crest slightly longer than a rod-shaped part of spermatheca, expanding towards apical with comparatively less sclerotized apex (Figs 2a,b). Rod-shaped part of spermatheca short and thick. Distal expansion of the spermateca circular. Basal transverse part of the anterior spermatheca narrow and V-shaped. "Anterior basal arc" wing-shaped. Anterior sides more strongly sclerotized than other parts. Transverse bar relatively straight, thin. Membranous sac extremely wide; about three times longer than anterior spermatheca (Figs. 2c,d).

Distribution: *Harpactea karaschkhan* sp. n., is only known from its type locality.

Comments: New species belongs to the genus *Harpactea* based on the position of cheliceral teeth and structure of the vulva. According to the classification of Deeleman-Reinhold (1993), the new species belongs to the *rubicunda* (D) group by having a wide membranous sac on the vulva and spines on coxa III and patella III. Presence of spines on the patellae and the tibiae of the second pair of legs and presence of retrolateral spines on the anterior femora are very unusual characters for the genus *Harpactea*.

Biospeleological notes: Yalandünya cave is located at the foothills of the Taurus Mountains. The cave is formed in bluish-gray Paleozoic (Permian) limestone. It is a 270 m long cave, open for tourists, which starts with a 50 m long corridor sloping from south to north. Following the entrance of the cave at the start of the corridor, the following spiders were observed: numerous *Loxosceles rufescens* (Dufour, 1820) (Sicariidae), several *Heteropoda variegata* (Simon, 1874) (Sparassidae), and some exuviae of *Chaetopelma* sp. (Theraphosidae). Also, funnel webs of *Tegenaria* sp. (Agelenidae) were seen along the corridor between or beneath large rocks. At the end of the corridor, a wide chamber continues eastward. Bat colonies were observed in this chamber [three species, according to Benda and Horáček (1998): *Eptesicus bottae* Peters, 1869; *Pipistrellus savii* (Bonaparte, 1837), and *Plecotus austriacus* (Fischer, 1829)], together with a dense population of a cricket *Ovaliptila beroni* (Popov, 1975) (Orthoptera: Gryllidae) on the cave floor. Terrestrial isopods were observed on the extensively damp walls of the chamber and also under rocks on the ground, together with troglomorphic silverfish *Coletinia* sp. (Zygentoma: Nicoletiidae). The cave continues northward with an initially narrow corridor. At the entrance of this corridor, webs of *Cataleptoneta* sp. (Araneae: Leptonetidae) were present on stone walls. One adult male of *Charinus ioanniticus* (Kritscher, 1959) (Amblypygi: Charinidae) was also collected in this corridor between large rocks on the ground on January 5, 2013. This corridor is followed by a second, terminal chamber, not as wide as the previous one.

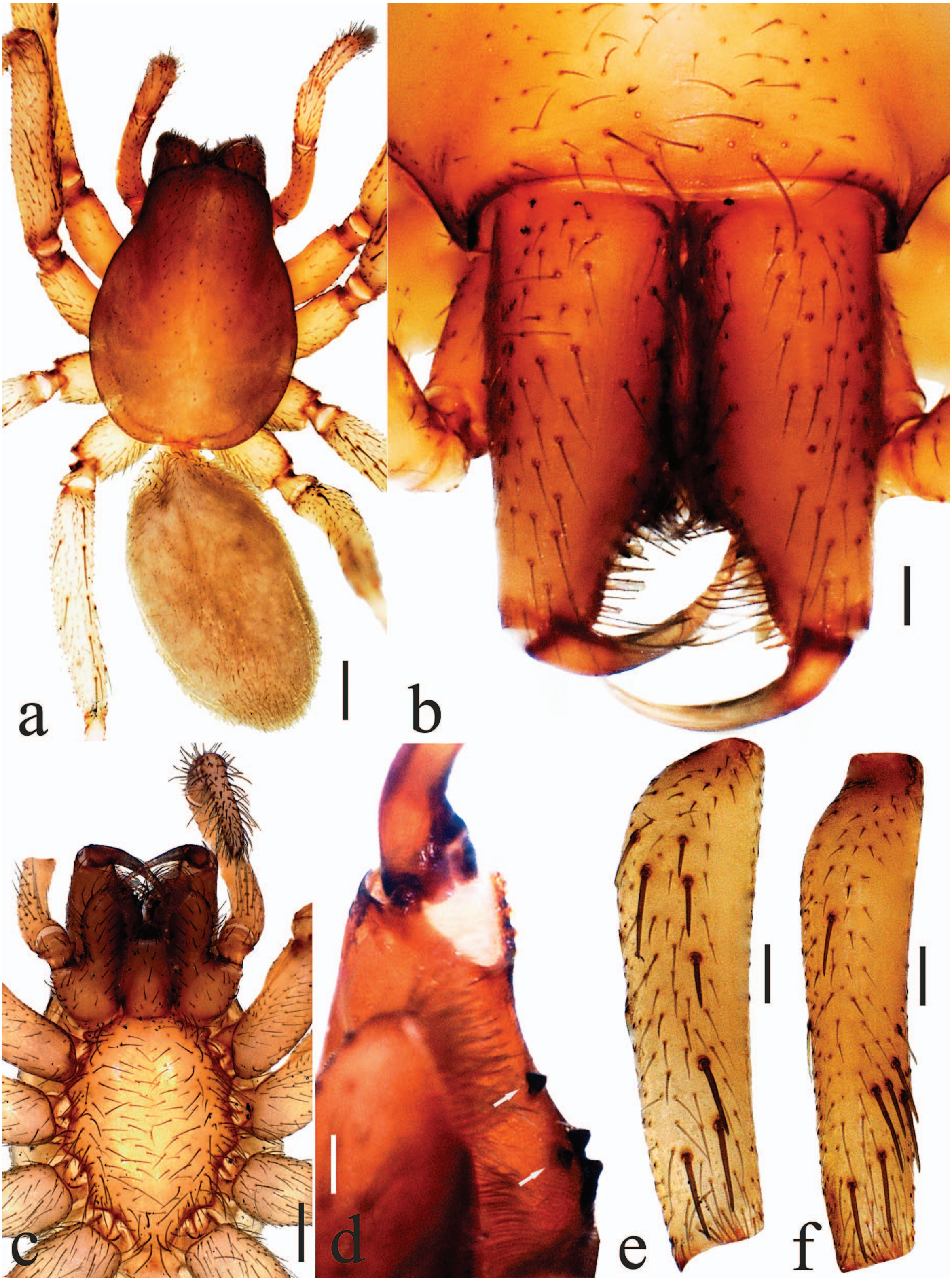

Figure 1. *Harpactea karaschkhan* sp. n. a. General habitus, dorsal view; b. Cephalic region of carapace and chelicera, anterior view; c. Sternum and mouthparts, ventral view; d. Cheliceral teeth, posterior-ventral view (arrows indicate the retromarginal teeth); e. Femur I, prolateral view; f. Femur II, prolateral view. Scale lines: a. 0.5 mm; b. and c. 0.4 mm; d. 0.1mm; and e. and f. 0.25 mm.

Table 2. Leg spination of *Harpactea karaschkhan* sp. n.

♀	Leg I	Leg II	Leg III	Leg IV
Cx	0	0	0-1 d	0
Fe	6-9 pl 2 d 1-2 rl	5-6 pl 3-4 d 2-3 rl	2-4 pl 7-8 d 3 rl	3 pl 6 d 3 rl
Pa	0	0-1 pl	1 pl 2 d 1 rl	1 pl 2 d 1 rl
Ti	0	1-2 pl 2 v	2-3 pl 1 d 2-3 rl 4 v	3 pl 1 d 2 rl 5 v
Me	0	0	2 pl 3 rl 6 v	3 pl 3 rl 5 v

Cx = coxa, Fe = femur, Pa = patella, Ti = tibia, and Me = metatarsus

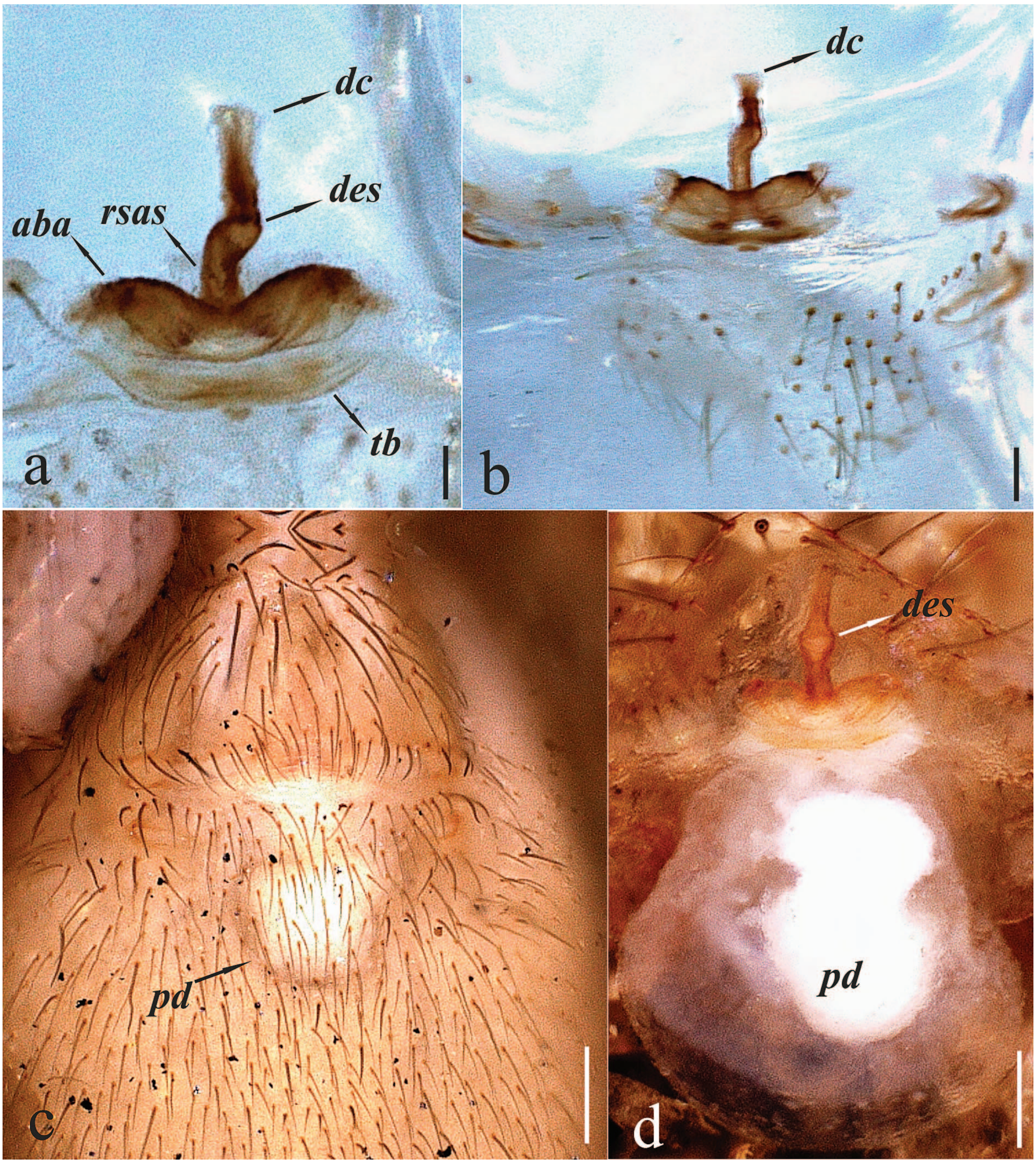

Figure 2. *Harpactea karaschkhan* sp. n. a,b. Vulva, dorsal view c,d. Vulva, ventral view *aba*, anterior basal arc; *dc*, distal crest; *des*, distal expansion of the spermatheca; *pd*, posterior diverticulum; *rsas*, rod-shaped part of the anterior spermatheca; *tb*, transverse bar. Scale lines: a. 0.05 mm; b. 0.1 mm; c. 0.2 mm; and d. 0.25 mm.

This terminal chamber is completely dark, while the rest of the cave is illuminated for tourists, and apparently more humid, with cave mud on the floor and several large columns inside. The specimens of *karaschkhan* sp. n. were collected as they were actively wandering on these columns.

Acknowledgements

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FUNGI ISOLATED AND QUANTIFIED FROM BAT GUANO AND AIR IN HARMANECKÁ AND DRINY CAVES (SLOVAKIA)

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Abstract: This study is the first mycological evaluation of bat guano and the air around it in Harmanecká and Driny Caves in Slovakia. These caves are the most important underground localities of bats in Slovakia. Samples were collected in July 2014 and cultivated for fungi. Harmanecká Cave had seven species of filamentous fungi and one yeast-like fungus isolated from bat guano, compared to six species of filamentous fungi in guano from Driny Cave. Air samples from Harmanecká Cave had twelve species of fungi, compared to nine species from Driny Cave. Fungal density was higher in guano from Driny Cave (4720.1 CFU/g guano) than from Harmanecká Cave (3498.3 CFU/g). The pattern was reversed with fungi from the air. Fungal density in air from Harmanecká Cave (211.3 CFU/m^3) was higher than that from Driny Cave (175.7 CFU/m^3). *Penicillium granulatum* was the most frequently isolated fungal species, except in the guano of Driny Cave, where *Mucor hiemalis* was most common. Bat guano is a very good substrate for the development and survival of fungi in the caves, and it can be a reservoir of fungi harmful for bats. However, air samples from both caves contained more species of fungi than the bat guano, because the majority of fungi are transferred to underground ecosystems with air bioaerosols from the external environment.

Introduction

Cave ecosystems and other underground habitats are characterized by low temperatures and low availability of nutrients (Ogórek et al., 2013). Bat guano is rich in carbon, nitrogen, and vital minerals, so it is used in agriculture for fertilization of plants (Shetty et al., 2013). Bat guano, plant debris, animal carcasses, and other organic debris are the most important substrates for fungi inside caves (Nieves-Rivera et al., 2009).

Fungal conidia or spores are commonly found in caves and other underground environments. These elements enter mainly with air currents from the external environment (Pusz et al., 2014; Ogórek et al., 2014a, 2014b, 2014c). They can also be carried into caves by water, by animals such as bats and arthropods, or by humans visiting underground spaces (Mulec, 2008; Chelius et al., 2009; Vanderwolf et al., 2013; Griffin et al., 2014). The cave mycobiota are very important for underground ecology, because the fungi present are decomposers or parasites and probably constitute the major food source for other organisms (Sustr et al., 2005; Walochnik and Mulec, 2009; Bastian et al., 2010). Evidence for microbial activity in a cave includes spots on the cave surfaces, unusual coloration of speleothems, precipitates, corrosion residues, structural changes, and the presence of biofilms (Barton, 2006).

Bats are mammals that lead nocturnal lives and play important roles in the ecosystem such as plant pollination, seed dissemination, forest regeneration, and insect control, so their health and factors that influence it should be of high concern. Investigating fungi in guano is appealing, because guano is found in the immediate neighborhood of bat roosts and is likely to be highly exposed to bat pathogens. Bat pathogens probably won't grow on guano, but can land on it. Moreover, sampling of guano is non-invasive, in contrast to direct examination of bats, and may be conducted at a time when bats are absent from their hibernacula (Mulec, 2008; Nováková, 2009).

Harmanecká and Driny Caves rank among the most important underground localities for bats in Slovakia. The dominant species in Harmanecká Cave are the greater mouse-eared bat (*Myotis myotis*) and the lesser mouse-eared bat (*M. blythii*), with 1000 to 1500 hibernating individuals. The dominant species in Driny Cave is the lesser horseshoe bat (*Rhinolophus hipposideros*), with 100 to 150 individuals (Lehotská and Lehotský, 2009).

Our research focused on two goals, the mycological analysis of the species composition of the fungi found in

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the guano of bats and airborne fungi around it in the Harmanecká and Driny Caves and the quantification of their concentrations.

Materials and Methods

Harmanecká Cave is located in the Harmanec Valley to the northwest of Banská Bystrica city, in the southern part of the Great Fatra Mountains. Geographic coordinates of the cave are 8°81′39″ N, 19°04′01″ E. Its entrance is situated on the northern side of Kotolnica Hill at 821 m a.s.l. Its length is 3123 m, and the length of the tourist path is 1020 m. The cave was discovered in 1932 by Bacúrik, and it was opened to the public in 1950. During World War II, the cave served as a shelter for local people (Bella et al., 2001; Lehotská et al., 2011). The air temperature in the cave is between 5.8 and 6.4 °C, and the relative humidity is between 94 and 97% (Bella et al., 2001). Driny Cave is located in the Smolenice Karst in the Lesser Carpathian Mountains, south-west from Smolenice, in the Trnava district and near the recreation resort Jahodník. Geographic coordinates of the cave are 48°50′04″ N, 17°40′20″ E. Its entrance is situated on the western slope of Driny Hill at 399 m a.s.l. and its length is 680 m. The cave was discovered in 1929 by Vajsábel and Banič, and it was opened to the public in 1935 with provisional electric lighting for 175 meters. Now the length of the tourist path is 410 m (Bella et al., 2001; Lehotská et al., 2011). The air temperature in the cave is between 7.1 and 7.8 °C, and the relative humidity is between 92 and 97% (Bella et al., 2001). In 2014, Harmanecká Cave was visited by 17,425 people, and Driny Cave was visited by 31,859 people.

The samples were taken on July 24, 2014 from Harmanecká Cave and on July 25, 2014 from Driny Cave, before tourists arrived. Bat guano, not fresh, was found only in a single location in Harmanecká Cave, in the Entrance Dome in front of an airlock door, and also only in a single location in Driny Cave, about 140 m into the cave in the Slovak Speleological Society Hall (Fig. 1). The guano samples were collected using sterile forceps and placed into sterile sampling bags (three samples per cave, about 8 g of guano per sample). The samples were transported in cool conditions (ca. 7 °C) to the laboratory and were stored there in the cold room. Mycological evaluation of the samples was carried out within three days from the date of sampling. The air samples were collected using an Air Ideal 3P sampler and Potato Dextrose Agar (PDA) medium. It was programmed for air sample volumes of 50 L, 100 L, and 150 L. Measurements in several locations around each guano pile were performed in three replicates for each volume. The sampler was positioned about 1.5 m from the bat guano.

Potato Dextrose Agar (PDA, Biocorp) was used for the isolation of fungi from the guano and from the air and for the identification of some species. Czapek-Dox Agar (1.2% agar, Biocorp) and Malt Extract Agar (MEA, Biocorp) were used for the identification of species belonging to the *Penicillium* and *Aspergillus* genera. Sabouraud Agar (4% dextrose, 2% agar, 1% peptone, A&A Biotechnology) medium was used for identification of yeast-like fungi.

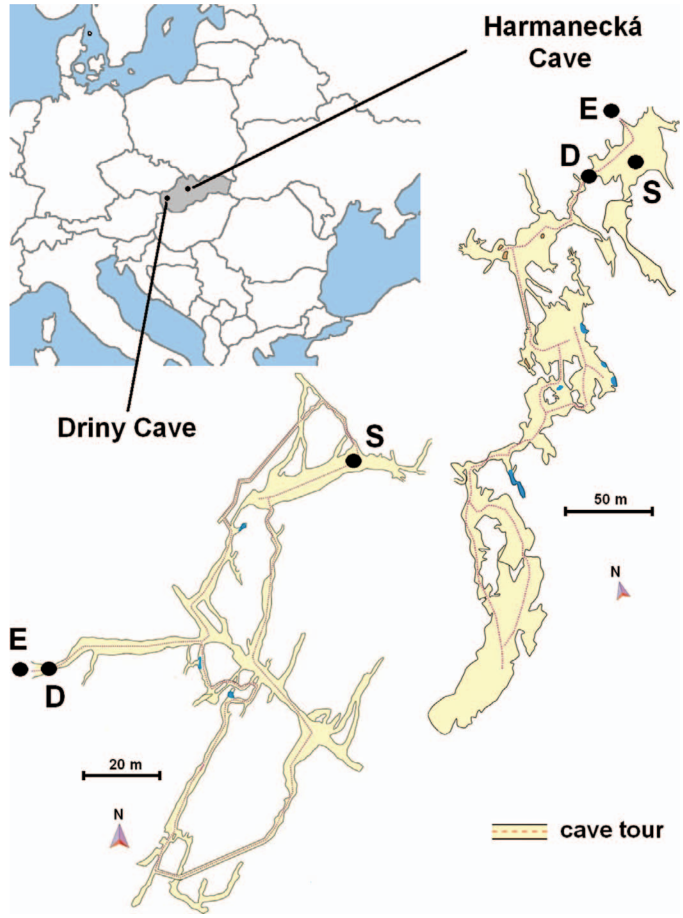

Figure 1. Geographic location of the caves and map of the tourist routes: E – entrance and exit of the cave; D – airlock door; S – sampling locations (Harmanecká Cave, before the airlock door in the Entrance Dome; Driny Cave, about 140 m into the cave, in the Slovak Speleological Society Hall).

One gram of guano (in three replicates) taken from each sample was shaken for 20 minutes in a 250-mL Erlenmeyer flask containing 9 mL of sterile distilled water. After shaking, samples were spread-plated using serial dilution onto PDA. Incubation was carried out at 15, 20, and 25 °C for 4 to 14 days in the dark. After incubation, the number of colony-forming units (CFU) per 1 g of guano was calculated as averages from the replicates at all incubation temperatures.

Similarly, the incubation of cultures from the air samples on PDA was carried out at 15, 20, and 25 °C for 4 to 14 days in the dark. After incubation, the number of CFU per m^3 of air was calculated as averages from the replicates at all incubation temperatures.

After incubation, the fungal colonies grown were counted and identified. The species identification was

Table 1. Filamentous and yeast-like fungi cultured from the bat guano and the air around it in the Harmanecká and Driny Caves. A + indicates that the species was found.

	Harmanecká		Driny	
Fungi Species	Guano	Air	Guano	Air
Absidia glauca Hagem	+	+		
Alternaria alternata (Fr.) Keissl.				+
Aspergillus foetidus Thom & Raper	+	+	+	
Aspergillus fumigatus Fresen.			+	+
Botrytis cinerea Pers. ex. Fr.		+		
Cladosporium herbarum (Pers.) Link		+		+
Epicoccum nigrum Link		+		+
Gliocladium roseum Bainier		+		
Mucor hiemalis Wehmer	+	+	+	+
Penicillium chrysogenum Thom	+	+	+	+
Penicillium granulatum Rainier	+	+	+	+
Penicillium lanosocoeruleum Thom		+		
Penicillium roseopurpureum Dierckx	+			
Penicillium urticae Bainier		+		+
Phoma fimeti Brunaud				+
Rhizopus stolonifer (Ehrenb.) Vuill.	+	+	+	
Rhodotorula glutinis (Fresen.) F.C. Harrison	+			
$\sum$ species	8	12	6	9

performed using macro- and microscopic observations, namely the morphology of hyphae, conidia, or spores and the colonies that had grown on culture media. The filamentous fungi were identified using diagnostic keys and descriptions by Pitt and Hocking (2009) and Watanabe (2010). The yeast-like fungi were identified using the diagnostic key and descriptions by Kurtzman and Fell (1998).

Results

The bat guano and the air around it in Harmanecká Cave contained more species of fungi than the samples from Driny Cave. Seven species of filamentous fungi and one of the yeast-like group of fungi were isolated from the guano of Harmanecká Cave and six species of filamentous fungi from Driny Cave. Air samples from Harmanecká Cave grew twelve species of filamentous fungi, and there were nine species from Driny Cave air. *Penicillium roseopurpureum* and *Rhodotorula glutinis* were isolated only from the guano sampled, whereas *Alternaria alternata*, *Botrytis cinerea*, *Cladosporium herbarum*, *Epicoccum nigrum*, *Gliocladium roseum*, *Penicillium lanosocoeruleum*, *Penicillium urticae*, and *Phoma fimeti* were found exclusively in the samples of air (Table 1).

The largest group of filamentous fungi in both caves constituted fungi of the genus *Penicillium*, whereas the yeast-like fungi were cultured only from guano sampled from Harmanecká Cave. The concentration of all fungi isolated from bat guano was 3498.3 colony-forming units per 1 g of guano from Harmanecká Cave and 4720.1 per 1 g of guano from Driny Cave. Air samples respectively grew 211.3 and 175.7 CFU per 1 m^3 of air (Tables 2 and 3).

Of the colonies cultured from guano, 75% from Harmanecká Cave were *Penicillium granulatum* and 54% from Driny Cave were *Mucor hiemalis* (Figs. 2 and 3). Fungal species such as *Absidia glauca*, *P. roseopurpureum* and *R. glutinis* were isolated only from the guano collected in Harmanecká Cave and *Aspergillus fumigatus* only from Driny Cave (Table 1).

The fungus most frequently cultured from the air was *P. granulatum* (Fig. 4) in both caves, and it constituted approximately 45% of fungi (Table 3, Figs. 2 and 3). *A. alternate*, *A. fumigatus* and *P. fimeti* were present only in the air of Driny Cave, whereas *A. glauca*, *Aspergillus foetidus*, *B. cinerea*, *G. roseum*, *P. lanosocoeruleum*, and *Rhizopus stolonifer* were found exclusively in the air of Harmanecká Cave (Table 1).

The air samples in both the caves contain both quantitatively and qualitatively more propagules of fungi than the bat guano. *P. granulatum* was the most frequently isolated fungus with the exception of the guano collected in Driny Cave, where *M. hiemalis* was the most common (Fig. 2 and 3).

Discussion

The most important factors affecting the survival of fungi in the environment are temperature and humidity. However, underground environments are characterized by a very specific, stable microclimate; therefore the most important factors that determine occurrence of fungi in

Table 2. Mean concentrations of filamentous and yeast-like fungi cultured from the bat guano collected in the Harmanecká and Driny Caves

		Guano (CFU/g)		
		Individual Species		
Cave	Fungi Species	Range of Values	Mean ± SD[a]	Total
Harmanecká				3498.3
	Absidia glauca	71.0 – 185.0	133.2 ± 44.3	
	Aspergillus foetidus	45.0 – 135.0	94.7 ± 34.4	
	Mucor hiemalis	133.0 – 297.0	225.8 ± 56.1	
	Penicillium chrysogenum	103.0 – 244.0	180.5 ± 56.1	
	Penicillium granulatum	1521.0 – 3810.0	2644.0 ± 879.3	
	Penicillium roseopurpureum	42.0 – 115.0	79.8 ± 26.8	
	Rhizopus stolonifer	60.0 – 181.0	120.3 ± 44.8	
	Rhodotorula glutinis	2.0 – 40.0	20.0 ± 14.2	
Driny				4720.1
	Aspergillus foetidus	14.0 – 40.0	25.0 ± 10.1	
	Aspergillus fumigatus	21.0 – 74.0	50.3 ± 20.9	
	Mucor hiemalis	1500.0 – 3800.0	2550.1 ± 869.7	
	Penicillium chrysogenum	146.0 – 339.0	249.4 ± 78.2	
	Penicillium granulatum	1026.0 – 2679.0	1825.0 ± 598.0	
	Rhizopus stolonifer	8.0 – 34.0	20.3 ± 9.6	

[a] SD = Standard Deviation.

them are airflow, the conditions prevailing in the neighboring external environment, such as the season, local flora, the geographical location, and especially the availability of organic matter (Ogórek et al. 2013, 2014a, 2014b; Pusz et al. 2014; Vanderwolf et al. 2013). In our study, the most species of fungi were isolated from Harmanecká Cave. The situation is probably because the samples were taken in Harmanecká Cave between the entrance and the air-lock door and in Driny Cave behind the door, so the samples in Harmanecká Cave were directly exposed to airborne fungi from the surrounding external environment.

The traditional assessment method for fungi based on the calculation of colony-forming units and microscopic analysis, which is more common and cheaper than molecular biological methods, gives the ability to identify colonies to the species level, and a huge reference database is available for the proper identification of strains (Pasanen 2001; Rastogi and Sani, 2011). However, several disadvantages of CFU analysis are also apparent in comparison to molecular methods. CFU analysis does not allow us to detect non-culturable fungi, and also it can overlook fungal species that are not easily culturable. Furthermore, it might underrepresent those fungal types that grow slowly because they are overtaken by faster growing colonies (MacNeil et al., 1995; Wu et al., 2000; Macher, 2001; Pasanen, 2001). In addition, the type of culture medium and temperature of incubation have an influence on the in vitro growth rate of fungi isolated from the environment, their species composition, and their concentrations (Kaufman et al., 1963; Marshall et al., 1998; Meletiadis et al., 2001; Ogórek et al., 2011a, 2011b). A variety of molecular methods based on direct isolation and analysis of nucleic acids or proteins from environmental samples could be a very good alternative to standard culture techniques for in-depth characterization of environmental microbial communities (Rastogi and Sani, 2011).

Littman (1947) reported that Sabouraud Agar medium is the most suitable for isolation of a large spectrum of fungal species from the environment. According to Ogórek et al. (2011a, 2011b), Potato Dextrose Agar medium demonstrates comparable efficacy. Therefore we used this medium for isolation of fungi from the guano and the air samples. Fungal response to temperature is quite varied. Active growth will usually be associated with a limited range of temperatures. However, many fungi remain alive for extended periods at temperatures unsuitable for growth (Smith, 1988). Generally, most fungi grow well at room temperature ranging from 20 to 25 °C, but, for example, the optimal temperatures for growth of *Geomyces destructans* (now *Pseudogymnoascus destructans*) is between 12.5 and 15.8 °C, and the upper critical temperature for growth is between 19.0 and 19.8 °C (Kaufman et al., 1963; Verant et al., 2012). This fungus causes white-nose syndrome in bats (Gargas et al., 2009; Minnis and Lindner, 2013). This is a widespread, epizootic disease affecting hibernating bats, and this disease is associated, inter alia, with an unprecedented bat mortality in the United States and Canada (Blehert et al., 2009). Therefore, we took into account incubation at different temperatures to obtain a wide spectrum of species of fungi.

Table 3. Mean concentrations of filamentous fungi cultured from the air around the bat guano collected in the Harmanecká and Driny Caves.

		Air (CFU/m^3)		
		Individual Species		
Cave	Fungi Species	Range of Values	Mean ± SD[a]	Total
Harmanecká				211.3
	Absidia glauca	4.0 – 10.0	6.7 ± 2.2	
	Aspergillus foetidus	1.0 – 3.0	2.1 ± 0.8	
	Botrytis cinerea	7.0 – 16.0	11.8 ± 3.0	
	Cladosporium herbarum	8.0 – 19.0	13.1 ± 3.6	
	Epicoccum nigrum	0.0 – 3.0	1.8 ± 1.1	
	Gliocladium roseum	1.0 – 3.0	2.1 ± 0.9	
	Mucor hiemalis	7.0 – 33.0	20.1 ± 9.5	
	Penicillium chrysogenum	9.0 – 26.0	18.3 ± 6.2	
	Penicillium granulatum	49.0 – 139.0	96.2 ± 33.4	
	Penicillium lanosocoeruleum	5.0 – 12.0	8.9 ± 2.1	
	Penicillium urticae	7.0 – 17.0	11.5 ± 3.5	
	Rhizopus stolonifer	9.0 – 27.0	18.7 ± 6.5	
Driny				175.7
	Alternaria alternata	1.0 – 3.0	1.9 ± 0.9	
	Aspergillus fumigatus	4.0 – 10.0	6.8 ± 2.0	
	Cladosporium herbarum	7.0 – 13.0	10.1 ± 1.9	
	Epicoccum nigrum	2.0 – 9.0	5.2 ± 2.7	
	Mucor hiemalis	29.0 – 88.0	59.1 ± 23.1	
	Penicillium chrysogenum	7.0 – 12.0	8.9 ± 1.9	
	Penicillium granulatum	44.0 – 115.0	82.2 ± 28.7	
	Penicillium urticae	0.0 – 3.0	1.3 ± 1.3	
	Phoma fimeti	0.0 – 1.0	0.2 ± 0.4	

[a] SD = Standard Deviation.

Bat guano is one of the most important energy inputs for caves in temperate climate zones for survival and development of fungi (Poulson and Lavoie, 2000). Our results agree with those previously described, because mostly the same species of fungi were isolated from both the guano and the air samples, especially *Penicillium granulatum*, which predominated (Fig. 4, Table 2).

We isolated more fungal species from the air than from the guano. This agrees with other reports that the most species of fungi were isolated from the air samples in underground sites (Ogórek et al., 2013, 2014a; Pusz et al., 2014). Fungi are transferred to mines, adits, and caves by airflow from the external environment, so the majority of fungal species are in the air. The overall mean concentrations of CFU found in the caves in the present study, 175.7 and 211.3 CFU/m^3, were similar to, or lower than, those observed by other studies inside underground enclosures or caves. For example, Ogórek et al. (2014c) isolated from 245.5 to 1040.3 CFU of fungi in 1 m^3 of air from the underground Rzeczka complex, and Pusz et al. (2014) collected from 92 to 259 CFU from the underground Osówka complex, while Ogórek et al. (2013) isolated a maximum of only 232 CFU from Niedźwiedzia Cave. The concentration of airborne fungi in the caves Harmanecká and Driny did not exceed official limits and norms for a health risk to tourists. According to the World Health Organization, air is not too contaminated unless it contains more than 1500 CFU of a mixture of species of fungi in 1 m^3 of air (World Health Organization, 1990).

According to Nováková (2009), the largest group of filamentous fungi isolated from bat guano constituted fungi of the genus *Penicillium*. We obtained similar results. In addition, we isolated filamentous fungi, such as *Absidia glauca*, *Aspergillus* spp., *Mucor hiemalis*, and *Rhizopus stolonifer* and one species from the yeast-like group, *Rhodotorula glutinis*. According to other researchers, bat guano is also a reservoir of fungi that may be dangerous for mammal health such as *Candida* spp., *Geomyces* spp., *Microsporum* spp. *Trichosporon* spp., and *Trichophyton* spp. (Nováková, 2009; Mulec et al., 2013). In our studies, we did not detect any of the above fungi in the bat guano. Especially important is the absence of detection of *Pseudogymnoascus destructans* (Gargas et al., 2009; Minnis and Lindner, 2013).

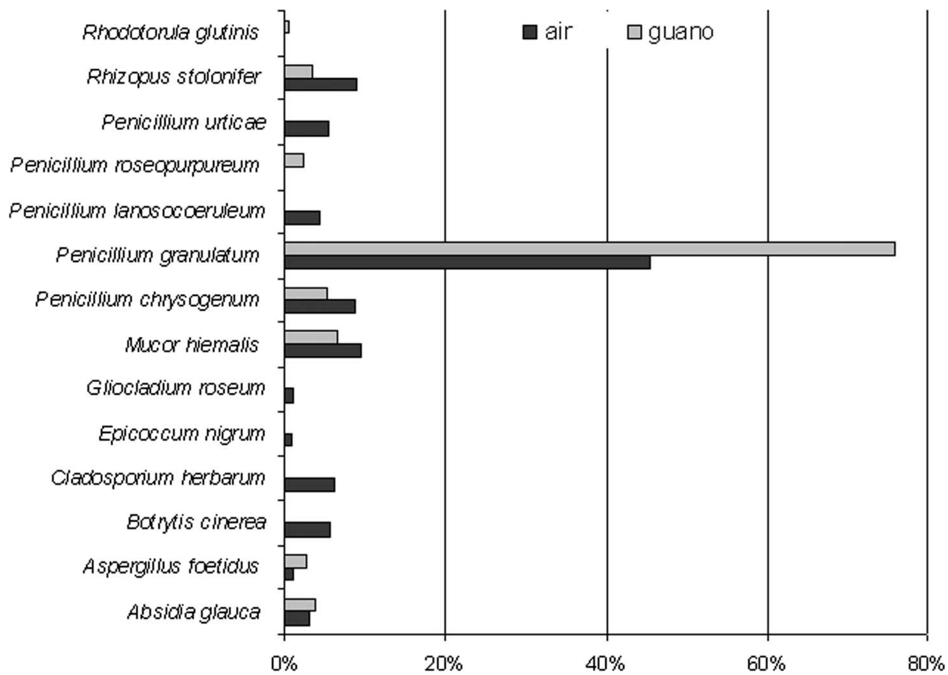

Figure 2. Relative percentages of colonies of filamentous fungi and yeast-like fungal species cultured from the bat guano and the air around it from Harmanecká Cave.

The most frequently isolated fungus from the guano and from the air collected in Harmanecká Cave and Driny Cave was *Penicillium granulatum*, with the exception of the guano collected in Driny Cave, where *Mucor hiemalis* was the most common. These species of fungi are common in many parts of the world. Their conidia and spores can be found in air, water, and soil and on animals and plants (Samson et al., 2011; Madden et al., 2012).

M. hiemalis may cause, for example, cutaneous and subcutaneous mucormycosis in humans belonging to the risk groups (Desai et al., 2013). *P. granulatum* may produce secondary metabolites, including mycotoxins, that are dangerous to animals and humans (Koteswara Rao et al., 2011). It can also cause allergic alveolitis, chronic respiratory symptoms, and sensitization by antigens from mold conidia (Qiu et al., 2014). Probably fungi such as

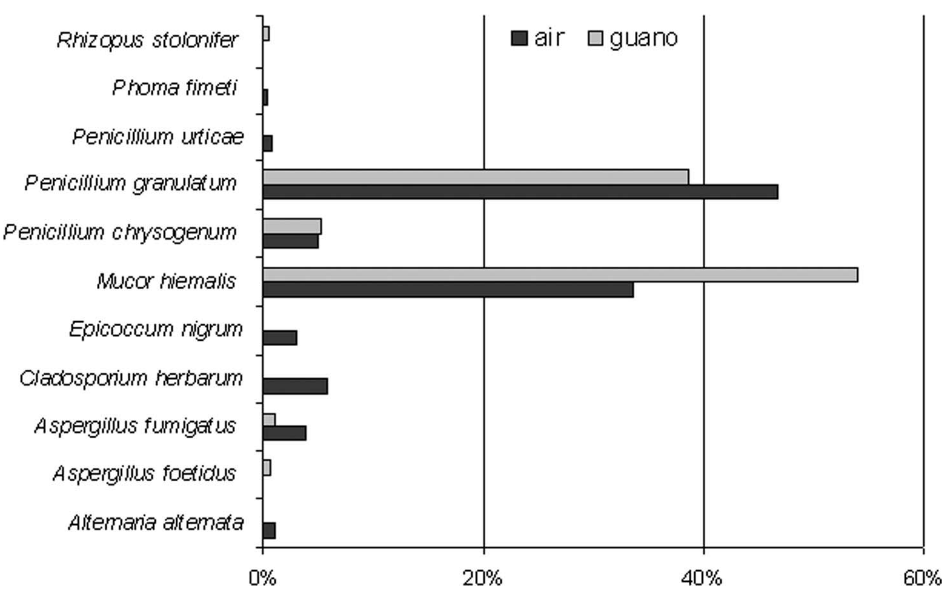

Figure 3. Relative percentages of colonies of filamentous fungal species cultured from the bat guano and the air around it in Driny Cave.

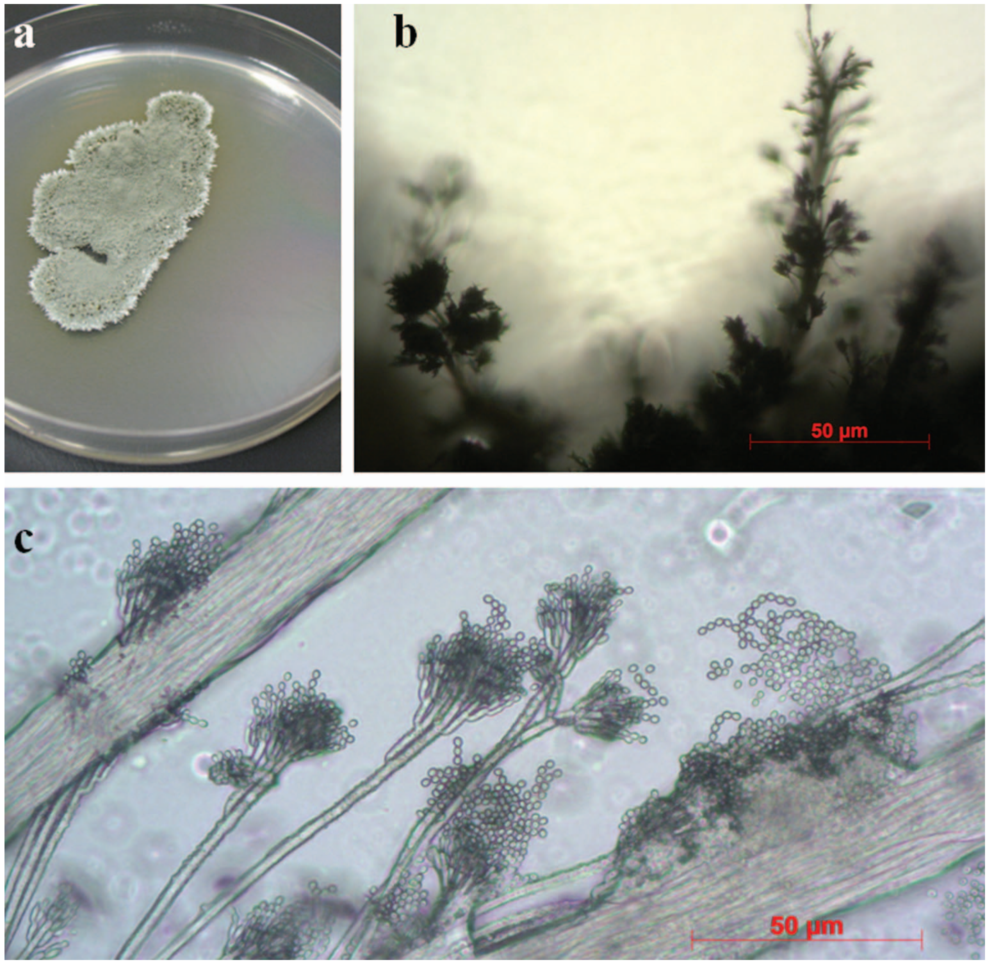

Figure 4. *Penicillium granulatum* culture on Potato Dextrose Agar medium, 10-day-old culture at 25 °C: a) top view of a colony; b) branched conidiophores under the optical microscope; c) branched conidiophores, phialides, and conidia under the optical microscope.

Penicillium spp. are also able to colonize membranes of hibernating bats. According to Johnson et al. (2013), fungi belonging to the *Penicillium* genus accounted for about 13% of all the strains isolated from hibernating bats. Nevertheless, the pathogenicity of species belonging to the *Penicillium* genus is controversial.

Conclusions

The results of the present study show that bat guano is a very good substrate for the development and survival of fungi inside caves, and it can also be a reservoir of fungi harmful to bats and humans. However, air samples from both caves contained more species of fungi than the bat guano, because the majority of fungi are transferred to underground ecosystems with bioaerosols from the external environment. Therefore, the external environment around an underground site and airflows are the major factors that affect the species composition and concentration of airborne fungi in indoor air of caves. The most frequently isolated species from the indoor and outdoor air and from the bat guano were fungi from the genus *Penicillium* (especially *P. granulatum*), except those isolated from guano of Driny Cave, where *Mucor hiemalis* was predominant. These fungi are common in many parts of the world. The observed concentrations and species composition of the fungi isolated from the air in both caves constitute no threat to the health of visiting tourists. We used only culture-based analysis of the fungal species, which did not allow us to detect non-culturable fungi. Therefore, in the near future, we are also going to use molecular techniques for in-depth characterization of environmental microbial communities in the case of underground mycobiota.

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