

Article

The Crystal-Wonder Cave System: A New Hotspot of Subterranean Biodiversity in the Southern Cumberland Plateau of South-Central Tennessee, USA

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Abstract: The Crystal-Wonder Cave System developed in the Western Escarpment of the southern Cumberland Plateau in the Interior Low Plateau karst region of south-central Tennessee, USA is a global hotspot of cave-limited biodiversity. We combined historical literature, museum accessions, and database occurrences with new observations from bio-inventory efforts conducted between 2005 and 2022 to compile an updated list of troglomorphic and stygobiotic biodiversity for the Crystal-Wonder Cave System. The list of cave-limited fauna includes 31 species (23 troglomorphic and 8 stygobiotic) with 28 and 18 species documented from the Crystal and Wonder caves, respectively, which represents five phyla, ten classes, nineteen orders, and twenty-six families (six arachnids, three springtails, two diplurans, three millipedes, six insects, three terrestrial snails, one flatworm, five crustaceans, and two vertebrates, respectively). The Crystal-Wonder Cave System is the type locality for six species—*Anillinus longiceps*, *Pseudanophthalmus humeralis*, *P. intermedius*, *Platylabus hatchi*, *Tolus appalachius*, and *Chitrella archeri*. The carabid beetle *Anillinus longiceps* is endemic to the Crystal-Wonder Cave System. Sixteen species are of conservation concern, including twelve taxa with NatureServe conservation ranks of G1–G3. The exceptional diversity of the Crystal-Wonder Cave System has been associated with several factors, including a high dispersal potential of cave fauna associated with expansive karst exposures along the Western Escarpment of the southern Cumberland Plateau, a high surface productivity, and a favorable climate throughout the Pleistocene.

Keywords: checklist; karst; species richness; stygobiont; troglomorphic

1. Introduction

The escarpments of the southern Cumberland Plateau of Tennessee, Alabama, and Georgia (TAG), USA in the Interior Low Plateau karst region contain thousands of caves that harbor an exceptional subterranean biodiversity [1–5]. The high density of cave systems and exposed karst in this region is a contributing factor for high levels of species richness and endemism in the southern Cumberland Plateau [1,2,6,7], which has been recognized as a global regional hotspot of subterranean biodiversity [1,2,7]. Several cave systems support a significant biodiversity at the local scale, including 19 caves with >12 cave-limited (i.e., permanent inhabitants of subterranean habitats) species documented [2,8], Niemiller

and Zigler, unpublished data]. In particular, two cave systems in the southern Cumberland Plateau region have been recognized as hotspots for cave-limited biodiversity [sensu 9]: Shelta Cave in Madison County, Alabama with 24 species [9], and Fern Cave in Jackson County, Alabama with 27 species, respectively [10]. Here, we add a third cave system—the Crystal-Wonder Cave System in Grundy County, Tennessee—that is the most speciose cave system with respect to the cave-limited fauna in the southern Cumberland Plateau.

1.1. Description of the Crystal-Wonder Cave System

The Crystal-Wonder Cave System in southwestern Grundy County, Tennessee, USA is located 6.4 km (4 mi) north of the former resort town Monteagle, at the base of Cedar Ridge on the north side of Layne Cove on the Western Escarpment of the Cumberland Plateau in the Upper Elk River watershed (Figure 1). The stream in the Wonder Cave (Tennessee Cave Survey no. TGD30), called the Mystic River, emerges from the main spring entrance, and then flows west for ca. 91 m (300 ft) before sinking into the insurgence entrance of the Wonder Natural Bridge Cave (TGD179) for ca. 46 m (150 ft), then resurging at the spring entrance of the Wonder Natural Bridge Cave, flowing on the surface for ca. 31 m (100 ft) before sinking into the insurgence entrance of the Crystal Cave (TGD10). In total, the Crystal-Wonder Cave System has ca. 5.74 km (18,828 ft) of passage, which includes 4474 m (14,678 ft) in the Wonder Cave, 46 m (150 ft) in the Wonder Natural Bridge Cave, and 1219 m (4000 ft) in the Crystal Cave, respectively.

The Wonder Cave is developed from the Mississippian-aged Monteagle Limestone. From the spring entrance (E1 black in Figure 1), the cave extends east for 2134 m (7000 ft). The Mystic River flows on the north side of passage; the passage averages 7.6 m (25 ft) wide and 2.4 m (8 ft) high for the first 335 m (1100 ft), respectively, and then enlarges to 13.7 m (45 ft) high with an extensive, well-decorated upper level with numerous dripstone formations. The upper level is 7.6 m (25 ft) above the Mystic River and ranges 4.5–6 m (15–20 ft) high, 9–24 m (30–80 ft) wide, and 107 m (350 ft) long, respectively with two main chambers—Statuary and Cathedral halls. At ca. 1402 m (4600 ft) from the entrance, the Mystic River flows from beneath a breakdown pile, marking the entry climb into the Pyramid Room, which is a dome chamber 37 m (120 ft) high and 61 m (200 ft) in diameter, respectively. Two passages continue from this room. One heads north for ca. 1500 m (5000 ft) in a dry upper-level avenue 6 m (20 ft) high that ranges 11–37 m (35–120 ft) wide. The Mystic River flows eastward from the Pyramid room for approximately 762 m (2500 ft) in passage that ranges 4–20 m (15–20 ft) high and 3–6 m (10–20 ft) wide, respectively, with several low air spaces before terminating the beneath breakdown.

The Crystal Cave represents a remnant of a lower portion of the Wonder Cave, which was also developed from the Monteagle Limestone. The Historic Section of the cave extends from the historic entrance (E1 white in Figure 1) for 229 m (750 ft) to the main stream passage. Steps were constructed at the entrance, and several areas in the historic section were excavated to provide access for visitors. Much of this section of the cave averages 1.5 m (5 ft) high and 3 m (10 ft) wide, respectively, with a small stream before lowering to a 0.6 m (2 ft) high crawl in water before connecting to the main stream passage. The main stream passage contains the same water that exits the Wonder Cave. From the junction of the historic section and main stream passage, the passage continues upstream following the Mystic River but gradually lowers in height, becoming a crawl in the stream and also over the breakdown toward the insurgence entrance (E4 white in Figure 1). Downstream of the junction, the main stream passage enlarges to 18 m (60 ft) wide and 2.4 m (8 ft) high, respectively, to a breakdown on the right leading to the quarry entrance (E3 white in Figure 1). The passage continues downstream past the quarry entrance to another junction, with the right passage continuing for ca. 91 m (300 ft) and the left continuing as a water-filled tube to the spring entrance (E2 white in Figure 1). The spring entrance is 8 m (26 ft) wide and 2.7 m (9 ft) high, respectively.

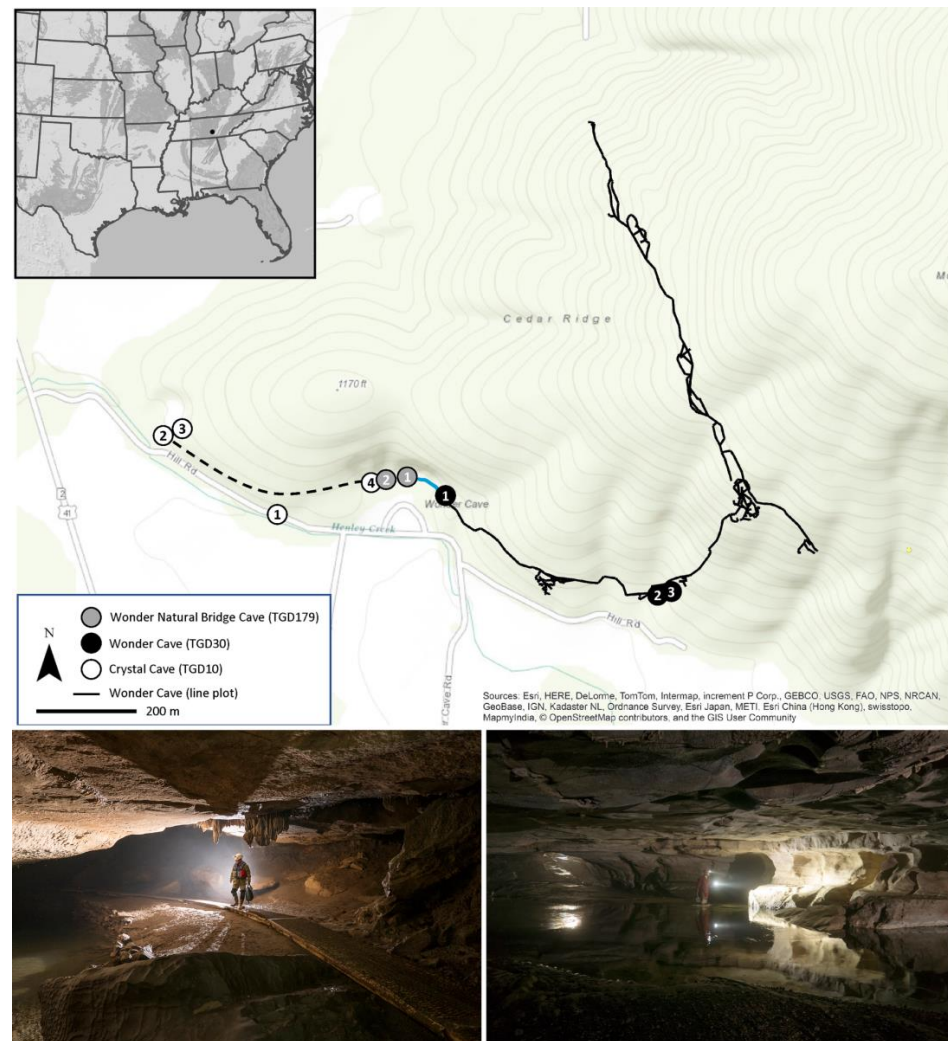


Figure 1. Line map and location of the Crystal-Wonder Cave System (top) in southwestern Grundy County, Tennessee, USA. Entrances to the three caves of the Crystal-Wonder Cave System are numbered and colored as follows: the Crystal Cave (TGD10) in white, the Wonder Cave (TGD30) in black, and the Wonder Natural Bridge Cave (TGD179) in gray, respectively. A map of the Crystal Cave is not available. The stream in the Wonder Cave (i.e., Mystic River) flows out of the Wonder Cave (E1 black) and on the surface briefly (blue line) before sinking into the resurgence entrance (E1 gray) of the Wonder Natural Bridge Cave, and then quickly emerging again (E2 gray) to briefly flow on the surface before sinking into the resurgence entrance (E4 white) of the Crystal Cave (TGD10). The stream flows through the Crystal Cave (general flow path shown as a dashed line) and finally resurges at the spring entrance (E2 white) of the Crystal Cave. The bottom photographs show the Mystic River along the former commercial cave tour in the Wonder Cave (left) and in the Crystal Cave near the connection of the Mystic River passage and Historical Section (right). Photographs by Amata Hinkle.

1.2. Discovery, Exploration, and History of the Crystal-Wonder Cave System

Descriptions of the Wonder Cave and details on the history of exploration, dye tracing, and commercial operations can be found in [11–15]. Native Americans likely camped at the spring based on the presence of artifacts nearby, while the Wonder Cave itself was named by the Vanderbilt University students Robert Nelson, Melville Anderson, and Will Fitzgerald when they “discovered” it in 1897 [11]. It was then quickly purchased by the local businessman R.M. Payne in 1898 as a potential attraction for his hotel—the Monteagle Hotel. Payne enlarged the entrance to allow for boat tours and constructed a new rock wall and walking trails. In addition, a large stream pump was installed to pump spring water from the cave to the hotel. The first commercial operation offered flat-bottom boat

tours via the Mystic River which runs through the cave and lantern tours along the walking trails. After more than a decade of boat tours, Payne created a new tunnel entrance into the cave in 1914 before passing away in 1917. A significant moonshine alcohol operation was located a few hundred meters inside the cave during the Prohibition era [16]. In 1929, Jefferson Jones Raulston, Payne's grandson-in-law and granddaughter Mary, took over the operations of the Wonder Cave, constructing a stone entry façade leading into the cave, and a log house near the entrance that contained a gift shop and ticket office. This cave was Tennessee's most popular commercial cave at that time, being ideally located just 0.4 km (0.25 mi) off of U.S. Route 41. After the construction of Interstate 24 in the 1960s, visitation plummeted by nearly 90 percent, as it was an 11 km (7 mi) detour from the interstate. The cave remained in the family until 1987 when it was sold to the Born family, who operated it commercially for a short time during the summer months until 2000. The Wonder Cave was one of the best-known historical commercial caves in the United States, with over two million people visiting between 1897 and 2000 and attracting more than 40,000 visitors a year at its peak [17].

The first partial map of the Wonder Cave was published by the State Geological Survey of Tennessee in 1912. Thomas Barr published a complete map of the known passage of the Wonder Cave in his book titled the "Caves of Tennessee" [12] based on a trip with Bill Cuddington, Roy Davis, Frank Raulston, and others in December 1953. In 2014, Jason Hardy began a resurvey of the Wonder Cave, and his map was archived with the Tennessee Cave Survey in 2020. Despite its proximity to and hydrological connections with the Wonder Cave [15], a detailed map of the Crystal Cave remains to be published.

1.3. Biological Investigation of the Crystal-Wonder Cave System

The Crystal-Wonder Cave System has a long history of biological interest dating to the 1930s, when the first biological collections were conducted by J.M. Valentine, C. Mohr, K. Dearolf, and colleagues. Valentine collected the specimens of several troglobiotic species that would be described over the next three decades (e.g., [18–22]). Dearolf [23] summarized the observations and collections of several species from 75 caves visited in the United States, including records from both the Crystal and Wonder caves. Dearolf reported six cave-limited species and several additional records for non-troglobiotic fauna. Additional historical collections were made by T.C. Barr, L.R. Hubricht, L.P. Woods, J.G. Armstrong, and R.A. Brandon in the 1950s and 1960s that were included in later taxonomic studies (e.g., Woods and Inger [24], Malcolm and Chamberlin [25], Hubricht [26], Peck [27,28], and Shear [29]). Lewis [30] reported a list of cave-limited fauna from the Crystal Cave, which included 16 species. Finally, Niemiller and Zigler [2] identified the Crystal-Wonder Cave System as the most biodiverse cave system with respect to the cave-limited fauna in Tennessee. These authors reported 24 cave-limited species but did not include a faunal list for the cave system.

Herein we present an updated comprehensive list of the terrestrial and aquatic cave obligate fauna (i.e., troglobionts and stygobionts, respectively) for the Crystal-Wonder Cave System based on a comprehensive search of the scientific literature and museum records, and from recent biosurveys of the cave system conducted by the authors and colleagues between 2005 and 2022. In addition to the species list, we include a comprehensive bibliography on the cave obligate fauna of the Crystal-Wonder Cave System, discuss factors potentially driving its biodiversity, and comment on the conservation status of the exceptional biodiversity of this North American and global hotspot of subterranean biodiversity.

2. Materials and Methods

2.1. Ecological Classification of the Troglobionts and Stygobionts

We recognized troglobionts (i.e., troglobites; terrestrial species) and stygobionts (i.e., stygobites; aquatic species) as species that are permanent inhabitants of subterranean habitats [31–33], and that are unable to complete their life cycles outside of such habi-

tats [34]. Troglobionts and stygobionts have source populations in subterranean habitats but may have sink populations in surface habitats from a metapopulation perspective [32]. While the use of morphology alone cannot definitively classify species ecologically [33], we used the presence of traits often observed in troglomorphic and stygobiotic fauna (i.e., troglomorphisms), such as reduced eyes and pigmentation, or hypertrophy of nonvisual sensory structures, but not found in surface-dwelling relatives, as evidence for isolation in subterranean habitats.

2.2. Cave Biosurveys

We conducted faunal bio-inventories of the Crystal-Wonder Cave System on six occasions since 2005 in association with other projects: the Crystal Cave on 12 May 2005, 21 November 2006, 21 June 2015, 22 April 2022, and 22 August 2022; and the Wonder Cave on 20 June 2015 and 22 April 2022, respectively. Bio-inventories consisted of time-constrained visual encounter surveys for cave life in terrestrial and aquatic habitats, including entrance areas and twilight zones, walls and ceilings, mud banks, rimstone pools, streams, and talus slopes. We searched underneath rocks and cover and within detritus and other organic debris, as well as searching through stream cobble. Each survey was conducted by two to seven researchers.

We identified common vertebrate and invertebrate species in the field. For many vertebrates, we field-identified taxa by direct observations without capture, or through taxonomically reliable indirect observations, such as the visual identification of mammal scat or footprints left in the mud. For many invertebrates, we collected specimens and identified them in the laboratory using the available taxonomic keys and literature. We outsourced identification to experts for taxa with which we had an insufficient taxonomic knowledge when possible. We took voucher photographs of the invertebrate and vertebrate taxa when possible.

2.3. Literature and Museum Searches

We conducted a search of the scientific literature to compile an updated list of troglomorphic and stygobiont species for the Crystal-Wonder Cave System. Scientific literature sources included journal articles, book chapters, books, conference proceedings, theses and dissertations, and government reports. Searches of these scientific literature sources included keyword queries on the ISI Web of Science, Google Scholar, and zoological records. Keywords used in these searches included “Crystal Cave”, “Wonder Cave”, “Grundy County”, “Tennessee”, “Monteagle”, “species”, “troglobite”, “stygobite”, “troglobiont”, “stygobiont”, “troglobiotic”, “stygobiotic”, “groundwater”, “subterranean”, “salamander”, “fish”, “vertebrate”, “snail”, “mollusk”, “insect”, “fly”, “beetle”, “arthropod”, “arachnid”, “spider”, “harvestman”, “pseudoscorpion”, “mite”, “crustacean”, “crayfish”, “isopod”, “amphipod”, “copepod”, “ostracod”, and “flatworm”. In addition, we also searched biodiversity databases, including the Global Biodiversity Information Facility (GBIF; <https://gbif.org>; accessed on 7 May 2023), VertNet (<http://www.vertnet.org>; accessed on 7 May 2023), Symbiota Collections of Arthropods Network (SCAN; <https://scan-bugs.org/portal/>; accessed on 7 May 2023), and InvertEBase (<http://www.invertebase.org/portal/index.php>; accessed on 7 May 2023). The list of cave obligate fauna includes the scientific name, authority, and conservation status of each species. Taxonomic nomenclature followed primarily the Integrated Taxonomic Information System (ITIS; <http://itis.gov>; accessed on 7 May 2023). For the conservation status, we included the International Union for Conservation of Nature (IUCN) red list of threatened species (<http://www.iucnredlist.org>; accessed on 8 May 2023) and NatureServe (<http://www.natureserve.org>; accessed on 8 May 2023) conservation statuses when they were available. The status of a species according to the United States list of threatened and endangered species under the U.S. Endangered Species Act was included (<http://www.fws.gov/endangered>; accessed on 8 May 2023), as well its con-

servation status in the state of Tennessee (Tennessee State Wildlife Action Plan; <http://twraonline.org/2015swap.pdf>; accessed on 8 May 2023).

3. Results

The list of cave-limited fauna documented within the Crystal-Wonder Cave System includes 31 species, with 23 troglobionts and eight stygobionts, respectively (Table 1; Figures 2 and 3). Twenty-eight species were known from the Crystal Cave, while eighteen species have been documented from the Wonder Cave, respectively. The Crystal Cave is the type locality for two cave-limited species (*Anillinus longiceps* and *Pseudanophthalmus humeralis*), while the Wonder Cave is the type locality for four species (Table 1): *Tolus appalachius*, *Chitrella archeri*, *Pseudanophthalmus intermedius*, and *Ptomaphagus hatchi*, respectively. *Anillinus longiceps* is known only from the Crystal-Wonder Cave System (Table 1). The cave-limited fauna represents five phyla, ten classes, nineteen orders, and twenty-six families.

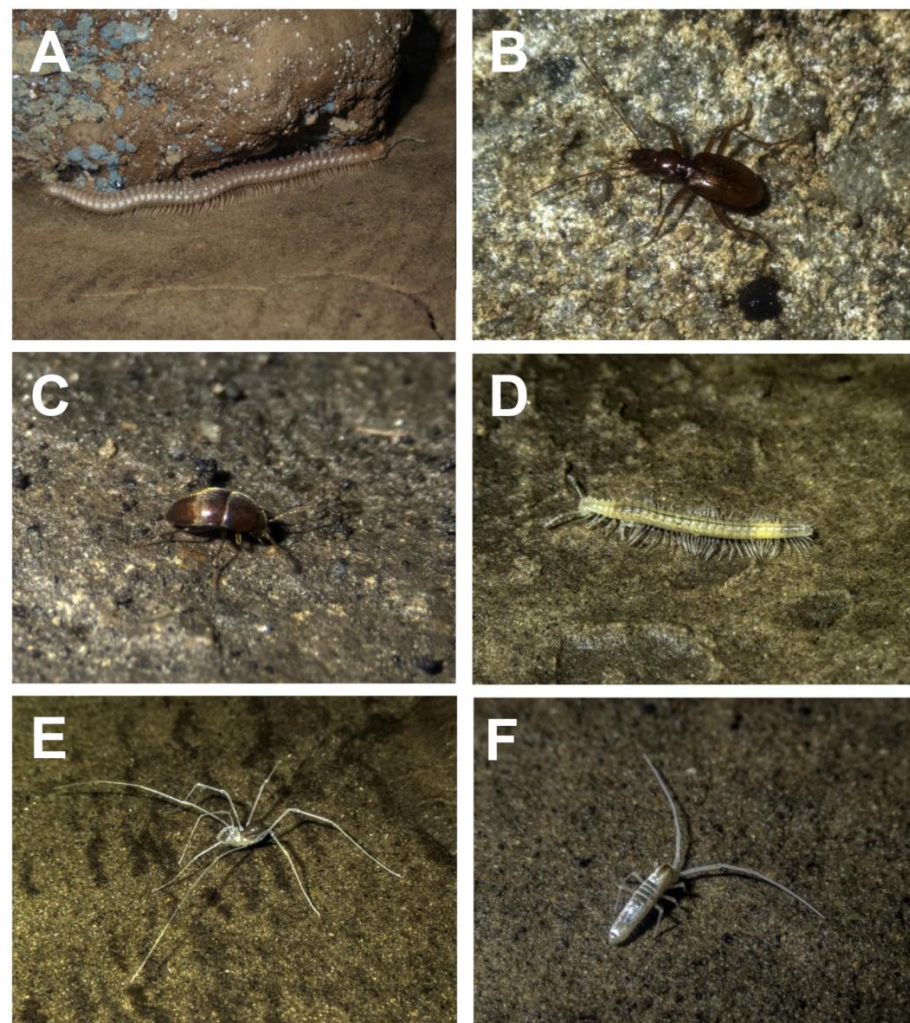


Figure 2. Representative terrestrial cave-limited fauna from the Crystal-Wonder Cave System, Alabama, USA: (A) *Tetracion tennesseensis*; (B) *Pseudanophthalmus intermedius*; (C) *Ptomaphagus hatchi*; (D) *Scoterpes ventus*; (E) *Tolus appalachius*; and (F) *Pseudosinella christianseni*. All photos were taken by Matthew L. Niemiller.

Table 1. Troglobionts and stygobionts of the Crystal-Wonder Cave System, Grundy County, Tennessee, USA. NatureServe conservation ranks include secure (G5), apparently secure (G4), vulnerable (G3), imperiled (G2), critically imperiled (G1), possibly extinct (GH), presumed extinct (GX), unranked (GNR), and unrankable (GU). T# is infraspecific taxon (i.e., subspecies) rank. A ? denotes an inexact numeric rank. State ranks for Tennessee are included in parentheses. IUCN red list categories include least concern (LE), near threatened (NT), vulnerable (VU), endangered (EN), critically endangered (CR), extinct in the wild (EW), and extinct (EX). Tennessee Wildlife Resources Agency state statuses include endangered (E), threatened (T), deemed in need of management (D), and special concern (S). Species of greatest conservation need in Tennessee are marked with an asterisk under State Status. No species are federally listed.

Taxon	Authority	NatureServe Status	IUCN Red List	State Status	Crystal Cave	Wonder Cave
TROGLOBIONTS						
Phylum Arthropoda						
Class Arachnida						
Order Araneae						
Family Linyphiidae						
<i>Phanetta subterranea</i> \$	(Emerton, 1875)	G5 (S4)			X	X
Family Zoropsidae						
<i>Liocranoides archeri</i> \$	Platnick, 1999	G2 (S2)			X	X
Order Opiliones						
Family Phalangodidae						
<i>Tolus appalachius</i> T,\$	Goodnight and Goodnight, 1942	G3G4 (S3)		*	X	X
Order Pseudoscorpiones						
Family Chernetidae						
<i>Hesperochernes mirabilis</i> \$	(Banks, 1895)	G5 (S3)			X	
Family Syarinidae						
<i>Chitrella archeri</i> T,\$	Malcolm & Chamberlin, 1960	G1G2 (S1S2)		*		X
Order Acari						
Family Rhagidiidae						
Unidentified genus and species \$					X	X
Class Collembola						
Order Entomobryomorpha						
Family Entomobryidae						
<i>Pseudosinella christianseni</i> \$	Salmon, 1964 (Delamare)	G5 (S2)		*	X	X
<i>Pseudosinella spinosa</i> \$	DeBoutteville, 1949)	G5 (S2)		*	X	X
Order Symphypleona						
Family Arrhopalitidae						
<i>Pygmarrhopalites pavo</i> \$	(Christiansen and Bellinger, 1996)	G3? (S1S2)			X	
Class Diplura						
Order Rhabdura						
Family Campodeidae						
<i>Litocampa cookei</i>	(Packard, 1871)	G5 (S3)				X
<i>Litocampa valentinei</i> \$	(Conde, 1949)	G5 (S2)		*	X	
Class Diplopoda						
Order Callipodida						
Family Abacionidae						
<i>Tetracion tennesseensis</i> \$	Causey, 1959	G2G3 (S2S3)		*	X	X
Order Chordeumatida						
Family Cleidogonidae						
<i>Pseudotremia barri</i> \$	Lewis, 2005	G2 (S2)			X	X
Family Trichopetalidae						
<i>Scoterpes ventus</i> \$	Shear, 1972	G3 (S1)		*	X	X
Class Insecta						
Order Coleoptera						
Family Carabidae						

Table 1. Cont.

Taxon	Authority	NatureServe Status	IUCN Red List	State Status	Crystal Cave	Wonder Cave
TROGLOBIONTS						
<i>Anillinus longiceps</i> ^{T,E}	Jeannel, 1963				X	
<i>Pseudanophthalmus humeralis</i> ^{T,\$}	Valentine, 1931	G2 (S2)		*	X	X
<i>Pseudanophthalmus intermedius</i> ^{T,\$}	(Valentine, 1931)	G2 (S2)			X	
Family Leiodontidae						
<i>Ptomaphagus hatchi</i> ^{T,\$}	Jeannel, 1933	G3 (S3?)			X	X
Family Staphylinidae						
Subfamily Pselaphinae						
<i>Batrissodes valentinei</i>	Park, 1951	G2G4 (S1?)		*	X	
Order Diptera						
Family Sphaeroceridae						
<i>Spelobia tenebrarum</i> ^{\$}	(Aldrich, 1897)	G5 (S4,S5)			X	X
Phylum Mollusca						
Class Gastropoda						
Order Ellobiida						
Family Ellobiidae						
<i>Carychium stygium</i>	Call, 1897	G3 (S2)		*	X	
Order Stylommatophora						
Family Helicodiscidae						
<i>Helicodiscus notius specus</i>	Hubricht, 1962	G5T2 (S1?)		*	X	
Family Zonitidae						
<i>Glyphyalinia specus</i>	Hubricht, 1965	G4 (S3)			X	
STYGOBIONTS						
Phylum Platyhelminthes						
Class Turbellaria						
Order Tricladida						
Family Kenkiidae						
<i>Sphalloplana percoeca</i> ^{\$}	(Packard, 1879)	G5 (S3?)			X	
Phylum Arthropoda						
Class Malacostraca						
Order Amphipoda						
Family Crangonyctidae						
<i>Stygobromus vitreus</i>	Cope, 1872	G4 (S2)			X	
<i>Stygobromus</i> nov. sp. 1						X
<i>Stygobromus</i> nov. sp. 2					X	
Order Decapoda						
Family Cambaridae						
<i>Orconectes australis</i> ^{\$}	(Rhoades, 1941)	G5 (S3)	LC		X	X
Order Isopoda						
Family Asellidae						
<i>Caecidotea bicrenata</i> ^{\$}	(Steeves, 1963)	G5 (na)			X	X
Phylum Chordata						
Class Actinopterygii						
Order Percopsiformes						
Family Amblyopsidae						
<i>Typhlichthys subterraneus</i> ^{\$}	Girard, 1859	G4 (S3)	NT	D *	X	X
Class Amphibia						
Order Caudata						
Family Plethodontidae						
<i>Gyrinophilus pallescens</i> ^{\$}	McCready, 1954	G2,G3 (S2)	VU B2ab(ii,v)	T *	X	

^T Type locality in the Crystal-Wonder Cave System; ^E Endemic to the Crystal-Wonder Cave System; ^{\$} Observed since 2015.

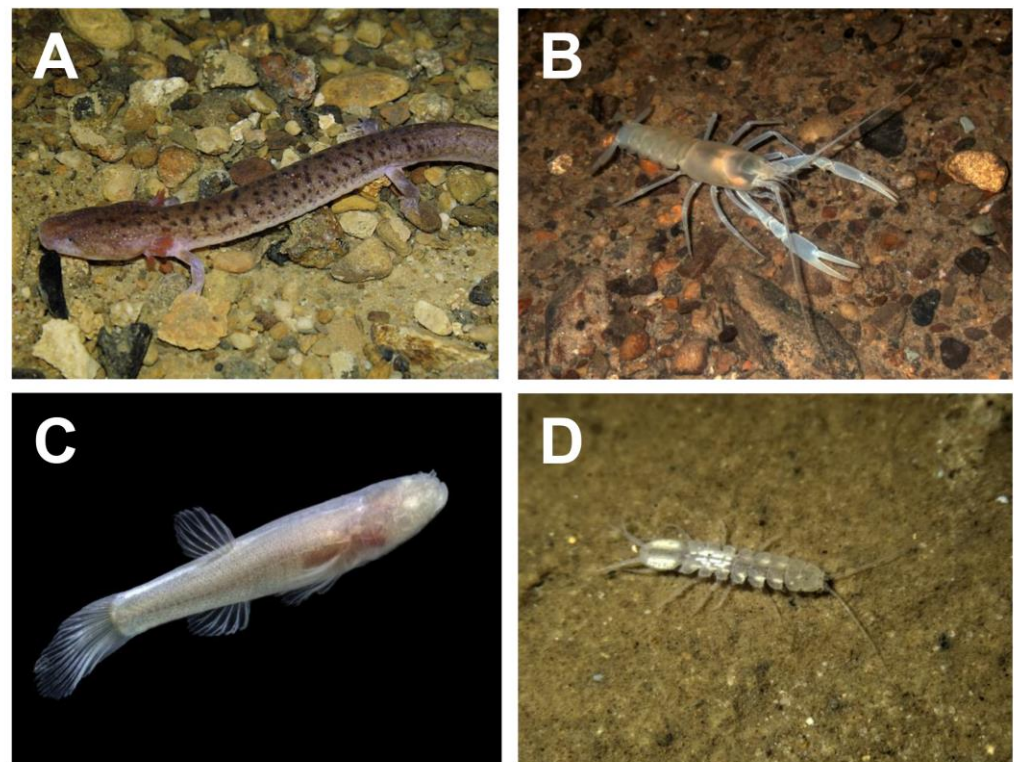


Figure 3. Representative aquatic cave-limited fauna from the Crystal-Wonder Cave System, Grundy County, Tennessee, USA: (A) *Gyrinophilus palleucus*; (B) *Orconectes australis*; (C) *Typhlichthys subterraneus*; and (D) *Caecidotea bicrenata*. All photos were taken by Matthew L. Niemiller.

3.1. Terrestrial Fauna

Troglobiotic spiders known from the Crystal-Wonder Cave System include linyphiids and zoropsids. The cave linyphiid *Phanetta subterranea* has one of the largest distributions of any troglobiont in North America [35,36]. Lewis [30] reported this species from the Crystal Cave, while we observed the spider at the Wonder Cave in 2022. *Liocranoides archeri* is known from several caves along the Western Escarpment of the Cumberland Plateau from southern Warren County, Tennessee southward into northeastern Alabama [30,37]. This species is pale in coloration but does not possess other obvious troglomorphic characters [37]; however, it has only been reported from caves [37] and has been treated as a troglobiont by other authors [2,3]. Platnick [37] reported this species from the Crystal Cave from a collection by Valentine and Beakley in 1935. We observed this spider in both the Crystal and Wonder caves in 2022.

The Wonder Cave is the type locality of the cave harvestman *Tolus appalachius* [19]. The holotype and paratypes have previously been collected from the Wonder Cave, with additional paratypes collected from the Crystal Cave by Valentine and Beakley in 1935. *Tolus appalachius* is a small, highly troglomorphic harvestman that occurs in several caves along the Western Escarpment of the Cumberland Plateau from Overton County, Tennessee southward into Jackson County, Alabama [3]. This species has also been reported from the Crystal-Wonder Cave System by Peck [38], Lewis [30], and Hedin and Thomas [39].

Two troglobiotic pseudoscorpions occur in the Crystal-Wonder Cave System. *Hesperochnes mirabilis* is a widely distributed chernetid species that is most abundant near entrances where it is associated with bat guano, rodent nests, and mammal scat [8,40,41]. We observed this species at the Crystal Cave in 2022 in the vicinity of raccoon scat near the main entrance. The Wonder Cave is the type locality for the syarinid *Chitrella archeri*. The male holotype was collected in 1938, while an allotype male and female paratype were collected in 1957 [25]. We collected a female from the top of the breakdown pile in the pyramid room of the Wonder Cave on 15 June 2015; this specimen represents the first reported occurrence of the species since its description in 1957. This species lacks eyes, has

attenuated appendages and is only known from three caves: the Wonder Cave [25,30], the nearby Trussell Cave in Grundy County [30], and the Piper Cave on the Eastern Highland Rim in Smith County, Tennessee [25,30]. The record obtained from the Trussell Cave is based on a tentative identification [30] and may not represent *C. archeri*.

One unidentified trogllobiotic rhagidiid mite is known from the Crystal-Wonder Cave System, where it was observed in both of these caves. This mite may be a species in the genus *Rhagidia* that has been reported from caves in northwestern Georgia [8,40].

Three trogllobiotic millipedes have been documented in the Crystal-Wonder Cave System, including one callipodidan and two chordeumatids. *Tetracion tennesseensis* is a large cave millipede (up to 8 cm in length) known from several caves along the Western Escarpment of the Cumberland Plateau from southern White County southward into southwestern Grundy County and northeastern Franklin County [30,42]. This species has previously been reported from the Crystal Cave [30,42], and we observed *T. tennesseensis* in the Crystal and Wonder caves in 2022. *Pseudotremia barri* occurs in caves along the Western Escarpment of the Cumberland Plateau from southern Warren County into northeastern Franklin County [43]. Lewis [43] reported collecting specimens in stream detritus and pitfall traps in riparian mudbanks in the Crystal Cave. We observed *P. barri* on mudbanks along the main streams in both the Crystal and Wonder caves in 2022. We attributed an early report of *Pseudotremia* sp. by Dearolf [23] from the Wonder Cave to *P. barri*. Lewis [30] also reported this species (as *Pseudotremia* sp. nov. 7) from the Crystal Cave. *Scoterpes ventus* has a broad distribution throughout the Cumberland Plateau and Eastern Highland Rim of Tennessee from the Jackson and Overton Counties southward to the Franklin, Grundy, and Marion Counties [29,30,44]. This small, blind, and unpigmented trichopetalid trogllobiont likely represents a species complex [29]. *Scoterpes ventus* has been previously reported from both the Crystal [29,30] and Wonder caves [23,29]. We observed this species in both caves in 2022, where it is most often found in moist habitats with organic matter comprising rotting wood, debris, and cricket frass (guano).

Three species of cave-limited collembolans (i.e., springtails) have been documented from the Crystal-Wonder Cave System. Both *Pseudosinella christianseni* and *P. spinosa* are broadly distributed across the Interior Low Plateau [45]. *Pseudosinella spinosa* is the largest of the trogllobiotic *Pseudosinella* in North America, and lacks eyes and pigmentation [45]. *Pseudosinella christianseni* also lacks eyes and pigmentation and may be a species complex [45]. Both species have been previously collected from the Crystal Cave, while *P. spinosa* has been collected from the Wonder Cave (Christiansen Springtail Collection), respectively. We observed both species in the Crystal and Wonder caves in 2022. Dearolf [23] reported *Pseudosinella* sp. from the Wonder Cave, which may be either or both of *P. christianseni* and *P. spinosa*. Lewis [30] reported *Pygmarrhopalites pavo* from the Crystal Cave. This small globular springtail is known from caves observed in Virginia [46], as well as caves from the Grundy and Overton Counties, Tennessee [30].

Two trogllobiotic diplurans have been reported from the Crystal-Wonder Cave System. Lewis [30] reported *Litocampa valentinei* from the Crystal Cave. This dipluran is known from several caves in northeastern Alabama and south-central Tennessee along the escarpments of the Cumberland Plateau [30,47], including several caves near the Crystal-Wonder Cave System [30]. Dearolf [23] reported *L. cookei* from the Wonder Cave. This species has the broadest distribution of any trogllobiotic dipluran in the eastern United States, ranging from western Kentucky to southwestern Virginia, and southward into south-central Tennessee [47]. The occurrence of two *Litocampa* species is notable, as syntopy of *Litocampa* is rare [47]. *Litocampa cookei* co-occurs with an undescribed species at the Goodmans Cave in Hancock County, Tennessee [47]. Ferguson [47] did not examine specimens from the Wonder Cave, but there is a nearby record at the Wet Cave in Franklin County [30]. We included both species in our list of trogllobiotic taxa, but also noted that additional survey efforts and a comprehensive phylogenetic study are needed to ascertain the species limits in this complex genus.

The trogllobiotic beetle fauna of the Crystal-Wonder Cave System is diverse, and includes three carabids, one leiodid, and one staphylinid. All three cave carabid species are blind and wingless. Two species of *Pseudanophthalmus* trechine cave beetles have been previously reported from both the Crystal and Wonder caves [18,30,48–54]. The Crystal Cave is the type locality of *P. humeralis* [18] of the *engelhardti* species group [54], while the Wonder Cave is the type locality of *P. intermedius* [18] of the *intermedius* species group [54]. Both species were first collected in 1931 from the Crystal-Wonder Cave System [18,48] and are known from caves along the Western Escarpment of the Cumberland Plateau from the Franklin and Grundy Counties [18,30,48,54]. *Pseudanophthalmus intermedius* is larger at 5–6 mm compared to 3.9–4.5 mm for *P. humeralis*, respectively [18,48]. Valentine [48,49] recognized two subspecies of *P. humeralis*—*P. h. humeralis* from the Crystal Cave and *P. h. brevis* from the Wonder Cave—but these subspecies were later synonymized by Jeannel [51]. Both species have been found primarily in association with rotting wood [48]. The Crystal Cave is the type locality for the bembidiine ground beetle *Anillinus longiceps* [22,55,56]. The holotype male was collected in 1931 by J.M. Valentine. Although many species in the genus are endogean, *A. longiceps* is considered as a trogllobiont [56].

The Wonder Cave is the type locality of the round fungus beetle *Ptomaphagus hatchi*. This beetle has been reported previously from both the Crystal and Wonder caves [21,27,28,51,52,57–59]. This species is the most broadly distributed trogllobiotic *Ptomaphagus* in the southern Cumberland Plateau [27,28]. The staphylinid cave ant beetle *Batrissodes valentinei* is known from the Crystal Cave [20,60,61]. The range of this trogllobiont is primarily in northern Alabama, with the presumably isolated population from the Crystal Cave. However, Park [62] noted that this specimen from the Crystal Cave should be reexamined, as it might represent a new subspecies or species. We observed both species of *Pseudanophthalmus* and *Ptomaphagus hatchi* during biosurveys in 2022; in contrast, *A. longiceps* and *B. valentinei* have not been observed in the Crystal-Wonder Cave System since 1931.

The only other trogllobiotic insect documented from the Crystal-Wonder Cave System is the cave dung fly *Spelobia tenebrarum*, which has been reported from many caves in the eastern United States [8,30,53,63,64], where it has been associated with scat. This species has reduced eyes and is the only known trogllobiotic fly in the United States [63,64]. This species was reported from the Crystal Cave by Lewis [30], and we observed this fly in both caves in 2022.

Three trogllobiotic snails occur in the Crystal-Wonder Cave System. *Carychium stygium* is a minute (<2 mm) terrestrial snail known from >75 caves throughout the Interior Low Plateau karst region of Kentucky and Tennessee [30,65,66], where it is often found in association with cricket guano [67]. Lewis [30] reported *C. stygium* from the Crystal Cave. Weigand et al. [68,69] suggested that *C. stygium* may be a morph of the troglophile *C. exile* based on mitochondrial COI sequence data. *Glyphyalinia specus* is a wide-ranging trogllobiotic glyph known from twenty-seven caves in five states [66], including the Crystal Cave [26], in association with cricket guano [65]. *Helicodiscus notius specus* also has a broad distribution but is known from just four caves in Kentucky and Tennessee [66], including the Crystal Cave [30], where it is associated with cricket guano [65].

3.2. Aquatic Fauna

One cave flatworm—*Sphalloplana percoeca*—has been previously reported from the Crystal-Wonder Cave System [28]. We observed this flatworm on occasion in isolated drip pools. This species has been reported from 34 caves in the TAG region, including several nearby caves in Grundy County [2,30,70].

The cave-limited crustacean fauna includes one crayfish, one isopod, and three amphipods. The stygobiotic crayfish *Orconectes australis* is common in pools in the cave streams in the Crystal-Wonder Cave System. This cave crayfish also has been reported previously by Mohr [71] (as *Cambarus hamulatus*), Hobbs and Barr [72], Hobbs et al. [73], Buhay and Crandall [74], and Lewis [30]. *Orconectes australis* is the most wide-ranging and common stygobiotic crayfish in the southern Cumberland Plateau, occurring in >250 caves

from Overton County in Tennessee southward to the Madison and Jackson Counties in Alabama [2,30,74]. We observed as many as 30 *O. australis* in the Crystal Cave in 2005 and 19 crayfish in the Wonder Cave in 2022, respectively.

The stygobiotic isopod *Caecidotea bicrenata* occurs throughout the Crystal-Wonder Cave System in several habitats, including stream riffles and pools, rimstone pools, and drip pools. *Caecidotea bicrenata* is widely distributed across a variety of subterranean habitats throughout the Interior Low Plateau [75] but may represent a cryptic species complex. This stygobiotic asellid was reported previously from the Crystal and Wonder caves by Dearolf [23] (as *C. nickajackensis*) and from the Crystal Cave by Lewis [30,75]. *Caecidotea bicrenata* was found to be common in both caves in 2022.

Dearolf [23] reported the cave amphipod *Stygobromus vitreus* from the Crystal Cave based on a collection in 1937. This species is known primarily from the Mammoth Cave region in central Kentucky with scattered occurrences in south-central Tennessee and northern Alabama [23,76]. Holsinger [76] noted that populations from Alabama morphologically differed slightly from populations in Kentucky, and potentially may represent a distinct species. An undescribed *Stygobromus* amphipod is known from the Wonder Cave [30]. This species apparently occurs in the southern Cumberland Plateau of south-central Tennessee and northern Alabama but extends southward to the north of Birmingham (Holsinger, pers. comm. in Lewis, [30]). Another undescribed *Stygobromus* amphipod is known from the Crystal Cave that is distinct from the taxon observed in the Wonder Cave. This species occurs in several caves along the Cumberland Plateau from the White County southwestward into the Franklin County (Holsinger, pers. comm. in Lewis [30]). Additional specimens have been collected from the Crystal Cave, which may be either of the two undescribed taxa, *S. vitreus*, or another species, such as *S. exilis*, which also has a broad distribution in the Interior Low Plateau karst region, including the southern Cumberland Plateau [30,76].

Two cave-limited vertebrates are known from the Crystal-Wonder Cave System. The southern cavefish *Typhlichthys subterraneus* has been observed in both the Crystal [24,30,77–80] and Wonder caves [30,77,78]. This cavefish is abundant in the main stream and tributaries in the Crystal Cave [78] and is considered as a top aquatic predator. *Typhlichthys subterraneus* is a cryptic species complex [79], with lineage B identified from the Upper Elk River watershed [79,80]. From our biosurveys of the Crystal Cave we observed six cavefish in 2005 and as many as 30 cavefish in 2006.

The Tennessee cave salamander *Gyrinophilus palleucus* was first observed in the Crystal Cave in 2006 [81,82]. This neotenic plethodontid salamander is considered as a top predator of cave streams and is known from several caves along the escarpments of the southern Cumberland Plateau in Tennessee, Alabama, and Georgia [81,83,84]; however, it has been presumed to be rare in the Crystal-Wonder Cave System. Only one individual has been observed on three occasions, most recently in 2022. *Gyrinophilus palleucus* has yet to be observed in the Wonder Cave.

4. Discussion

The cave-limited fauna of the Crystal-Wonder Cave System is remarkably diverse with 31 troglobionts and stygobionts, making it the most diverse cave system in the southern Cumberland Plateau region, and one of the most diverse cave systems in all of North America. With 31 cave-limited species, the Crystal-Wonder Cave System trails only the San Marcos artesian Well in central Texas (55 species; [85]) and the Mammoth Cave System in central Kentucky (49 species; [86]) and ranks ahead of the Fern Cave System in Alabama (27 species; [10]) and Sistema Huautla in Oaxaca, Mexico (27 species; [87]). In particular, the terrestrial fauna is exceptionally rich with 23 species, trailing only the Mammoth Cave System (32 troglobionts; [86]) and Sistema Huautla (27 species; [87]) in North America. The stygofauna of the Crystal-Wonder Cave System is also diverse (8 species), but not remarkable compared to other hotspot subterranean communities in North America, such as the San Marcos Artesian Well in Texas (55 species; [85]), Mammoth Cave System in Kentucky (17 species; [86]), and Shelta Cave in Alabama (12 species, [9,88]). The high

number of troglobionts relative to the stygobionts in the Crystal-Wonder Cave System is not surprising given the cave's location in Tennessee, where troglobionts outnumber stygobionts by roughly four to one [2]. The Crystal-Wonder Cave System is the most speciose cave system in the southern Cumberland Plateau region ahead of the Fern Cave (27 species; [10]), the Shelta Cave (24 species; [9]), and the Big Mouth-Big Room System nearby in Grundy County, Tennessee (20 species; [2]).

The remarkable level of cave biodiversity observed in the Crystal-Wonder Cave System can largely be attributed by its location. The cave system sits at the junction of the Cumberland Plateau escarpment and the Eastern Highland Rim, the two ecoregions supporting the most cave biodiversity in Tennessee [2]. The cave's location in southern Tennessee also contributes, as cave biodiversity increases towards the southern interface of the Cumberland Plateau escarpment and the Eastern Highland Rim [2]. An additional factor is its history as a highly visited commercial cave, which attracted early cave biologists and increased the likelihood of the detection of exceptionally rare species (e.g., the single-cave endemic *Anillinus longiceps*), and explains how the cave came to be the type locality for six troglobionts.

The physical structure of the Crystal-Wonder Cave System also contributes to its remarkable troglobiont community. The cave system is relatively large (5.8 km), and hosts a variety of terrestrial and aquatic habitats, including a large cave stream that flows through much of the cave system. This stream, along with the multiple entrances, increases the opportunity for nutrients to enter and disperse throughout the cave system, thereby supporting a diverse troglobiont community. Although it has a long history as a commercial cave, the Wonder Cave has been closed to the public for more than two decades, reducing recent human impacts. Although the cave's watershed has not been mapped, the slopes of the adjacent Cumberland Plateau escarpment are forested, and the areas around the cave entrances are largely undeveloped, which benefits the cave community.

Of the thirty-one cave-limited species known from the Crystal-Wonder Cave System, eighteen species (sixteen troglobionts and two stygobionts) are of conservation concern (i.e., G1–G3 NatureServe conservation rank, state status). None of the species have federal status. Many of these species are at an increased risk of extinction due to their restricted distributions, or are known from few occurrences, such as *Anillinus longiceps*, which is known only from the Crystal-Wonder Cave System. The cave-limited fauna of the Crystal-Wonder Cave System are also facing potential threats related to changes in land use within the cave's watershed, particularly logging, home building, and a proposed sand quarry, as all of the land near the cave system is privately owned. Changes in the land use within the cave's watershed could increase sedimentation and otherwise negatively impact the water quality and quantity in the cave stream. In addition, the Crystal-Wonder Cave System is privately owned, and the various entrances to the cave system are owned by different individuals, thereby limiting the public's ability to monitor and manage the cave system with biodiversity in mind.

Despite its exceptional cave biodiversity, the list of cave-limited fauna that occur in the Crystal-Wonder Cave System is likely incomplete. Several sections, particularly within the Wonder Cave, remain to be comprehensively bio-inventoried, and several habitats, such as the epikarst and stream sediments, have not been adequately sampled and may harbor additional species. Several taxonomic groups are notably absent from the cave-limited fauna of the Crystal-Wonder Cave System, including stygobiotic copepods and troglobiotic woodlice, all of which may be discovered during future biosurveys. For example, two species of copepods—*Diacyclops yeatmani* and *D. indianensis*—are known from nearby the Big Mouth Cave [30] and may occur in the Crystal-Wonder Cave System. Other taxonomic groups have not been particularly well-studied in the Crystal-Wonder Cave System, including mites, spiders, pseudoscorpions, and springtails. More intensive biosurvey efforts on these groups may uncover additional taxa, as several species yet to be documented in the Crystal-Wonder Cave System have reported from other nearby caves in Grundy County [30]. Finally, comprehensive sampling within the Crystal-Wonder System coupled with genetic analyzes has the potential to uncover cryptic diversity, which

is an increasingly common discovery of phylogenetic studies in cave-limited taxa [79,89]. Phylogeographic studies have incorporated specimens from the Crystal-Wonder Cave System, including the studies of *Orconectes australis* [74], *Gyrinophilus palleucus* [82], *Tetracion tennesseensis* [42], *Typhlichthys subterraneus* [79], and *Ptomaphagus hatchi* [59], but several taxonomic groups, such as stygobiotic isopods and amphipods, remain to be studied from a genetic perspective.

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