Status Report for the Southern Cavefish, *Typhlichthys subterraneus* in Arkansas

Final Report

By

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Executive Summary

This preliminary report summarizes the results of our study on the status of the southern cavefish (*Typhlichthys subterraneus*) in Arkansas. Its presence in the state represents the western-southern limits of its distribution. Three localities have been confirmed that contain individuals of this species: Richardson Cave (Fulton County), Alexander Cave/ Clark Spring (Stone County) and Ennis Cave (Stone County). A fourth locality has been cited as a well in Randolph County, but because the exact location is unknown, its presence has not been confirmed. There are a number of unconfirmed localities for "cavefishes" in the region which are listed in this report. Populations of this species in Arkansas seem to be small (less than 100 individuals) which is common among populations of hypogean amblyopsids elsewhere. All the confirmed localities are in areas either under controlled access by the private owners or by the federal government. No immediate threat to these populations was found by either overcollecting or other anthropogenic causes. Yet long-term monitoring of the recharge areas is recommended since pollution of these areas has been the major ecological problem for this and other hypogean amblyopsids species elsewhere. Current work suggests that the populations in Arkansas may represent a new species of *Typhlichthys*. If that were the case, then a reconsideration of the conservation status of this purported new species needs to be carried out.

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1. Introduction

1.1. Systematic position of the family Amblyopsidae within the order Percopsiformes

The systematic position of the Family Amblyopsidae has been a source of debate (see Murray 1994:7-9 for a summary). Although the amblyopsids have been commonly placed within the Order Percopsiformes (trout-perches, sand rollers, pirate perches, and cavefishes) (Romero 2003), several other authors have contended that such order is not monophyletic (e.g., Rosen 1985, Patterson and Rosen 1989). Based on a cladistic analysis of morphological characters, Murray and Wilson (1999) proposed to remove the Family Amblyopsidae from the Percopsiformes and created a new order: Amblyopsiformes. Currently A. Romero, R. Johnson, and M. Niemiller are carrying out a project aimed at clarifying this issue from both a molecular and morphological viewpoint using morphometrics for the latter.

1.2. The biology of the family Amblyopsidae

1.2.1. Family name

This family was first named by Bonaparte (1846) based on the description of *Amblyopsis spelaea*, originally described as *A. spelaeus* by DeKay (1842) (for a description of the history of the discovery of this species see Romero 2002, Romero and Woodward 2005).

1.2.2. Morphological characteristics

Amblyopsids are characterized by: (a) large, flattened head, (b) oblique mouth, (c) strongly protruding lower jaw, (d) large branchial cavity (possibly for oral incubation), (e) a jugular vent (anus) in between gill membranes, (f) small embedded cycloid scales, except in the head which is naked, (g) rows of neuromasts (sensory papillae) on the head, body, and caudal fin, (h) myodome (the eye muscle canal) lost, (i) presence of a swimmbladder, (j) toothed vomer, (k) . segmented premaxilla, and (l) absent or incomplete lateral line.

Dorsal and anal fins have similar shapes. Single dorsal fin originates anteriorly to the origin of the anal fin with 7-12 soft rays. Anal fin with 7-11 soft rays. Pectoral fins with 9-12 soft rays. Pelvic fins present only in *A. spelaea* and not in all individuals with 0-8 soft rays. Caudal fin shape variable with 9-22 rays all of which are branched except in *Speoplatyrhinus poulsoni* (Table 1). 27-35 vertebrae.

Their eyes range from small (microphthalmic) in the epigean and troglophilic species to vestigial (remnant eye tissue under the skin) in the troglomorphic ones. Troglomorphic species are also characterized for: (a) being depigmented (looking pinkish because of the blood vessels showing through the translucent skin, with only a few, mostly non-functional melanophores), (b) low metabolism, and (c) low fecundity. Because of the jugular position of the genital papilla and the attachment of the gill membranes to the isthmus, Woods and Inger (1957) proposed that all amblyopsids carry eggs and yolk-sac fry in their gill cavities; however, such a behavior has been observed only in the genus *Amblyopsis*. All of the above morphological characters are based on Romero 2003, 2004, Poly and Proudlove 2004, Nelson 2006 and references therein.

1.2.3. Diversity and distribution

There are five genera and six species within this family, with one being an occupant of swamps, another being a facultative cave dweller, and the other four being obligate cave dwellers. Thus, the six species of this family represent a transition from epigean to hypogean waters: *Chologaster cornuta* (epigean), *Forbesichthys agassizii* (troglophilic or facultative cavernicole) and *Typhlichthys subterraneus*, *Amblyopsis rosae*, *A. spelaea*, and *S. poulsoni*, all troglomorphic (blind and depigmented) (Figure 1) (Cooper and Kuhene 1974, Page and Burr 1991, Romero 2001a). There are two species of this family represented in Arkansas: *Amblyopsis rosae* and *Typhlichthys subterraneus* found in the northwest and northeast-central parts of the state respectively. Comparative characteristics are summarized in Table 1. Therefore, there is substantial morphological variation among the species of the family (Romero 2004). The similarities that do exist are thought to reflect the outcome of convergent evolution (Romero and Green 2005).

The Amblyopsidae is a freshwater fish family distributed in the southern and eastern, unglaciated United States (Figure 2). The range of each amblyopsid species is limited, and little geographic overlap occurs among the taxa. The six species are: (1) the swampfish, *Chologaster cornuta* Agassiz 1853, limited to quiet acidic waters within the Atlantic Coastal Plain, ranging from Virginia to Georgia; (2) The spring cavefish, *Forbesichthys agassizii* (Putnam 1872) a facultative cave dweller, as it emerges from caves to the mouths of springs at night and found on both sides of the Mississippi River in Kentucky, Tennessee, Illinois and Missouri; (3) the southern cavefish, *Typhlichthys subterraneus* Girard 1859, present on both sides of the Mississippi River, with populations in Alabama, Georgia, Tennessee, Kentucky, Missouri and Arkansas (the possibility of this species found in Indiana has been mentioned, see page 7 of this report); (4) the northern cavefish, *Amblyopsis spelaea* DeKay 1842, found in Kentucky and Indiana; (5) the Ozark cavefish, *Amblyopsis rosae* (Eigenmann 1898), whose distribution is limited to the Springfield Plateau of Arkansas, Missouri and Oklahoma; and, (6) the Alabama cavefish, *Speoplatyrhinus poulsoni* Cooper and Kuehne 1974, which has the smallest range within the family and is restricted to a single cave in Alabama (Romero 2004).

The systematics of this family needs revision since genetic studies have shown it to be much more complex that previously believed (Bergstrom 1997). Taxonomically, the inclusion of *A. rosae* and *A. spelaea* within the same genus (*Amblyopsis*) has been questioned (Figg and Bessken 1995). The taxonomic status of *A. rosae* is unclear; given that at least two subspecies are identifiable (Bergstrom 1997).

Table 1. Summary information on the amblyopsid species. Sources: Robison and Buchanan 1988, Page and Burr 1991, Means and Johnson 1995, Pflieger 1997, Romero 1998a,b,c, Romero and Bennis 1998.

Species	**Max. SL (Standard Length, mm)**	**Eyes**	**Pigmentation**	**Number of rays (soft) in fins**			**Pelvic fins**	**Number of rows of papillae in the caudal fin**
				Dorsal	**Anal**	**Caudal**		
C. cornuta	68	microphthalmic	Yes	9-12	9-10	9-11 (branched)	Absent	0-2
F. *agassizii*	75	microphthalmic	Yes	9-11	9-11	11-16 (branched)	Absent	0-2
T. subterraneus	90	Vestigial	No	7-10	7-10	10-15 (branched)	Absent	0-2
A. spelaea	110	Vestigial	No	9-11	8-11	11-13 (branched)	Absent/ very reduced, 0-8 rays	4-6
A. rosae	65	Vestigial	No	7-9	8	9-11 (branched)	Absent	4-6
S. poulsoni	72	No vestiges?	No	9-10	8-9	21-22 (unbranched)	Absent	4

2. *Typhlichthys subterraneus*

2.1. Taxonomy

The first specimen of the southern cavefish, *Typhlichthys subterraneus*, was collected in a well near Bowling Green, Warren County, Kentucky, USA, and sent to the Smithsonian Institution by J. E. Younglove and later described by Charles F. Girard (1859). Carl H. Eigenmann (1905) described a new species of the genus *Typhlichthys* (*T. osborni*) by differentiating it from *T. subterraneus* based on the dissimilar head width and eye diameter. Eigenmann (1905) also described a third species as *T. wyandotte*. Carl Hubbs (1938) described a fourth species *T. eigenmanni*. Parenti (2006) based on neuroanatomical characters, proposed that *T. eigenmanni* Charlton (1933) is a subjective synonym of *T. subterraneus*. All of the species described by Eigenmann and Hubbs are now considered synonyms of *T. subterraneus* (Woods and Inger 1957) This is a species that shows a great deal of variability, in part, due to its extensive distribution; it is possible that this "species" is actually composed of two or more actual biological species (Barr and Holsinger 1985:330). Preliminary genetic studies suggest that the genetic structure of the southern cavefish is quite complex probably being an artificial mosaic of unrelated (paraphyletic) populations (Bergstrom *et al.* 1995). The populations in Arkansas may well belong to a new species within the genus as suggested by both morphological characters and ND2 phylogeny (Figure 3, Romero *et al*. unpublished).

Woods and Inger (1957) noted that the type specimens of *Typhlichthys wyandotte* had been lost and the type-locality destroyed. However, a well-like entrance into a cave, on the property of a car dealership in Corydon, Indiana, was discovered recently and is speculated to be the type-locality of *T. wyandotte* (Lewis 2002). That does not mean that the southern cavefish is found in Indiana: a year-long study of the Binkley Cave System and associated caves in the Corydon area by Lewis and Sollman (1998) found only individuals of *Amblyopsis spelaea*. A survey of about 200 caves in the same drainage basin also failed to find *Typhlichthys subterraneus* (Lewis 1998). Poly and Proudlove (2004) reported that they had rediscovered the holotype of *T. wyandotte* (CAS 91988) and some of the syntypes of *T. orboni* (CAS 78370, 91980, 91981, 91982) in the California Academy of Sciences collection. Yet, the finding of the specimen does not confirm the presence of this species in Indiana.

2.2. Morphology

Individuals of *T. subterraneus* reach 90 mm of standard length (SL). They have a large, broad head. Fin ray counts is as follows: dorsal soft rays: 7-10; anal soft rays: 7-10; lacks pelvic fins. Caudal rays: 10-15 (all branched). The number of vertebrae is 28-29. The caudal fin has from 0 to 2 rows of sensory papillae (one on the upper half and the other on the lower half) and a vertical basal row. The anus is well in front of the anal fin in adults and it has a port-cleithrum bone. They display rudimentary eyes hidden under the skin (Woods and Inger 1957).

Their body is depigmented with few non-functional pigment cells. Their cycloid scales are minute and embedded. Under exposure to white light some individuals develop epidermal melanophores (Woods and Inger 1957) which suggests that they are subject to phenotypic plasticity, a phenomenon that has been observed among other cave fishes (Romero and Green 2005). This species has a swimmbladder (Schubert 1993:130) and they show enlarged olfactory and acoustico-lateralis in their brain as well as reduced optic centers. They have numerous neuromasts with round bulbous cupulae, laying in a row and alternating left and right of center. Recent scanning electron microscope photographs show that individuals of this species have numerous small and unicuspid teeth (Figure 4, Romero *et al*. unpublished).

2.3. Life history and reproduction

Members of this species are long-lived (possibly even for decades) and slow growing (Noltie and Wicks 2001). Breeding probably occurs in late spring (April and May) in association with rising water levels (Robison and Buchanan, 1988). Branchial (i.e., gill chamber) brooding is possible because of the position of the jugular vent and the size and shape of the gill chamber (Poulson 1963) but this has yet to be proven. As many as 50% of the adult females of a population may breed in any one year. They develop the first scales at three months of age and the first annulus at 7-10 months. They become reproductively active at 22-24 months of age. Fecundity is very low, perhaps fewer than 50 ova per female. It takes about two months for free swimming young to develop from the zygote stage. Females display parental care (Poulson 1961).

2.4. Food and feeding

Members of this species feed mostly on copepods (60-80% of their diet), but remains of other organisms have been found in their digested system such as amphipods, isopods, decapods, ostracods, cladocerans, non-annelid worms, aquatic insects (both adults and larvae), algae, and debris (Poulson 1961, Cooper and Beiter 1972). Schubert (1993) found that the daily mean consumption rate is 2.68 amphipods/day or 11.8 mg amphipod/g fish/day.

2.5. Behavior and physiology

Individuals of the southern cavefish do not respond to light (Green and Romero 1997). They tend to rest motionless on the bottom for long periods of time, show a wide range of agonistic behaviors (Bechler 1983) and weak rheotaxic responses (Noltie and Wicks 2001). This species has about 28,600 lamellae on gill filaments, their gill area is 169.1 mm^2 and its metabolic rate is 0.016 O_2 ml g^{-1} h^{-1}. Their branchial volume is 50 mm^3, ventilation frequency is 19 (15-21), and amplitude 0.8 (0.4-1.0). All these values as well as their thyroid activity fall into the median for other species of amblyopsids (Poulson 2001).

2.6. Habitat

T. subterraneus inhabits water bodies that range in depth from near the water table to deep pools and streams of more than 200 m (Noltie and Wicks 2001). They are found mostly in flowing waters (Smith 1980) and seem to be attracted to point sources of water efflux (Schubert and Noltie 1995). They display a strong preference for large substrate sizes. They may use the larger interstices in between the pebbles for protection from both, predators and strong currents (Schubert 1993) which, in turn may facilitate the fish dispersal (Schubert *et al*. 1993).

An individual of *T. subterraneus* was reported in Key Cave, Alabama, the only known cave inhabited by another species of amblyopsid, *Speoplatyrhinus poulsoni* (Kuhajda and Mayden 2001).

2.7. Distribution and abundance

The southern cavefish is found in the subterranean waters of two major disjunct ranges separated by the Mississippi River: in the Ozark Plateau of central and southeastern Missouri and northeastern Arkansas (Jones and Taber 1985); and the Cumberland and Interior Low plateaus of northwest Alabama, northwest Georgia, central Tennessee and Kentucky (Rice *et al*. 1983, Cooper and Iles 1971, Cooper and Beiter 1972). Its presence in southern Indiana (ca. 37^{o} 00' N, 86^{o} 29' W) has yet to be confirmed. A citation for Oklahoma is incorrect: it was based on a single, poorly preserved, 19.2-mm SL specimen from Cave Spring, Ottawa County. Mayden and Cross (1983) reidentified the original specimen, and one specimen collected later from the same locality, as *Amblyopsis rosae*, the Ozark cavefish. Their identification was based on six characters utilized to distinguish *Typhlichthys* from *Amblyopsis*. Appendix 1 contains a list of the known museum specimens of *T. subterraneus*.

Wicks and Noltie (1998) and Noltie and Wicks (2001) described the geological setting in which this species is found. They concluded that the southern cavefish resides at considerable depths (mostly between 175 and 240 m below the land surface). This is further supported by an observation reported by Schubert *et al*. (1993) of “gas bubble disease” or “decompression sickness” in *T. subterraneus* collected from a Missouri spring. Mohr and Poulson (1966) also reported this species from deep subterranean channels during a drilling operation. This makes this species an almost phreatic species and implies that sightings at caves just represent but a minimal sample of the actual population. In fact, at least 13 out of the 124 specimen-based museum records for this species (including one in Arkansas) are from wells (Appendix 1).

Given the above, it is not surprising that estimations of population sizes based on sightings at cave, yield very low numbers: usually fewer than 150 individuals. The population in Wayne County, Missouri, has been estimated to be at least 90 individuals (Pflieger 1997). Tryon (1971) asserted that when visible, most amblyopsid cave fish populations consist of 2-3 observable individuals for total populations of about 70 individuals in caves. Yet, accidents such as a contaminant spill at Maramec Spring, Missouri, caused nearly a thousand of individuals to exit another Missouri site (Crunkilton 1985). This suggests that populations for a particular phreatic area may be much higher than what one can assume based only on cave counts.

3. Materials and methods

We tried to obtain information on every single record for T. subterraneus in the state of Arkansas. Data were compiled from both scientific and nontechnical literature, from collections of museums and similar institutions, and from unpublished sightings by reliable observers, including those using photographs or videotape recordings. We include in this compilation only those reports from scientific publications and popular accounts that provide sufficient information, such as clear descriptions, videorecordings or photographs, to permit unambiguous species identification. Original sources were used wherever possible. All unpublished material has been deposited in the libraries of one of us (Romero). We tried to verify independently the identification of every specimen in museum collections. Appendix 1 shows both the list of specimens and institutions from which specimens were studied for Arkansas, their abbreviations and locations. We also visited every single locality for which there had been a record of the southern cavefish at least once and interviewed all people who claim to have first hand knowledge of this fish for that locality. Visits to the caves took place during 2006 and 2007 (see Table 2). Every single body of water that might support individuals of the southern cavefish was visited. Each one of those pools and/or streams was observed by two or more observers for at least 30 min. During that time we used flashlights, infrared goggles (Bushnell) and a digital video camera recorder with infrared capabilities (SONY Wide LCD, 3.0 mega pixels). We also look for any signs of human disturbance that may affect the quality of the underground water in the areas adjacent to the caves visited. During this study some evidence suggested that the *Typhlichthys* found at Ennis and Alexander caves may represent a new species. Until such an possibility can be confirmed though the publication of the data in a peer-reviewed journal, we will treat those specimens as *T. subterraneus*.

4. Results: presence and status in Arkansas

4.1. Confirmed localities for *T. subterraneus* in Arkansas

4.1.1. Fulton County: Richardson Cave

This cave also known as Martin Cave has been visited four times in search for *T. subterraneus*. On 3 February 1979 a team from Arkansas State University (ASU) visited and collected one specimen and saw 20 more. The specimen collected is at the fish collection of ASU under the catalog number ASUMZ 9064. The same team visited this cave a week later and could not see a single individual (Paige *et al*. 1981). Dunivan *et al*. (1982) reported one individual. We visited this cave twice, on 22 and 23 of June 2007. The second of this visit consisted in a reconnaissance of the entire cave including all of its bodies of water. No fish were seen.

4.1.2. Randolph County: "A well"

This is one of the most mysterious records for *T. subterraneus* in Arkansas. This is based on at least one specimen at the Museum of Zoology of the University of Michigan (UMMZ 133844). According to the letter accompanying the two specimens that were sent to that institution, these specimens were collected "from a well, Randolph County., Arkansas, collected in the spring of 1940". Since the letter requested that one of the specimens be returned to the sender, that might explain why despite the fact that two were sent to the museum, only one is in its collection. The letter was signed by Byron C. Marshall, the proprietor of a company called Ozark Biological Laboratories. According to Douglas W. Nelson, Collection manager of the Fish Division of the Museum of Zoology at the University of Michigan (UMMZ), that company might have been in the business of supplying schools with specimens. The specimen was sent to the then curator of the UMMZ, Carl Hubbs, in November 1940. Two specimens were received. Their standard length was 53 and 58 mm respectively. The smallest one was returned to Marshall who did not want to divulge the locality of the well where these specimens were collected.

D.W. Nelson (pers. comm.) reported to us that "Interestingly, Marshall had previously sent to Hubbs two specimens (of 4 total) of *Amblyopsis rosae* from a collection that he had made in Downer's Cave, Sarcoxie, Missouri. Marshall wanted these returned, and they were returned to him in November 1935. Marshall had been sending most of his material to Barton Bean at the

USNM. Bean may have become tired of doing this guy's work for him and referred him to Hubbs (just speculation on my part: I have not seen the correspondence)." Therefore we may never learn what is the exact location for this (these) specimen(s). We visited Pocahontas on 18 June 2007 and interviewed several local officials and archivists. Nobody has heard of any well from which blind/depigmented fish have been pumped out.

4.1.3. Stone County: Alexander Cave

Also known as Castle Cave, this is the location in which *T. subterraneus* has been seen more frequently and in larger numbers for the state of Arkansas. This cave is connected with a water resurgence known as Clark Spring that has been explored in several occasions using SCUBA equipment and that has resulted in several sightings including some recorded on video tape. The first recorded sighting is from 1975 by Mr. Tim Ernst (in Robinson and Buchanan 1988, Harvey 1975). Other visits are detailed in Table 1. The largest numbers of individuals are seen when scuba diving through Clark Spring and the sightings at the pool location in the cave produces numbers between two and five. We spoke to the steward of this cave, Mr. R.C. Schroeder, and he told us that every time he visits the cave he sees at least two individuals at the pool.

4.1.4. Stone County: Ennis Cave

This is the most recent locality record for *Typhlichthys* in Arkansas. This cave is owned by the Rose Family and the steward is Mr. Tim McClain. This is a gated cave with a perennial stream where the fish can be seen. Ennis Cave was visited by G.O. Graening, D. Fenolio and E. Corfey on 7 May 2004 and collected an individual (Graening *et al*. 2005). We visited it on 26 May 2007 and observed one fish which quickly disappeared through a crevice.

4.2. Unconfirmed sites for "cave fishes" nearby the above-referenced localities

There have been a number of claims of cavefishes in the areas nearby to confirmed localities for the southern cavefish. Some of those are described below.

4.2.1. Baxter County: Riley's Springbox

This as a location for a subterranean fish that has yet to be confirmed. The landowner of the location is Mr. Chuck Riley, who claims having seen fish pumped out of a well in his property in 2001.

4.2.2. Sharp County: Cave City Cave/Crystal River Cave

This location was visited by Graening and others based on some rumors that there might be some cavefishes in there. They saw none.

4.2.3. Stone County: Cave River Cave

Graening and Brown reported (2000) rumors about cavefishes in this cave.

Table 2. Confirmed localities of *T. subterraneus* in Arkansas.

County	Location	Date	# of individuals	Source
Fulton	Richardson Cave	3 Feb. 1979	21	Paige *et al*. 1981
	Richardson Cave	10 Feb. 1979	0	Paige *et al*. 1981
	Richardson Cave	1982	1	Dunivan *et al*. 1982
	Richardson Cave	22-23 June 2007	0	This report
Randolph	A well	1940	2	Woods and Inger 1975
Stone	Alexander Cave	1975	20	T. Ernst in Robison and Buchanan 1988
	Alexander Cave	14 Dec. 2002	3	G.O. Graening, per. comm.
	Alexander Cave	22 May 2004	4	M. Slay, pers. comm.
	Alexander Cave/ Clark Spring	1975	3	Harvey 1975
	Alexander Cave/ Clark Spring	1999	23	T. Ernst (SCUBA)
	Alexander Cave/ Clark Spring	13 Aug. 2005	5	D. Kampwerth, pers. comm.
	Alexander Cave/ Clark Spring	27 Jan. 2001	0	Graening *et al*. 2001
	Alexander Cave	28 Oct. 2006	2	This report
	Ennis Cave	7 May 2004	1	G.O. Graening, pers. comm.
	Ennis Cave	26 May 2007	1	This report

Table 3. Some unconfirmed reports of cave fishes in localities nearby confirmed ones for *T. subterraneus* in Arkansas. None of those sightings could be confirmed by the authors of this report. See also Figure 5.

County	Location	Date	Source
Baxter	Riley’s Springbox	1 Jan. 1996	Chuck Riley, G.O. Graening
Sharp	Cave City Cave (=Crystal River Cave)	1950	Graening et al. 2005
Stone	Cave River Cave		Graening and Brown, 2000

5. Conservation status

T. subterraneus is classified as Vulnerable (VU D2) by the World Conservation Monitoring Centre (WCMC) (Romero 1998b). Global Rank: G4 apparently secure. The global rank of G4 is usually assigned to species that have been recorded from more than 100 localities. Although this species is known from sufficient localities to merit the rank of G4, its position in cave ecosystems as a predator suggests a lower (G3) rank.

Its protection status in states of the United States is as follows: Alabama: Threatened, Protected; Arkansas: Inventory Element; Georgia: Rare; Indiana: Endangered; Kentucky: Special concern; Missouri: Watch List; Oklahoma: Extirpated; Tennessee: Deemed in need of management (Noltie and Wicks 2001). The Missouri State Rank: S2/S3 imperiled/vulnerable; The state rank of S2 is typically assigned to species that have been recorded from between 6-20 localities. The state rank of S3 is assigned to species that have been recorded from between 21-100 localities.

The actual conservation status of this species in Arkansas is unknown. Harvey (1975) considered at that time *T. subterraneus* that was not really endangered because the only locality known there besides the unnamed well was Alexander Cave that was well protected.

Recent genetic and morphological analyses in progress suggest that the populations for Alexander and Ennis caves may represent a new species of *Typhlichthys* (Romero *et al*. in prep., Fig. 3).

6. Potential threats

Potential threats to aquatic hypogean fauna in general and cave fishes in particular has been listed by Keith (1988). Below we present a modified version of that list:

6.1. Water quantity: changes in the hydrological regime due to impoundments, quarrying, welling and/or water extraction.

6.2. Water quality:
6.2.1. Groundwater pollution by agrochemicals, sewage, accidental spills of hazardous materials, oil and/or gas exploration/exploitation, and intentional dumping of hazardous waste into sinkholes and sinking streams.
6.2.2. Sedimentation and runoff as a consequence of farming activities, logging and/or deforestation, road and building construction (urbanization) as well as runoff and erosion from rainfall.
6.3. Overcollecting
6.4. Cave habitat alteration to either facilitate recreational activities and/or gating that may prevent bats and other fauna to come into the cave and carry with them potential nutrients.

During our visits to the three known localities for *Typhlichthys* in Arkansas, we did not observe any of the above-ground anthropogenic activities listed above. That does not mean that there is no potential for environmental impacts in the future due to water pollution in the recharge areas of surrounding areas (Aley and Aley 1997). Such an occurrence can cause a large mortality event as the one that took place in November of 1981 when about 1,000 southern cavefish individuals and ca. 10,000 Salem cave crayfish (*Cambarus hubrichti*) were killed in Meramec Spring, Missouri, because of a fertilizer pipeline failure that released 80,000 L of liquid ammonium nitrate (Crunkilton 1982, 1985, Weaver 1992).

T. subterraneus appear to be a fish found deep into the karst system and that leads us to make a number of considerations regarding its current status and conservation outlook for this species. In the first place, despite the fact that the counts for this fish are very low, that might be due fact that both springs and caves that can be visited by humans may represent fringe habitats for this species that seems to be more abundant in phreatic layers of its range (see p. 9 of this report). Wicks and Noltie (2001) suggested that because the depth at which these fish are found, rainfall must reach the layers where the fish in found after achieving at least certain degree of thermal and chemical stability which, in turn, makes the physico-chemical environment of the southern cavefish more stable. By the same token, these authors argued, larger chemical spills or sedimentation events into smaller bodies of water would likely generate greater below-ground concentrations than would the dilution of smaller spills or sedimentation events into larger streams.

Access to all three caves (Alexander, Richardson, and Ennis) is controlled in varying degrees by the cave owners by gating (Alexander), constant human presence (Ennis) or special permit (Richardson). The Clark Spring entrance to Alexander Cave can only be utilized under special permit by the U.S. Forest Service since that entrance is on land under the management of that federal agency[1]. That spring can only be penetrated using SCUBA equipment and because of its characteristics only experienced scuba divers can explore it. Therefore overcollecting does not seem to be a concern. Only Alexander and Ennis caves had been slightly altered by the owners in order to facilitate exploration and that is only at certain points. None of those alterations are at/or seem to have impacted the bodies of water where the fish could be found.

7. Conclusions: recommendations

We found no indication that the populations of Typhlichthys in Arkansas are in need of urgent actions by either federal or state agencies and/or private individuals. Yet, because of the potential impact that some activities may have in the future, we recommend to maintain a periodic monitoring of the anthropogenic activities listed above that may impact the conservation status of those populations.

Also, if the existence of a new species of *Typhlichthys* in Arkansas is confirmed, a recommendation for giving it a federally protected status needs to be seriously considered given the very few locations where it has been recorded and its low population numbers.

8. Acknowledgements

Danté Fenolio, Department of Biology, University of Miami, Coral Gables, FL, provided the color picture of a *Typhlichthys* on the cover of this report. G.O. Graening, The Nature Conservancy Arkansas Field Office, provided very valuable first hand information collected by him from his field work in Arkansas. David Kampwerth from the U.S. Fish and Wildlife Service

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[1] Mr. James Whalen from the USDA Forest Service required us to have a permit to even observe the fish and to go in to the Alexander Cave via Clark Spring.

provided useful information an encouragement. Bob Koch provided us with an underwater video of *Typhlichthys* in Clark Spring. Ron Lather took us to the pool at Ennis Cave where fish have been reported. Douglas W. Nelson, Collections Manager, Fish Division, Museum of Zoology, provided information about the specimen of *T. subterraneus* from a well in Randolph County deposited at the University of Michigan. Matt Niemiller, Department of Ecology and Evolutionary Biology, University of Tennessee, provided with the provisional phylogenetic tree for the Amblyopsids of North America. Randy Rose, owner of Ennis Cave, provided us with access to Ennis Cave. R.C. Schroeder, Association of Arkansas Cave Studies, served as a guide during our visit to Alexander Cave. Stanley Trauth, Department of Biological Sciences, Arkansas State University, produced the scanning electron microscope photograph. James Whalen, United States Department of Agriculture Forest Service, provided us with the permit to even observe the fish and visit Alexander Cave via Clark Spring. Darrel Williams, owner of Richardson Cave, allowed us to visit that cave.

Figure 1. Drawings of (a) *Chologaster cornuta*, (b) *Forbesichthys* agassizii, (c) *Typhlichthys subterraneus*, (d) *Amblyopsis rosae*, (e) *Amblyopsis spelaea*, and (f) *Speoplatyrhinus poulsoni*. Drawing by John Ellis.

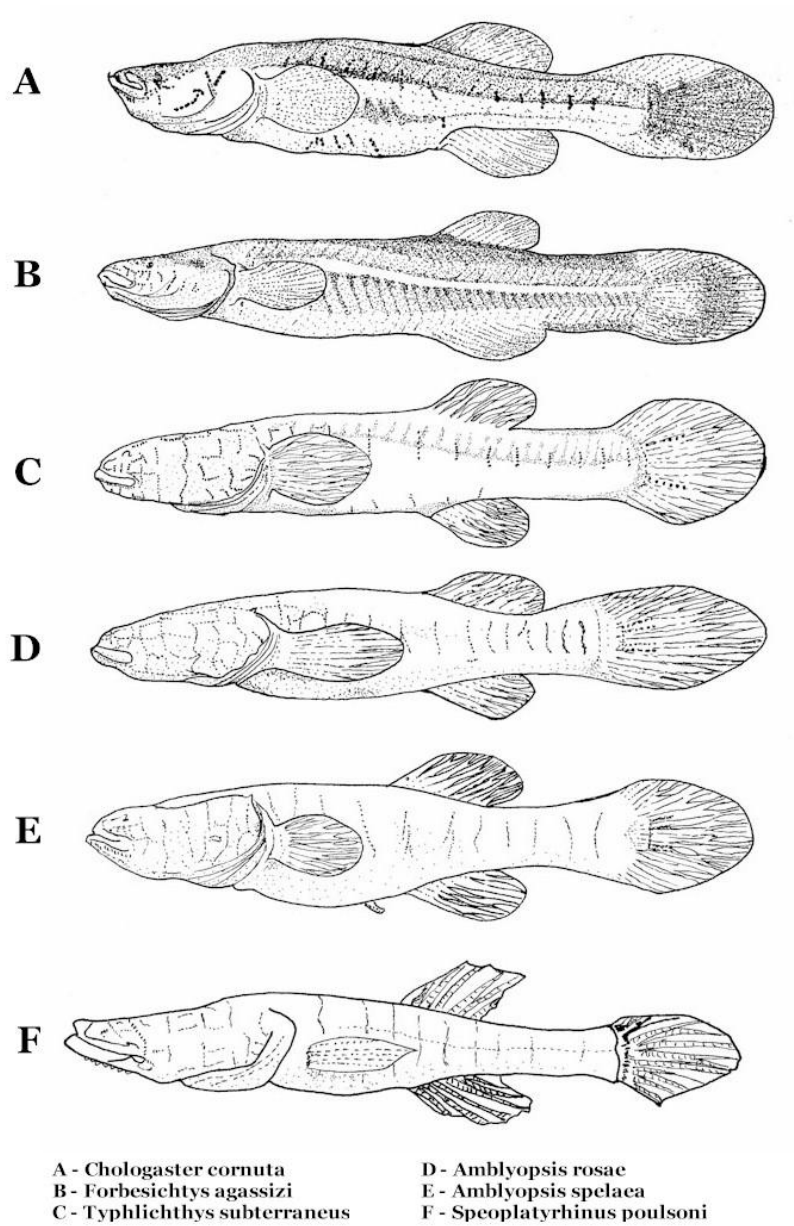


Figure 2. Distribution of the species of the family Amblyopsidae.

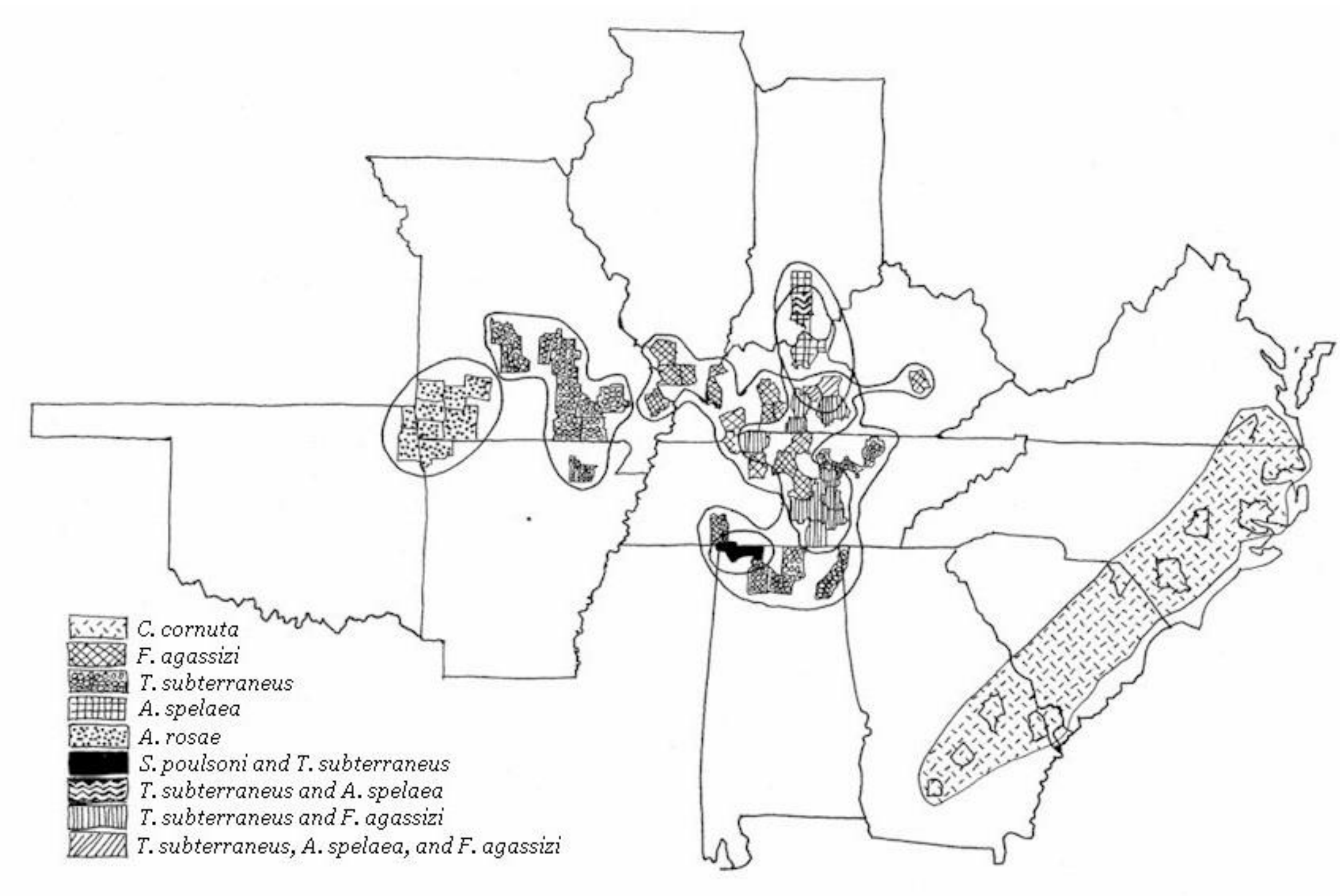
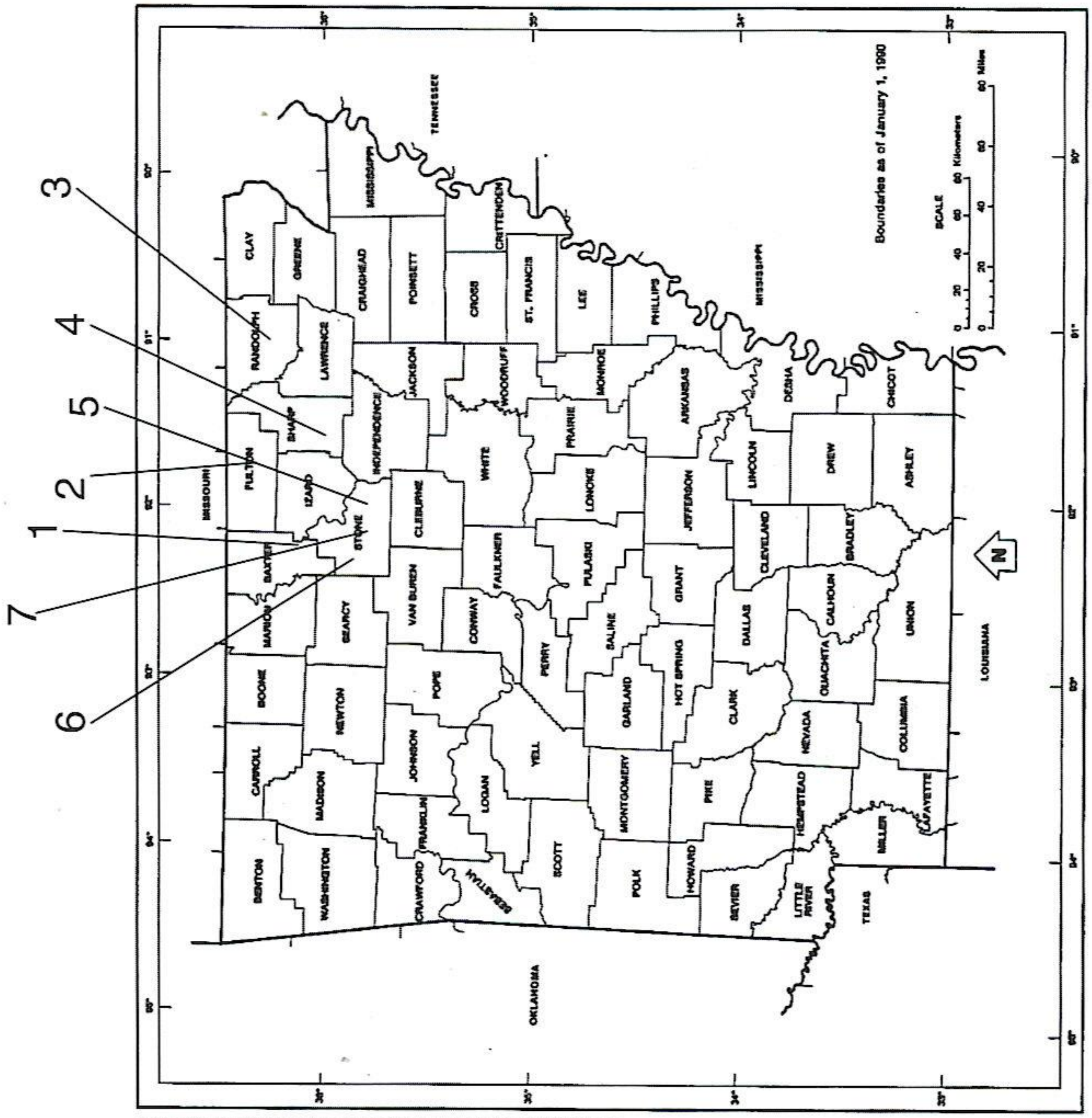


Figure 3. Preliminary ND2 phylogeny of the family Amblyopsidae (Courtesy of M. Niemiller)

Figure 4. Photograph using a scanning electron microscope of the head region of a specimen of *Typhlichthys* from Ennis Cave, Arkansas. Photograph by Stanley Trauth.

Figure 5. Map of localities in Arkansas mentioned in the text. Legend: 1. Riley's Spring Box, Baxter County, AR. UTM NAD83 Northing=3999789, Easting=564759; 2. Richardson's Cave, Fulton County, AR. UTM NAD83 Northing=4017654, Easting=614218; 3. A well, Randolph County, AR. Coordinates unknown; 4. Cave City Cave, Sharp County, AR. UTM NAD83 Northing=3978593, Easting=630803; 5. Alexander Cave, Stone County, AR. UTM NAD83 Northing=3978765, Easting=563827; 6. Ennis Cave, Stone County, AR. UTM NAD83 Northing=3969290, Easting=602194; 7. Cave River Cave, Stone County, AR. UTM NAD83 Northing=3974286, Easting=590875. Only localities 2, 3, 5,and 6 have had confirmed presence of the southern cavefish.

Counties


1
2
3
4
5
6
7
Boundaries as of January 1, 1990
SCALE
TENNESSEE
MISSOURI
MISSISSIPPI
LOUISIANA
TEXAS
OKLAHOMA
CLAY
GREENE
RANDOLPH
LAWRENCE
SHARP
FULTON
IZARD
BAXTER
STONE
MARION
BOONE
CARROLL
BENTON
WASHINGTON
MADISON
NEWTON
SEARCY
VAN BUREN
CLEBURNE
INDEPENDENCE
JACKSON
CRAIGHEAD
POINSETT
MISSISSIPPI
CRITTENDEN
CROSS
ST. FRANCIS
LEE
PHILLIPS
MONROE
WOODRUFF
WHITE
PRAIRIE
LONOKE
ARKANSAS
DESHA
CHICOT
JEFFERSON
LINCOLN
DREW
ASHLEY
CLEVELAND
BRADLEY
GRANT
PULASKI
FAULKNER
CONWAY
POPE
JOHNSON
FRANKLIN
CRAWFORD
SEBASTIAN
LOGAN
YELL
PERRY
SALINE
GARLAND
HOT SPRING
DALLAS
CALHOUN
OUACHITA
UNION
CLARK
MONTGOMERY
SCOTT
POLK
PIKE
HOWARD
SEVIER
LITTLE RIVER
HEMPSTEAD
NEVADA
COLUMBIA
LAFAYETTE
MILLER

Appendix 1. Known museum specimens of *T. subterraneus*. Yellow background is for specimens from Arkansas. Blue backgrounds is for specimens obtained from wells.

Catalog Number	# specimens	State	County	Locality	Collection Year
AMNH 8103		KY	Edmonson	Mammoth Cave	?
AMNH 18715		KY	Edmonson	Mammoth Cave	1903
AMNH 18176		KY	Hart	Horse Cave	?
AMNH 22693		?	?	?	?
ANSP 148699	1	KY	Barren	Mitchell's Cave	1937
ASUMZ 9064		AR	Fulton	Richardson's Cave	1979
AUM 2067	4	AL	DeKalb	Sell's Cave 3 mi W of Collinsville; T9S R7E S4	?
AUM 16045	2	KY		Green or Barren R.	?
AUM 35501	1 & 2	AL	Madison	Hering Cave, 4 mi NE Owens Crossroads, T5S R2E S10NE	?
BMNH 1898.30.31.19-21	3	MO	Jasper	Day's Cave	1898
BMNH 1898.30.31.22-23	2	MO	Jasper	Day's Cave	1898
CAS 78370	1	KY	Hart	Horse Cave	1902
CAS 91980	4	KY	Hart	Horse Cave	1902
CAS 91981	1	KY	Hart	Horse Cave	1902
CAS 91982	2	KY	Hart	Horse Cave	?
CAS 91983	1	KY	Edmonson	Mitchell's Cave	1902
CAS 91984	1	KY	Edmonson	Mitchell's Cave	1902
CAS 91985	1	KY	Edmonson	Cave City	1900
CAS 91986	1	KY	Edmonson	Cave City, Glasgow or Mammoth Cave	?

CAS 91987	1	KY		Small cave near Mammoth Cave	1873
CAS 91988	1	IN	Harrison	Corydon	?
CAS/SU 103928	1	IN		Cave near Wyandotte	?
CAS/SU 1310	2	IN	Harrison	D.S. Jordan	?
CAS/SU 25283	1	KY	Edmonson	Mammoth Cave, River Styx	1905
CMNH 62046		KY	Hart	?	?
CMNH 62325		TN	Grundy	?	?
CU 21726	2	KY	Edmonson	Mammoth Cave	1952
CU 32901	2	MO	Camden	River Cave at Hahatonka Spring	1952
FMNH 3871	2	KY	Hart	Horse Cave	1900
FMNH 62046	10 & 4	KY	Hart	Hidden River Cave, Horse Cave	1905
FMNH 62047	2	KY	Edmonson	Floyd Collins Crystal Cave	?
FMNH 62048	3	TN		Crystal Cave near Wonder Cave, 5 miles from Monteagle near Rt. 41."	1956
FMNH 62050	1	KY	Edmonson	Mammoth Cave	1905
FMNH 62051	6	KY		Mammoth Cave National Park, Stillhouse Hollow Cave	1905
FMNH 62052	1	TN		Sink Hole Cave near Wonder Cave	1905
FMNH 62053	1	TN	Coffee	Blowing Spring	1905
FMNH 62054	3	TN	Coffee	Blowing Spring	1905
FMNH 62055	3	TN	Coffee	Blowing Spring Cave, 3.5 miles N. of Pelham	1905
FMNH 62056	8	TN	Coffee	Sink Hole Cave near Wonder Cave, or Blowing Spring	1905
FMNH 62325	5	TN	Coffee	Sink Hole Cave near Wonder Cave	1905
FMNH 86306	2			?	?
INHS 50142		TN	Putnam	?	1965
INHS 60575					?

INHS 60576		AL	Madison		1964
KU 3210	1	OK	Ottawa	Peoria: Cave Spring: 2 mi. W and 1 mi. S. Peoria	1954
KU 12853	1	KY	Edmonson	?	1934
KU 14007	1	OK	Ottawa	Cave Springs Creek, 2 mi. W and 1 mi. S Peoria	1966
NLU 9659	1	TN	Montgomery	Cave on Austin Peay State College Farm	?
NLU 15000		AR	Randolph		
NLU 24998	3	TN	Montgomery	Cave on Austin Peay State College Farm, Clarksville	1972
NLU 28534		TN	Decatur		?
NLU 28535		TN	Decatur		?
NLU 28536	1	TN	Decatur	Baugus Cave, Hwy 69, 1.5 mi N of Jeannette	1973
NLU 28537	1	TN	Perry	Blowing Cave #1, 5 mi. E of Hwy 50, near Breadstown	1973
NLU 28538	1	TN	Perry	Blowing Cave #1, 5 mi. E of Hwy 50, near Breadstown	1973
NLU 28539	1	TN	Hickman	Cave Branch Cave on Caine Cr. Rd., N of Linden	1973
NLU 28540		TN	Hickman		?
NLU 28541	1	TN	Decatur	Stewman Cr. Cave, 3/4 mi. W of Pleasant Grove	1973
NLU 28542	1	TN	Decatur	Stewman Cr. Cave, 3/4 mi. W of Pleasant Grove	1973
NLU 28543	1	TN	Lewis	Blowing Cave 1.25 mi. S of Riverside	1973
NLU 28544	1	TN	Perry	Cave, 3.1 mi. from Jct. of TN Hwy. 13 & Lost Cr. Rd.	1973
NLU 25545	1	TN	Perry	Cave, 2.7 mi. from Jct. of TN Hwy13 & Lost Cr. Rd.	1973
NLU 28546	1	TN	Montgomery	Dunbar Cave, E. edge of Clarksville, Country Club	1973
NLU 28547	1	TN	Montgomery	Dunbar Cave, E. edge of Clarksville, Country Club	1973
MCZ 780	7	KY	Edmonson	Mammoth Cave	1859
MCZ 781	1	AL	Lawrence	Moulton	?
MCZ 782	1	TN	Wilson	Lebanon	b. 1855
MCZ 27585	8	MO	Jasper	Wilson's Cave, Sarcoxie	b. 1889

MCZ 27586	3	MO	Jasper	From a well in Sarcoxie	b. 1889
MCZ 27587	1	MO	Jasper	From brook outside of Wilson's Cave near Sarcoxie; 50 feet from entrance to cave.	b. 1889
MCZ 35058	1	KY	Edmonson	Mammoth Cave	1859
TMNH 22766	11	AL	Madison		?
TMNH 22765	39	AL	Madison		?
TMNH 19381	34	AL	Madison		?
TMNH 16675	1	AL	Morgan		?
TMNH 16679	2	AL	Morgan		?
TMNH 6268	1	KY	Edmundson		?
UAIC 656.01	8	AL	De Kalb	Sells Cave, 3 mi W of Collinsville	1959
UAIC 1052.02	4	TN	Smith	Taylor Farm Cave at Beasley Bend on Cumberland River near Rome, 8 mi W of Caruhage on US Hwy 70 N	1963
UAIC 1053.01	4	TN	Smith	Seay-White Farm Cave, 3 mi S of Rome on Flat Rock Road, tributary to Lick Creek	1963
UAIC 1977.01	2	TN	Grundy	Crystal Cave complex, underground streams 0.5 mi from Wonder Cave attraction	1966
UAIC 1999.01	11	AL	Madison	Shelta Cave, underground lake between Cave and Link Avenues in Huntsville	1967
UAIC 2148.01	3	AL	Madison	Shelta Cave, underground lake between Cave and Link Avenues in Huntsville	1964
UAIC 3958.01	5	TN	Grundy	Wonder Cave (Crystal Cave) near Pelham	1963
UAIC 3959.01	3	KY	Warren	Friendship Cave, 1 mi NW of Allen, near Scottsville	1949
UAIC 4134.01	3	AL	Madison	Hering Cave (Flint River); No. Al-6, Geological Survey of Alabama Circular 52	1974
UAIC 10863.01	3	AL	Madison	Matthews Cave (AL 23) next to I-565 on Redstone Arsenal	1993
UAIC 11124.01	0	AL	Lauderdale	Key Cave, 6.5 mi SW of Florence	1995
UAIC 12302.01	0	AL	Colbert	McKinney Pit Cave, 1.7 mi E of Pride	1999

UAIC 12313.01	0	AL	Lauderdale	Davis Bat Cave, 1 mi NW of Thortontown	1999
UMMZ 103473	1	AL	Madison	Shelta Cave, near Huntsville	1936
UMMZ 144606	1	AL	Madison	Cave Spring Cave, N of New Hope	1939
UMMZ 146990	3	AL	Madison	Pond in Shelta Cave, 2 mi N of Huntsville	1939
UMMZ 133844	1	AR	Randolph	Well, Randolph Co.	?
UMMZ 157008	2	KY	Edmonson	Mitchell's Cave, Glasgow	?
UMMZ 88027	1	KY	Hart	Hidden River Cave, at Horse Cave	1929
UMMZ 156795	1	MO	Camden	River Cave near Hahatonka; Osage River Dr	1930
UMMZ 156796	6	MO	Camden	River Cave near Hahatonka; Osage River Dr	1930
UMMZ 150421	1	MO	Laclede	Bennett Spring source, at Bennett State Park	1939
UMMZ 136379	4	MO	Shannon	Welch's Cave, on Current River	1941
UMMZ 105667	3	TN	Hardin	Cave near Dry Creek	1938
UMMZ 196194	1	TN	Montgomery	Austin Peay Pit Cave, Austin Peay University Farm	1972
UMMZ 174850	2	TN	Putnam	Cave 1 mi N of Monterey-Sparta Hwy., on farm; Tennessee River Dr	1953
UMMZ 133264	2	TN	Rutherford	Well in Murfreesboro (E Castle St.) - Lee Jenkin’s well	?
UMMZ 133544	2	TN	Rutherford	Well in Murfreesboro (E Castle St.) - Lee Jenkin's well	1941
UMMZ 103552	1	TN	Wilson	Well at Lebanon	1937
USNM 0008563	3	KY		Well near Bowling Green	?
USNM 0036632	4	KY	Hart	Mammoth Cave	1884
USNM 0036806	1	KY	Hart	Mammoth Cave	1884
USNM 0045490	6	KY		Mitchell's Cave	1894
USNM 0089417	1	AL	Lauderdale	Well owned by A.V. Pallerson	1929
USNM 0091585	1	AL	Lauderdale	Well at Hines	1930
USNM 0092298	1	AL	Lauderdale	Well at Hines	1932

USNM 0093518	1	AL	Lauderdale	Well at Hines	1934
USNM 0101172	3	KY		Mammoth Cave: Roaring River	?
USNM 0101501	1	AL	Lauderdale	Well at Hines	1935
USNM 0109468	1	AL	Lauderdale	Well near Florence	1939
USNM 0162700	3	MO	Shannon	Eminence, 150 mi. E. of Springfield, deep well	1952
USNM 0175248	3	AL	Madison	Huntsville, 500 yds., NNE of intersection of Pulaski Pike & Oakwood Rd., LGE. Lake in cave	1957
USNM 0199401	1	MO		Welch Cave, 27 mi. SW of Salem, 1 mi. of Akers	1965
USNM 0232538	8	TN	Putnam	Cave 1.8 mi. SW of Calfkiller School in the floor of long hollow at an elevation of 950 ft.	1969
YPM 8000	1	AL	Madison	Huntsville	?
ZMUC * 2		KY		Mammoth Cave	1879
ZMUC * 3		KY		Mammoth Cave	1879

Museum acronyms

AMNH: American Museum of Natural History (New York)
ANSP: Academy of Natural Sciences of Philadelphia
ASUMZ: Arkansas State University Museum of Zoology (Jonesboro)
AUM: Auburn University Museum (Alabama)
BMNH: British Museum of Natural History (London)
CAS: California Academy of Sciences (San Francisco)
CMNH: Cleveland Museum of Natural History)Ohio)
CUMV: Cornell University Museum of Vertebrates (Ithaca)
FMNH: Field Museum of Natural History (Chicago)
INHS: Illinois Natural History Survey (Champaign)
KU: Kansas University (Lawrence)
MCZ: Museum of Comparative Zoology, Harvard University (Cambridge, MA)

MNHN: Musée Nationale d'Histoire Naturelle (Paris)
NLU: Northeast Louisiana University (Monroe)
UAIC: University of Alabama Ichthyological Collection (Tuscaloosa)
UMMZ: University of Michigan Museum of Zoology (Ann Arbor)
USNM: United States National Museum (Washington, DC)
YPM: Yale Peabody Museum, Yale University (New Heaven, CT)
ZMUC: Zoological Museum of the University of Copenhagen (Denmark)

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