Status and Distribution of the Amblyopsid Fishes *Forbesichthys agassizii* and *Typhlichthys subterraneus* in Tennessee

Final Report

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Summary

The amblyopsid cavefishes are a small family of specialized fishes endemic to eastern North America. Although this family has been known to science since the 1840s, we still no little about the demography, life history, conservation, and genetics of most of the six recognized species in the family. Two species of amblyopsids occur in Tennessee: the Spring Cavefish (*F. agassizii*), which inhabits caves and springs within the Highland Rim of the Tennessee River drainage and the middle and lower Cumberland River drainage, and the obligate cave-dwelling Southern Cavefish (*T. subterraneus*) which has a discontinuous distribution throughout the karst regions of the Highland Rim, Central Basin, and the western escarpment and southern sections of the eastern escarpment of the Cumberland Plateau. Currently, the Spring Cavefish is listed as "Apparently Secure" (S4) in Tennessee; whereas the Southern Cavefish is listed as "Vulnerable" (S3) by NatureServe. The Southern Cavefish is also designated as "Deemed in Need of Management" by state agencies. The primary objective of this study was to determine the status and distribution of historic and putative localities of the Southern Cavefish and Spring Cavefish in Tennessee. Moreover, we provide information on relative abundance, demography, habitat requirements, and aspects of life history for both species. Lastly, we discuss the intra- and interspecific genetic relationships of both species.

We surveyed for Southern Cavefish and Spring Cavefish from May 2004 through June 2009 in caves, springs, and spring-fed streams and ponds throughout the Interior Low Plateau of central Tennessee. This includes surveys conducted from May 2004 through June 2006 conducted by Miller and Niemiller (2005, 2007). In addition to acquiring data on ecology and life history, we collected voucher specimens or tissue samples for phylogenetic analyses. Within Tennessee, Southern Cavefish are found in 107 caves, two springs, and one well in 26 counties. The distribution extends throughout much of the Interior Low Plateau in the central part of the state from the Tennessee River in west Tennessee east to the Cumberland Plateau. The distribution of the Southern Cavefish includes 17 HUC8 Watersheds in Tennessee. Spring Cavefish have been reported primarily from surface streams and springs in 13 counties in Tennessee throughout the Eastern Highland Rim, Western Pennyroyal Karst, and portions of the Western Highland Rim of the Interior Low Plateau in the central part of the state. Spring Cavefish also have been reported from the Nashville Basin in Bedford, Davidson, and Wilson counties. The distribution of the Spring Cavefish includes nine HUC8 Watersheds in Tennessee.

Because of the extensive distribution of Southern Cavefish and the results of molecular studies of other troglobites, several authors have speculated that this species represents several independent invasions and, therefore, distinct lineages (reviewed in Niemiller and Poulson in press). Our phylogenetic analyses revealed significant genetic divergence and both mitochondrial and nuclear DNA variation was structured among hydrological drainages, supporting the hypothesis that

morphological similarity is the result of parallel evolution rather than significant dispersal and gene flow across major drainage and river divides. There was a clear pattern of correspondence between mtDNA lineages and surface hydrological boundaries with sequence divergence up to 11.6% among lineages. Little evidence of contemporary gene flow was found, particularly among drainages. Each of the watersheds in Tennessee and Alabama should be considered ESUs or at least as demographically separate management units because the lack of haplotype sharing and deep divergences among haplotypes suggest that each drainage harbors a unique and historically significant portion of the evolutionary diversity of *Typhlichthys* and dispersal among drainages is insignificant. Phylogenetic analyses of the Spring Cavefish indicate that populations from Illinois and Tennessee are distinct and likely are isolated from each other. Populations sampled from the Eastern Highland Rim are genetically distinct at multiple loci from Illinois populations, and, therefore, likely represent distinct species. However, samples from the Western Pennyroyal Karst in Tennessee in and around the Clarksville area have not been genetically analyzed; consequently, the taxonomic affinities of these populations remains unknown. Populations sampled across the Upper Elk, Upper Duck, Collins, and Caney Fork watersheds in Tennessee show little divergence, suggesting that populations are more connected that previously thought and these populations should be designated as a single ESU and management unit.

Southern Cavefish and Spring Cavefish populations in Tennessee face a number of threats, including habitat degradation and loss, hydrological manipulations, environmental pollution, overexploitation, and impacts of introduced aquatic animals. However, both species are apparently secure in the state at this time. Southern Cavefish are widespread in Tennessee with reports from 107 caves, two springs, and a well. Several caves are known to contain large populations At this time, we recommend that the status of Southern Cavefish be elevated to S4 (Apparently Secure) in Tennessee, given the large number of occurrences, estimated abundance, and number of apparently protected cave systems occurring on state, federal, or cave conversation organization land. Likewise, Spring Cavefish have been reported from several localities in the state, and several localities, particularly in the Barrens of the Eastern Highland Rim, yield large numbers of fish during surveys. At this time, we do not recommend any change to the status of the Spring Cavefish in Tennessee. However, the status of both species (as currently recognized) should be reevaluated on a regular basis (e.g., 10 year intervals).

Introduction

The Appalachians and Interior Low Plateau support the highest aquatic subterranean biodiversity within the continental United States (Culver et al. 2003). Much of this diversity is the product of independent invasion and isolation of past surface-dwelling populations. Although endemism has resulted in high biodiversity, more than 95% of subterranean fauna in North America are considered vulnerable or imperiled (Culver et al. 2000) due, in large part, to habitat degradation (Elliott 2000; Danielopol et al. 2003; Boulton 2005) and restricted geographic ranges

(Culver et al. 2000; Culver et al. 2003). Unfortunately, the distribution and status of local populations for many species is incomplete or lacking entirely, making conservation and management decisions by federal, state, and local agencies difficult. Here we investigate the distribution, demography, status, and threats to populations of two cave-associated fish species in the family Amblyopsidae in Tennessee: the Spring Cavefish (*Forbesichthys agassizii*) and the Southern Cavefish (*Typhlichthys subterraneus*). Despite widespread distributions throughout the karst regions of middle Tennessee, little is known regarding the demography and ecology of these species.

The Amblyopsidae is a small family of specialized fishes endemic to the unglaciated regions of the eastern United States. The six species (in five genera) in the family represent the transition from epigean to subterranean habitats and are an excellent system to investigate the evolution of troglomorphic characters and speciation in subterranean environments. Four species are obligate subterranean inhabitants that exhibit troglomorphic features (*Typhlichthys subterraneus*, *Amblyopsis rosae*, *A. spelaea*, and *Speoplatyrhinus poulsoni*), whereas *Forbesichthys agassizii* (facultative cavernicole) and *Chologaster cornuta* (epigean) do not. Although fishes from this family have been known to science since the early 1840s, little is known about the demography and persistence of local populations of the subterranean species, systematic relationships among species, or systematic relationships among populations within species.

Two species of amblyopsids occur in Tennessee: the Spring Cavefish (*F. agassizii*, Fig. 1), which inhabits caves and springs within the Highland Rim of the Tennessee River drainage and the middle and lower Cumberland River drainage, and the cave-dwelling Southern Cavefish (*T. subterraneus*, Fig. 2) which has a discontinuous distribution throughout the karst regions of the Highland Rim, Central Basin, and the western escarpment and southern sections of the eastern escarpment of the Cumberland Plateau (Etnier and Starnes 1993). The Spring Cavefish is listed as "Apparently Secure" (S4) in Tennessee by NatureServe (2009) and considered locally abundant in ideal habitats (Etnier and Starnes 1993). The Spring Cavefish is not listed in Tennessee at this time (Withers et al. 2004); however, studies explicitly investigating the distribution and demography of local populations are lacking from Tennessee. Etnier and Starnes (1993) show 25 localities in Tennessee with concentrations in the Barrens region of the Eastern Highland Rim and the Land-between-the-Lakes area on the northern Western Highland Rim.

Conversely, the Southern Cavefish is listed as Vulnerable (S3) in Tennessee (Natureserve 2009) and "Deemed in Need of Management" by state agencies (Withers et al. 2004). Etnier and Starnes (1993) show 27 localities in Tennessee. However, a survey of the literature and unpublished data from the Tennessee Cave Survey and the Tennessee Department of Environment and Conservation, Natural Heritage Program resulted in a list of 78 localities from 25 counties in Tennessee (Table 1). Miller and Niemiller (2005) discovered eight new localities during surveys for the Tennessee Cave Salamander (*Gyrinophilus palleucus*). Despite a nearly three-fold increase in the number of localities from Etnier and Starnes (1993), many of the localities listed are unconfirmed observations by local cavers, and data on number of cavefish observed, seasonal variation in abundance, water conditions, etc. are lacking. Furthermore, because of its large distribution, several researchers have suggested that the Southern Cavefish may actually represent a species complex of morphologically cryptic, but related species. Preliminary genetic evidence supports this hypothesis. Although the species is not considered to be in any immediate danger (Etnier and Starnes 1993), local populations may be threatened by urbanization, groundwater pollution, and silviculture practices in the vicinity of recharge areas (Etnier and Starnes 1993; Aley and Aley 1997). All of the preceding perturbations of habitat are known or suspected to adversely affect populations of other amblyopsid species. Thus, there is a need to document the distribution of the species statewide and to assess demographic parameters of, and threats to, local populations, particularly if genetic and morphometric analyses reveal hidden diversity within the species.

The primary objective of this study is to determine the status and distribution of historic and suspected (based on unconfirmed reports) localities of the Southern Cavefish and Spring Cavefish in Tennessee. Moreover, we provide information on relative abundance, demography, habitat requirements, aspects of life history, conversation, and genetics for both species.

Methods

Surveys. We searched for Southern Cavefish (*T. subterraneus*) and Spring Cavefish (*F. agassizii*) from May 2004 through June 2009 in caves, springs, and spring-fed streams and ponds throughout the Interior Low Plateau of central Tennessee. This includes surveys conducted from May 2004 through June 2006 conducted by Miller and Niemiller (2005, 2007). We conducted surveys during every month of the year, but concentrated searches during periods of favorable stream conditions (i.e., shallow, clear water with little flow). To locate Southern Cavefish, we donned wetsuits and slowly walked along, waded through, or crawled in the cave stream channel and thoroughly scanned the streambed with the beams of our headlamps. We also carefully lifted flat rocks, small cobble, and detritus under which smaller individuals might seek refuge. Lifted rocks were returned to their original positions to minimize habitat disturbance. A similar approach was taken in surface springs, streams, and ponds while surveying for Spring Cavefish. We used large dipnets to search through aquatic vegetation and detritus where Spring Cavefish might seek refuge during the day. We also searched beneath rocks, logs, and other potential cover objects. A tally of each individual found was kept, and a concerted effort was made to capture, with small bait nets, each cavefish encountered. Captured fish were placed in clear plastic bags until their total length (TL) and standard length (SL) were measured to the nearest mm using a small metric rule or digital calipers. Other data were gathered from each captured fish if possible, including sex, condition (e.g., injuries, growths, or presence of parasites), habitat (aquatic: stream pool, stream riffle, rimstone pool; terrestrial: mud bank, bank-cut, crevice), substrate (mud, sand, cobble, gravel, bedrock, organic debris, artificial), cover type (rock, log, crevice, organic debris), and other aspects of life history (diet, behavior, community associates). Additionally, we excised a small tissue sample from the right pectoral fin or caudal fin of one or more cavefish captured at each locality for subsequent genetic analyses.

Phylogenetic analyses. Tissue samples (fin clips) or voucher specimens were collected from 53 Southern Cavefish localities throughout the species' range in Alabama, Arkansas, Georgia, Kentucky, Missouri, and Tennessee (Appendix 1). Additionally, samples of Spring Cavefish from five localities were collected from Illinois, Kentucky, and Tennessee (Appendix 2). Tissue samples or DNA for the other amblyopsid species and eight Southern Cavefish localities were provided by T. Near (Yale University), D. Neely and B. Kuhajda (University of Alabama), and Aldemaro Romero and Ron Johnson (Arkansas State University). Voucher specimens were deposited into the University of Tennessee Ichthyological Collection.

DNA was extracted using standard methods and polymerase chain reaction (PCR) was used to amplify portions of one mitochondrial gene, ~1218bp of NADH dehydrogenase subunit 2 (ND2) including the entire coding region and portions of flanking tRNAs, and one nuclear gene, 820bp of ribosomal protein S7. Sequencing reactions were performed using original PCR primers and run on an ABI Prism 3730 at the Molecular Biology Resource Facility at the University of Tennessee. The Swampfish (*Chologaster cornuta*) and Ozark Cavefish (*Amblyopsis rosae*) were used as outgroups. Sequences were aligned to each other and to outgroup sequences for each locus.

Gene trees were constructed using Bayesian analyses with the ND2 and S7 datasets analyzed separately. The optimal model of sequence evolution for each dataset was determined using Akaike's information criterion (AIC) implemented in MODELTEST 3.7 (Posada and Crandall 1998). Bayesian posterior probabilities were estimated in MRBAYES 3.1 (Ronquist and Huelsenbeck 2003). Two independent runs using four Markov chains and temperature profiles at the default setting of 0.2 were conducted for 10 million generations, sampling every 100th generation. MODELTEST selected different models of sequence evolution for first, second, and third position codons of ND2. Therefore, the ND2 dataset was partitioned accordingly and unlinked allowing values for transition/transversion ratio, proportion of invariable sites and among-site rate heterogeneity to vary across codon positions during analysis. Random trees were used to begin each Markov chain and a molecular clock was not enforced. The first 1.5 million generations were discarded as "burn-in" to ensure stationarity after examination of the posterior probability. Samples from the stationary distribution of trees were used to generate 50% majority-rule consensus trees for each locus.

Results and Discussion

Morphology

All amblyopsids are characterized by possessing (i) a large, flat head and a tubular, non-streamlined body, (ii) an oblique mouth with the lower jaw protruding beyond the upper jaw, (iii) a segmented premaxilla, (iv) jugular position of the anus and urogenital pore, (v) reduced head lateral line canals on the head, and absence of the lateral line canal on the trunk, but the presence of superficial papillae (neuromasts) arranged in distinct rows on the head and body, and papillae of unknown function in 2–4 rows on the caudal fin, (vi) small, embedded cycloid scales except on the head, (vii) six branchiostegal rays, (viii) presence of a swim bladder, (ix) tubular anterior nostrils, (x) absence of pelvic fins (except rudimentary in *A. spelaea*). The troglobitic species also are characterized by (i) lack of externally visible eyes, (ii) reduced pigmentation, (iii) hypertrophy of the superficial lateral line system that includes an extensive system of elevated neuromasts arranged in distinct ridges, (iv) hypertrophy of the semicircular canals and otoliths, and (v) presence of highly-developed caudal sensory papillae. Amblyopsids are also characterized by having dorsal and anal fins that are similar in shape with the dorsal fin origin anterior to the anal fin origin. All fins lack spines, although the first ray in the dorsal fin of the Southern Cavefish has been reported as spinuous by some authors. Dorsal fin rays range 7–12, anal fins 7–11 rays, and pectoral fins 9–12 rays. Pelvic fins are absent except in *A. spelaea* that have 0–6 rays. The caudal fin may be elliptical, lanceolate, or rounded with 9–22 branched rays. No fin rays are branched (except in the Alabama Cavefish). The urogenital pore is positioned just anterior to the anal fin at hatching and migrates anteriad until it occupies a jugular position in adults (Woods and Inger 1957). Southern Cavefish have 7–10 dorsal fin rays, 7–10 anal fin rays, 9–12 pectoral fin rays, and 10–15 caudal fin rays. Pelvic fins are absent. Spring Cavefish have 9–11 dorsal fin rays, 9–11 anal fin rays, 9–11 pectoral fin rays, and 11–17 caudal fin rays. Pelvic fins are absent in both species. Both species cannot be sexed using external morphology. However, Spring Cavefish can be sexed by observing the gonads through the translucent body wall during the breeding season (Weise 1957).

Troglobitic amblyopsids are known for their degenerate eyes. However, Southern Cavefish have the least degenerate eyes of the cave amblyopsids but lack eye muscles, scleral cartilages, and pigment epithelium. However, variation in eye degeneration among populations has yet to be examined. Ontogenetically, the eyes of Southern Cavefish develop to a certain state of tissue differentiation and then become more simplified and degenerate with advancing age (Eigenmann 1897). Older cavefish also have more variable eye development and there is more right to left side variation of eyes in the same individual. Varying degrees of eye degeneration are exhibited in Tennessee populations of Southern Cavefish. The eyes of Spring Cavefish examined by Eigenmann (1897) lack ciliary muscles. Both eye size, optic lobe length, and optic lobe volume is smaller in Spring Cavefish than in the Swampfish (Niemiller and Poulson in press). The eyes and optic lobe of

Southern Cavefish are about 80% and 30% smaller respectively than those of Spring Cavefish.

Contrary to popular belief, Southern Cavefish are not albinos; rather they have distinct, albeit small, melanophores. Live Southern Cavefish from across the range appear white to pearly opalescent and in high-resolution photographs outlines of the tiny embedded scales are visible as slightly grayer in color (Figs. 2 and 3). Several populations in Kentucky and Tennessee have visible blobs of fat around the vestigial eyes (Fig. 2); whereas others lack adipose tissue around the vestigial eyes (Fig. 3). Southern Cavefish examined have comparable densities of melanophores to Swampfish but have much smaller surface areas of each pigmented melanophore, such that adjacent melanophores rarely overlap (Niemiller and Poulson in press). In preserved specimens, the visible melanophores are concentrated along the dorsal myomere borders and scattered elsewhere on the body. Melanophores are not visible on the cheek. In the laboratory, Southern Cavefish exhibit increased pigmentation with exposure to light (Woods and Inger 1957; Niemiller and Poulson in press). Spring Cavefish are dull brown dorsally to slightly lighter ventrally (Fig. 1) with only one kind of chromatophore–melanophores. Spring Cavefish have poorly defined stripes, one mid-dorsal and two lateral, and slightly more melanophores along the edges of the myomeres than elsewhere.

Distribution – Southern Cavefish

The Southern Cavefish is the most widely distributed troglobitic fish in North America. Its range is discontinuous and divided into two main parts east and west of the Mississippi River: an eastern component that extends along the Cumberland Plateau and through Interior Low Plateau from central Kentucky (Mammoth Cave region) southward into central Tennessee, northern Alabama, and northwestern Georgia, and a western component that includes the Ozark Plateau of central and southeastern Missouri and northeastern Arkansas (Fig. 4). The distribution of the species was once thought to include southern Indiana and northeastern Oklahoma. These records are now thought to be erroneous.

Within Tennessee, Southern Cavefish have been found in 107 caves, two springs, and one well in 26 counties (Fig. 5; Table 1). The distribution extends throughout much of the Interior Low Plateau in the central part of the state from the Tennessee River in west Tennessee east to the Cumberland Plateau (Fig. 6). The number of Southern Cavefish localities averages 4.2 per county with a maximum of 12 in Van Buren County (Table 3). The highest density of Southern Cavefish localities occurs on the Western Escarpment of the Cumberland Plateau, particularly in Putnam, Van Buren, and Grundy counties. Included in the distribution of the Southern Cavefish are 17 HUC8 Watersheds in Tennessee (Fig. 7). The number of Southern Cavefish localities averages 6.5 per watershed, with a maximum of 25 localities within the Caney Fork Watershed (Table 4). Below we discuss the distribution of Southern Cavefish in each HUC8 watershed.

Buffalo River Watershed.—The Buffalo River watershed drains into the Duck River just before the Duck River drains into the Tennessee River. Southern Cavefish have been reported from four caves in this watershed: Cave Branch Cave in Hickman Co., Allens Creek Cave in Lewis Co., and Blowing Caves and Greer Hollow Cave in Perry Co. We observed Southern Cavefish in Cave Branch Cave (1 cavefish) and Allens Creek Cave (4 cavefish) during our surveys. No new localities were discovered. We did not observe any cavefish in Greer Hollow Cave and could not gain permission to access Blowing Caves where as many as 16 cavefish have been observed (TNHD 2009). A small maternity colony of Gray Bats is present in Allens Creek Cave.

Caney Fork River Watershed.—The Caney Fork River watershed drains into the Cumberland River. Southern Cavefish have been reported from 24 caves and one artesian spring in this watershed, including several localities where we found more than 100 cavefish have been observed during a single survey (e.g., Jacques Cave in Putnam Co. and Swamp River Cave, Thunder Run Cave, and Camps Gulf Cave in Van Buren Co.; Table 1). Southern Cavefish are known from one cave in DeKalb Co., seven caves in Putnam Co., 12 caves in Van Buren Co., four caves in White Co., and an artesian spring by the Rock Island Dam in Warren Co. We observed 218 Southern Cavefish in five caves during our surveys. Camps Gulf Cave No. 2 in Van Buren Co. is a new locality. Several cave systems in this watershed are afforded some protection, as at least portions of their drainage basins occur on government-owned land, including Anderson Spring Cave, Camps Gulf Cave, Camps Gulf Cave No. 2, Rumbling Falls Cave, and Rose Cave. We could not gain permission to access to Cripps Mill Cave in DeKalb County.

Collins River Watershed.—The Collins River watershed drains into the Caney Fork River, which then drains into the Cumberland River. The Collins River drains portions of the Cumberland Plateau and Eastern Highland Rim in Cannon, Coffee, Grundy, Sequatchie, Van Buren, and Warren counties. Southern Cavefish have been reported from six caves in this watershed, including Nunley Mountain Cave in Grundy Co., and Blowing Cave, Hazel Ward Cave, Hickey Pot, Jaco Spring Cave, and Panter Cave in Warren Co. Hazel Ward Cave and Jaco Spring Cave are part of the same drainage basin. We observed 31 cavefish during two surveys of Jaco Spring Cave and three cavefish during two surveys of Blowing Cave. Both of these caves represent new records within the watershed. We could not gain permission to access Hazel Ward Cave or Panter Cave. Jaco Spring Cave houses a significant maternity colony of Gray Bats.

Cumberland River (Lower Cumberland) Watershed.—The Lower Cumberland Watershed of the Cumberland River drains portions of the Western Highland Rim and Western Pennyroyal Karst in Cheatham, Dickson, Houston, Montgomery, and Stewart counties. Southern Cavefish have been reported from four caves in this watershed: Jewel Cave and Ruskin Cave in Dickson County and Bellamy Cave and Darnells Cave in Montgomery County. We did not survey any caves within this watershed during this study.

Cumberland River (Lower Cumberland-Old Hickory Lake) Watershed.—The Lower Cumberland-Old Hickory Lake Watershed drains portions of the Inner and Outer Nashville Basin in Macon, Smith, Sumner, Trousdale and Wilson counties. Southern Cavefish have been reported from three caves in this watershed: Flat Rock Cave and Wet Weather Spring Cave in Smith County and Lackey Cave in Sumner County. We observed ten cavefish during a single survey of Flat Rock Cave. Larry Matthews has reportedly observed up to 100 Southern Cavefish in Lackey Cave, but the entrances to this cave system are now covered by a housing development.

Cumberland River (Upper Cumberland-Cordell Hull Reservoir) Watershed.— The Upper Cumberland-Cordell Hull Reservoir Watershed of the Cumberland River drains portions of the Outer Nashville Basin and Eastern Highland Rim in Clay, Jackson, Macon, Overton, and Smith counties. Southern Cavefish have been reported from four caves in this watershed: East Water Supply Cave and Mill Spring Cave in Overton County and Bartlett Cave and Talent Hollow Cave in Putnam County. We observed eight cavefish during a single survey of Bartlett Cave.

Harpeth River Watershed.—The Harpeth River watershed drains portions of the Inner and Outer Nashville Basin, and Western Highland Rim from southwestern Rutherford County northwest through much of Williamson County, southwestern Davidson County, southern Cheatham County, and eastern Dickson County before flowing into the Cumberland River. Southern Cavefish have been reported from one cave (The Kitchen Sink) in Rutherford County just on the other side from the Stones River drainage divide. We did not survey this locality.

Obey River Watershed.—The Obey River is a major tributary of the Cumberland River draining portions of the Cumberland Plateau and Eastern Highland Rim in Clay, Cumberland, Fentress, Pickett, and Putnam counties. Southern Cavefish have been reported from four caves in this watershed, including Wolf River Cave in the Wolf River drainage and Lott Dean Cave (part of the Mountain Eye Cave system), Xanadu Cave, and Zarathrustras Cave in the East Fork Obey River drainage. We searched Lott Dean Cave and did not observe any Southern Cavefish. We could not gain permission to access Zarathrustras Cave. Wolf River Cave is owned by the Southeastern Cave Conservancy, Inc. and contains a large hibernaculum of Indiana Bats and Gray Bats in the summer.

Red River Watershed.—The Red River is a major tributary of the Cumberland River and drains much of the northern Highland Rim in Tennessee. Southern Cavefish have been reported from eight caves in this watershed: three in Montgomery County and five in Robertson County. This includes Dunbar Cave located in Dunbar Cave State Park in Montgomery County. We observed one cavefish during a single survey of Sinking Ridge Cave in this watershed, which represents a new locality record.

Sequatchie River Watershed.—The Sequatchie River drains the Sequatchie Valley, which is a long, nearly linear anticline valley extending from southern

Cumberland County south-southwest into northeastern Alabama. The valley is bounded the east by the Cumberland Plateau and to the west by Walden Ridge in Tennessee. The Sequatchie River ultimately flows into the Guntersville Lake section of the Tennessee River. Southern Cavefish have been reported from four caves in this watershed, all within in Little Sequatchie River valley in eastern Marion County. We observed two Southern Cavefish from two caves: Butterfly Cave and Pryor Spring Cave. Pryor Spring Cave represents a new locality record.

Stones River Watershed.—The Stones River Watershed drains into the Cumberland River and its basin includes the Inner and Outer Nashville, and portions of the Eastern Highland Rim in southern Wilson, most of Rutherford, and west-central Cannon counties. Southern Cavefish have been reported from 12 caves and one well in this watershed. We observed 250 cavefish during 24 surveys of six caves, including a new locality record for Pattons Cave in Rutherford County. The main entrance to Snail Shell Cave in Rutherford County is owned by the Southeastern Cave Conservancy, Inc. Jackson Cave is located within Cedars of Lebanon State Park; whereas Burnt House Cave, Canyon Cave, Cedar Forest Cave, and Hurricane Cave are located within Cedars of Lebanon State Forest. Significant maternity colonies of Gray Bats exist within Pattons Cave and Herring Cave in Rutherford County.

Tennessee River (Lower Tennessee-Beech) Watershed.—The Lower Tennessee-Beech Watershed of the Tennessee River drains portions of the Western Highland Rim and Southeastern Plains (Transitional Hills) in west Tennessee. Southern Cavefish have been observed in five caves in this watershed, including four caves west of the Tennessee River. Only Dry Creek Cave lies east of the Tennessee River in Hardin County. We observed 16 cavefish during two surveys of Baugus Cave in Decatur County. We could not gain permission to access Woodlawn Shores Cave. We also were unable to locate Dry Creek Cave, even though coordinates are listed within the TCS database.

Tennessee River (Guntersville Lake) Watershed.—The Guntersville Lake drainage of the Tennessee River drains portions of the Eastern Escarpment of the Cumberland Plateau in Franklin and Marion counties. Southern Cavefish have been reported from six caves in this watershed, including Buggytop Cave, Garner Spring Cave, Little Crow Creek Cave, and Salt River Cave in Franklin County and Lost Pig Cave and Shakerag Cave in Marion County. We observed 64 cavefish in five caves, including new locality records at Garner Spring Cave, Little Crow Creek Cave, Lost Pig Cave, and Shakerag Cave. Buggytop Cave is located on the Carter State Natural Area.

Tennessee River (Middle Tennessee-Chickamauga) Watershed.—The Middle Tennessee-Chickamauga watershed encompasses the Tennessee River drainage between Nickajack Dam and Watts Bar Dam. Only Nickajack Cave in Marion County is a confirmed Southern Cavefish locality in this watershed. Nickajack Cave is partially inundated by Nickajack Reservoir restricting access to the portion of the cave where Southern Cavefish have been observed. Nickajack Cave also is home to a large maternity colony of Gray Bats.

Tennessee River (Pickwick Lake) Watershed.—The Pickwick Lake Watershed of the Tennessee River drains portions of the Western Highland Rim and Transitional Hills in southern Hardin, Lawrence, and Wayne counties. Southern Cavefish have been reported from two caves in this watershed, Pickwick Pot in Hardin County and Hound Dog Drop in Wayne County. We did not observe any cavefish during a single survey of the twilight zone of Pickwick Pot. This cave is only penetrable for about 18 m. Kuhajda and Mayden (2001) observed two cavefish during a single survey of Hound Dog Drop.

Upper Duck River Watershed.—The Duck River flows from the Eastern Highland Rim westward across the southern Central Basin and into the Western Highland Rim before emptying into the Tennessee River in west Tennessee. Southern Cavefish have been reported from six caves in this watershed, including Riley Creek Cave (known locally as Duke Cave) in Coffee Co., Gallagher Cave, Gallagher Cave South, Gropp Cave, and Macedonia River Cave in Marshall Co., and Pompie Cave in Maury Co. Southern Cavefish are not known from Bedford County. All but one cave (Riley Creek Cave) occur within the Central Basin. Normandy Reservoir now inundates Riley Creek Cave and the status of this population is unknown. We observed 39 cavefish in Gallagher Cave, Gallagher Cave South, and Pompie Cave during our surveys. All three caves represent new locality records. Pompie Cave is located on the Yanahli Wildlife Management area.

Upper Elk River Watershed.—The Elk River flows from the Western Escarpment of the Cumberland Plateau in southeastern Coffee and western Grundy Cos. west through the southern Eastern Highland Rim before entering the Tennessee River in northern Alabama. Southern Cavefish have been reported from 13 caves and one spring in this watershed, the majority occurring in the rich beds of Mississippian-aged limestones along the base of the Western Escarpment of the Cumberland Plateau. Two caves with Southern Cavefish are found in the Eastern Highland Rim: Caney Hollow Cave and Powers Cave. We observed 224 cavefish in eight caves including new locality records at Lusk Cave in Coffee Co., and Big Room Cave, Elkhead Shelter Cave, Jay Creek Spring Cave, and Trussell Cave in Grundy Co. We could not gain permission to access Wonder Cave in Grundy Co. The spring where Southern Cavefish have been reported, Blue Spring, is the resurgence of the cave stream in Elkhead Shelter Cave.

Distribution – Spring Cavefish

The Spring Cavefish occurs from south-central Tennessee northward into central and western Kentucky, then west following the Shawnee Hills of southern Illinois and the Benton Hills west of the Mississippi River in southeastern Missouri (Fig. 4). In Kentucky and Tennessee, Spring Cavefish occur in springs and caves, including the Mammoth Cave system, from the Eastern Highland Rim ecoregion of the Tennessee

River drainage, middle and lower Cumberland River drainage, and the upper Barren-Green River system of Kentucky (Etnier and Starnes 1993).

Within Tennessee, Spring Cavefish have been reported primarily from surface streams and springs in 13 counties (Fig. 8; Table 4) throughout the Eastern Highland Rim, Western Pennyroyal Karst, and portions of the Western Highland Rim of the Interior Low Plateau in the central part of the state (Fig. 9). Spring Cavefish also have been reported from the Nashville Basin in Bedford, Davidson, and Wilson counties. However, most Spring Cavefish localities occur in the Barrens of the Eastern Highland Rim in Coffee, Cannon, and Warren counties with another cluster of localities occurring in the Western Pennyroyal Karst of Montgomery and Stewart counties. Included in the distribution of the Spring Cavefish are nine HUC8 Watersheds in Tennessee (Fig. 10). This includes the Caney Fork, Collins, Red, the Kentucky Lake section of the Tennessee, Upper Duck, Upper Elk, and the Lower Cumberland, Lower Cumberland-Old Hickory Lake, and the Lower Cumberland-Sycamore sections of the Cumberland River (Table 5). Spring Cavefish also are known from the Barren River watershed but not within Tennessee.

Habitat – Southern Cavefish

Most Southern Cavefish habitat is at or near the water table. Southern Cavefish inhabit cool, 10–15ºC subterranean waters, including vadose streams (those above the water table) as well as phreatic waters (those at the water table). However, most observations of Southern Cavefish during the current study occurred in large pools within major stream passages or in smaller, infeeder streams with little current. Southern Cavefish are indifferent to light (Eigenmann 1909; Verrier 1929; Green and Romero 1997). Our observations are consistent with past researchers. Southern Cavefish do not respond directly to illumination by headlamps. Likewise, we observed Southern Cavefish in the twilight zones of several caves. Water velocity varies tremendously among seasons and years for most Southern Cavefish habitats. However, Southern Cavefish are positively rheotaxis and prefer habitats with little or no current. This species is sensitive to the slightest increase in water velocity, and is known to seek shelter hours before humans can detect an oncoming flood (W. Pearson, pers. comm. in Niemiller and Poulson in press). The composition of substrates also vary a great deal within and among caves inhabited by Southern Cavefish, ranging from bedrock to mud to thick deposits of organic debris. However, most of our observations occurred in pools with mud to slit substrates with some organic debris. Moreover, Southern Cavefish are typically observed at depths less than one meter; although we observed a cavefish descend to at least four meters in Butterfly Cave before it was lost from view. We do not know to what depths Southern Cavefish occur, but anecdotal evidence from Missouri suggests this species may utilize deeper waters than currently recognized.

Habitat – Spring Cavefish

Spring Cavefish occur at the interface of epigean and subterranean habitats bridging the threshold toward a troglobitic life. Although the species have been reported from caves throughout most of its range, Spring Cavefish are most abundant in springs, spring runs, spring-fed ponds, and seeps. Both cave and surface habitats are used throughout much of its range; fish often emerge from subterranean haunts at dusk to feed and then later retreat back underground before dawn. Spring Cavefish also can be found underneath rocks in springs and spring runs during the day. In Tennessee, Spring Cavefish are common in dense vegetation associated with springs and spring-fed ponds and streams, particularly on the Barrens of the Eastern Highland Rim. Spring Cavefish are negatively phototactic (Poulson 1963; Niemiller and Poulson in press) and hide in vegetation or under objects even in low levels of ambient light. This species also has been collected in very low numbers in caves of the Western Pennyroyal Karst in Tennessee. We did not observe any Spring Cavefish in caves associated with the Eastern Highland Rim. However, Spring Cavefish have been reported from caves in Dickson County (UMMZ 97211). Like Southern Cavefish, Spring Cavefish are positively rheotactic and avoid strong currents. Although this species has been collected from streams, collections usually occur underneath cover or within dense vegetation in pools or at the margins of streams.

Spring Cavefish might be only marginal troglophiles because there is no documentation of the species reproducing and living permanently in caves. The best-studied populations emerge nightly or seasonally from food-poor caves to feed in spring runs that have abundant live prey. Spring Cavefish presumably spawn in caves, at least in southern Illinois and central Kentucky, as adults disappear from surface springs for a few months beginning in late autumn (Weise 1957; Hill 1966). Both adults and small juveniles have been found on the surface in early spring. However, gut content analyses reveal that cave populations of Spring Cavefish predominantly have empty guts and have poor condition factors (Niemiller and Poulson in press).

Taxonomy and Systematics

Typhlichthys subterraneus. The Southern Cavefish was described by Girard (1859) from a well near Bowling Green, Warren Co., Kentucky. Later, Eigenmann (1905) described both *T. osborni* and *T. wyandotte* based on differences in head width and eye diameter. *Typhlichthys osborni* was described from Horse Cave, Kentucky. *Typhlichthys wyandotte* was described from a well near Corydon, Indiana, that was later destroyed. Recently, a well-like entrance into a cave on the property of a car dealership in Corydon was discovered and is believed to represent the type locality (Black in Lewis 2002). Regardless, this species is generally considered invalid and was not listed as a locality in Woods and Inger (1957). Recent surveys in the vicinity of Corydon have failed to document *T. subterraneus*, finding only *A. spelaea* (Lewis 1998; Lewis and Sollman 1999). *Typhlichthys eigenmanni* (*nomen nudeum*) was described as a fourth species in the genus from Camden Co., Missouri (likely River Cave). Recently, Parenti (2006) proposed that *T. eigenmanni* Charlton

(1933) is a subjective synonym of *T. subterraneus*. Woods and Inger (1957) synonymized all species under *T. subterraneus* on the basis of lack of any clear geographic pattern in morphological variation. All populations in Tennessee have been considered as *T. subterraneus*.

Because of the extensive distribution of Southern Cavefish and the results of molecular studies of other troglobites, several authors have speculated that this species represents several independent origins and, therefore, distinct lineages (reviewed in Niemiller and Poulson in press). Electrophoretic analyses by Swofford (1982) showed considerable differentiation among morphologically similar populations of *Typhlichthys* including populations from Tennessee, indicative of multiple, independent lineages and limited gene flow. However, owing to small sample size, Swofford's study was limited in its ability to distinguish modular or hierarchical subdivision from a continuous relationship between genetic and geographic distance.

Niemiller and Fitzpatrick (2008; this study) examined genetic variation among eastern populations of *Typhlichthys* in Alabama, Georgia, and Tennessee. Significant genetic divergence was observed and both mitochondrial and nuclear DNA variation was structured among hydrological drainages. Molecular and morphological evidence also indicate that Arkansas populations warrant recognition of a distinct species (Graening et al., unpublished data). These studies support the hypothesis that morphological similarity is the result of parallel evolution rather than significant dispersal and gene flow across major drainage and river divides (Barr and Holsinger 1985). Bayesian analyses of both the mitochondrial ND2 and nuclear S7 datasets support the monophyly of Southern Cavefish (Fig. 11). Within *Typhlichthys*, 65 haplotypes were recovered for the mitochondrial ND2 dataset (Fig. 12). There was a clear pattern of correspondence between mtDNA lineages and surface hydrological boundaries with sequence divergence up to 11.6% among lineages. Almost all haplotypes from a given hydrological unit grouped within the same lineage. Exceptions included haplotype TsubAE from Marion County, Tennessee, that grouped with haplotypes from northwest Georgia and haplotypes from caves in Overton and Putnam counties in the Upper Cumberland River drainage of Tennessee that grouped with haplotypes from the Upper Caney Fork River drainage in Van Buren County rather than other Cumberland River haplotypes downstream. Little evidence of contemporary gene flow was found, particularly among drainages. Only two localities located in Franklin County, Tennessee, and separated by 2.5 km shared ND2 haplotypes. The nuclear S7 dataset also supported a monophyletic *Typhlichthys* but relationships among drainages were not nearly as resolved (Fig. 11). Twenty-five S7 haplotypes were recovered with uncorrected sequence divergence up to 2.6% observed among drainages.

Forbesichthys agassizii. The Spring Cavefish was described as *C. agassizi* by Putnam (1872) from a well near Lebanon in Wilson Co., Tennessee. Later, Forbes (1882) described *C. papilliferus* from a spring in western Union Co., Illinois, on the basis of coloration differences between the Tennessee and Illinois populations.

Jordan and Evermann (1927) erected a new genus, *Forbesella*, citing that the subterranean nature of spring cavefish warrants separate recognition. Jordan (1929) later replaced *Forbesella* with *Forbesichthys*, as the former was preoccupied in tunicates. This genus is still considered a junior synonym of *Chologaster* by some authors, however. Woods and Inger (1957) noted that the slight differences among populations of Spring Cavefish from southern Illinois, central Kentucky, and central Tennessee did not warrant specific or subspecific designation. Therefore, they synonymized *C. papilliferus* with *C. agassizi*, a revision that has been followed by most subsequent authors. Exceptionally, Clay (1975) maintained that *C. agassizi* and *C. papilliferus* are specifically distinct. More recently, allozyme analyses by Swofford (1982) revealed considerable differentiation between populations, which later justified resurrection of the genus *Forbesichthys* by Page and Burr (1991).

Spring Cavefish have not been the subject of phylogenetic studies since Swofford (1982). Swofford found that Spring Cavefish show slightly less allozyme differentiation than Swampfish with an average heterozygosity of 0.028 compared to 0.040. Swofford's data imply that Spring Cavefish utilize surface corridors for dispersal, at least in central Kentucky and the Eastern Highland Rim in Tennessee. However, molecular evidence indicates that populations of *Forbesichthys* from Illinois and Tennessee are distinct and likely isolated from each other, suggestive that long distance dispersal is rare (Near et al., unpubl. data). Populations sampled from the Eastern Highland Rim are genetically distinct at multiple loci from Illinois populations, and, therefore, likely represent distinct species. However, samples from the Western Pennyroyal Karst in Tennessee in and around the Clarksville area have not been genetically analyzed; consequently, the taxonomic affinities of these populations remains unknown at this time. Although cave populations of Spring Cavefish exist, subterranean dispersal seems unlikely, given low abundance in caves, low tolerance to starvation, relatively poor food finding ability, and short life span. However, surface dispersal along rivers between springs might be possible. Indeed, little mtDNA sequence divergence was observed across the Barrens of the Eastern Highland Rim from DeKalb County south to Coffee County (Fig. 12).

Population Size and Relative Abundance

Few studies have attempted to quantify population sizes and relative abundance of amblyopsids, including Southern Cavefish and Spring Cavefish. The few studies that have attempted to quantify population sizes via techniques such as mark-recapture or survey removal have focused on caves that are known to contain relatively large populations. Other studies for which the most reliable estimates of abundance have been obtained have focused on the species of conservation concern. Additional demographic studies, including long-term censuses, are needed for both epigean and subterranean populations. In general, the majority of Southern Cavefish localities yield few fish observations during single surveys, including results of the current study. Almost 74% of Southern Cavefish localities have yielded fewer than 10 cavefish during a single survey (Fig. 13). Indeed, only 14 of 91 caves with survey data have reported more than 20 fish during a single survey.

The top four caves during our surveys (Herring Cave, Jacques Cave, Blowing Springs Cave, and Stamps Cave) account for 47% of the 871 cavefish censused in 45 reported Southern Cavefish caves surveyed during this study (Table 1). Jacques Cave in Putnam County, Tennessee, had 121 fish in a 400 m stream section with an estimated 864 per hectare. In several caves, we routinely see > 40 fish per visit and in two caves these moderate numbers are consistent over a period of years. From most to least numbers per trip, Herring Cave in Rutherford County had 47, 39, 37, and 32 cavefish. Blowing Springs Cave in Coffee County had 52, 37, 31, and 26 cavefish including surveys from the 1960s by Poulson (unpub. data). In Shelta Cave, Alabama, 64 Southern Cavefish were observed during a single survey in an area under a gray bat colony by Poulson (1960) with an estimated 229 per hectare.

Although these results might be a reflection of actual abundance in some instances, the distribution and abundance of troglobitic species likely is greater than currently realized. Localities for which Southern Cavefish have been reported represent but a fraction of total available habitat accessible to cavefish. This was clearly illustrated during a fertilizer pipeline break within the recharge zone of Maramec Spring that resulted in the death of at least 1,000 Southern Cavefish and likely many more (see discussion in Noltie and Wicks 2001). This unfortunate kill is informative because the drainage basin had few records documented previously. The problem with inferring population densities from such fish kills is that we do not know the volume or extent of habitat impacted.

Most observations of Southern Cavefish are restricted to caves near the surface and there is some controversy as to whether even the best Southern Cavefish caves are sources or sinks (Niemiller and Poulson in press). Habitats where few or no cavefish are observed likely represent population sinks and not sources. Wells and short stream segments encountered in an otherwise dry cave may not be representative of the habitat that most Southern Cavefish inhabit. Cavefish can disperse through and occupy submerged passages inaccessible to humans but these habitats are probably neither usual for the fish nor optimal. These habitats likely act as corridors for dispersal. Given their longevity, low metabolic rates, and foraging efficiency, Southern Cavefish likely can move long distances.

Our best survey of 121 cavefish observed and 88 of which were measured at Jacques Cave in Putnam County showed a unimodal distribution of sizes with the majority of individuals between 41 and 60 mm total length (Fig. 14). This pattern is consistent with a stable population. Although this survey was conducted on 25 October, no gravid females were documented. However, two small individuals (< 25 mm TL) were observed and likely represent the preceding year’s reproductive cohort.

Historically, Spring Cavefish has been considered rare to uncommon throughout much of its range. Smith and Welch (1978) estimated less than a thousand individuals from eight springs in Union County, Illinois, and around 40 Spring

Cavefish at Cave Springs Cave in Union County, Illinois. However, many hundreds of specimens have been accessioned from a large spring run at Rich Pond in Warren County, Kentucky. In Tennessee, Spring Cavefish can be locally abundant in ideal habitats (Etnier and Starnes 1993; this study), and is easily overlooked because of its nocturnal and reclusive habits. Indeed, we failed to dipnet any Spring Cavefish in spring-fed streams, and museum collections from streams in Tennessee are few. Spring Cavefish are most abundant in spring-fed ponds with abundant aquatic vegetation on the Barrens of the Eastern Highland Rim. We did not observe Spring Cavefish during any cave surveys. However, Spring Cavefish have been reported from caves in the Western Pennyroyal Karst of Tennessee. Estimates of population density range from as many as 8.0 fish per m^2 in spring runs in the Pine Hills of Illinois (Weise 1957) to 0–0.01 fish per m^2 in Mammoth Cave (Poulson 1969). We estimated 1.1 fish per m^2 at Rigsby Pond in Coffee County, Tennessee (57 fish in 10 x 5 m area).

Ecology, Life History, and Behavior

Reproduction

Troglobitic species in many systems are subject to pronounced seasonality in food availability and water levels (flooding). Accordingly, cave amblyopsids have peaks in reproduction just after spring floods (Poulson and Smith 1969; reviewed in Niemiller and Poulson in press). Synchronization of reproduction with spring flooding is adaptive because offspring survival is maximized. Young are produced shortly after spring floods when food availability is still high, yet mortality due to extreme flow is reduced (Poulson and Smith 1969). However, timing of these cues is unpredictable and may occur from late fall into spring. Rises in water level and alkalinity, coupled with subtle drops in water temperature, may be triggers to reproduction and synchronization of circannian rhythms of reproductive readiness (Poulson 1963; Jegla and Poulson 1970).

Breeding in the Southern Cavefish likely occurs during spring, associated with higher water levels from later winter and early spring rains. However, data is scant regarding reproduction in this species despite its broad distribution. Hatchlings have never been observed or collected. Poulson (1960) observed 15–20 mm TL fish in Shelta Cave in Madison Co., Alabama. We captured two and observed six other Southern Cavefish in the 15–25 mm TL size class at Jacques Cave in Putnam County in October 2007. Likewise, we have observed similar-sized fish in small tributaries to the main stream in Big Mouth Cave, Grundy County during several trips in autumn and winter. These individuals likely represent first-year fish and are consistent with reproduction occurring in spring. However, more data are needed to better understand the reproductive biology of this species.

Unlike Southern Cavefish, the timing of reproduction in Spring Cavefish is fairly clear. In the Illinois populations studied by Weise (1957) and Smith and Welch

(1978), most adults presumably spawn underground during late winter. This assumption is based on the nearly complete disappearance of adults from springs during this season. Populations of Spring Cavefish in the Western Pennyroyal Karst of Tennessee apparently also breed underground; however, data from the Eastern Highland Rim populations are lacking. These latter populations might breed and oviposit in springs or in other surface habitats, given the paucity of subterranean observations of Spring Cavefish in this ecoregion. Ova begin to enlarge in autumn reaching mature size in January when adults move underground (Weise 1957; Poulson 1963). Subterranean spawning is believed to occur from January through April and peaks in February when water levels typically are at their maximum. Fry appear and adults return to the surface by early May (Niemiller and Poulson in press). Spring Cavefish as small as 8 mm SL have been collected in March in Tennessee (Etnier and Starnes 1993).

Cave amblyopsids, including Southern Cavefish, have fewer, larger, and potentially more nutrient-rich eggs than their surface counterparts (Poulson 1985). Compared to smaller eggs, larger eggs contain more yolk and produce larger larvae with greater starvation tolerance, greater swimming ability when foraging and when avoiding predation, and can accommodate a wider range of prey sizes when all yolk reserves are depleted. All of these are adaptations that increase survival in a food-poor environment (Niemiller and Poulson in press).

Egg diameter in Southern Cavefish averages 2.3 mm (Niemiller and Poulson in press) and clutch size is reportedly low, perhaps fewer than 50 eggs. Ovarian eggs of Spring Cavefish range from 1.5–2.0 mm in diameter (Niemiller and Poulson in press) and clutch sizes average about 100 eggs. Hatchling fry after yolk sac absorption are about 6.0 mm long (Hill 1966). The jugular position of the urogenital pore in all amblyopsids is circumstantial evidence for branchial brooding of eggs. Some have speculated that branchial brooding reduces egg predation in cave habitats (Noltie and Wicks 2001). Indeed, this has been observed in the Northern Cavefish. However, Niemiller and Poulson (in press) argue against branchial brooding in most amblyopsid species, citing that, except for the Northern Cavefish, total egg volume exceeds branchial volume.

Growth and Development

Generally, cave organisms exhibit reduced growth rates and delayed development and maturity compared to related surface species. Reduced growth rates represent an adaptive response to low food supplies in cave environments because less energy over a given amount of time is needed (Hüppop 2000). Within the Amblyopsidae, growth and developmental rates decrease with increasing cave adaptation (Poulson 1963). First year Spring Cavefish grow 10–20 mm per year on average and also exhibit variable growth rates from season to season (Smith and Welch 1978; Hill 1966). Hill (1971) studied squamation and pigmentation development in Spring Cavefish. Scale primordia first appear on the caudal peduncle at around six weeks. By 12 weeks, both squamation and pigmentation pattern are well developed. Vent migration is characteristic of the Amblyopsidae and Aphredoderidae (Poulson 1963). Hill (1966) found that vent migration to the jugular position was complete in 16-18 mm SL Spring Cavefish. Growth rates for troglobitic amblyopsids are substantially slower with estimates of 1.0–1.25 mm month^{-1} for Southern Cavefish. Sexual maturity is also delayed in cave amblyopsids. Spring Cavefish reach sexual maturity around 12 months of age, whereas Southern Cavefish may take four years or longer to reach sexual maturity.

Longevity

Increased longevity of cave organisms compared to their surface relatives is one of several life history adaptations toward a low-r strategy by which cave organisms cope with limited food resources. Prolonged life spans, coupled with a trend from semelparity to iteroparity, increases the chance of population persistence over time, as a population is less likely to be extirpated during times of extremely low food supplies that result in little to no recruitment (Hüppop 2000). This pattern is evident in the Amblyopsidae as longevity inferred from scale marks increases with increased adaptation to cave environments (Poulson 1963). Spring Cavefish are known to live up to three years (Hill 1966; Smith and Welch 1978). It is likely that, as in many short-lived species, death occurs after a single reproductive attempt (semelparity) in this species. Therefore, older individuals are those that simply did not acquire enough resources to reproduce at a younger age. Troglobitic species live considerably longer. Conservatively based on scale marks, Southern Cavefish were estimated to live 3–4 years (Poulson 1963); however, individuals have been maintained in captivity for over a decade and likely live considerably longer than initial estimates in nature (Noltie and Wicks 2001). However, Poulson (2001) later questioned his original longevity estimates of the troglobitic species stating they may be off by a factor of 3–4, partly because of the difficulty in determining scale annuli in larger individuals, but primarily because of observed growth rates of marked individuals in nature (see above Growth Rates). Accordingly, Southern Cavefish may live 16–24 years or longer.

Diet

All amblyopsids eat live, moving prey, with invertebrates comprising most of the diet. However, cannibalism has been documented and small amounts of nonliving food, such as bat guano and detritus have been observed in the stomach contents of some species. Indeed, three Southern Cavefish located in a pool beneath a Gray Bat roost had bat guano visible in their guts. These are probably ingested along with live prey and would provide much lower nutritional benefit per volume than live prey.

The diet of Southern Cavefish consists largely of copepods and isopods, but rare larger food items, such as young crayfish, salamander larvae, or conspecifics may result in high growth efficiency and a burst in growth rate (Poulson 2001). A variety of prey have been reported in stomach contents of *T. subterraneus*, including copepods, amphipods, isopods, trichopteran and tendepedid larvae, cladocerans, isopods, and crayfish (Poulson 1960, 1963; Cooper and Beiter 1972); however, copepods are the primary food source accounting for 60–90% of the diet by volume (Poulson 1963).

The diet of Spring Cavefish varies geographically and between cave and surface populations. Illinois populations feed almost exclusively on *Gammarus* amphipods (Forbes and Richardson 1908; Layne and Thompson 1952; Gunning and Lewis 1955; Weise 1957), although amphipods (Weise 1957), insect remnants (Gunning and Lewis 1955), and detritus (Gunning and Lewis 1955) also have been found in stomachs. In Kentucky, Spring Cavefish living in surface habitats feed primarily on chironomids, but also copepods, oligochaetes, nematodes, and ostracods (Hill 1969). On the contrary, individuals of the same population are strongly cannibalistic on younger individuals when in the cave part of the habitat. In caves cannibalism may represent an alternative feeding strategy in response to competition for more typical but extremely rare invertebrate food sources. Data are lacking for Tennessee populations.

Predators

Southern Cavefish are at the top of the food chain in most cave systems that they inhabit, and, therefore, have few natural predators. Epigean fishes potentially prey on Southern Cavefish, as well as crayfish, and Tennessee Cave Salamanders; however, direct evidence is lacking. Young Southern Cavefish likely are susceptible to cannibalism by larger adults, as has been documented in the related Northern Cavefish (Poulson 1963). Cannibalism has been reported in at least one cave population of the Spring Cavefish. Cannibalism may serve as one means to regulate population densities in a food-limited environment (Poulson 1969). Epigean populations of Spring Cavefish likely are occasional prey for other surface fish, snakes, birds, and mammals (Smith and Welch 1978). At Rich Pond in Kentucky, natural predation is seasonally heavy (J.E. Cooper in Smith and Welch 1978), but data are lacking for Tennessee populations.

Parasites

Like many cave vertebrates, few parasites have been reported afflicting Southern Cavefish and its surface relative, the Spring Cavefish. Proteocephalan cestodes have been collected from the pyloric caeca of Spring Cavefish. Whittaker and Hill (1968) described *Proteocephalus chologasteri* from the Spring Cavefish. In southern Illinois, 71 percent of Spring Cavefish examined were parasitized by cestodes and other internal parasites (G. Garoian in Smith and Welch 1978). Small, unidentified leeches also have been reported on Spring Cavefish in Illinois (Smith and Welch 1978). Data are lacking from Tennessee populations of Spring Cavefish and throughout the distribution of Southern Cavefish.

Diseases

Few diseased amblyopsid cavefishes have been reported in nature. Fournie and Overstreet (1985) reported on an adult Spring Cavefish from Union County, Illinois, with a retinoblastoma on the right side of the head. This condition may be related to chromosomal abnormalities. At least one other individual collected at the same spring had a similar tumor in appearance and eventually died after the tumor involved the entire head. However, this specimen was not available for histological examination (Bechler, pers. comm. in Fournie and Overstreet 1985). Gas bubble disease has been documented in recently collected Southern Cavefish at a spring site in Missouri (Schubert et al. 1993). Southern Cavefish from Missouri may be particularly susceptible to this disease because of the depths at which individuals reside (Schubert et al. 1993; Noltie and Wicks 2001). No data is available from Tennessee populations of either species.

Community Associates

Southern Cavefish are commonly found in the same cave systems with other cave macrofauna, including cave crayfish (*Cambarus australis*, *C. barri*, *C. hamulatus*, and *C. incomptus*), and Tennessee Cave Salamanders (*G. p. palleucus* and *G. p. necturoides*). The only surface fish species commonly found in the same cave systems with Southern Cavefish is the Banded Sculpin (*Cottus carolinae*). Although Southern Cavefish are often found in the same pools as Tennessee Cave Salamanders, these species appear to exhibit habitat partitioning within the same cave systems, as the highest densities for each respective species are located in different areas. Tennessee Cave Salamanders prefer rockier substrates where individuals are often encountered underneath rocks; whereas Southern Cavefish prefer silt-sand-mud substrates that typically lack any appreciable cover. However, this observation requires further scientific scrutiny.

Spring Cavefish are associated with several fish species, including the Barrens Topminnow (*Fundulus julisia*), Western Mosquitofish (*Gambusia affinis*), Flame Chub (*Hemitremia flammea*), Southern Redbelly Dace (*Phoxinus erythrogaster*), Fringed Darter (*Etheostoma crossopterum*), Creek Chub (*Semotilus atromaculatus*), Central Stoneroller (*Campostoma anomalum*) Bluntnose Minnows (*Pimephales notatus*), Banded Sculpin (*Cottus carolinae*), Bluegill (*Lepomis macrochirus*), Green Sunfish (*Lepomis cyanellus*), and Largemouth Bass (*Micropterus salmoides*) (Goldsworthy and Bettoli 2006; current study). Other nonavian and nonmammalian vertebrate associates include the Long-Tailed Salamander (*Eurycea longicauda*), Red Salamander (*Pseudotriton ruber*), Green Frog (*Rana clamitans*), Bullfrog (Rana catesbeiana), Stinkpot (*Sternotherus odoratus*), and Red-Eared Slider (*Trachemys scripta elegans*).

Agonistic Behavior

Agonistic behavior of amblyopsids has been investigated by Bechler (1980, 1981, 1983). Both Spring Cavefish and Southern Cavefish exhibit two submissive acts, “freeze” and “escape.” Adult Spring Cavefish cannibalize smaller conspecifics (Hill

1966) and freezing should be an excellent defense because amblyopsids use only their lateral line to detect other fish and prey. Thus, it is not surprising that fish that perceive that they are losing in an agonistic encounter ‘freeze’ more often than escaping by fleeing. This allows avoidance of the most intense kinds of acts. Both Spring Cavefish and Southern Cavefish always initiated agonistic acts from under or next to rock shelter. This is consonant with the high importance of thigmotaxis to both species.

Territoriality and Social Groups

Although epigean species are often territorial or form social groups, troglobitic species generally are found in low population densities and are usually solitary with a large home range (Langecker 2000). In general, populations Southern Cavefish are low in density (but see Poulson 1969; Niemiller and Poulson in press) and individuals are irregularly distributed over suitable habitat (Poulson 1963). Individual cavefish have large home ranges, cover long distances in search of food, and never defend areas (Poulson 1963; Mohr and Poulson 1966). However, during aggression trials by Bechler (1983), both Spring Cavefish and Southern Cavefish established distinct territories in aquaria with rocks ("stations"). Neither species displays schooling behavior, although individuals are sometimes observed in close proximity to conspecifics. These loose aggregations are typically around food sources, such as underneath a bat roost (in Southern Cavefish) or aggregated in dense vegetation (in Spring Cavefish).

Conservation

The conservation status of subterranean fishes has received increasing attention in the past few years. According to Proudlove (2006), 63 of the 104 known species of subterranean fishes are listed by the International Union for the Conservation of Nature and Natural Resources (IUCN 1996, 2000). The Southern Cavefish is included on this list. In this section we review the conservation status of the Spring Cavefish and Southern Cavefish, examine the major threats facing each species, and conservation measures that have either been implemented or proposed. A more in depth review of threats to all cave amblyopsid is found in Niemiller and Poulson (in press).

Conservation status. Troglobitic amblyopsids are considered vulnerable or endangered across their respective distributions, including the Southern Cavefish. Southern Cavefish (as currently recognized) is the most widely distributed and least cave-adapted (Poulson 1963) of the troglobitic amblyopsids. As such, it is considered the most secure (although it is afforded protection in several states) and is considered endangered only in Georgia where it ranges only into the extreme northwest corner of the state. In Tennessee, Southern Cavefish are currently “Deemed in Need of Management” and have an S3 Natureserve status designation. Indeed, Southern Cavefish are widespread in Tennessee and most populations apparently are secure. This species has been reported from 107 caves, two springs, and a well in the state. Moreover, several caves are known to contain large populations of Southern Cavefish (more than 40 cavefish observed during a single survey). At this time, we recommend that the status of Southern Cavefish be elevated to S4 (Apparently Secure) in Tennessee, given the large number of occurrences, estimated abundance, and number of apparently protected cave systems occurring on state, federal, or cave conservation organization land.

Spring Cavefish are apparently secure throughout their range, although disjunct populations in southeast Missouri are listed as endangered (Missouri Natural Heritage Program 2008). In Tennessee, Spring Cavefish are not listed and have an S4 (Apparently Secure) Natureserve status designation. Spring Cavefish have been reported from several localities in the state. Several localities, particularly in the Barrens of the Eastern Highland Rim, yield large numbers of fish (>50 individuals) during surveys. At this time, we do not recommend any change to the status of the Spring Cavefish in Tennessee.

Threats. Proudlove (2006) listed five general threats that subterranean fishes can face. This list includes: (1) habitat degradation, (2) hydrological manipulations, (3) environmental pollution, (4) overexploitation, and (5) impacts of introduced aquatic animals. Many of the threats discussed below are interrelated because of their wide range of potential effects. For example, dam construction can result in direct destruction and degradation of cavefish habitat, alter hydrological patterns, and allow surface species to colonize and either compete or prey on existing cavefish populations. Here we generally follow the broad classification of threats listed by Proudlove (2006) and review the threats to populations of Southern Cavefish and Spring Cavefish populations. We focus on the first four of Proudlove's list as little work has investigated the effects of introduced species on cavefish populations. The Western Mosquitofish (*Gambusia affinis*) has been introduced to many localities where Spring Cavefish also exist. However, unlike the Barrens Topminnow, it is currently thought that Spring Cavefish are unlikely to be strongly impacted by *Gambusia* (Goldsworthy and Bettoli 2006; current study), given the cooccurrence of these two species at several localities in the Barrens of the Eastern Highland Rim. Nonetheless, experiments should be conducted to assess any potential influence (i.e., predation and competition) Western Mosquitofish have on Spring Cavefish populations.

Habitat degradation and alteration

Habitat degradation and alteration can result from the direct destruction or manipulation of habitat during quarrying and mining operations, highway construction, and urban development. The majority of habitat degradation and alteration threats are indirect, resulting in loss of habitat because of siltation, sedimentation, and alteration of hydrological flow patterns and levels. Many caves in the eastern Interior Low Plateau have massive silt banks along streams that are likely associated with farming that began in the 1800s (Poulson, personal. communication). However, some caves contain cavefish populations that are found entirely on silt substrates and have high population sizes and frequent reproduction.

Likewise, on a much longer time scale, huge changes in habitat composition and food availability must have occurred with glacial cycles during the Pleistocene. Studies are needed to assess the actual rather than speculative impacts on cavefish populations from increased siltation and sedimentation.

Land development within cave recharge zones can alter surface runoff patterns or even block or destroy major recharge points. This can result in dramatic habitat alteration because of increased or decreased water volume, water velocity, sedimentation, or stream scouring depending on local hydrological patterns. In forested areas, increased erosion and production of sediment because of logging can result in increased siltation and sedimentation or the complete blockage of a cave passage. For Spring Cavefish, loss of forested areas can cause the decline or loss of local populations. Removal of the surrounding forest causes increased insolation and drying of aquatic habitat. Indeed, many spring habitats in the Barrens of the Eastern Highland Rim have been altered during the development of nurseries and farmlands.

Reduced input of surface runoff in recharge zones could potentially have impacts on Southern Cavefish reproduction. Southern Cavefish are believed to rely on increased flow and small temperature changes associated with cave flood events during winter and spring to coordinate reproduction and spawning (Poulson 1963, 1969). Reductions in surface runoff may disrupt the environmental cues necessary for successful reproduction leading to greater susceptibility to extirpation. The entrance to at least one significant Southern Cavefish locality in Tennessee (Lackey Cave in Sumner County) has been covered and sealed by housing development.

Impoundments are another serious threat for Southern Cavefish populations. A primary example is the construction of Lock and Dam #6 constructed on the Green River below Mammoth Cave in 1906. Although the Green River naturally back-floods into the cave system, flood levels have increased since dam construction (Lisowski and Poulson 1981). The Styx and Echo River areas in Mammoth Cave experienced an apparent decline in cave biota, including cavefish, from the late 1800s to the 1920s (Elliott 2000). Impoundments have inundated portions of at least three Southern Cavefish localities in Tennessee. However, their impacts remain to be elucidated.

Hydrological manipulations

Hydrological manipulations can include underground water removal for human consumption, irrigation, or industry. However, some hydrological manipulations, such as impoundments or increased surface runoff, can raise water tables and alter habitats (see above). Lowering of the water table resulting from direct human consumption, irrigation, or industrial use may threaten cavefish populations. Unfortunately, data are lacking on how Southern Cavefish respond to hydrological manipulations and the impacts of such manipulations on local populations.

Groundwater pollution

Groundwater pollution has been listed as factor negatively affecting populations for all cave amblyopsids, including Southern Cavefish. This threat includes eutrophication and contamination from agricultural and industrial runoff containing pesticides, fertilizers, and heavy metals, sewage effluent, spills and illegal dumping of hazard materials, and thermally altered runoff. Although few studies have examined the direct effects of groundwater pollution on cavefish populations in detail, several studies implicate this threat in population declines. Certainly, Spring Cavefish populations are likely to be exposed to runoff from agricultural fields, but data are lacking that link chemical runoff and Spring Cavefish population declines.

Groundwater pollution may be acute in nature, such as a toxic spill resulting in a large impulse of contaminants, or chronic occurring over several months to years (Proudlove 2001). Both forms have been attributed to cavefish declines or extirpations from cave systems. Nearly 1,000 dead or dying Southern Cavefish were expelled from Meramec Spring in Missouri after a fertilizer pipeline rupture in November 1981 caused acute, catastrophic deoxygenation of groundwater (Vandike 1984; Crunkilton 1985). In contrast, several decades worth of gross pollution by decomposable organic matter (creamery waste) and heavy metal contamination (electroplating waste) is the suspected cause of the apparent extirpation of Southern Cavefish along with other cave life at Hidden River Cave in Kentucky (Lewis 1996). However, cavefish and other cave life have re-colonized areas in Hidden River Cave previously affected from far upstream refuges.

Heavy metal and hazardous chemical contamination of groundwater also are threats to Southern Cavefish populations. Heavy metal runoff from a local landfill may threaten populations of *T. subterraneus* in Pulaski Co., Kentucky (Tercafs 1992). However, known examples from Tennessee are lacking. At least four of these threats, industrial effluents, underground storage tank leaks and sinkhole dumping, have been connected to the decline of Southern Cavefish and other cave life from Hidden River Cave (Pearson and Boston 1995; Lewis 1996). Organic enrichment from sewage treatment plant effluents and septic tank leaks also have been implicated at Hidden River Cave and other caves with amblyopsids. Organic enrichment can increase nutrients in an otherwise low-nutrient environment and drastically alter food web dynamics, increase risk of disease, and dramatically decrease dissolved oxygen levels.

Collection and cave visitation.

The collection of cavefish, illegal or otherwise, for the aquarium trade or scientific purposes may pose a threat to Southern Cavefish. Because of their uniqueness to hobbyists and the ease at which individuals can be captured, Southern Cavefish can be easily exploited. Over-collection of fish can potentially reduce or even eliminate local populations. The rarity of Northern Cavefish in the Echo River and River Styx sections of Mammoth Cave system and its presumed absence from adjacent caves to the north have led some to speculate that the species was either introduced or decimated during the 1800s when it was sold as a novelty (Poulson 1968; Elliott 2000). However, this threat appears to be minimal in Tennessee. Few museum accessions of Southern Cavefish (Table 6) and Spring Cavefish are known from Tennessee.

Commercial exploitation of caves can alter or even destroy considerable amounts of cavefish habitat. Commercial caves increase human traffic and disturbance in addition to increased light levels. At least two populations of Southern Cavefish are indirectly affected by commercial cave tours in Tennessee (historically or currently) including Wonder Cave and Crystal Cave in Grundy County. However, the exact impacts and long-term effects of commercial cave operations remains to be examined. Human disturbance caused by increased traffic is more of a concern than commercial exploitation. The activities of even the most cautious caver may have serious impacts on cave organisms in shallow, silt-bottomed streams. Disturbance caused by substantial cave visitation may alter breeding of cavefish populations, disturb food sources, and unknowingly stress individual fish by increasing fish activity. However, no evidence has been obtained for any of the above potential threats.

Several Southern Cavefish localities also are home to significant Grey Bat (*Myotis grisescens*) colonies (Table 7). Increased disturbance caused by human activity is thought to negatively affect grey bat colonies by increasing bat mortality or the eventual abandonment of a cave. If bat colonies are extirpated, Southern Cavefish populations may lose an important source of food and nutrients; however, data and anecdotal information are largely lacking. This could be a very serious threat given that the caves with the largest *A. rosae* populations are also grey bat maternity colony caves. However, more studies are needed to assess the impacts and lasting effects of intense cave visitation on cave fauna. The huge decline in the rich aquatic fauna of Shelta Cave, Alabama, including Southern Cavefish, has been attributed to the loss of a major grey bat roost (H. Hobbs, pers. comm.).

Cryptic diversity and conservation implications. The identification of cryptic species and ESUs (evolutionary significant units) has important implications for conservation and management. The occurrence of cryptic species in endangered nominal species requires special consideration in conservation planning (Bickford et al. 2007). First, species already having a conservation listing might be comprised of multiple species that may be more rare than previously thought. Second, these species might require different conservation strategies (Schonrogge et al. 2002). Our analyses of genetic diversity and structure in the wide-ranging Southern Cavefish revealed a diversity of deeply divergent lineages within this species and support provisional recognition of ESUs and even new species with more restricted geographic distributions than *T. subterraneus sensu lato*.

A definite pattern of correspondence existed between mitochondrial lineages and surface hydrological drainages within *Typhlichthys*. This pattern also has been observed for several other aquatic cave-dwelling species (Verovnik *et al.* 2004; Finston *et al.* 2007). No haplotypes were shared among drainages and pairwise sequence divergence between some drainages was as high as 11.6%. Lower levels of sequence divergence between some surface drainages east of the Mississippi River indicate a more recent connection. However, we found little evidence of contemporary gene flow among drainages and among populations within drainages. Haplotype sharing was observed only between two caves separated by 2.5 km in southern Franklin County, Tennessee. However, sampling for this dataset is sparse within localities and within some drainages. Therefore, more thorough collections are needed to elucidate contemporary gene flow among *Typhlichthys* populations.

It is important not to employ a single source of data, even molecular, when identifying units for conservation and management. Some sources, such as morphology for many wide-ranging cave organisms like the Southern Cavefish, may not offer much valuable information when discerning taxonomic or conservation units. Therefore, multiple sources of data, if available, should be utilized when identifying units for conservation and management. For many cave organisms, sources may be limited to geography, geology, morphology and a few molecular markers. The monophyly of several lineages within *Typhlichthys* that correspond to distinct drainages provides evidence that these lineages have evolved independently for considerable amounts of time. Some lineages have been separated since the late Miocene. Relying on genetic evidence alone, many of these lineages would be considered distinct species despite lack of morphological differences. Many of these lineages also inhabit different geological units and physiographic regions. However, can we demonstrate that genetic variation among lineages corresponds to speciation? Life history and behavioral evidence for reproductive isolation among lineages are lacking and remain to be demonstrated. However, several lineages can be defined as “genealogical species” under the genealogical species concept (Avise and Ball 1990; Baum and Shaw 1995) based on concordance of genetic, geographic, and geologic datasets. Likewise, these lineages can be considered “diagnosable species” under the criteria of the phylogenetic species concept (de Queiroz and Donoghue 1990) and as ESUs (*sensu* Moritz 1994) for conservation and management.

At this time, we offer three provisional recommendations. First, the *Typhlichthys* found on the Ozark Plateau west of the Mississippi are geographically and genetically distinct and should be designated an ESU or potentially a separate species. Second, *Typhlichthys* north of Tennessee must be studied further, as our single sample from the Red River drainage appears to be sister to the Ozark group and deeply divergent from all other eastern samples. Third, each of the other watersheds in Tennessee and Alabama should be considered ESUs or at least as demographically separate management units (Palsboll et al. 2007) because the lack of haplotype sharing and deep divergences among haplotypes suggest that each drainage harbors a unique and historically significant portion of the evolutionary diversity of *Typhlichthys* and dispersal among drainages is insignificant.

Although our sampling of Spring Cavefish is limited compared to that of Southern Cavefish, a different phylogeographic pattern is evident. First, populations from southern Illinois are genetically distinct from populations sampled in the Eastern Highland Rim of Tennessee (Fig. 12). Uncorrected pairwise mtDNA sequence divergence between these two groups averages 4.3% and supports recognition of two species: *F. papilliferus* from southern Illinois and *F. agassizii* from the Eastern Highland Rim of Tennessee. What remain in doubt presently are the taxonomic affinities of populations from Kentucky and the Western Pennyroyal Karst of Tennessee. Populations sampled across several watersheds in Tennessee (Upper Elk, Upper Duck, Collins, and Caney Fork) show little divergence suggesting that populations are more connected that previously thought and these populations should be designated as a single ESU and management unit.

Conservation measures. Several conservation measures have been proposed or implemented for populations of cave amblyopsids, including Southern Cavefish. Fencing or gating of cave entrances have been proposed or implemented to reduce and control human visitation to sensitive cave ecosystems. Special bat gates are needed to allow entry and exit by bats but stop human entry. Bat Conservation International and The National Speleological Society have been leaders in the improvement and installation of such gates on an increasing number of bat caves.

Protection of cave surface and subsurface watersheds is probably the most important intervention for Southern Cavefish caves. Watershed protection has included establishing preserves as well as institution of best land management practices around sinkholes and sinking creeks. This includes reforestation. Indeed, a number of cave systems receive some protection by occurring on state or federally owned land or are owned or leased by conversation agencies. In other cases, water tracing has identified the source of pollutants and so allowed legal action that remedied the situation. Hidden River Cave in Kentucky is one example. We suggest that demographic source caves (see Niemiller and Poulson in press) deserve complete protection of their watersheds. Only a few caves have the vast majority of all Southern Cavefish ever censused. Attention to protecting these caves is a number one priority for the near future. Likewise, source populations of Spring Cavefish should be identified and protected.

Introduction of Southern Cavefish and Spring Cavefish to new localities or to caves that were historic localities is worth considering in the event of dramatic large-scale population declines. However, until we learn to breed amblyopsids, the only source for introductions is caves with thriving populations. To protect natural patterns of genetic variation, these source caves should only be in watersheds that include the recipient cave.

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Table 1. Localities and survey results of Southern Cavefish in Tennessee compiled from literature sources, the Tennessee Natural Heritage Database (TNHD), the Tennessee Cave Survey (TCS), and the current study. Ecoregions: CB – Central Basin, CP – Cumberland Plateau, HR – Eastern or Western Highland Rim.

Date	Cave	Cave ID	Eco-region	Watershed	Lat	Long	County	No. Seen	Observers	Source	Comments
1957	Blowing Springs Cave	TCF18	CP	Upper Elk	35:21:34	85:53:38	Coffee	na	Woods & Inger	TNHD; Woods & Inger (1957)	
10/2/04	Blowing Springs Cave	TCF18	CP	Upper Elk	35:21:34	85:53:38	Coffee	26	ML Niemiller, BT Miller, N Mann, G Moni	Current Study	
7/21/07	Blowing Springs Cave	TCF18	CP	Upper Elk	35:21:34	85:53:38	Coffee	52	ML Niemiller, BT Miller, N Mann, G Reynolds	Current Study	
8/25/04	Lusk Cave	TCF8	CP	Upper Elk	35:25:29	85:54:49	Coffee	1	ML Niemiller, BT Miller, N Mann	Current Study	
10/18/04	Lusk Cave	TCF8	CP	Upper Elk	35:25:29	85:54:49	Coffee	0	ML Niemiller, BT Miller, BM Glorioso, GR Wyckoff	Current Study	
3/11/05	Lusk Cave	TCF8	CP	Upper Elk	35:25:29	85:54:49	Coffee	0	ML Niemiller, BT Miller, J Todd	Current Study	
8/26/05	Lusk Cave	TCF8	CP	Upper Elk	35:25:29	85:54:49	Coffee	1	ML Niemiller, BM Glorioso	Current Study	
1968	Riley Creek Cave	TCF9	HR	Upper Duck	35:29:14	86:12:46	Coffee	na	na	TNHD	
1985	Riley Creek Cave	TCF9	HR	Upper Duck	35:29:14	86:12:46	Coffee	na	S Jones	TCS	Inundated by Normandy Reservoir
1972	Baugus Cave	TDC1	HR	Tennessee-Lower Tennessee-Beech	35:45:09	88:05:07	Decatur	1	B Henne, J Ledbetter	Bechler (1974)	1 specimen collected
1974	Baugus Cave	TDC1	HR	Tennessee-Lower Tennessee-Beech	35:45:09	88:05:07	Decatur	na	J Ledbetter	TCS	
7/16/73	Baugus Cave	TDC1	HR	Tennessee-Lower Tennessee-Beech	35:45:09	88:05:07	Decatur	10	DL Bechler	Bechler (1974)	5 specimens collected
7/22/73	Baugus Cave	TDC1	HR	Tennessee-Lower Tennessee-Beech	35:45:09	88:05:07	Decatur	10	DL Bechler	TNHD; Bechler (1974)	3 specimens collected
7/22/86	Baugus Cave	TDC1	HR	Tennessee-Lower Tennessee-Beech	35:45:09	88:05:07	Decatur	1	Pride	TNHD	
7/17/05	Baugus Cave	TDC1	HR	Tennessee-Lower Tennessee-Beech	35:45:09	88:05:07	Decatur	7	ML Niemiller, N Mann, G Moni	Current Study	
5/2/08	Baugus Cave	TDC1	HR	Tennessee-Lower Tennessee-Beech	35:45:09	88:05:07	Decatur	9	ML Niemiller, RG Reynolds	Current Study	
1974	Stewman Creek Cave	TDC12	HR	Tennessee-	35:25:21	88:10:08	Decatur	2	J Ledbetter	TCS	

				Lower Tennessee-Beech							
8/4/73	Stewman Creek Cave	TDC12	HR	Tennessee-Lower Tennessee-Beech	35:25:21	88:10:08	Decatur	4	DL Bechler	TNHD	2 specimens collected
1976	Unnamed Cave		HR	Tennessee-Lower Tennessee-Beech	35:44:28	88:03:33	Decatur	1	J Ledbetter	TNHD	Cave not listed in TCS Database
1973	Woodlawn Shores Cave	TDC13	HR	Tennessee-Lower Tennessee-Beech	35:44:56	88:00:57	Decatur	1	J Ledbetter	TCS	Cave may be partially inundated by Kentucky Lake; access likely only by boat
2002	Cripps Mill Cave	TDK8	HR	Caney Fork	35:56:33	85:54:11	DeKalb	1	J Douglas	TCS	Landowner would not allow access in 2008
1960	Jewel Cave	TDI12	HR	Cumberland-Lower Cumberland	36:10:04	87:31:30	Dickson	na	na	TNHD	
1985	Jewel Cave	TDI12	HR	Cumberland-Lower Cumberland	36:10:04	87:31:30	Dickson	na	S Jones	TCS	
1994	Ruskin Cave	TDI16	HR	Cumberland-Lower Cumberland	36:09:39	87:31:13	Dickson	1	K Oeser	TCS	
1996	Ruskin Cave	TDI16	HR	Cumberland-Lower Cumberland	36:09:39	87:31:13	Dickson	1	K Oeser	TCS	
1/21/09	Lott Dean Cave	TFE116	CP	Obey	36:16:36	85:02:23	Fentress	0	ML Niemiller, N Mann	Current Study	
2007	Wolf River Cave	TFE12	CP	Obey	36:31:58	84:56:41	Fentress	2	K Bobo	TCS	Cave owned by SCCi; could not obtain access because of White-Nose Syndrome concerns
1980	Xanadu Cave	TFE94	CP	Obey	36:19:08	85:00:12	Fentress	12	J Hoffelt	TCS	
1984	Xanadu Cave	TFE94	CP	Obey	36:19:08	85:00:12	Fentress	6	East Tennessee Grotto and Louisville Grotto	TNHD	
10/3/84	Xanadu Cave	TFE94	CP	Obey	36:19:08	85:00:12	Fentress	1	M Swindoll	TNHD	1 specimen collected (UT 62.15)
9/26/96	Xanadu Cave	TFE94	CP	Obey	36:19:08	85:00:12	Fentress	0	na	TNHD	
pre1960	Zarathrustras Cave	TFE60	CP	Obey	36:18:13	85:00:39	Fentress	na	T Barr	T Barr pers. comm.	
1985	Buggytop Cave	TFR16	CP	Tennessee-Guntersville Lake	35:07:09	85:54:36	Franklin	na	S Jones	TCS	
2006	Buggytop Cave	TFR16	CP	Tennessee-Guntersville Lake	35:07:09	85:54:36	Franklin	1	G Moni	TCS	Cave owned by TDEC
6/9/04	Buggytop Cave	TFR16	CP	Tennessee-Guntersville Lake	35:07:09	85:54:36	Franklin	0	ML Niemiller, BT Miller, N Mann, J Corser, C Davis	Current Study	
10/18/04	Buggytop Cave	TFR16	CP	Tennessee-Guntersville Lake	35:07:09	85:54:36	Franklin	0	ML Niemiller, BT Miller, BM Glorioso, GR Wyckoff	Current Study	
2007	Caney Hollow Cave	TFR2	HR	Upper Elk	35:07:16	86:15:49	Franklin	1	K Oeser	TCS	
2003	Garner Spring Cave	TFR199	CP	Tennessee-	35:01:43	85:54:26	Franklin	3	J Buhay	TCS	

				Guntersville Lake							
8/28/04	Garner Spring Cave	TFR199	CP	Tennessee-Guntersville Lake	35:01:43	85:54:26	Franklin	0	ML Niemiller, G Moni	Current Study	Water level too high to pentrate cave more than 50 m
8/2/06	Garner Spring Cave	TFR199	CP	Tennessee-Guntersville Lake	35:01:43	85:54:26	Franklin	6	ML Niemiller, BT Miller	Current Study	
8/6/06	Garner Spring Cave	TFR199	CP	Tennessee-Guntersville Lake	35:01:43	85:54:26	Franklin	4	ML Niemiller, BT Miller	Current Study	
8/13/06	Garner Spring Cave	TFR199	CP	Tennessee-Guntersville Lake	35:01:43	85:54:26	Franklin	2	ML Niemiller, BT Miller	Current Study	
9/20/08	Garner Spring Cave	TFR199	CP	Tennessee-Guntersville Lake	35:01:43	85:54:26	Franklin	18	ML Niemiller, BT Miller, N Mann, J Miller	Current Study	
9/20/08	Little Crow Creek Cave	TFR15	CP	Tennessee-Guntersville Lake	35:00:45	85:58:00	Franklin	8	ML Niemiller, BT Miller, N Mann, J Miller	Current Study	
1944	Pearson Spring Cave	TFR228	CP	Upper Elk	35:11:16	85:57:26	Franklin	3	HT Kirby-Smith	TCS	
2003	Pearson Spring Cave	TFR228	CP	Upper Elk	35:11:16	85:57:26	Franklin	na	J Buhay, N Mann	TCS	
1/31/55	Powers Cave	TFR292	HR	Upper Elk	35:13:42	86:07:58	Franklin	4	HC Yeatman	TNHD	4 specimens collected; cave listed as Schwan Cave in TNHD
5/20/07	Powers Cave	TFR292	HR	Upper Elk	35:13:42	86:07:58	Franklin	0	ML Niemiller, BT Miller, N Mann, M Venarsky, TD Niemiller	Current Study	
1992	Salt River Cave	TFR23	CP	Tennessee-Guntersville Lake	34:59:18	85:58:32	Franklin	2	K Oeser	TCS	
8/6/06	Salt River Cave	TFR23	CP	Tennessee-Guntersville Lake	34:59:18	85:58:32	Franklin	18	ML Niemiller, BT Miller	Current Study	
5/20/07	Salt River Cave	TFR23	CP	Tennessee-Guntersville Lake	34:59:18	85:58:32	Franklin	4	ML Niemiller, BT Miller, N Mann, M Venarsky, TD Niemiller	Current Study	
1/4/76	Big Mouth Cave	TGD2	CP	Upper Elk	35:19:58	85:49:37	Grundy	na	na	TNHD	
6/29/04	Big Mouth Cave	TGD2	CP	Upper Elk	35:19:58	85:49:37	Grundy	0	ML Niemiller, BT Miller, N Mann, C Davis	Current Study	
7/21/04	Big Mouth Cave	TGD2	CP	Upper Elk	35:19:58	85:49:37	Grundy	1	ML Niemiller, BT Miller, BM Glorioso	Current Study	
1/26/05	Big Mouth Cave	TGD2	CP	Upper Elk	35:19:58	85:49:37	Grundy	1	ML Niemiller, BT Miller, BM Glorioso	Current Study	
2/6/05	Big Mouth Cave	TGD2	CP	Upper Elk	35:19:58	85:49:37	Grundy	1	ML Niemiller, BT Miller, BM Glorioso, J Todd	Current Study	
3/10/05	Big Mouth Cave	TGD2	CP	Upper Elk	35:19:58	85:49:37	Grundy	2	ML Niemiller, BT Miller, BM Glorioso, J Todd	Current Study	
4/17/05	Big Mouth Cave	TGD2	CP	Upper Elk	35:19:58	85:49:37	Grundy	7	BT Miller, GR Wyckoff, J Todd	Current Study	
6/16/05	Big Mouth Cave	TGD2	CP	Upper Elk	35:19:58	85:49:37	Grundy	16	ML Niemiller, J Todd	Current Study	
8/25/05	Big Mouth Cave	TGD2	CP	Upper Elk	35:19:58	85:49:37	Grundy	0	ML Niemiller, BT Miller	Current Study	
11/10/05	Big Mouth Cave	TGD2	CP	Upper Elk	35:19:58	85:49:37	Grundy	1	ML Niemiller, BT Miller, BM Glorioso	Current Study	
4/16/06	Big Mouth Cave	TGD2	CP	Upper Elk	35:19:58	85:49:37	Grundy	1	ML Niemiller, BT Miller	Current Study	

7/15/06	Big Mouth Cave	TGD2	CP	Upper Elk	35:19:58	85:49:37	Grundy	16	ML Niemiller, BT Miller	Current Study	
3/10/07	Big Mouth Cave	TGD2	CP	Upper Elk	35:19:58	85:49:37	Grundy	14	ML Niemiller, BT Miller	Current Study	
3/29/08	Big Mouth Cave	TGD2	CP	Upper Elk	35:19:58	85:49:37	Grundy	1	ML Niemiller, BT Miller	Current Study	
7/20/08	Big Mouth Cave	TGD2	CP	Upper Elk	35:19:58	85:49:37	Grundy	17	ML Niemiller, BT Miller, CD Hulsey	Current Study	
7/7/04	Big Room Cave	TGD3	CP	Upper Elk	35:19:53	85:49:30	Grundy	1	ML Niemiller, N Mann, C Davis	Current Study	
1979	Blue Spring			Upper Elk	35:21:27	85:50:12	Grundy	na	J Cooper	TNHD; Cooper (1979)	Spring outflow for Elkhead Shelter Cave (TGD165)
2004	Crystal Cave	TGD10	CP	Upper Elk	35:16:22	85:51:13	Grundy	12	G Moni	TCS	
12/19/66	Crystal Cave	TGD10	CP	Upper Elk	35:16:22	85:51:13	Grundy	na	Armstrong and Williams	TNHD; Armstrong and Williams (1976)	
5/12/05	Crystal Cave	TGD10	CP	Upper Elk	35:16:22	85:51:13	Grundy	24	ML Niemiller, BT Miller, N Mann, J Todd	Current Study	
11/21/06	Crystal Cave	TGD10	CP	Upper Elk	35:16:22	85:51:13	Grundy	30	ML Niemiller, BT Miller	Current Study	
5/25/05	Elkhead Shelter Cave	TGD165	CP	Upper Elk	35:21:30	85:50:13	Grundy	2	ML Niemiller, N Mann, J Todd	Current Study	
9/9/07	Jay Creek Spring Cave	TGD55	CP	Upper Elk	35:21:52	85:49:47	Grundy	3	BT Miller, K Ulicny	Current Study	
10/2/07	Jay Creek Spring Cave	TGD55	CP	Upper Elk	35:21:52	85:49:47	Grundy	4	BT Miller, N Mann	Current Study	
1990	Nunley Mountain Cave	TGD91	CP	Collins	35:30:09	85:43:01	Grundy	1	J Douglas	TCS	
8/28/04	Trussell Cave	TGD26	CP	Upper Elk	35:15:31	85:52:15	Grundy	0	ML Niemiller, G Moni	Current Study	
9/28/04	Trussell Cave	TGD26	CP	Upper Elk	35:15:31	85:52:15	Grundy	0	ML Niemiller, BT Miller	Current Study	
11/14/06	Trussell Cave	TGD26	CP	Upper Elk	35:15:31	85:52:15	Grundy	0	BT Miller	Current Study	
11/21/06	Trussell Cave	TGD26	CP	Upper Elk	35:15:31	85:52:15	Grundy	1	ML Niemiller, BT Miller	Current Study	
12/17/06	Trussell Cave	TGD26	CP	Upper Elk	35:15:31	85:52:15	Grundy	1	ML Niemiller, BM Fitzpatrick, B Sheffer	Current Study	
1944	Wildman Cave	TGD29	CP	Upper Elk	35:15:48	85:51:05	Grundy	30	HT Kirby-Smith	TCS	
1960	Wildman Cave	TGD29	CP	Upper Elk	35:15:48	85:51:05	Grundy	na	na	TNHD	
1985	Wildman Cave	TGD29	CP	Upper Elk	35:15:48	85:51:05	Grundy	na	S Jones	TCS	
1985	Wonder Cave	TGD30	CP	Upper Elk	35:16:24	85:50:59	Grundy	2	S Jones	TCS	Could not gain permission to enter cave in 2008
12/18/66	Wonder Cave	TGD30	CP	Upper Elk	35:16:24	85:50:59	Grundy	2	Armstrong and Williams	Armstrong and Williams (1971)	
1985	Dry Creek Cave	THR4	HR	Tennessee-Lower Tennessee-Beech	35:12:30	87:59:10	Hardin	na	S Jones	TCS	Could not gain permission to enter cave in 2008
pre1958	Dry Creek Cave	THR4	HR	Tennessee-Lower Tennessee-Beech	35:12:30	87:59:10	Hardin	na	na	TNHD	
1975	Pickwick Pot	THR6	TH	Tennessee-Pickwick Lake	35:01:37	88:10:28	Hardin	2	J Ledbetter	TCS	Could not gain permission to enter cave in 2007
7/18/73	Cave Branch Cave	THI3	HR	Buffalo	35:40:37	87:42:20	Hickman	2	DL Bechler	TNHD	
7/22/06	Cave Branch Cave	THI3	HR	Buffalo	35:40:37	87:42:20	Hickman	1	ML Niemiller, BT Miller, G Moni, A Moni	Current Study	
1999	Allens Creek Cave	TLS3	HR	Buffalo	35:26:15	87:35:47	Lewis	6	J Douglas	TCS	

7/25/73	Allens Creek Cave	TLS3	HR	Buffalo	35:26:15	87:35:47	Lewis	1	na	TNHD	1 specimen collected; list as Blowing Spring Cave in TNHD
8/9/74	Allens Creek Cave	TLS3	HR	Buffalo	35:26:15	87:35:47	Lewis	1	DL Bechler	TNHD; Bechler (1974)	1 specimen collected; list as Blowing Spring Cave in TNHD
7/22/06	Allens Creek Cave	TLS3	HR	Buffalo	35:26:15	87:35:47	Lewis	4	ML Niemiller, BT Miller, G Moni, A Moni	Current Study	
1999	Butterfly Cave	TMN160	CP	Sequatchie	35:12:34	85:35:26	Marion	20	A Cressler	TCS	
4/20/08	Butterfly Cave	TMN160	CP	Sequatchie	35:12:34	85:35:26	Marion	1	ML Niemiller, BT Miller, N Mann, J Miller	Current Study	
1985	Dancing Fern Cave	TMN8	CP	Sequatchie	35:07:58	85:35:26	Marion	na	S Jones	TCS	
7/12/08	Dancing Fern Cave	TMN8	CP	Sequatchie	35:07:58	85:35:26	Marion	0	ML Niemiller, BT Miller, N Mann	Current Study	
4/21/07	Lost Pig Cave	TMN20	CP	Tennessee-Guntersville Lake	35:03:18	85:45:23	Marion	2	ML Niemiller, N Mann	Current Study	
1985	Mandys Cave	TMN132	CP	Sequatchie	35:13:30	85:36:30	Marion	na	S Jones	TCS	
1901	Nickajack Cave	TMN26	CP	Tennessee-Middle Tennessee-Chickamauga	34:59:23	85:36:38	Marion	1	WP Hay	TNHD	Cave inundated by Nickajack Reservoir; Reported as Wine House Cave which is probably part of the Nickajack Cave
7/16/08	Pryor Spring Cave	TMN129	CP	Sequatchie	35:05:38	85:37:08	Marion	1	ML Niemiller, BT Miller	Current Study	
5/4/05	Shakerag Cave	TMN371	CP	Tennessee-Guntersville Lake	35:03:45	85:46:41	Marion	2	ML Niemiller, BT Miller	Current Study	
6/18/05	Gallagher Cave	TMS23	CB	Upper Duck	35:31:08	86:44:51	Marshall	1	ML Niemiller, G Moni, J Todd	Current Study	
8/9/05	Gallagher Cave	TMS23	CB	Upper Duck	35:31:08	86:44:51	Marshall	2	ML Niemiller, BM Glorioso	Current Study	
1/2/06	Gallagher Cave	TMS23	CB	Upper Duck	35:31:08	86:44:51	Marshall	0	ML Niemiller, BT Miller	Current Study	
6/18/05	Gallagher Cave South	TMS24	CB	Upper Duck	35:31:07	86:44:49	Marshall	23	ML Niemiller, G Moni, J Todd	Current Study	
2000	Gropp Cave	TMS31	CB	Upper Duck	35:39:18	86:48:06	Marshall	8	K Oeser	TCS	
2004	Macedonia River Cave	TMS42	CB	Upper Duck	35:39:16	86:48:49	Marshall	1	L Roebuck	TCS	
6/18/05	Pompie Cave	TMU19	CB	Upper Duck	35:32:42	86:53:25	Maury	0	ML Niemiller, G Moni, J Todd	Current Study	
8/9/05	Pompie Cave	TMU19	CB	Upper Duck	35:32:42	86:53:25	Maury	8	ML Niemiller, BM Glorioso	Current Study	
1/2/06	Pompie Cave	TMU19	CB	Upper Duck	35:32:42	86:53:25	Maury	0	ML Niemiller, BT Miller	Current Study	
6/16/06	Pompie Cave	TMU19	CB	Upper Duck	35:32:42	86:53:25	Maury	5	ML Niemiller, BT Miller, JA Miller, JH Miller	Current Study	
1975	Austin Peay Cave	TMY10	HR	Red	36:34:06	87:20:14	Montgomery	3	D McDowell	TCS	
11/22/68	Austin Peay Cave	TMY10	HR	Red	36:34:06	87:20:14	Montgomery	1	D Harker, Alsop, DA Etnier	UTKIC	1 specimen collected (UT 62.1)
4/28/79	Austin Peay Cave	TMY10	HR	Red	36:34:06	87:20:14	Montgomery	na	na	TNHD	
12/28/85	Bellamy Cave	TMY1	HR	Cumberland-Lower Cumberland	36:29:39	87:34:14	Montgomery	1	T Mann	TNHD; TCS	
pre1978	Bellamy Cave	TMY1	HR	Cumberland-Lower	36:29:39	87:34:14	Montgomery	na	Snyder	TNHD	

				Cumberland							
2007	Darnells Cave	TMY13	HR	Cumberland-Lower Cumberland	36:31:10	87:33:36	Montgomery	1	J Richards	TCS	
1961	Dunbar Cave	TMY5	HR	Red	36:33:11	87:18:22	Montgomery	1	T Barr	TNHD; T Barr pers. comm.	
1976	Dunbar Cave	TMY5	HR	Red	36:33:11	87:18:22	Montgomery	3	D McDowell	TCS	
2002	Dunbar Cave	TMY5	HR	Red	36:33:11	87:18:22	Montgomery	1	K Oeser	TCS	
2003	Silvey Cave	TMY41	HR	Red	36:33:27	87:12:41	Montgomery	na	M Silvey	TCS	
11/16/75	Silvey Cave	TMY41	HR	Red	36:33:27	87:12:41	Montgomery	na	na	TNHD	
1957	East Water Supply Cave	TOV15	CP	Cumberland-Upper Cumberland-Cordell Hull Reservoir	36:22:55	85:20:22	Overton	na	Woods & Inger	TNHD; Woods & Inger (1957)	
2005	East Water Supply Cave	TOV15	CP	Cumberland-Upper Cumberland-Cordell Hull Reservoir	36:22:55	85:20:22	Overton	1	G Moni	TCS	
2006	Mill Spring Cave	TOV153	CP	Cumberland-Upper Cumberland-Cordell Hull Reservoir	36:18:02	85:19:39	Overton	1	K Bobo	TCS	
7/17/73	Blowing Caves	TPR4	HR	Buffalo	35:42:02	87:44:28	Perry	16	D Bechler	TNHD; TCS	2 specimens collected; could not gain permission to enter cave in 2007
7/21/86	Blowing Caves	TPR4	HR	Buffalo	35:42:02	87:44:28	Perry	12	Pride	TNHD	
1985	Greer Hollow Cave	TPR50	HR	Buffalo	35:48:18	87:45:36	Perry	na	S Jones	TCS	
8/10/73	Greer Hollow Cave	TPR50	HR	Buffalo	35:48:18	87:45:36	Perry	2	na	TNHD	2 specimens collected; cave listed as Unnamed Cave in TNHD
7/22/06	Greer Hollow Cave	TPR50	HR	Buffalo	35:48:18	87:45:36	Perry	0	ML Niemiller, BT Miller, G Moni, A Moni	Current Study	
10/25/07	Anderson Spring Cave	TPU431	CP	Caney Fork	36:08:03	85:26:47	Putnam	2	ML Niemiller, BT Miller, M Thurman, N Mann, J Miller	Current Study	
2/21/09	Anderson Spring Cave	TPU431	CP	Caney Fork	36:08:03	85:26:47	Putnam	5	ML Niemiller, BM Fitzpatrick	Current Study	
1985	Arch Cave	TPU66	CP	Caney Fork	36:01:58	85:15:58	Putnam	na	S Jones	TCS	
1/22/02	Barlett Cave	TPU2	HR	Cumberland-Upper Cumberland-Cordell Hull Reservoir	36:13:41	85:44:37	Putnam	1	N Mann, J Buhay	TNHD; TCS	
5/28/06	Barlett Cave	TPU2	HR	Cumberland-Upper Cumberland-Cordell Hull Reservoir	36:13:41	85:44:37	Putnam	8	ML Niemiller, BT Miller, G Moni	Current Study	
1961	Blind Fish Cave	TPU4	CP	Caney Fork	36:03:21	85:20:31	Putnam	2	T Barr	TCS	

1990	Blind Fish Cave	TPU4	CP	Caney Fork	36:03:21	85:20:31	Putnam	2	K Oeser	TCS	
6/26/76	Blind Fish Cave	TPU4	CP	Caney Fork	36:03:21	85:20:31	Putnam	na	na	TNHD	
3/21/78	Blind Fish Cave	TPU4	CP	Caney Fork	36:03:21	85:20:31	Putnam	1	Burkhead, Nieland, Beets, Ryon, and Harris	UTKIC	1 specimen collected (UT 62.8)
8/4/08	Blind Fish Cave	TPU4	CP	Caney Fork	36:03:21	85:20:31	Putnam	18	ML Niemiller, BT Miller, J Miller	Current Study	
1982	Blondefish Spring Cave	TPU222	CP	Caney Fork	36:03:14	85:19:57	Putnam	na	D Anthony	TCS	
1988	Bridge Creek Cave	TPU7	CP	Caney Fork	36:02:12	85:16:25	Putnam	na	K Oeser	TCS	
1988	Jacques Cave	TPU128	CP	Caney Fork	36:06:51	85:26:29	Putnam	1	J Douglas	TCS	
10/25/07	Jacques Cave	TPU128	CP	Caney Fork	36:06:51	85:26:29	Putnam	121	ML Niemiller, BT Miller, M Thurman, N Mann, J Miller	Current Study	
2007	Stamps Cave	TPU55	CP	Caney Fork	36:07:34	85:23:54	Putnam	1	J Douglas	TCS	
4/15/08	Stamps Cave	TPU55	CP	Caney Fork	36:07:34	85:23:54	Putnam	58	ML Niemiller, M Thurman	Current Study	
2007	Talent Hollow Cave	TPU130	CP	Cumberland-Upper Cumberland-Cordell Hull Reservoir	36:12:36	85:22:52	Putnam	4	K Bobo	TCS	
2000	Bagwells Borehole	TRB39	HR	Red	36:34:23	86:56:45	Robertson	1	K Oeser	TCS	
pre1960	Jesse James Cave No. 1	TRB8	HR	Red	36:34:38	86:42:53	Robertson	na	T Barr	T Barr pers. Comm	
1987	Mint Spring Cave	TRB65	HR	Red	36:36:41	87:05:43	Robertson	6	G Hrepta	TCS	
2006	Sinking Ridge Cave	TRB88	HR	Red	36:34:13	86:55:52	Robertson	2	R Van Fleet	TCS	
3/17/08	Sinking Ridge Cave	TRB88	HR	Red	36:34:13	86:55:52	Robertson	1	ML Niemiller, G Moni	Current Study	
pre1960	Yates Cave	TRB13	HR	Red	36:33:57	86:42:22	Robertson	na	T Barr	T Barr pers. Comm	
2007	Guy James Cave	TRU140	CB	Stones	35:52:56	86:16:44	Rutherford	10	J Richards	TCS	Underwater cave requiring SCUBA gear
1986	Herring Cave	TRU8	CB	Stones	35:56:19	86:18:27	Rutherford	1	P Hamel, P Hamel	TNHD	
2003	Herring Cave	TRU8	CB	Stones	35:56:19	86:18:27	Rutherford	na	L Roebuck	TCS	
7/27/94	Herring Cave	TRU8	CB	Stones	35:56:19	86:18:27	Rutherford	1	na	TNHD	
7/29/04	Herring Cave	TRU8	CB	Stones	35:56:19	86:18:27	Rutherford	37	ML Niemiller, BT Miller, H Garland, DI Withers, J Douglas	Current Study	
8/27/04	Herring Cave	TRU8	CB	Stones	35:56:19	86:18:27	Rutherford	47	ML Niemiller, BT Miller	Current Study	
6/25/05	Herring Cave	TRU8	CB	Stones	35:56:19	86:18:27	Rutherford	32	ML Niemiller, BT Miller, J Todd	Current Study	
1/9/06	Herring Cave	TRU8	CB	Stones	35:56:19	86:18:27	Rutherford	0	H Garland. S Stroebel	TNHD	Incomplete survey
7/20/06	Herring Cave	TRU8	CB	Stones	35:56:19	86:18:27	Rutherford	39	ML Niemiller, BT Miller, G Call	Current Study	
2007	Lewis Sinking Creek Cave	TRU143	CB	Stones	35:56:44	86:27:16	Rutherford	10	J Richards	TCS	Underwater cave requiring SCUBA gear
7/28/04	Pattons Cave	TRU12	CB	Stones	36:03:00	86:26:48	Rutherford	1	ML Niemiller, BT Miller	Current Study	
7/29/04	Pattons Cave	TRU12	CB	Stones	36:03:00	86:26:48	Rutherford	1	ML Niemiller, BT Miller, H Garland, DI Withers, J Douglas	Current Study	
5/3/05	Pattons Cave	TRU12	CB	Stones	36:03:00	86:26:48	Rutherford	4	ML Niemiller, BM Glorioso	Current Study	

12/10/05	Pattons Cave	TRU12	CB	Stones	36:03:00	86:26:48	Rutherford	0	ML Niemiller, J Todd	Current Study	
3/6/06	Pattons Cave	TRU12	CB	Stones	36:03:00	86:26:48	Rutherford	33	ML Niemiller, BT Miller	Current Study	
8/7/06	Pattons Cave	TRU12	CB	Stones	36:03:00	86:26:48	Rutherford	3	ML Niemiller, BT Miller	Current Study	
3/11/07	Pattons Cave	TRU12	CB	Stones	36:03:00	86:26:48	Rutherford	17	ML Niemiller, BT Miller, N Mann	Current Study	
1961	Snail Shell Cave	TRU16	CB	Stones	35:46:56	86:32:12	Rutherford	1	T Barr	TCS	
1988	Snail Shell Cave	TRU16	CB	Stones	35:46:56	86:32:12	Rutherford	2	na	TNHD	
7/18/04	Snail Shell Cave	TRU16	CB	Stones	35:46:56	86:32:12	Rutherford	6	ML Niemiller, BT Miller, N Mann, B Biddix	Current Study	
8/4/04	Snail Shell Cave	TRU16	CB	Stones	35:46:56	86:32:12	Rutherford	17	ML Niemiller, BT Miller, N Mann, J Douglas, C Davis	Current Study	
1988	The Kitchen Sink	TRU91	CB	Harpeth	35:46:23	86:34:27	Rutherford	1	D Plemons	TCS	
8/24/94	Well		CB	Stones	35:52:36	86:29:35	Rutherford	2	M Taylor	TNHD	2 individuals videotaped 64' below surface
1985	West Fork Cave	TRU60	CB	Stones	35:54:17	86:26:38	Rutherford	2	D Plemons	TCS	
1963	Flat Rock Cave	TSM66	CB	Cumberland-Lower Cumberland-Old Hickory Lake	36:14:06	86:05:57	Smith	4	W Brode	TNHD	4 specimens collected; cave listed as Seay-White Farm Cave in TNHD
1992	Flat Rock Cave	TSM66	CB	Cumberland-Lower Cumberland-Old Hickory Lake	36:14:06	86:05:57	Smith	2	H Love	TCS	
5/28/06	Flat Rock Cave	TSM66	CB	Cumberland-Lower Cumberland-Old Hickory Lake	36:14:06	86:05:57	Smith	10	ML Niemiller, BT Miller, G Moni	Current Study	
1963	Wet-Weather Spring Cave	TSM91	HR	Cumberland-Lower Cumberland-Old Hickory Lake	36:16:05	86:05:17	Smith	4	W Brode	TNHD	4 specimens collected; listed as Taylor Farm Cave in TNHD; coordinates put Taylor Farm Cave N of Cumberland River whereas TSM91 is S of river and closest known cave to coordinates
2006	Wet-Weather Spring Cave	TSM91	CB	Cumberland-Lower Cumberland-Old Hickory Lake	36:16:05	86:05:17	Smith	3	G Moni	TCS	
1977	Lackey Cave	TSN10	CB	Cumberland-Lower Cumberland-Old Hickory Lake	36:22:23	86:27:58	Sumner	24	L Matthews	TCS	Cave entrances covered by housing development
1987	Lackey Cave	TSN10	CB	Cumberland-Lower Cumberland-Old Hickory	36:22:23	86:27:58	Sumner	100	L Matthews	TNHD	Cave entrances covered by housing development

				Lake							
9/20/78	Lackey Cave	TSN10	CB	Cumberland-Lower Cumberland-Old Hickory Lake	36:22:23	86:27:58	Sumner	na	L Matthews	TNHD	Cave entrances covered by housing development
12/7/80	Lackey Cave	TSN10	CB	Cumberland-Lower Cumberland-Old Hickory Lake	36:22:23	86:27:58	Sumner	2	L Matthews, K McLean	TNHD	Cave entrances covered by housing development; 2 specimens collected
1987	Camps Gulf Cave	TVB2	CP	Caney Fork	35:44:54	85:22:50	Van Buren	na	S Carroll	TNHD	
2007	Camps Gulf Cave	TVB2	CP	Caney Fork	35:44:54	85:22:50	Van Buren	100	J Hutchison	TCS	
12/20/97	Camps Gulf Cave	TVB2	CP	Caney Fork	35:44:54	85:22:50	Van Buren	6	L Roebuck	TNHD	
10/2/05	Camps Gulf Cave No. 2	TVB197	CP	Caney Fork	35:44:57	85:22:28	Van Buren	14	ML Niemiller, G Moni, A Moni	Current Study	
1979	Davis Tire Cave	TVB227	CP	Caney Fork	35:42:09	85:22:44	Van Buren	1	J Smyre	TCS	
2000	Hillis Cave	TVB413	CP	Caney Fork	35:42:29	85:34:16	Van Buren	6	G Moni	TCS	
2004	Horrid Hole	TVB244	CP	Caney Fork	35:44:55	85:22:46	Van Buren	1	G Gould	TCS	
2006	Keystone River Cave	TVB341	CP	Caney Fork	35:42:42	85:31:36	Van Buren	3	D Titus	TCS	
1974	Pennywinkle Cave	TVB173	CP	Caney Fork	35:45:11	85:32:15	Van Buren	1	J Smyre	TCS	
2002	Pennywinkle Cave	TVB173	CP	Caney Fork	35:45:11	85:32:15	Van Buren	na	J Lewis	TNHD; Lewis (2002)	
2000	Rumbling Falls Cave	TVB588	CP	Caney Fork	35:44:28	85:25:01	Van Buren	25	MO Smith	TCS	
10/29/01	Rumbling Falls Cave	TVB588	CP	Caney Fork	35:44:28	85:25:01	Van Buren	na	J Lewis	TNHD; Lewis (2001)	
1999	Swamp River Cave	TVB657	CP	Caney Fork	35:47:54	85:27:01	Van Buren	100	H Love	TCS	
1999	Swamp River Cave	TVB657	CP	Caney Fork	35:47:54	85:27:01	Van Buren	100+	several cavers	TNHD	
10/13/01	Swamp River Cave	TVB657	CP	Caney Fork	35:47:54	85:27:01	Van Buren	na	J Lewis	TNHD; Lewis (2001)	
1991	Thunder Run Cave	TVB515	CP	Caney Fork	35:47:58	85:25:46	Van Buren	1	J Hutchison	TCS	
1998	Thunder Run Cave	TVB515	CP	Caney Fork	35:47:58	85:25:46	Van Buren	100+	several cavers	TNHD	
1999	Thunder Run Cave	TVB515	CP	Caney Fork	35:47:58	85:25:46	Van Buren	2	H Love	TCS	
10/13/01	Thunder Run Cave	TVB515	CP	Caney Fork	35:47:58	85:25:46	Van Buren	1	J Lewis	TNHD; Lewis (2001)	
1997	Upper Cane Creek Cave	TVB631	CP	Caney Fork	35:42:38	85:22:53	Van Buren	1	J Hutchison	TCS	
1987	Windy River Cave	TVB352	CP	Caney Fork	35:43:35	85:23:31	Van Buren	1	J Hutchison	TCS	
2000	Windy River Cave	TVB352	CP	Caney Fork	35:43:35	85:23:31	Van Buren	1	J Douglas	TCS	
1960	Artesian Spring by Rock Island Dam		HR	Caney Fork	35:48:07	85:37:24	Warren	na	na	TNHD	
3/6/05	Blowing Cave	TWR4	CP	Collins	35:34:17	85:39:40	Warren	1	BT Miller, N Mann	Current Study	
6/16/07	Blowing Cave	TWR4	CP	Collins	35:34:17	85:39:40	Warren	2	ML Niemiller, BT Miller, K Ulicny	Current Study	
1997	Hazel Ward Cave	TWR315	HR	Collins	35:46:53	85:39:39	Warren	na	D Hunter	Tag-Net	Upstream of Jaco Spring Cave (TWR317)
1992	Hickey Pot	TWR95	CP	Collins	35:36:38	85:39:33	Warren	na	MO Smith	TCS	
8/5/04	Jaco Spring Cave	TWR317	HR	Collins	35:47:12	85:39:38	Warren	3	ML Niemiller, BT Miller, N Mann, J Douglas, B Walter	Current Study	

6/22/07	Jaco Spring Cave	TWR317	HR	Collins	35:47:12	85:39:38	Warren	28	ML Niemiller, BT Miller	Current Study	
1957	Panter Cave	TWR21	CP	Collins	35:34:18	85:38:18	Warren	na	Woods & Inger	TNHD; Woods & Inger (1957)	Could not gain permission to enter cave in 2006
1982	Hound Dog Drop	TWY8	HR	Tennessee-Pickwick Lake	35:02:47	87:35:28	Wayne	na	Garrett	TNHD	
1999	Hound Dog Drop	TWY8	HR	Tennessee-Pickwick Lake	35:02:47	87:35:28	Wayne	2	B Kuhajda and Mayden	Kuhajda and Mayden (2001)	
1997	Blue Spring Cave	TWH2	CP	Caney Fork	35:57:57	85:23:12	White	2	H Love	TCS	
2007	Blue Spring Resurgence Cave	TWH979	CP	Caney Fork	35:57:26	85:22:59	White	4	C Richards	TCS	
1999	Great Big Bottom Cave	TWH734	CP	Caney Fork	35:49:00	85:21:05	White	15	J Greene	TCS	
2000	Great Big Bottom Cave	TWH734	CP	Caney Fork	35:49:00	85:21:05	White	3	K Oeser	TCS	
2002	Rose Cave	TWH36	CP	Caney Fork	35:49:26	85:16:02	White	na	J Lewis	TNHD; Lewis (2002)	
5/23/05	Burnt House Cave	TWL35	CB	Stones	36:05:06	86:17:47	Wilson	0	ML Niemiller, J Todd	Current Study	
5/10/97	Canyon Cave	TWL8	CB	Stones	36:02:32	86:20:44	Wilson	1	K Oeser	TNHD; TCS	
9/14/93	Cedar Forest Cave	TWL9	CB	Stones	36:05:23	86:23:09	Wilson	1	L Walthers and D Mullen	TNHD	1 specimen collected
8/28/01	Cedar Forest Cave	TWL9	CB	Stones	36:05:23	86:23:09	Wilson	4	K Oeser	TNHD; TCS	
5/26/05	Cedar Forest Cave	TWL9	CB	Stones	36:05:23	86:23:09	Wilson	0	ML Niemiller, J Todd	Current Study	
2009	Hurricane Creek Cave	TWL80	CB	Stones	36:04:14	86:18:10	Wilson	3	R Van Fleet	TCS	
12/18/05	Hurricane Junction Cave	TWL73	CB	Stones	36:04:06	86:22:12	Wilson	0	ML Niemiller, BT Miller, J Todd	Current Study	
1/3/00	Jackson Cave	TWL20	CB	Stones	36:05:08	86:19:29	Wilson	4	K Oeser	TNHD; TCS	
2/5/00	Jackson Cave	TWL20	CB	Stones	36:05:08	86:19:29	Wilson	na	K Oeser	TNHD	
6/8/04	Jackson Cave	TWL20	CB	Stones	36:05:08	86:19:29	Wilson	1	BT Miller, C Davis	Current Study	
6/12/04	Jackson Cave	TWL20	CB	Stones	36:05:08	86:19:29	Wilson	1	ML Niemiller, TD Niemiller	Current Study	
11/11/04	Jackson Cave	TWL20	CB	Stones	36:05:08	86:19:29	Wilson	2	ML Niemiller, BT Miller	Current Study	
5/3/05	Jackson Cave	TWL20	CB	Stones	36:05:08	86:19:29	Wilson	1	ML Niemiller, BM Glorioso	Current Study	
5/23/05	Jackson Cave	TWL20	CB	Stones	36:05:08	86:19:29	Wilson	2	ML Niemiller, J Todd	Current Study	
6/29/05	Jackson Cave	TWL20	CB	Stones	36:05:08	86:19:29	Wilson	2	ML Niemiller, J Todd	Current Study	
9/8/05	Jackson Cave	TWL20	CB	Stones	36:05:08	86:19:29	Wilson	1	ML Niemiller, R Timmons	Current Study	
7/8/06	Jackson Cave	TWL20	CB	Stones	36:05:08	86:19:29	Wilson	1	ML Niemiller, BT Miller, JA Miller	Current Study	
9/13/08	Jackson Cave	TWL20	CB	Stones	36:05:08	86:19:29	Wilson	2	ML Niemiller, BT Miller, J Miller	Current Study	

Table 2. Distribution of Southern Cavefish (*T. subterraneus*) localities and maximum number of cavefish observed during a single survey by county.

County	Localities	Maximum Fish Observed
Coffee	3	52
Decatur	4	10
DeKalb	1	1
Dickson	2	1
Fentress	4	12
Franklin	7	18
Grundy	10	30
Hardin	2	2
Hickman	1	2
Lewis	1	6
Marion	7	20
Marshall	4	23
Maury	1	8
Montgomery	5	3
Overton	2	1
Perry	2	16
Putnam	9	121
Robertson	5	6
Rutherford	8	47
Smith	2	10
Sumner	1	>100
Van Buren	12	>100
Warren	6	28
Wayne	1	2
White	4	15
Wilson	6	4

Table 3. Distribution of Southern Cavefish (*T. subterraneus*) localities and maximum number of cavefish observed during a single survey by HUC8 watershed.

HUC8 Watershed	Localities	Maximum Fish Observed
Buffalo	4	16
Caney Fork	25	121
Collins	6	28
Cumberland-Lower Cumberland	4	1
Cumberland-Lower Cumberland-Old Hickory Lake	3	>100
Cumberland-Upper Cumberland-Cordell Hull	4	8
Harpeth	1	1
Obey	4	12
Red	8	6
Sequatchie	4	20
Stones	13	47
Tennessee-Lower Tennessee-Beech	5	10
Tennessee-Guntersville Lake	6	18
Tennessee-Middle Tennessee-Chickamauga	1	1
Tennessee-Pickwick Lake	2	2
Upper Duck	6	23
Upper Elk	14	52

Table 4. Distribution of Spring Cavefish (*F. agassizii*) localities by county based on museum collections, literature records, and the current study.

County	Localities
Bedford	1
Cannon	4
Coffee	10
Davidson	2
DeKalb	5
Dickson	1
Franklin	2
Grundy	1
Montgomery	8
Stewart	5
Sumner	1
Warren	2
Wilson	1

Table 5. Distribution of Spring Cavefish (*F. agassizii*) localities by HUC8 watershed based on museum collections, literature records, and the current study.

HUC8 Watershed	Localities
Caney Fork	5
Collins	10
Cumberland-Lower Cumberland	3
Cumberland-Lower Cumberland-Old Hickory Lake	2
Cumberland-Lower Cumberland-Sycamore	2
Red	7
Tennessee-Kentucky Lake	3
Upper Duck	8
Upper Elk	3

Table 6. Museum accessions of Southern Cavefish (*T. subterraneus*) from Tennessee.

Museum	Mus. No.	Date	Locality	County	No.	Collectors
CMNH	62325			Grundy		
FMNH	62048	10/23/1950	Crystal Cave	Grundy		Woods and Inger
FMNH	62052	10/26/1950	Crystal Cave	Grundy	1	LP Woods, RH Kanazawa
FMNH	62053	10/29/1950	Blowing Springs Cave	Coffee	1	LP Woods, HT Kirby-Smith, K Kanazawa, Harrison
FMNH	62054	10/29/1950	Blowing Springs Cave	Coffee	3	LP Woods, HT Kirby-Smith, K Kanazawa, Harrison
FMNH	62055	10/29/1950	Blowing Springs Cave	Coffee	3	LP Woods, HT Kirby-Smith, K Kanazawa, Harrison
FMNH	62056	10/26/1950	Crystal Cave	Grundy	8	LP Woods, RH Kanazawa
FMNH	62325	10/26/1950	Crystal Cave	Grundy	5	LP Woods, RH Kanazawa
FMNH	62048	10/23/1956	Crystal Cave	Grundy	3	LP Woods, RH Kanazawa, E Raulston
INHS	50142	1965	Blind Fish Cave	Putnam	1	
MCZ	782	1854	Lebanon	Wilson	1	JM Safford
UAIC	1052.02	1963	Taylor Farm Cave at Beasley Bend on Cumberland River near Rome, 8 mi W of Carthhage on US Hwy 70 N	Smith	4	W. Brode
UAIC	1053.01	1963	Seay-White Farm Cave, 3 mi S of Rome on Flat Rock Road, tributary to Lick Creek	Smith	4	W. Brode
UAIC	1977.01	12/20/1970	Crystal Cave complex, underground streams 0.5 mi from Wonder Cave attraction	Grundy	2	J.G. Armstrong, J.D. Williams, F. Raulston
UAIC	3958.01	6/23/1967	Wonder Cave (Crystal Cave) near Pelham	Grundy	5	R.A. Brandon, J.E. Huhley
UFFC	697	11/23/1953		Grundy	2	RB Cumming, MV Protherae
UMMZ	103552	3/10/1937	Well at Lebanon	Wilson	1	AR Cahn
UMMZ	105667	2/10/1938	Cave near Dry Creek	Hardin	3	LR Miller & Bryan
UMMZ	133264		Well in Murfreesboro (E Castle St.) - Lee Jenkins well	Rutherford	2	For GM Edney
UMMZ	133544	4/16/1941	Well in Murfreesboro (E Castle St.) - Lee Jenkin's well	Rutherford	2	For GM Edney
UMMZ	174850	6/4/1953	Cave 1 mi N of Monterey-Sparta Hwy. on farm; Tennessee River dr	Putnam	2	N Benson, Fetteroff, *
UMMZ	196194	8/17/72	Austin Peay Pit Cave, Austin Peay University Farm	Montgomery	1	D Bechler
USNM	232538	7/30/69	Blind Fish Cave	Putnam	8	R Bouchard, A Gnilka, R Sayres
UTKIC	62.1	11/22/1968	Sink Hole Cave on Austin Peay College	Montgomery	1	Don Harker, Alsop, Etnier

			Farm in Clarksvile			
UTKIC	62.8	3/21/1978	Blind Fish Cave off county road just off of SR 84, 14.0 air miles N of Sparta, ca 10.9 air miles ESE of Cookeville	Putnam	1	Burkhead, Nieland, Beets, Ryon, and Harris
UTKIC	62.15	10/3/1984	Xanadu Cave, ca. 100 yds from Obey River	Fentress	1	Cathy Justus and Mike Swindoll

Table 7. Southern Cavefish localities that also house significant Grey Bat (Myotis grisescens) colonies.

Cave	Cave ID	County
Allens Creek Cave	TLS3	Lewis
Jaco Spring Cave	TWR317	Warren
Caney Hollow Cave	TFR2	Franklin
Lusk Cave	TCF8	Coffee
Trussell Cave	TGD26	Grundy
Bellamy Cave	TMY1	Montgomery
Dunbar Cave	TMY5	Montgomery
Herring Cave	TRU8	Rutherford
Pattons Cave	TRU12	Rutherford
Wolf River Cave	TFE12	Fentress
Nickajack Cave	TMN26	Marion

Fig. 1. The Spring Cavefish (*F. agassizii*) from Coffee County, Tennessee. Photo by Matthew Niemiller.

Fig. 2. The Southern Cavefish (*T. subterraneus*) from Grundy County, Tennessee. Photo courtesy of Dante Fenolio.

Fig. 3. The Southern Cavefish (*T. subterraneus*) from Marion County, Tennessee. Note the lack of adipose tissue around the vestigial eyes. Photo by Matthew Niemiller.

Fig. 4. Distribution by county of the Amblyopsidae in the eastern United States. Southern Cavefish and Spring Cavefish are the only amblyopsid cavefishes that occur in Tennessee.

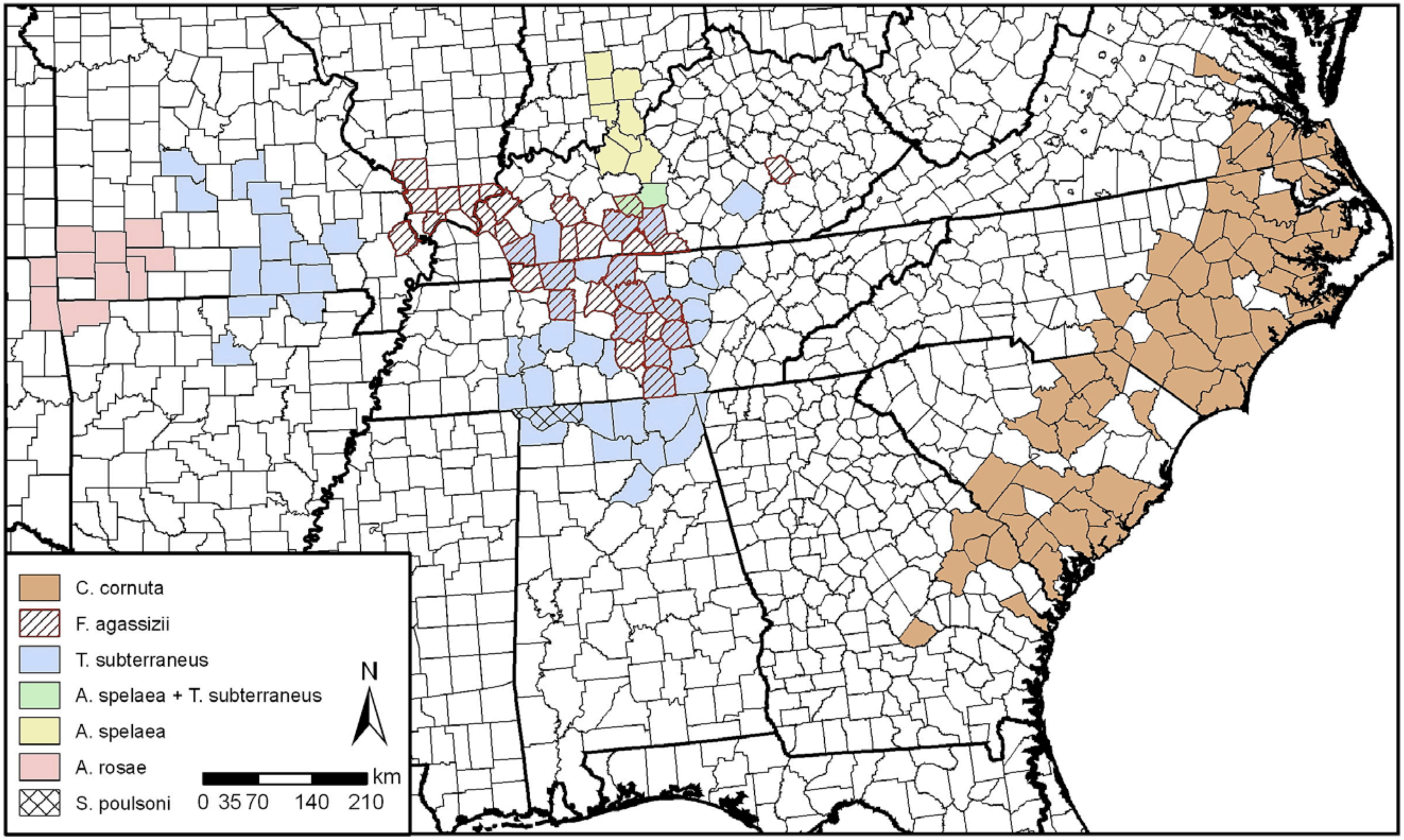


Fig. 5. The distribution of the Southern Cavefish (*T. subterraneus*) in Tennessee. Southern Cavefish have been reported from 107 caves, two springs, and one well in 26 counties.

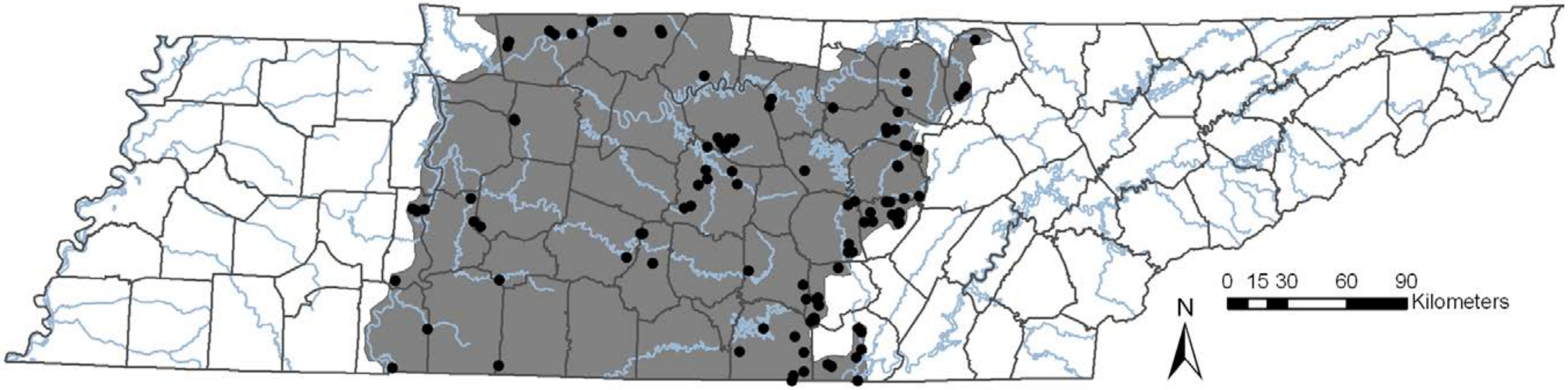


Fig. 6. The distribution of the Southern Cavefish (*T. subterraneus*) in Tennessee overlaid on ecoregion. Southern Cavefish are found primarily in the Interior Low Plateau and escarpments of the Cumberland Plateau.

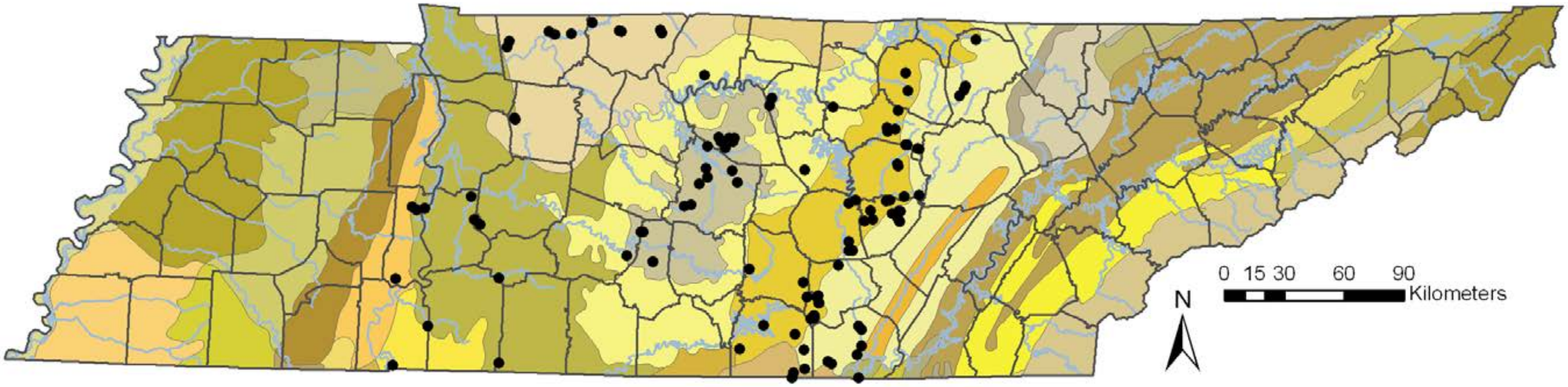


Fig. 7. The distribution of the Southern Cavefish (*T. subterraneus*) in Tennessee overlaid on HUC8 watershed. Southern Cavefish have been reported from 17 watersheds in the state.

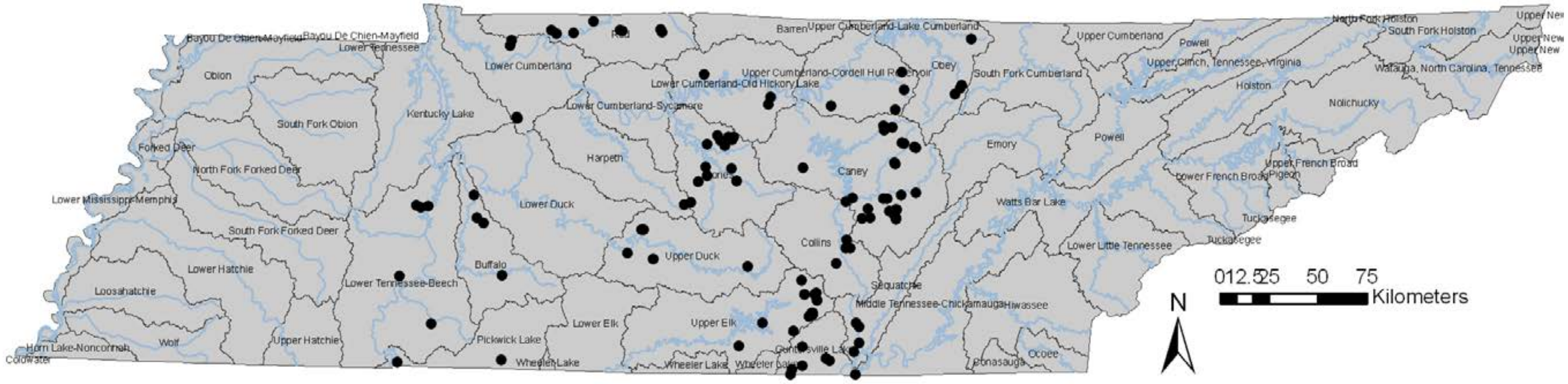


Fig. 8. The distribution of the Spring Cavefish (*F. agassizii*) in Tennessee. Spring Cavefish have been reported from 13 counties in the state.

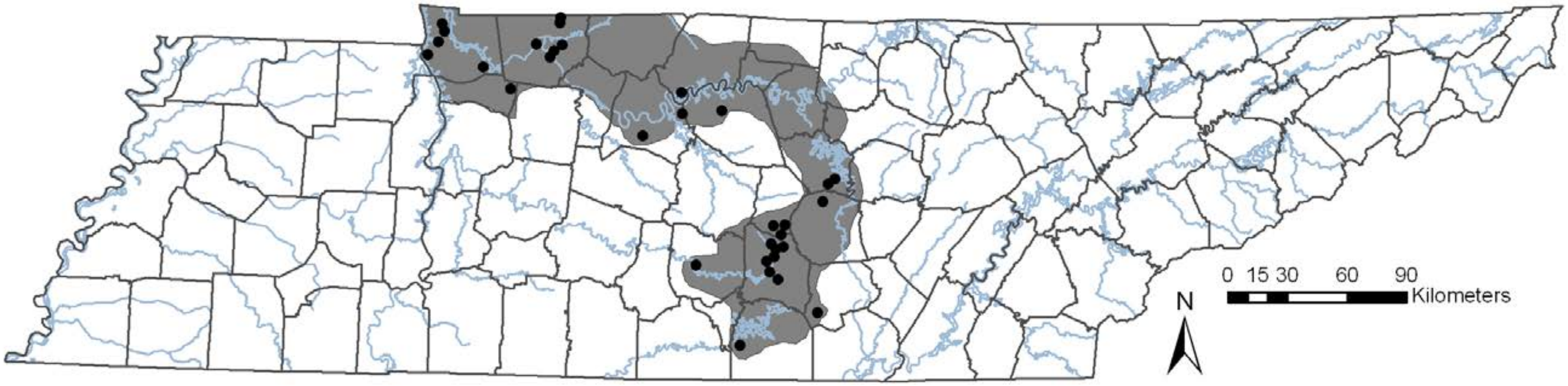


Fig. 9. The distribution of the Spring Cavefish (*F. agassizii*) in Tennessee overlaid on ecoregion. Spring Cavefish are principally confined to the Barrens of the Eastern Highland Rim and the Western Pennyroyal Karst.

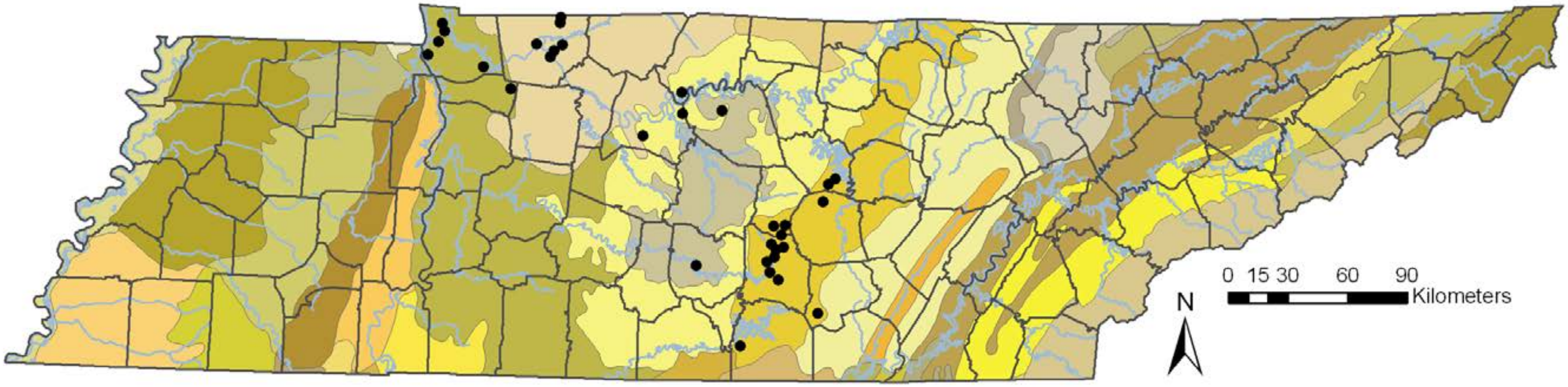


Fig. 10. The distribution of the Spring Cavefish (*F. agassizii*) in Tennessee overlaid on HUC8 watershed. Spring Cavefish have been reported from 9 watersheds in the state and from the Barren River watershed in Kentucky.

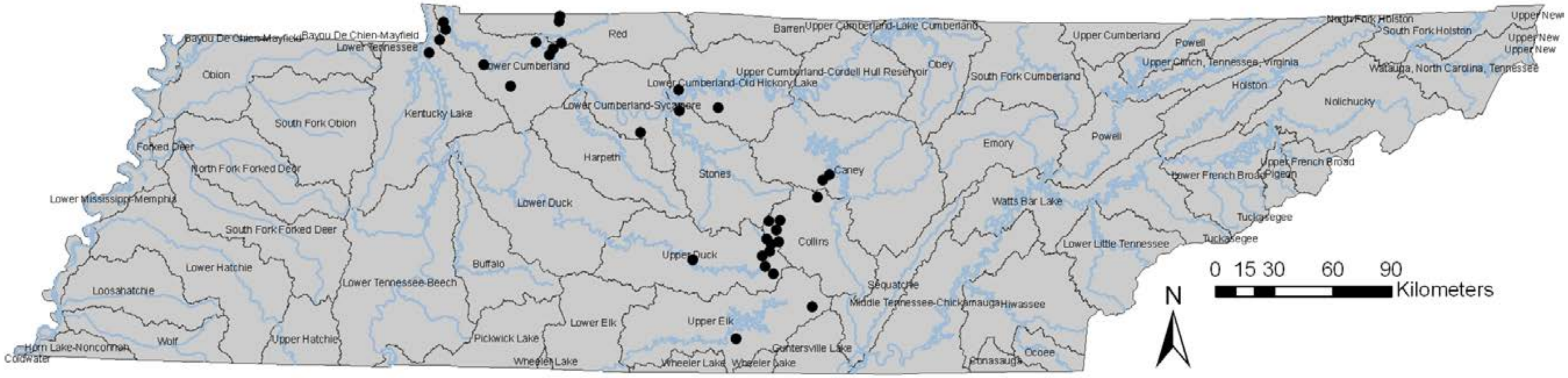


Fig. 11. Phylogenetic relationships of Southern Cavefish (*T. subterraneus*) based on mitochondrial DNA (left) and the nuclear S7 intron (right). Black circles at nodes correspond to > 0.95 Bayesian posterior probabilities. Numbers at node represent divergence date estimates in millions of years before present based on fossil calibration. Colors represent haplotypes from the same hydrological drainage.

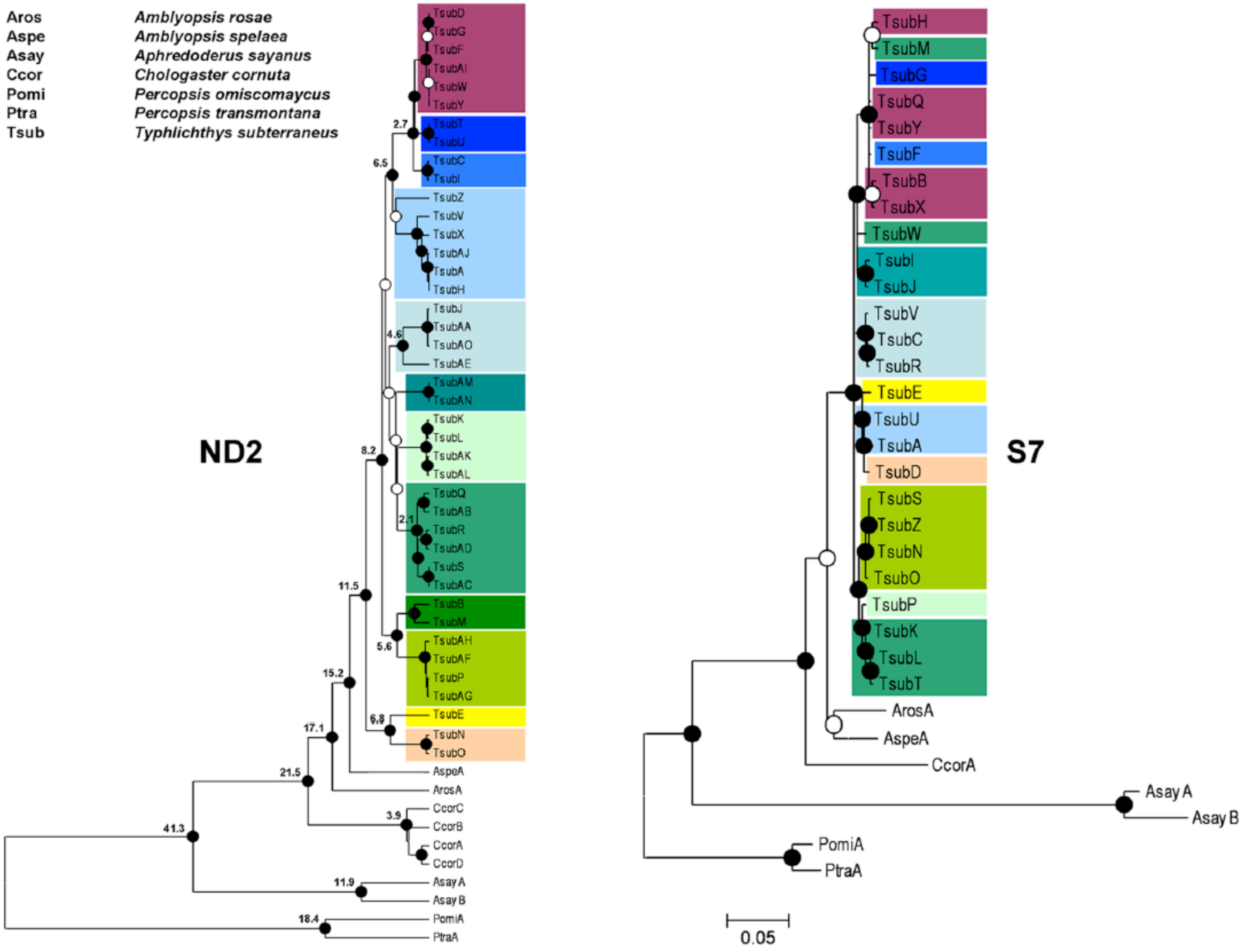


Fig. 12. Phylogram of mtDNA ND2 gene showing genealogical relationships of sampled populations (Appendix 1).

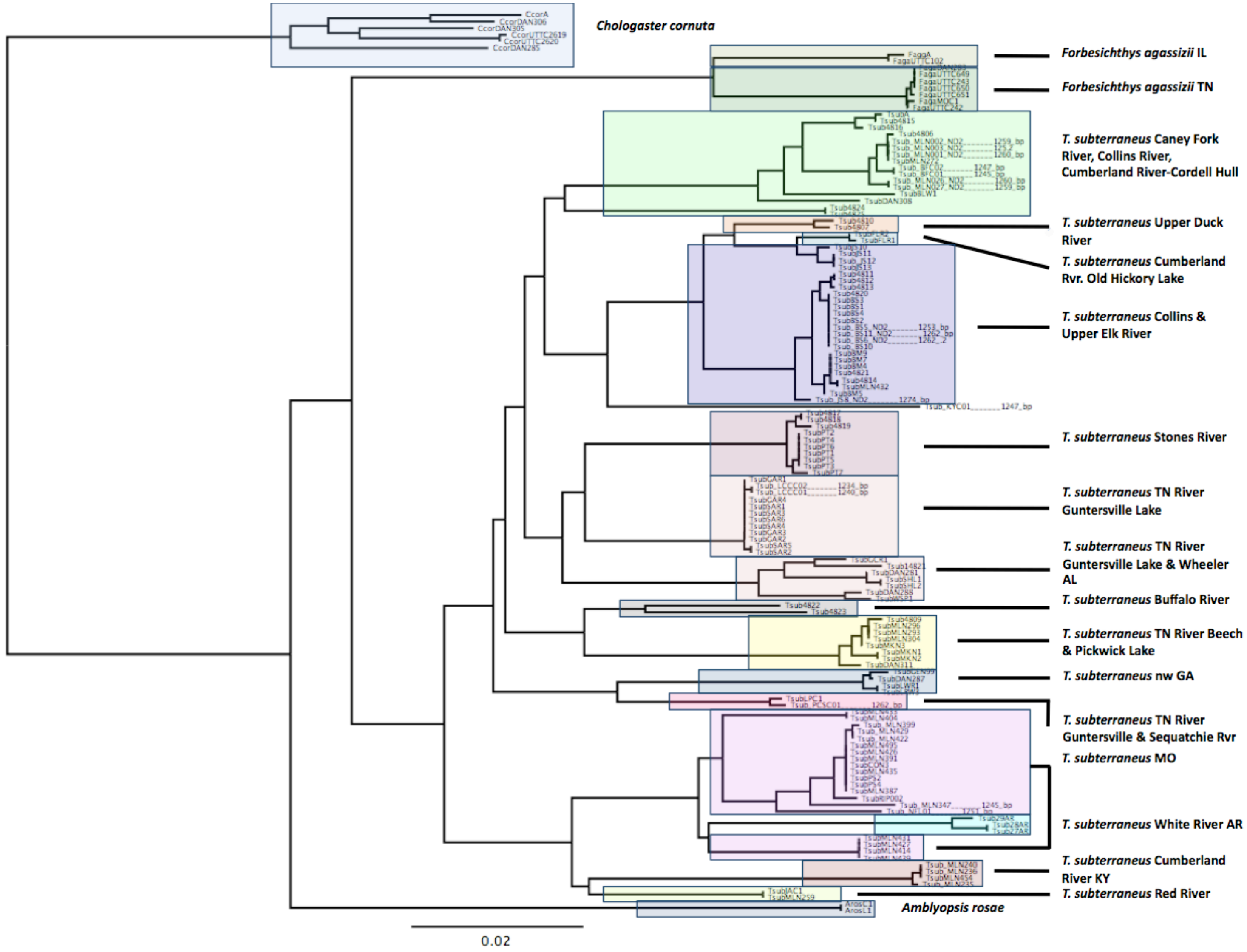


Fig. 13. Pie chart illustrating the proportion of Southern Cavefish (*T. subterraneus*) cave localities with survey data (n = 91) with the maximum number of cavefish observed during a single survey. Nearly 74% of cave localities have yielded ten or fewer cavefish during a given survey.

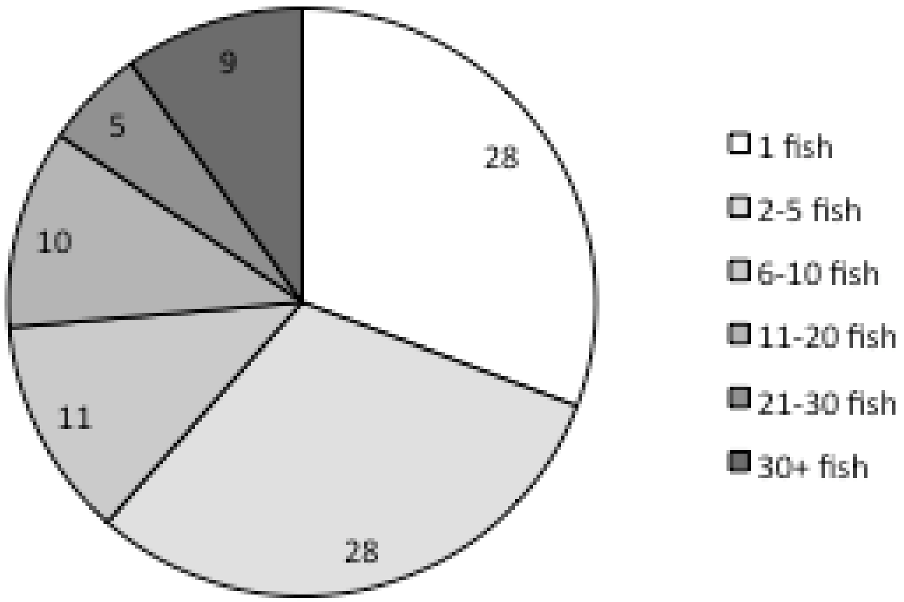
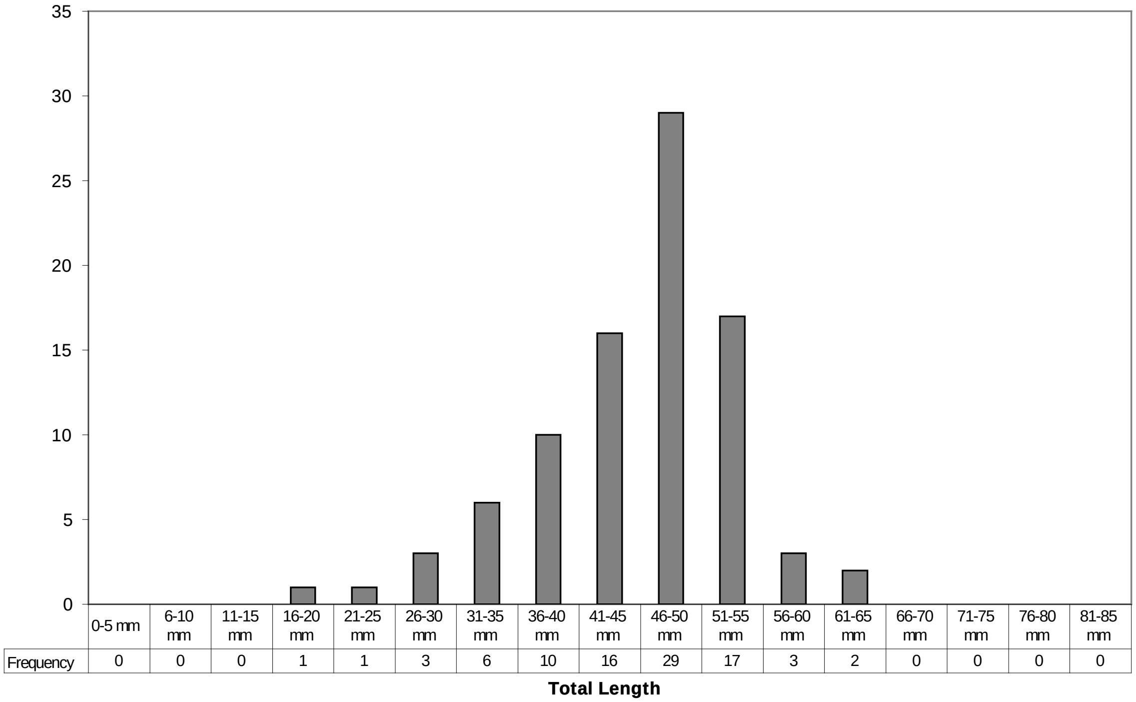


Fig. 14. Histogram of total length for 88 cavefish measured on 25 October 2007 at Jacques Cave, Putnam Co., Tennessee.



	0-5 mm	6-10 mm	11-15 mm	16-20 mm	21-25 mm	26-30 mm	31-35 mm	36-40 mm	41-45 mm	46-50 mm	51-55 mm	56-60 mm	61-65 mm	66-70 mm	71-75 mm	76-80 mm	81-85 mm
Frequency	0	0	0	1	1	3	6	10	16	29	17	3	2	0	0	0	0

Appendix 1. Southern Cavefish (*T. subterraneus*) localities sampled for genetic analyses.

Locality	County	State	No. Samples	ND2	S7
McKinney Pit	Colbert	AL	4	*	*
Guess Creek Cave	Jackson	AL	1	*	*
Davis Bat Cave	Lauderdale	AL	1	*	*
Key Cave	Lauderdale	AL	1	*	
White Spring Cave	Limestone	AL	1	*	*
Bobcat Cave	Madison	AL	1	*	
Muddy Cave	Madison	AL	1		*
Shelta Cave	Madison	AL	2	*	*
Beech Spring Cave	Marshall	AL	1	*	*
Cave Spring Cave	Morgan	AL	1		*
Alexander Cave	Stone	AR	2	*	*
Ennis Cave	Stone	AR	1	*	*
Limestone Caverns	Dade	GA	2	*	*
Longs Rock Wall Cave	Dade	GA	2	*	*
Dave’s Cave	Pulaski	KY	3	*	
Wells Cave	Pulaski	KY	1	*	
Carroll Cave	Camden	MO	4	*	
Coalbank Cave	Carter	MO	1	*	
Concolor Cave	Howell	MO	2	*	
Falling Spring Cave	Oregon	MO	2	*	
Posy Spring Cave	Oregon	MO	3	*	
Roaring Spring Cave	Oregon	MO	2	*	
Panther Cave	Ripley	MO	1	*	
Brawley Cave	Shannon	MO	1	*	
Flying W Cave	Shannon	MO	2	*	
Blowing Springs Cave	Coffee	TN	15	*	*
Baugus Cave	Decatur	TN	2	*	*
Garner Spring Cave	Franklin	TN	4	*	*
Little Crow Creek Cave	Franklin	TN	2	*	
Salt River Cave	Franklin	TN	6	*	*
Big Mouth Cave	Grundy	TN	10	*	*
Crystal Cave	Grundy	TN	3	*	*
Trussell Cave	Grundy	TN	1	*	*
Cave Branch Cave	Hickman	TN	1	*	*
Allens Creek Cave	Lewis	TN	1	*	*
Lost Pig Cave	Marion	TN	1	*	*
Pryor Cave Spring Cave	Marion	TN	1	*	
Gallagher Cave South	Marshall	TN	2	*	*
Pompie Cave	Marshall	TN	1	*	*
East Water Supply Cave	Overton	TN	1	*	*
Anderson Spring Cave	Putnam	TN	2	*	
Bartlett Cave	Putnam	TN	2	*	*
Blindfish Cave	Putnam	TN	2	*	
Jacques Cave	Putnam	TN	3	*	
Stamps Cave	Putnam	TN	1	*	
Sinking Ridge Cave	Robertson	TN	2	*	*
Herring Cave	Rutherford	TN	3	*	*
Pattons Cave	Rutherford	TN	13	*	*
Flat Rock Cave	Smith	TN	2	*	*
Camps Gulf Cave	Van Buren	TN	1	*	*
Camps Gulf Cave No. 2	Van Buren	TN	2	*	*
Blowing Cave	Warren	TN	1	*	
Jaco Spring Cave	Warren	TN	10	*	*

Appendix 2. Spring Cavefish (*F. agassizii*) localities sampled for genetic analyses.

Locality	County	State	No. Samples	ND2	S7
Cave Spring Cave	Union	IL	2	*	*
Fultz Pond	Coffee	TN	3	*	*
Rigsby Pond	Coffee	TN	10	*	*
Blue Springs	DeKalb	TN	5	*	*
Mountain Creek	Warren	TN	1	*	*