



OPEN Surprising cryptic cavefish diversity in a long-studied karst cave ecosystem of northern Alabama

Matthew L. Niemiller^{1,2}✉, Jonathan W. Armbruster³, Michael W. Sandel^{4,5}, Eric C. Maxwell¹, Orran M. Bierstein⁶, C. Lael Anderson¹, Jacob Schaefer¹, Coral I. Quering¹, Amata Hinkle¹, Matt Candeias⁷, K. Denise Kendall Niemiller^{1,2} & Pamela B. Hart⁵✉

Subterranean ecosystems host highly specialized and often cryptic biodiversity, yet even intensively studied landscapes may conceal deeply divergent vertebrate lineages. Here we describe *Demogorgonichthys arcanus* gen. et sp. nov., a new genus and species of cave-obligate fish discovered in Bobcat Cave, a long-monitored karst system on Redstone Arsenal in northern Alabama, USA. Phylogenetic analyses based on mitochondrial *nd2*, complete mitochondrial genomes, and the nuclear gene *rhodopsin* place *D. arcanus* within Amblyopsidae but reveal deep divergence from all described genera, including extensive lineage-specific degeneration of a vision-related gene. Notably, *D. arcanus* occurs in syntopy with the Southern Cavefish (*Typhlichthys subterraneus*) despite lacking a close phylogenetic relationship, providing evidence for multiple independent evolutionary origins of cave adaptation within a single groundwater system. This discovery highlights persistent detection bias in groundwater ecosystems and demonstrates that cryptic vertebrate diversity can persist even in well-characterized environments. Extreme endemism and restriction to a single cave-aquifer system further underscore the vulnerability of subterranean biodiversity and the importance of integrating evolutionary and conservation perspectives.

Keywords Cave adaptation, Conservation biology, Groundwater, Karst aquifer, Speciation, Subterranean biodiversity

Extreme environments impose strong physiological and ecological constraints that limit organismal persistence, yet life has repeatedly colonized habitats once thought to be inhospitable. Across the Tree of Life, organisms occur in environments characterized by extreme temperatures, pressures, chemical toxicity, hypoxia, and resource limitation, often exhibiting remarkable evolutionary innovations that permit survival under these conditions^{1–6}. These systems have increasingly emerged as focal points for biological discovery, yielding disproportionate insights into adaptation, resilience, and cryptic biodiversity, including the continued discovery of undescribed taxa even in regions considered well explored^{7–11}.

Among vertebrates, teleost fishes represent one of the most evolutionarily versatile lineages to occupy extreme environments. As the most diverse group of vertebrates, teleosts have colonized nearly all aquatic habitats on Earth, including the deep sea^{12,13}, polar waters^{14,15}, sulfidic springs^{4,16}, and subterranean ecosystems^{17–21}. Subterranean habitats impose particularly severe constraints, including perpetual darkness, lack of primary productivity, and reliance on allochthonous nutrient inputs^{11,17–19,22,23}. Despite these challenges, cave- and groundwater-dwelling fishes have evolved repeatedly across teleost lineages, with representatives documented from more than ten orders worldwide^{18,20,24,25}. In several regions, including eastern North America, subterranean systems have been colonized multiple times independently^{21,26–29}, sometimes even within the same cave or aquifer system^{20,30–32}.

North America harbors one of the world's most intensively studied cavefish faunas, specifically within the family Amblyopsidae. This family includes surface-dwelling, facultative cave-associated, and obligate subterranean species distributed primarily across the Interior Low Plateau and Ozarks karst regions of the

¹Department of Biological Sciences, The University of Alabama in Huntsville, Huntsville, AL 35899, USA. ²Niemiller Nature LLC, Huntsville, AL 35756, USA. ³Auburn University Museum of Natural History, Auburn University, Auburn, AL 36849, USA. ⁴Forest and Wildlife Research Center, Mississippi State University, Mississippi State, MS 39762, USA. ⁵Department of Wildlife, Fisheries, and Aquaculture, Mississippi State University, Mississippi State, MS 39762, USA. ⁶Department of Biological Sciences, The University of Alabama, Tuscaloosa, AL 35401, USA. ⁷Directorate of Public Works, Cultural and Natural Resources Branch, Redstone Arsenal, Huntsville, AL 35808, USA. ✉email: matthew.niemiller@uah.edu; matthew.niemiller@niemillernature.com; pbhart@ua.edu

eastern United States^{30,33,34}. Amblyopsids have long served as model organisms for studies of cave adaptation, sensory evolution, and regressive traits^{17,21,22,27,29,30,35–38}, and their taxonomy, phylogenetic relationships, and systematics are comparatively well resolved relative to many other subterranean vertebrate groups^{9,21,27,29,32,38–41}. As a result, the discovery of novel amblyopsid diversity, particularly at the generic level, has been assumed to be increasingly unlikely in this region.

Here, we describe a new genus and species of cave-obligate amblyopsid cavefish discovered in a cave within the Tennessee River Valley of northern Alabama: *Demogorgonichthys arcanus* gen. et sp. nov., the Demon Cavefish. This species was first documented at Bobcat Cave (Alabama Cave Survey no. AMD1283), located on Redstone Arsenal near Huntsville, Madison County, Alabama, USA, during biological surveys conducted in February 2025. Remarkably, *D. arcanus* gen. et sp. nov. occurs in syntopy with the Southern Cavefish (*Typhlichthys subterraneus*), another obligate subterranean amblyopsid, yet phylogenetic analyses demonstrate that the two taxa are not sister lineages. This co-occurrence represents an independent evolutionary origin of cave adaptation within the same subterranean system, underscoring the complexity of colonization history and lineage persistence in karst aquifers.

The discovery of *Demogorgonichthys arcanus* gen. et sp. nov. from a cave that has been visited repeatedly and from a landscape widely regarded as biologically well characterized highlights the persistence of hidden vertebrate diversity even in intensively studied regions. More broadly, this finding challenges assumptions about biological survey completeness in subterranean ecosystems and emphasizes the role of access-restricted lands, including military installations, as inadvertent refugia for undescribed biodiversity. Through morphological and molecular analyses, we describe the diagnostic features, phylogenetic placement, and ecological context of this new genus and species, and discuss its implications for cavefish evolution, biogeography, and conservation in eastern North America.

Results

Class Actinopterygii Klein, 1885

Superorder Paracanthopterygii Greenwood et al., 1966

Order Percopsiformes Berg, 1940

Family Amblyopsidae Bonaparte, 1846.

Demogorgonichthys Hart, Niemiller, Sandel, & Armbruster, gen. nov.

<https://www.zoobank.org/FE7064B2-4A26-4160-9465-215A2355DC1A>

Figure 1 (middle of top and bottom), Fig. 2 (map and live photos), Fig. 3A–C

Diagnosis. Character differences are summarized in Supplementary Table 1. *Demogorgonichthys* can be separated from all Amblyopsidae except *Speoplatyrhinus* by the absence of branching in all fin rays (although it appears *Typhlichthys* may gain branches later in development); from *Chologaster* and *Forbesichthys* by the complete lack of eyes; from *Chologaster*, *Forbesichthys*, and *Typhlichthys* by a lack of melanophores (note that *Typhlichthys* will develop melanophores when exposed to light (Figs. 1 and 3); and from *Typhlichthys* by the absence of a supraopercular sensory papilla (vs. presence of papilla as long, thin, tubular structure just dorsal to opercle (SOS in Fig. 4); this structure may not be visible in poorly preserved specimens); presence of a single dorsal opercular neuromast column that is slightly anterior but almost contiguous with the medial opercular neuromast column (vs. antero- and posterodorsal opercular neuromast columns that are widely separated from the medial opercular neuromast column (Fig. 4B vs. A); a pronounced nuchal hump (vs. nuchal region straight to dorsal fin; Fig. 3), lower jaws approximately equal (vs. lower jaw slightly longer visible as a definite lip protuberance), and lower jaws running almost parallel with sides of snout and most medial portions well separated (vs. lower jaws angled medially such that they touch or nearly touch at approximate level of jaw angle and then separating slightly before end of snout; Fig. 4D vs. C).

Demogorgonichthys is most similar to *Speoplatyrhinus* and can be separated by rostral flaring (i.e., duckbill shape) less pronounced (snout nearly as flat with the distance from the tip of snout to the point of the narrowest point of the snout about the same length as the distance from the narrowest point of the snout to the supraoccipital vs. much longer); by having a convex hump immediately posterior to supraoccipital (vs. dorsal surface gradually increasing to dorsal fin), the lack of a posterodorsal column of neuromasts on the opercle (vs. presence), a near contiguous anterodorsal and medial column of neuromasts on the opercle (vs. well separated), and little overlap of the anterodorsal and medial columns (vs. the two columns overlapping across greater than 30% of their lengths). Embedded scales on *Demogorgonichthys* increase in size from dorsal to midline, while scales remain relatively the same size in *Typhlichthys* species and *Speoplatyrhinus*.

Molecular diagnosis. Mean uncorrected pairwise genetic distance between the *Demogorgonichthys* gen. nov. mitogenome and other amblyopsid genera is 11.0% relative to *Typhlichthys*, 10.9% to *Speoplatyrhinus*, 12.3% to *Amblyopsis*, 14.5% to *Troglichthys*, 13.4% to *Forbesichthys*, and 17.7% to *Chologaster*. All sequenced individuals of *Demogorgonichthys* gen. nov. possess unique mitochondrial *nd5* and nuclear *rhodopsin* haplotypes among amblyopsids. The *nd5* locus is characterized by a 3-bp deletion near the 3' terminus. *Rhodopsin* is characterized by a 284-bp deletion that results in a frameshift and multiple premature stop codons, as well as an additional 12-bp deletion not observed in other stygobiotic amblyopsids.

Type species. *Demogorgonichthys arcanus* sp. nov.

Etymology. Demogorgon is the name of an underworld phantom demon in Greek mythology, and is additionally, the name of the Demon Prince in the role-playing game “Dungeons and Dragons” and a monster in the Netflix television series “Stranger Things,” where the creature can be found in the Upside-Down, a cave-like version of the real world. *Ichthys* is Greek for fish; gender masculine.

Distribution. This genus is known from only a single cave system on the north side of the Tennessee River Valley in southwestern Madison County, Alabama, within the Highland Rim section of the Interior Low Plateau physiographic province (Fig. 2A).



Fig. 1. Comparative images of amblyopsid cavefishes in northern Alabama. Profile views of the full body (TOP) and dorsal views of the head (BOTTOM) of *Typhlichthys subterraneus* and *Demogorgonichthys arcanus* gen. et sp. nov. from Bobcat Cave, Madison County, Alabama, and *Speoplatyrhinus poulsoni* from Key Cave, Lauderdale County, Alabama. Photographs by M.L. Niemiller.

Demogorgonichthys arcanus Hart, Niemiller, Sandel, & Armbruster, sp. nov.
<https://zoobank.org/11F514EF-D422-40FA-B0AA-3C5D5DE087A7>

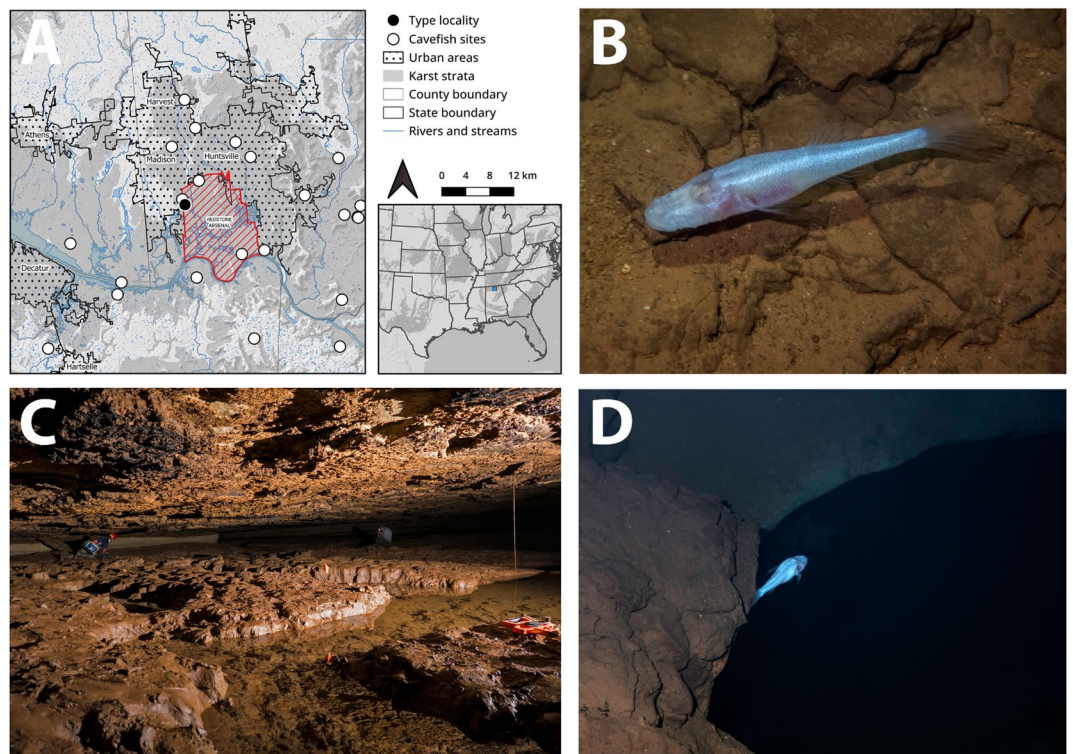


Fig. 2. Distribution map, photos in life of *Demogorgonichthys arcanus* gen. et sp. nov. and habitat. Distribution of *D. arcanus* gen. et sp. nov. from the type locality—Bobcat Cave—and occurrences of *Typhlichthys subterraneus* in the Huntsville metropolitan area in north-central Alabama (A). The boundary of Redstone Arsenal is shown in red hatching. Photos of *Demogorgonichthys arcanus* sp. nov. in situ on 5 February 2025 at Bobcat Cave (B and D). Pools in the Shrimp Room at Bobcat Cave where *D. arcanus* sp. nov. was discovered (C). Photographs by M.L. Niemiller (B and D) and A. Hinkle (C).

Type material. Holotype: UAIC 52236.01, 42.1 mm standard length (SL), Bobcat Cave, Redstone Arsenal, Madison County, Alabama, USA, Alabama Cave Survey (ACS) no. AMD1283, 34.6686°, -86.7130°, 10 February 2025, E.C. Maxwell, C.L. Anderson, M. Candeias, and M.L. Niemiller.

Paratypes. AUM 89691, 1, 36.8 mm SL, same collection information as holotype; AUM 89692, 1, 27.7 mm SL and UAIC 52237.01, 26.9 mm SL, same locality as holotype, 10 July 2025, E.C. Maxwell and C.L. Anderson.

Diagnosis. Same as genus.

Description. Measurements in Table 1. Lacking visible eyes and pigment. Snout concave dorsally then rising to supraoccipital and convex to dorsal fin with marked hump; highest point approximately midway between supraoccipital and dorsal fin. Body concave from dorsal-fin origin to caudal fin. Ventral surface of head angled ventrally to the end of head. Abdomen largely flat, unless distended by food. Caudal peduncle concave, very short; caudal peduncle narrowest approximately halfway between dorsal and caudal fins. Snout widest approximately at end of maxilla and then tapering before widening to opercle. Opercle strongly convex such that the widest point of head well anterior to opercular opening; head and body width decreasing evenly from maximum width in middle of opercles to caudal fin.

Body fully scaled, but scales deeply embedded in skin making counts impractical. All fins consist only of unbranched rays. Dorsal fin largely rounded posteriorly with ix(3*) or x(1) rays. Pectoral fin long and pointed, longest ray almost reaching dorsal fin, with xiv rays. Pelvic fin absent. Anal fin rounded, beginning approximately opposite mid-dorsal, with ix rays. Dorsal and anal fins extending approximately equally posteriorly. Caudal fin rounded with vi (1) or vii (3*) upper rays and vii lower rays; caudal-fin procurent rays extending just slightly anterior of caudal fin; no dorsal adipose ridge present (*Typhlichthys* often with dorsal surface of caudal peduncle raised as fleshy ridge).

Anterior nostril extended as tube, length of tube approximately three quarters width. Posterior nostril teardrop-shaped approximately two times length of first nostril tube from first nostril. Gape extending approximately opposite posterior nostril. Maxilla extending about one quarter length of gape beyond gape. Jaws approximately equal. Mouth slightly upturned. From ventral view, mandible mostly parallel with head, coming closest to one another at narrowest point of snout, and then distance widening anteriorly until jaws curve medially at end of. Gills attached to isthmus anteriorly, not broadly connected to one another. Vent located just posterior to isthmus and slightly posterior to where contralateral branchiostegals meet at midline. All individuals examined with the vent in anterior position, approximately halfway through opercular opening in adults.

Lateralis system present as exposed neuromasts that form lines on head, body, and caudal fin (Figs. 1, 3 and 4). Neuromasts relatively short. Lateralis system of body not readily apparent but consisting of several vertical



Fig. 3. Holotype of *Demogorgonichthys arcanus* gen et sp. nov., UAIC 52236.01, in dorsal (A), lateral (B), and ventral (C) views and *Typhlichthys subterraneus*, AUM 89701 in lateral view (D). Scale = 1 cm.

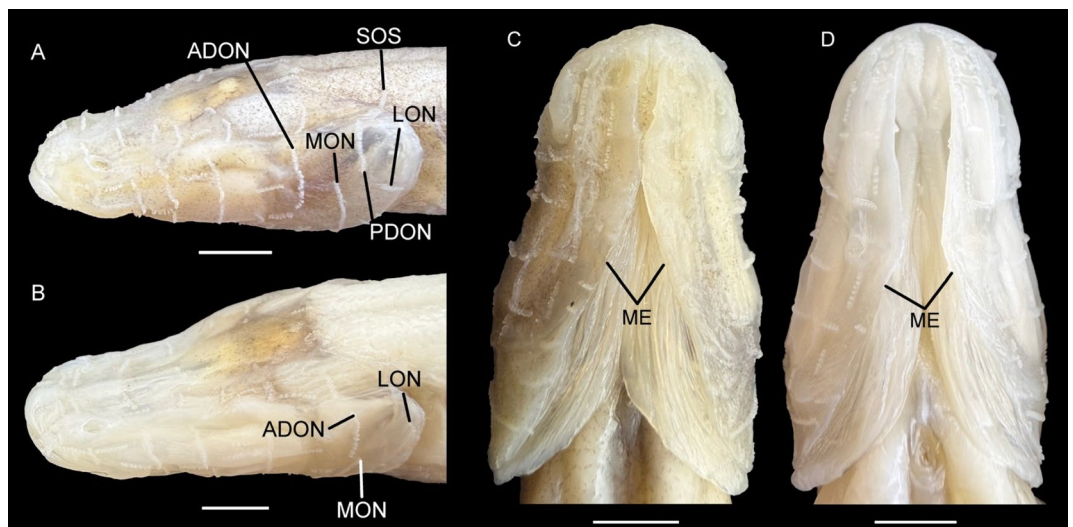


Fig. 4. Head, dorsolateral and ventral views of (A,C) *Typhlichthys subterraneus*, AUM 89701 and (B,D) *Demogorgonichthys arcanus*, gen. et sp. nov., paratype, AUM 89691. ADON – anterodorsal opercular neuromasts, DON – dorsal opercular neuromasts, LON – longitudinal opercular neuromasts, ME – mandible edge, MON – medial opercular neuromasts, PDON – posterodorsal opercular neuromasts, SOS – supraopercular sensory papilla. Scale = 2.5 mm.

Measurement	Holotype	Mean	SD	Min	Max
SL (mm)	42.1	33.4	7.4	26.9	42.1
Head length	34.9	35.9	0.9	34.9	37.0
Head width	15.6	15.3	0.3	14.9	15.6
Head depth	13.5	13.1	1.2	11.4	14.4
Snout breadth	13.0	13.0	1.1	11.7	14.4
Snout depth	5.7	5.6	0.1	5.4	5.7
Body width	8.6	8.7	0.2	8.5	8.8
Body depth	14.5	14.6	0.3	14.2	14.8
Predorsal length	58.3	59.9	1.2	58.3	61.2
Preanal length	58.8	58.5	1.3	56.7	59.8
Prepectoral length	37.0	36.0	1.8	33.4	37.1
Vent to anal fin	30.5	33.3	2.7	30.5	36.4
Longest dorsal ray	25.0	24.9	2.0	22.7	27.5
Pectoral fin length	19.7	19.5	1.1	17.9	20.4
Longest anal ray	26.2	25.5	2.0	23.3	27.9
Caudal length	22.5	25.9	3.9	22.5	30.2
Caudal peduncle depth	7.5	7.8	0.3	7.5	8.2
Upper jaw length	13.3	13.4	0.1	13.3	13.6

Table 1. Morphometrics of the four specimens of *Demogorgonichthys arcanus*. Values except standard length (SL) given as %SL.

columns of neuromasts; more apparent anteriorly. Neuromasts continue in two rows onto caudal fin on either side of midline and separated by 4(1) or 5(3*) rays. Neuromasts of head more readily apparent, with individual papillae length about equal to the width. Superficial neuromasts of dorsal portion of snout consisting of two short longitudinal rows between anterior nostrils; two transverse rows between nostrils; transverse row approximately dorsal from jaw angle; few transverse patches on occiput; and short longitudinal row approximately on parietal. Lateral surface of head with series of roughly vertical columns of neuromasts along snout angled anterodorsally; opercular neuromasts consist of two vertical lines (dorsal opercular neuromast column and median opercular neuromast column; Fig. 4B), almost contiguous, one dorsal and one ventral. Longitudinal row of superficial neuromasts running medially along mandible with some vertical lines running to mouth. One large vertical line of neuromasts running from mandible to dorsal surface of snout followed by two other vertical lines alongside of head and some patches of longitudinal lines in front of opercle.

Coloration in life. No melanophores present (Figs. 1 and 2B and D). Body white to light or electric blue; some red visible from blood under opercle and from gut. Scales with centers whiter and grayish edges between them.

Coloration in preservative. Body eggshell, skull translucent with muscles visible below skin (Fig. 3). Posterodorsal portion of neurocranium light tan in areas not covered by muscle. Brain and olfactory tract visible as white structures through cranium. Rays of fins white and membranes transparent.

Etymology. The specific epithet *arcanus* (Latin adjective meaning hidden, secret, or mysterious) refers to the long-undetected existence of this cavefish despite decades of intensive biological study at the type locality, as well as its occurrence within a subterranean environment largely concealed from human observation. The name further alludes to the species’ probable primary occupancy of the underlying karst aquifer rather than the accessible cave passages themselves, emphasizing its cryptic nature. The recommended common name is Demon Cavefish.

Distribution. *Demogorgonichthys arcanus* sp. nov. is currently known only from the type locality, Bobcat Cave, on Redstone Arsenal in southwestern Madison County, Alabama, USA (Fig. 2A). Bobcat Cave has a small entrance that opens within a low knoll near the western boundary of the installation. The cave is developed in the Tuscumbia Limestone and consists of a short entrance passage that slopes downward into a network of low (approximately 1–2 m high), water-filled tunnels interconnected by narrow crawlways⁴².

The mapped length of Bobcat Cave is approximately 454 m, with much of the cave floor covered by thick mud and limited exposed bedrock. The largest chamber, known as the Shrimp Room, is approximately 60 m in length and up to 30.5 m wide (Fig. 2C). Hydrologic conditions within the cave are highly dynamic^{42–45}. Water levels may fluctuate rapidly by as much as 3.9 m in response to heavy precipitation events, during which floodwaters inundate all but the entrance area. During drought conditions, standing water is reduced to a series of isolated pools, primarily within the Shrimp Room.

Dye-tracing studies conducted in the vicinity of Bobcat Cave indicate that the recharge basin is shallow and localized, potentially less than 1 km², likely restricted to areas immediately west of Indian Creek, with most recharge derived from shallow groundwater flowing primarily in a northward direction^{43–46}. The rapid hydrologic response of the cave to precipitation events suggests strong connectivity between surface runoff and the subterranean system^{44–46}. Trace metals, including cadmium, chromium, and lead, have been detected at elevated concentrations in water samples from Bobcat Cave over the past two decades^{43,44,46,47}, potentially

reflecting historical groundwater contamination. The cave occurs within a former industrial area of Redstone Arsenal associated with the disposal of excess conventional and chemical munitions.

Several additional cave systems in the surrounding region support populations of *Typhlichthys* (Fig. 2A), yet *D. arcanus* has not been detected at these sites despite repeated surveys. While previous dye-tracing studies suggest a shallow and localized recharge basin for Bobcat Cave, genetic evidence from co-occurring subterranean taxa indicates the possibility of broader regional hydrologic connectivity^{9,48–50}. Based on currently available information, we estimate the extent of occurrence (EOO) and area of occupancy (AOO) for *D. arcanus* to be 2 km² and 4 km², respectively. Given that the species is presently known from a single cave system and that the recharge basin has not been comprehensively delineated, all geographic range metrics represent minimum estimates and are expected to be refined as additional hydrologic and biological surveys are conducted.

Habitat. *Demogorgonichthys arcanus* has been observed in quiet, silt- to mud-bottomed pools within Bobcat Cave (Fig. 2C). Water levels within the cave are highly dynamic and fluctuate in response to recent precipitation events. Individuals have been observed in water depths ranging from approximately 5 cm to 1.5 m. Water quality data for Bobcat Cave are derived from monthly measurements collected between October 2019 and July 2022⁴⁷. During this period, water temperature ranged from 14.0 to 16.0 °C (median 15.0 °C), dissolved oxygen from 3.3 to 9.4 mg/L (median 5.3 mg/L), pH from 6.9 to 8.2 (median 7.4), and specific conductance from 171 to 386 µS/cm (median 267 µS/cm).

Ecology and life history. Most aspects of the ecology and life history of *Demogorgonichthys arcanus* remain unknown. Based on count data from four surveys conducted in 2025 (Supplementary Table 2), the abundance of *D. arcanus* within Bobcat Cave appears comparable to that of the syntopic Southern Cavefish (*Typhlichthys subterraneus*), with an approximate ratio of 1:1.12. Across these surveys, we observed 8–18 individuals (mean = 12.3) of *D. arcanus* and 6–19 individuals (mean = 13.8) of *T. subterraneus*. Estimating long-term population trends is complicated by the historical conflation of the two species in previous cavefish censuses. However, if a similar ratio has persisted historically, up to approximately 25 individuals of *D. arcanus* may have been present during some surveys conducted since the early 1990s^{42–44,51,52}, suggesting relative population stability over the past four decades.

Individuals as small as 8 mm standard length were observed in late December 2025, indicating the presence of multiple size classes and potentially signaling reproductive episodes in late autumn/early winter. The diet of *D. arcanus* is unknown but is likely similar to that of *Typhlichthys*, which feeds on live copepods, amphipods, isopods, cladocerans, and small crayfishes^{17,30}. We observed a juvenile attempting to feed on stygobiotic copepods in a shallow, isolated pool on 30 December 2025. The cavefish attempted to snap at three copepods that swam within 0.5 cm or touched its head. Documented predators are unknown but may include larger stygobiotic crayfishes (*Cambarus speleocoopi* and *Orconectes australis*), stygophilic crayfishes (*Cambarus tenebrosus*), other cavefishes (including *T. subterraneus* and conspecifics), and Tennessee Cave Salamanders (*Gyrinophilus pallescens*). Community associates of *D. arcanus* at Bobcat Cave include *Typhlichthys subterraneus* (lineages E and F), *Gyrinophilus pallescens*, *Palaemonias alabamiae*, *Orconectes australis*, *Cambarus speleocoopi*, *Cambarus tenebrosus*, *Stygobromus* sp., and stygobiotic cyclopoid copepods.

Conservation. Based on its extremely restricted distribution, confinement to a single subterranean system, and small estimated geographic range, *Demogorgonichthys arcanus* is considered highly vulnerable to extinction. Using the NatureServe conservation status framework, the species qualifies for a global rank of G1 (Critically Imperiled). Under the IUCN Red List framework, *D. arcanus* meets thresholds for Critically Endangered (CR) under criterion B, based on an estimated extent of occurrence (EOO) of approximately 2 km² and area of occupancy (AOO) of approximately 4 km², coupled with occurrence at a single location. The species is restricted to a single cave system and its associated aquifer, rendering it vulnerable to localized threats affecting groundwater quantity and quality. Hydrologic connectivity to surface runoff, documented fluctuations in water levels, and the presence of elevated trace metals in cave waters indicate potential exposure to environmental stressors. These assessments should be regarded as preliminary and conservative, pending further delineation of the recharge basin and additional surveys to better characterize the species' distribution and population status.

Remarks. Bobcat Cave was discovered in the early 1970s and rapidly gained biological significance following the discovery of the largest known population of the federally endangered Alabama Cave Shrimp (*Palaemonias alabamiae*) during a mapping expedition conducted by the Huntsville Grotto of the National Speleological Society in 1973^{42,43}. Since 1990, the cave has been the focus of long-term biological and hydrological monitoring, including nearly monthly water-quality measurements and shrimp population censuses conducted by the Geological Survey of Alabama and partners^{42–44,47,51–54}, as well as numerous additional biological investigations^{9,32,48–50,55,56}. Despite this sustained attention and long history of biological study, the cavefish described herein had not been previously documented.

Morphological distinctiveness

In many ways, *Demogorgonichthys arcanus* is intermediate in morphology between *Typhlichthys* and *Speoplatyrhinus* in that its snout is elongated and compressed compared to *Typhlichthys* but not to the same extent as *Speoplatyrhinus*. *Demogorgonichthys* does differ in shape in that the region between the supraoccipital and dorsal fin is elevated with the highest point roughly halfway (vs. slightly raised in *Typhlichthys* and a gradual slope to the dorsal fin in *Speoplatyrhinus*). *Demogorgonichthys* also has relatively smaller and less numerous superficial neuromasts. For example, both *Typhlichthys* and *Speoplatyrhinus* have three columns of neuromasts on the opercle, an antero- and posterodorsal column and medial column with the space roughly equal between the three columns (Fig. 4). *Typhlichthys* and *Speoplatyrhinus* also each have a largely longitudinal row of neuromasts that proceed from the ventral edge of the posterodorsal column with the longitudinal row at a right angle and well separated in *Typhlichthys* and at an obtuse angle and almost connected in *Speoplatyrhinus*. *Demogorgonichthys* lacks the posterodorsal column of neuromasts, the medial row is almost contiguous with the anterodorsal

column, and the longitudinal row is reduced (although also angled ventrally). In addition, the anterodorsal column only slightly overlaps the medial column in *Typhlichthys* and barely overlaps in *Demogorgonichthys* while there is over 30% overlap of each column in *Speoplatyrhinus*. *Typhlichthys* has a unique, tubular sensory structure just dorsal to the opercular opening, *Speoplatyrhinus* has a small column of neuromasts in the same region, and *Demogorgonichthys* lacks any obvious structures.

Osteology. *Demogorgonichthys* and *Speoplatyrhinus* have much taller orbital processes of the anguloarticular and dentary than *Typhlichthys* with the interspace between the two narrow in *Demogorgonichthys* (approximately the width of the coronoid process of the articular; similar to that of *Typhlichthys*) and wide in *Speoplatyrhinus* (approximately three times wider than the width of the coronoid process of the articular and even wider than that of the dentary) (Fig. 5, Supplementary Fig. 1, Supplementary Video 1–3). The severe constriction of the snout in *Speoplatyrhinus* causes the mesial laminae of the anguloarticulars to overlap at the midline (vs. a gap between them in other amblyopsids; Supplementary Fig. 2). The quadrate condyle to the anguloarticular is unique in *Demogorgonichthys* in that there are two bumps that are visible in ventral view; these bumps appear to be more developed in *Demogorgonichthys* (although this may be an artifact of scanning) and are angled at a 90° or less angle to one another vs. a strongly obtuse angle in *Speoplatyrhinus* and *Typhlichthys* (Supplementary Fig. 2, Supplementary Video 1–3).

Armbruster et al.³⁷ describe a lengthening of the anterodorsal arm of the hyomandibula, and this portion contains a large, longitudinally oval opening (Fig. 5, Supplementary Fig. 3). The anterodorsal arm of the hyomandibula is particularly narrow in *Demogorgonichthys* with the width of the arm narrower than the length of the opening (vs. about the same width as the length of the opening in *Speoplatyrhinus* and wider than the length of the opening in *Typhlichthys*). The dorsal lamina above the opening is narrowest in *Demogorgonichthys* and widest in *Speoplatyrhinus*. In addition, when viewed anteriorly, the hyomandibula has a medial, ventrally oriented process and a laterally directed process (Supplementary Fig. 4). The lateral process is wider in *Demogorgonichthys* and *Speoplatyrhinus* with it widest in *Demogorgonichthys*. The lateral process is oriented almost completely lateral in *Demogorgonichthys* and *Typhlichthys* and ventrolaterally in *Speoplatyrhinus*.

Armbruster et al.³⁷ describe the mesethmoid as being elongate in *Speoplatyrhinus* and *Typhlichthys* with the length greatest in *Speoplatyrhinus*. Although the computed tomography (CT) imagery does not allow for resolution of the posterior border of the mesethmoid with confidence, it appears to be as elongate as in *Speoplatyrhinus*. The mesethmoid flares anteriorly into a roughly rectangular shape, and there are a couple of differences here in *Demogorgonichthys* (Supplementary Fig. 2). In *Speoplatyrhinus* and *Typhlichthys*, there is a lateral concavity in the anterior portion of the mesethmoid with the anterior edge wider than the posterior; however, the anterior portion of the mesethmoid tapers anteriorly in *Demogorgonichthys* and is widest posteriorly. Also, in *Speoplatyrhinus* and *Typhlichthys*, the anterior portion of the neurocranium and the mesethmoid taper to the anterior portion of the mesethmoid while there is a constriction posterior to the anterior portion of the mesethmoid in *Demogorgonichthys* at about the posterior edge of the mesethmoid and then the mesethmoid begins to flare laterally before the enlarged anterior portion of the mesethmoid.

Demogorgonichthys and *Speoplatyrhinus* share an increased size of the fifth epibranchial, which is visible from the posterior in the CT scans (Supplementary Fig. 4). Epibranchial 5 is bent in the middle forming a dorsal lamina that is especially large in *Demogorgonichthys* and *Speoplatyrhinus*. The laterally-directed ramus of epibranchial 5 from this dorsal lamina is greater than half the width of the medially-directed ramus in *Speoplatyrhinus* and *Typhlichthys* and about quarter the width in *Demogorgonichthys*.

Phylogenetic placement and genetic divergence

The complete mitochondrial genome of *Demogorgonichthys arcanus* ranged from 17,794 to 17,795 bp, which is intermediate between *Speoplatyrhinus poulsoni* (17,901 bp) and *Typhlichthys* from Bobcat Cave (17,681 bp). Gene number, order, and orientation were conserved across all taxa and matched the inferred ancestral condition for Percopsiformes. *Demogorgonichthys* is further distinguished from all other percopsiforms by a 3-bp deletion near the 5' terminus of the *nd5* locus.

Maximum-likelihood analyses of the complete mitochondrial genomes recovered *Demogorgonichthys arcanus* as sister to (*Speoplatyrhinus poulsoni* + *Typhlichthys subterraneus*), although support for deeper nodes was low (Fig. 6). Using this topology, RelTime analyses estimated the time to the most recent common ancestor (TMRCA) of these three lineages at 11.6 ± 1.6 Ma (95% CI: 10.0–13.2 Ma). The estimated divergence between *Speoplatyrhinus* and *Typhlichthys* was 10.9 ± 1.7 Ma, overlapping the TMRCA of *Demogorgonichthys*. These divergence time estimates should be interpreted with caution, as they are derived from a relatively small mitogenomic dataset and are sensitive to assumptions regarding among-lineage rate variation and the calibration point applied; broader taxon sampling and additional fossil or biogeographic calibrations will be necessary to refine the temporal framework for amblyopsid diversification. Both the maximum likelihood (ML) mitogenome and *nd2* phylogenetic analyses recovered *Demogorgonichthys arcanus* as a strongly supported, monophyletic lineage distinct from *Typhlichthys* (including individuals co-occurring at Bobcat Cave and other previously defined lineages) and *Speoplatyrhinus poulsoni*. *Demogorgonichthys* was recovered as sister to (*Typhlichthys* + *Speoplatyrhinus*) in the mitogenome phylogeny (Fig. 6), whereas relationships among these lineages were poorly resolved in the *nd2* topology (Supplementary Fig. 5).

Mean uncorrected pairwise genetic distances (p-distances) among mitochondrial genomes ranged from 8.7% between *D. arcanus* and *S. poulsoni* to 9.4% between *D. arcanus* and *Typhlichthys* from Bobcat Cave. At the mitochondrial *nd2* locus, uncorrected p-distance was 10.8% between *D. arcanus* and syntopic *Typhlichthys*, and 10.9% between *D. arcanus* and *S. poulsoni*. In contrast, divergence at the nuclear *rhodopsin* locus was lower, with p-distances of 1.4% between *D. arcanus* and *Typhlichthys* from Bobcat Cave and 1.3% between *D. arcanus* and *S. poulsoni*. Individuals of *Typhlichthys* from Bobcat Cave grouped within two previously defined mitochondrial lineages, whereas all *D. arcanus* individuals formed a single, deeply divergent lineage across loci.

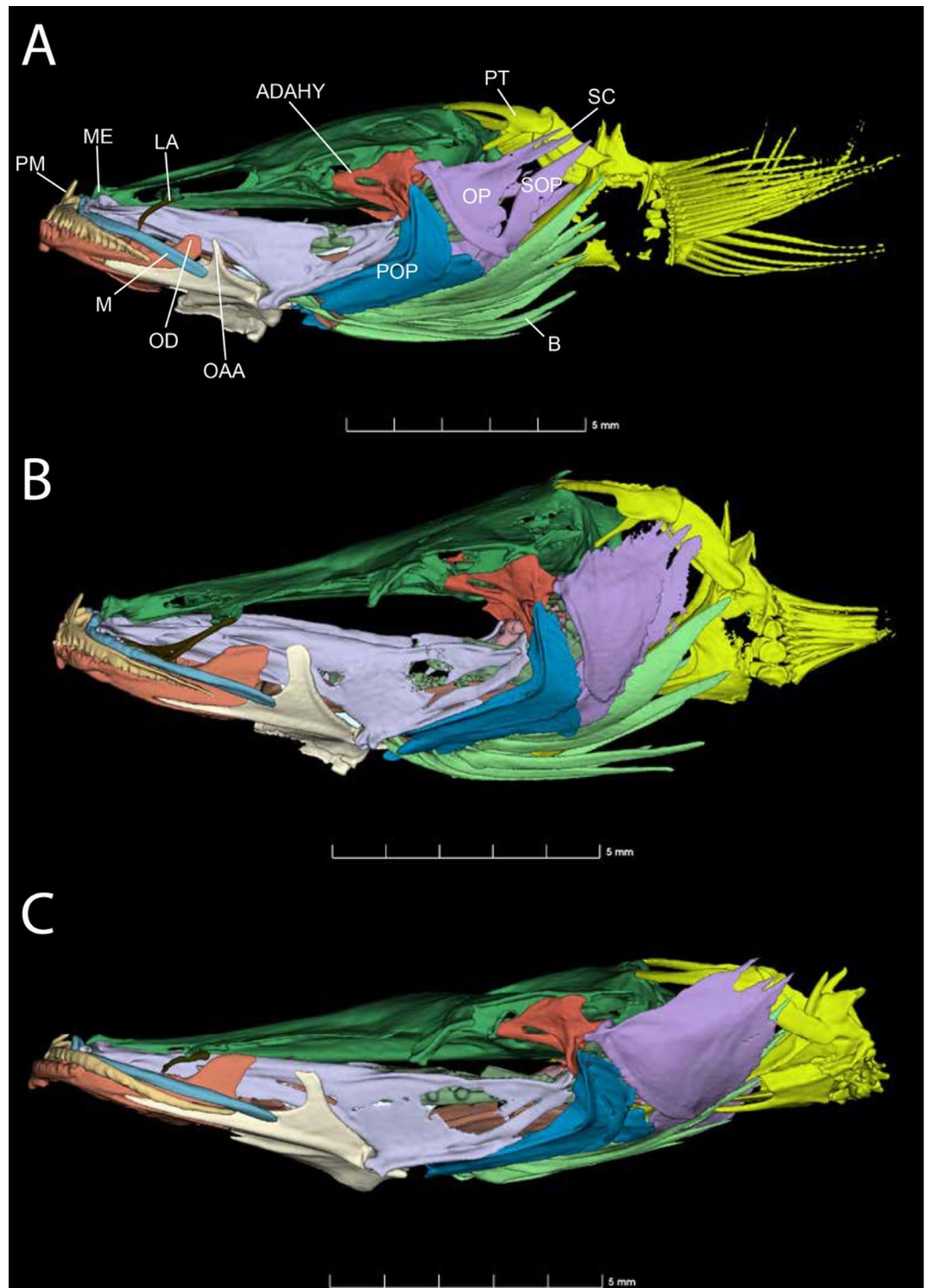


Fig. 5. Lateral views of head CT scans of (A) *Typhlichthys subterraneus*, UAIC 04134, (B) *Demogorgonichthys arcanus*, gen. et sp. nov., paratype, AUM 89691, and (C) *Speoplatyrhinus poulsoni*, UAIC 11125. ADAY - anterodorsal arm of hyomandibula, B - branchiostegals, LA - lacrimal, M - maxilla, ME - mesethmoid, OAA - orbital process of anguloarticular, OD - orbital process of dentary, OP - opercle, PM - premaxilla, POP - preopercle, PT - posttemporal, SC - supracleithrum, SOP - subopercle.

A single 572-bp *rhodopsin* haplotype was observed among the seven *D. arcanus* individuals sequenced. The coding region contained two deletions: a 284-bp deletion (94 amino acids) resulting in a frameshift, and a second 12-bp deletion (4 amino acids). The larger deletion introduces a frameshift that generates three premature stop codons, yielding a truncated open reading frame (Fig. 6) and providing strong evidence for pseudogenization.

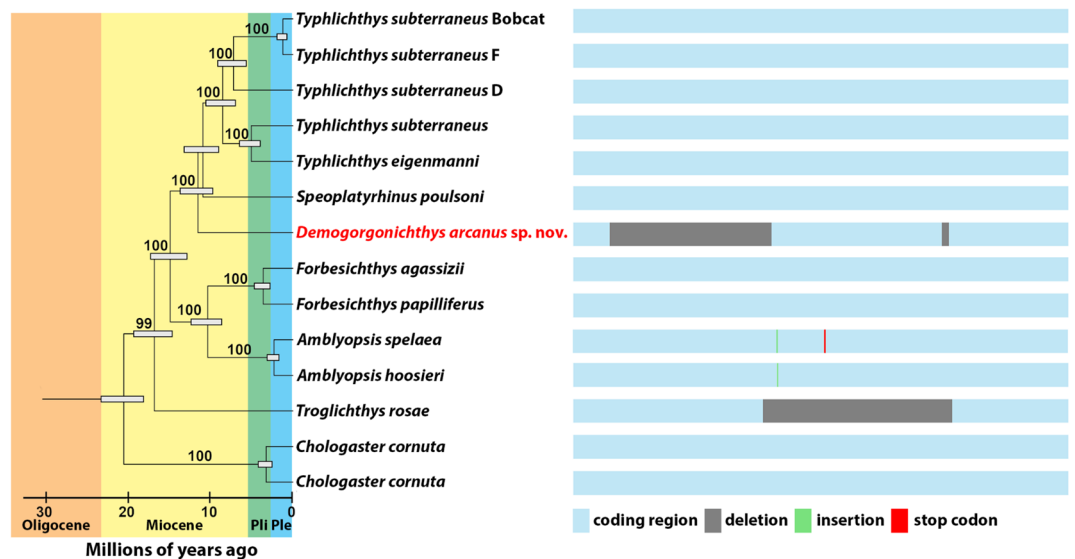


Fig. 6. Maximum-likelihood chronogram inferred from amblyopsid mitogenomes, including the new species (highlighted in red) under the General Time Reversible substitution model using IQ-TREE as implemented in the CIPRES Science Gateway. Divergence times were estimated using the RelTime method in MEGA. The log-likelihood of the inferred ML tree was $-111,253.57$. Bootstrap node support shown above and to the left of nodes. Outgroup taxa are not shown. Alignment of the vision gene *rhodopsin* is shown to the right highlighting the positions of putative loss-of-function mutations including deletions, insertions, and premature stop codons.

of the *rhodopsin* locus in this lineage, consistent with relaxation of selective constraint following colonization of an aphotic environment.

Discussion

Discovery in plain sight: hidden vertebrate diversity in a well-studied cave

The discovery of *Demogorgonichthys arcanus* is notable not just because it represents a new genus and species of North American cavefishes, but also because it was detected in a cave system that has been repeatedly visited and intensively monitored for decades. Bobcat Cave has been the focus of long-term biological and hydrological monitoring associated with the federally endangered Alabama Cave Shrimp (*Palaemonias alabamiae*), including nearly monthly water-quality measurements and shrimp censuses since 1990^{42–44,47,51–54}, as well as numerous targeted biological investigations^{9,32,48–50,55,56}. In many regions, such a sustained history of study would be expected to greatly reduce the likelihood of discovering additional vertebrate lineages, particularly within a family as well-characterized as the Amblyopsidae. The presence of an undescribed cavefish lineage under these circumstances underscores a persistent and underappreciated source of discovery bias in subterranean ecosystems: even when cave systems and other subterranean sites are well studied, detection probability for cryptic aquatic vertebrates can remain low.

Several non-mutually exclusive factors may explain why *D. arcanus* remained undocumented. First, the hydrologic regime of Bobcat Cave is highly dynamic, with rapid and substantial water-level fluctuations in response to precipitation and prolonged periods of reduced standing water during drought. Such variability likely affects both the spatial distribution and detectability of cavefishes, particularly if individuals spend much of their time in the underlying aquifer and enter human-accessible cave passages only under certain hydrologic conditions. Second, the cave's extensive mud substrate and episodic turbidity can limit visibility and hinder effective visual surveys. Third, researchers have often minimized entry into and disturbance of aquatic habitats in Bobcat Cave to avoid impacts on the federally endangered Alabama Cave Shrimp, which can constrain capture effort and reduce opportunities to closely examine fishes encountered in pools and submerged passages. Finally, *D. arcanus* occurs in syntopy with *Typhlichthys subterraneus*, and historical monitoring efforts have treated cavefish observations as a single taxonomic unit. This is a common challenge in subterranean biodiversity surveys, where morphologically similar or behaviorally cryptic taxa may be conflated unless detailed morphological or genetic examination is undertaken^{7,57}. Four previous collections (7 specimens in total) have been made of amblyopsids at Bobcat Cave: UAIC 45381.01 (1 specimen), UAIC 45378.01 (1), UAIC 49098.01 (3), and UAIC 14577.01 (2); all of which are *Typhlichthys*. Together, these considerations suggest that additional undescribed diversity may persist even in more intensely sampled karst regions, particularly in groundwater habitats with strong surface connectivity and challenging survey conditions.

Multiple independent evolutionary origins of cave adaptation within a single karst cave system

A striking result of this study is the co-occurrence of *Demogorgonichthys arcanus* with *Typhlichthys subterraneus* at Bobcat Cave despite the absence of a sister-group relationship between these taxa. Although syntopy of stygobiotic fishes has been documented elsewhere^{20,30–32}, such cases remain uncommon within North American amblyopsids and carry important implications for understanding the evolutionary assembly of subterranean communities. The presence of two deeply divergent amblyopsid lineages within a single cave system indicates that Bobcat Cave, or its associated aquifer network, has been colonized independently by at least two cave-adapted lineages that have persisted together over ecological timescales.

Comparable instances of amblyopsid syntopy are known from Key Cave in Lauderdale County, Alabama, where *Speoplatyrhinus poulsoni* and *T. subterraneus* occur within the same cave system^{20,58}, and from the Mammoth Cave system, where *Amblyopsis spelaea* and *Typhlichthys* co-occur^{17,20,30,33}. However, in the Mammoth Cave system, the two species rarely occupy the same aquatic microhabitats. In contrast, *D. arcanus* and *T. subterraneus* occur in close syntopy within Bobcat Cave, demonstrating that independently derived cavefish lineages can not only colonize the same subterranean system but also overlap spatially at fine ecological scales.

At present, limited resolution among major amblyopsid lineages in the mitochondrial topology constrains stronger inference about deeper relationships among *Demogorgonichthys*, *Typhlichthys*, and *Speoplatyrhinus*. Nonetheless, the strong monophyly and deep genetic divergence of *Demogorgonichthys* support its recognition as a distinct evolutionary lineage. The unresolved placement of *Demogorgonichthys* relative to *Typhlichthys* and *Speoplatyrhinus* in the *nd2* topology reflects well-recognized limitations of single-locus mitochondrial phylogenetics, which can be subject to stochastic lineage sorting, among-lineage rate heterogeneity, and insufficient phylogenetic signal at deeper divergence depths. The polytomous placement of *D. arcanus* in the *nd2* topology thus reflects genuine uncertainty in the divergence order of these lineages rather than evidence against its distinctiveness.

We acknowledge that the current phylogenetic framework relies primarily on mitochondrial markers, and that the rapid divergence of these lineages in the late Miocene means incomplete lineage sorting cannot be excluded as a contributor to topological uncertainty. Although *rhodopsin* was analyzed as a nuclear marker, its utility for resolving deeper relationships is limited by the extensive structural degeneration documented in *D. arcanus*. As such, *rhodopsin* is best interpreted here as evidence for unique visual system degeneration rather than as a primary phylogenetic marker. Phylogenomic analyses incorporating multiple conserved nuclear loci will be essential for definitively resolving the placement of *Demogorgonichthys* within Amblyopsidae, testing whether the mitogenomic topology reflects the true species tree, and differentiating between alternative historical scenarios, including whether *Demogorgonichthys* represents an ancient relict lineage within the Interior Low Plateau karst region or an early divergence associated with regional karst evolution in the Tennessee River Valley. Whole-genome sequencing data are currently being generated for *Demogorgonichthys arcanus* and other amblyopsid taxa as part of an ongoing study, and these data will provide the phylogenomic resolution needed to fully address remaining uncertainty in deeper amblyopsid relationships. A formal description of this lineage now provides a stable taxonomic foundation for that forthcoming work.

Evolutionary distinctiveness and convergent cave adaptation

The morphology of *Demogorgonichthys arcanus* exhibits classic hallmarks of obligate subterranean life, including complete loss of externally visible eyes, depigmentation, and elaboration of mechanosensory systems, all traits that have evolved repeatedly and convergently in cavefishes worldwide^{17–20,59–61}. Despite these shared troglomorphic features, *Demogorgonichthys* is readily distinguished from *Typhlichthys* and *Speoplatyrhinus* by a suite of cranial, jaw, neuromast, and fin characters. This mosaic of convergence and lineage-specific divergence underscores that cave adaptation in amblyopsids is not expressed as a single morphological endpoint, but rather reflects multiple evolutionary solutions shaped by ancestry and ecological context.

The temporal framework inferred from mitogenomic data suggests that *Demogorgonichthys*, *Speoplatyrhinus*, and *Typhlichthys* diverged during a relatively narrow interval in the Miocene, consistent with a rapid diversification of amblyopsid cavefishes rather than a protracted, stepwise accumulation of lineages. Comparable Miocene diversification time frames, including the split between *Speoplatyrhinus* and *Typhlichthys*, have been inferred in other amblyopsid studies^{21,27,29,40}, and independent subterranean colonization events have been documented in other cave and groundwater vertebrates, including Texas *Eurycea* groundwater salamanders^{62,63} and the *Eurycea spelaea* grotto salamander complex of the Ozark Highlands⁶⁴. Together, these patterns point to a shared evolutionary history shaped by regional geological and climatic change. The middle–late Miocene transition was characterized by increasing climatic seasonality and landscape restructuring^{65,66}, processes that likely destabilized surface habitats while favoring the persistence of groundwater refugia. Under this scenario, repeated invasions of subterranean environments by surface ancestors may have been facilitated by expanding karst connectivity and ecological release within caves and aquifers.

The nuclear vision-related gene *rhodopsin* provides an independent molecular perspective on this evolutionary history. Although *D. arcanus* lacks externally visible eyes, its *rhodopsin* coding region is still present but exhibits extensive structural disruption, including a large deletion that causes a frameshift and multiple premature stop codons, as well as an additional deletion not observed in other amblyopsid cavefishes examined to date (Fig. 6). Degeneration of vision-related genes has been documented repeatedly in cave-adapted fishes and is widely interpreted as a consequence of relaxed selective constraint following colonization of aphotic environments^{21,27,29,40,67–71}.

Importantly, the specific mutational pattern observed in *D. arcanus* differs from that reported in co-occurring *Typhlichthys* and other amblyopsid genera, suggesting that *rhodopsin* degeneration in *Demogorgonichthys* did not arise from a shared ancestral pseudogenization event. Instead, these molecular patterns are consistent with an

independent evolutionary trajectory following subterranean colonization. When viewed alongside pronounced morphological differentiation and deep mitochondrial divergence, the *rhodopsin* results reinforce the inference that multiple amblyopsid lineages have independently adapted to life underground within the same karst system.

Together, these findings demonstrate how subterranean environments can act simultaneously as arenas for strong phenotypic convergence and as long-term evolutionary filters that preserve deep genetic independence among co-occurring lineages. This dual role helps explain the surprising persistence of cryptic diversity in karst aquifers and highlights the complexity of evolutionary processes shaping groundwater communities.

Conservation implications: extreme endemism in a dynamic and vulnerable groundwater system

From a conservation perspective, *D. arcanus* exemplifies the vulnerability of narrowly endemic groundwater vertebrates occupying small, hydrologically dynamic karst systems. The species is currently known from a single cave and its associated aquifer and is inferred to occupy a highly restricted geographic range. These conditions support a NatureServe global rank of G1 (Critically Imperiled) and meet IUCN thresholds for Critically Endangered under criterion B, based on a very small extent of occurrence and area of occupancy coupled with occurrence at a single site. As with many described stygobiotic taxa^{72–74}, these designations reflect both genuine biological risk and substantial uncertainty regarding the full spatial extent of occupied habitat.

Although Bobcat Cave lies within a restricted-access military installation that likely limits direct anthropogenic disturbance within the cave itself, the primary threats to *D. arcanus* operate at the scale of groundwater processes extending beyond the human-accessible cave passages. Hydrologic studies demonstrate strong and rapid connectivity between surface runoff and the cave's groundwater, resulting in pronounced water-level fluctuations following precipitation events^{44–46}. Such connectivity increases susceptibility to surface-derived stressors, including contamination, sediment inputs, and altered hydrologic regimes within the recharge basin. The repeated detection of elevated concentrations of trace metals, including cadmium, chromium, and lead, over multiple decades suggests potential legacy contamination associated with historical industrial activities on Redstone Arsenal^{43,44,46,47}. While the biological effects of these contaminants on *D. arcanus* remain unknown, chronic exposure may pose sublethal or long-term risks typical of groundwater-dependent fauna.

In addition to chronic groundwater stressors, stochastic events such as extreme flooding or prolonged drought may further constrain habitat availability and impose demographic bottlenecks in species with small population sizes and limited distributions^{75,76}. These risks highlight a common paradox in subterranean conservation: occurrence on protected or access-restricted lands can reduce some immediate threats, such as disturbance from recreational caving, yet does not eliminate vulnerability arising from processes operating at the aquifer scale. Continued water-quality monitoring and biological surveys at Bobcat Cave, combined with protection of surface recharge areas and maintenance of existing access controls, are therefore likely to be critical for the long-term persistence of *D. arcanus*. Further hydrologic delineation and targeted biological surveys will be necessary to refine conservation priorities and management strategies.

Future directions

Several lines of investigation would substantially refine understanding of the evolution and conservation status of *Demogorgonichthys arcanus*. First, fine-scale delineation of the recharge basin and hydrologic connectivity of Bobcat Cave is needed to define the likely spatial extent of the population and to identify priority areas for groundwater protection. Second, targeted surveys of nearby cave and groundwater habitat, particularly using eDNA approaches, may reveal additional occurrences or confirm strict endemism. Third, phylogenomic analyses with broader sampling across amblyopsid lineages may resolve deeper relationships and enable divergence-time estimation, thereby placing *Demogorgonichthys* within the temporal framework of karst landscape evolution in the Interior Low Plateau karst region. Finally, monitoring programs at Bobcat Cave should explicitly distinguish *Demogorgonichthys* from *Typhlichthys* to generate species-specific abundance datasets and better evaluate population trends through time.

Conclusions

The discovery of *Demogorgonichthys arcanus* demonstrates that substantial vertebrate diversity can remain undocumented even within intensively studied karst systems and long-monitored landscapes. The occurrence of this lineage in syntopy with *Typhlichthys subterraneus*, despite deep mitochondrial divergence and lineage-specific degeneration of the vision gene *rhodopsin*, provides strong evidence that subterranean fish communities can assemble through multiple independent colonization events within a single groundwater system. Together, these findings reveal how caves and aquifers promote extreme phenotypic convergence while preserving deep evolutionary independence among co-occurring lineages. From a conservation perspective, *D. arcanus* exemplifies the vulnerability of narrowly endemic groundwater vertebrates and reinforces the importance of protecting karst aquifers and recharge areas, even where long-term monitoring and restricted access are already in place.

Methods

Survey approach and specimen collection

We conducted comprehensive biological inventories of terrestrial and aquatic fauna at Bobcat Cave on nine occasions between 2019 and 2025: 26 November 2019, 6 October 2020, 13 September 2023, 22 February 2024, 5 February 2025, 10 February 2025, 18 April 2025, 10 July 2025, and 30 December 2025. These visits were conducted in conjunction with other ongoing research and monitoring efforts on Redstone Arsenal.

Surveys consisted of time-constrained visual encounter surveys (VESs) of all human-accessible passages within the cave, using headlamps and handheld dive lights to illuminate aquatic habitats. Survey effort focused on visually scanning pools, submerged passage margins, and structural features while minimizing physical disturbance. During surveys conducted in 2025, cavefishes were captured using handheld dip nets. Captured individuals were transported alive to the surface in water-filled 500-mL Nalgene bottles for processing. Following examination and tissue sampling (see below), specimens were either preserved or released as appropriate under permit conditions.

Morphological analyses

Collection codes are AUM for the Auburn University Museum of Natural History and UAIC for the University of Alabama Ichthyological Collection. Morphometric measurements followed Cooper and Kuehne⁷⁷ and Chakrabarty et al.⁷⁸. Measurements were taken to the nearest 0.1 mm using digital calipers and include: standard length (SL; tip of snout to posterior margin of hypurals), head length (tip of snout to posterior edge of opercle), head width (measured across posterior margins of opercles), snout breadth (head width at its narrowest point), snout depth (vertical depth at the narrowest constriction of the snout), body width (width at dorsal-fin origin), body depth (depth at dorsal-fin origin), predorsal length (snout tip to dorsal-fin origin), preanal length (snout tip to anal-fin origin), prepectoral length (snout tip to insertion of the ventral pectoral-fin ray), vent-to-anal-fin distance (distance from center of vent to anal-fin origin), longest dorsal-fin ray (distance from dorsal-fin origin to maximum fin extent), pectoral-fin length (base of pectoral fin to maximum extent), longest anal-fin ray (anal-fin origin to maximum extent), caudal length (base of caudal fin at midline to maximum extent of fin), caudal peduncle depth (measured at the narrowest point), and upper jaw length (tip of snout to posterior angle of the jaw).

Fin-ray counts are reported using lowercase Roman numerals to indicate that all rays are unbranched. For meristic characters exhibiting variation, ranges are provided along with the number of individuals exhibiting each count; an asterisk denotes the count observed in the holotype. Scale counts were not attempted, as amblyopsid scales are deeply embedded, irregularly arranged, and do not form consistent rows, rendering counts unreliable.

Computed tomography of *Demogorgonichthys* was made at the Auburn University Center for Polymer and Advanced Composites with a Zeiss Xradia 520 Versa micro-CT scanner. Bones were segmented with 3D Slicer⁷⁹.

Molecular analyses

DNA extraction, amplification, and sequencing. Genomic DNA was extracted from fin clips obtained from voucher specimens and individuals released following processing using the Qiagen DNeasy Blood and Tissue Kit (Qiagen, Germantown, Maryland, USA) according to the manufacturer's protocols. We amplified a 1,041-bp fragment of the mitochondrial NADH dehydrogenase subunit 2 (*nd2*) locus using primers and PCR conditions described by Kocher et al.⁸⁰ and employed in previous phylogenetic studies of amblyopsid fishes^{9,27,40,41,81}. In addition, we amplified a 874-bp fragment of the nuclear vision-related gene *rhodopsin*, which consists of a single exon in teleost fishes⁸², using primers rho-1 (5'-GTCCATATGAATACCCTCAGTACTACC-3') and rho-2 (5'-TCTTCCGCAGCACACGTTGG-3') following protocols in Niemiller et al.^{27,40}. PCR products were purified using ExoSAP-IT (Thermo Fisher Scientific, Waltham, Massachusetts, USA) and sequenced in both forward and reverse directions at Eurofins Genomics (Louisville, Kentucky, USA). Raw reads were trimmed, ambiguous base calls verified by inspection of electropherograms, and contigs assembled in Geneious Prime v2026.0.1 (<https://www.geneious.com>). The mitochondrial *nd2* locus provides high phylogenetic resolution at shallow to moderate divergence depths in amblyopsids, whereas *rhodopsin* offers an independent, biparentally inherited nuclear marker that has been widely used to investigate cavefish evolution and visual system degeneration^{27,40,68}.

For a subset of representatives and selected outgroups, complete mitochondrial genome sequences were generated using two complementary approaches: (i) long-range PCR followed by Oxford Nanopore sequencing of whole-mitochondrial amplicons, and (ii) reference-guided assembly from subsampled Illumina whole-genome shotgun reads obtained from the NCBI Sequence Read Archive (SRA). Long-range PCR amplification and Nanopore sequencing followed methods described by Alley et al.⁸³. Amplicons were prepared using manufacturer-recommended library preparation protocols and sequenced on Nanopore flow cells at Plasmidsaurus (Louisville, Kentucky, USA). Raw Nanopore reads were basecalled, quality filtered, and assembled into circular mitochondrial genomes using de novo assembly tools in Geneious Prime. Sequence metadata and assembly statistics for each sample are presented in Supplementary Data 1.

For Illumina-based assemblies, paired-end reads were downloaded from the SRA, randomly subsampled to enrich mitochondrial coverage while reducing nuclear background, and assembled using reference-guided mapping to complete mitochondrial genomes of closely related percopsiform taxa. Iterative read mapping with medium-high sensitivity parameters was used to improve assembly completeness and consensus accuracy. Assembly circularity was confirmed by overlap of terminal regions. All mitochondrial genome assemblies were validated by confirming gene order, absence of frameshifts or premature stop codons in protein-coding genes, and consistency with known vertebrate mitochondrial genome structure. To minimize incorporation of nuclear mitochondrial pseudogenes (NUMTs), assemblies were screened for anomalous coverage patterns, internal stop codons, and frameshifts. Accession numbers for all previously published sequences are provided in Supplementary Data 1.

Phylogenetic analyses. Previously published sequences representing other amblyopsid taxa, along with outgroup sequences of *Aphredoderus sayanus* and *A. gibbosus*, were downloaded from NCBI GenBank (Supplementary Data 1). Newly generated sequences were aligned with existing data using MUSCLE⁸⁴ as implemented in MEGA v12⁸⁵. All novel sequences generated in this study have been deposited in GenBank (Supplementary Data 1).

Uncorrected pairwise sequence divergences (p-distances) were calculated for each locus in MEGA. Optimal models of nucleotide substitution were selected using jModelTest⁸⁶ under the corrected Akaike Information Criterion (AICc), with separate partitions specified for first, second, and third codon positions for *nd2*. Maximum-likelihood (ML) gene genealogies were reconstructed for each locus using PhyML v3.3⁸⁷, and nodal support was assessed using 1,000 nonparametric bootstrap replicates. The mitogenome phylogeny was inferred under the General Time Reversible substitution model using IQ-TREE as implemented in the CIPRES Science Gateway. Divergence times were estimated using the RelTime method⁸⁸ in MEGA, yielding an ultrametric timetree calibrated at the node representing the divergence of *Percopsis* from the rest of Percopsiformes. This split corresponds to the deepest internal split within the order 60 ± 3 Ma (95% CI = 54.12–64.88 Ma)^{27,40}.

Conservation assessment

We conducted conservation status assessments for the newly described cavefish species using both the NatureServe and IUCN Red List frameworks. Both systems assign taxa to one of seven categories along a continuum of increasing extinction risk, based on standardized criteria incorporating geographic range size, population status, threats, and trends. NatureServe conservation status was evaluated using the NatureServe Rank Calculator v3.186⁸⁹, following the methodology and criteria outlined in Master et al.⁹⁰. This assessment integrates measures of rarity, population size, geographic restriction, environmental specificity, and vulnerability to threats to derive a global conservation rank. The IUCN Red List category was determined using RAMAS Red List v3.0⁹¹ in accordance with the most recent IUCN Red List Categories and Criteria⁹². Assessments focused primarily on geographic range metrics and inferred threats, given the limited information available for population size and trends typical of newly described subterranean taxa. Geographic range size was quantified using the web-based program GeoCAT⁹³. Extent of occurrence (EOO) was calculated as a minimum convex polygon encompassing all known localities, and area of occupancy (AOO) was estimated using a 2×2 km grid (4 km^2) following IUCN-recommended standards^{89,92}. Potential threats were identified and categorized using the standardized threat classification scheme of Salafsky et al.⁹⁴. These threat assessments were used to inform both the NatureServe and IUCN evaluations.

Permits, animal care, and ethical compliance

All fieldwork and specimen collection were conducted in accordance with applicable federal, state, and institutional regulations. Access to Bobcat Cave and all biological surveys on Redstone Arsenal were authorized by the U.S. Army Garrison–Redstone Arsenal. Collection, handling, and tissue sampling of fishes were conducted under scientific collection permits issued by the Alabama Department of Conservation and Natural Resources (permit nos. 2025112916068680 and 2025112916268680). Animal handling protocols followed established ethical guidelines for the use of fishes in research and were approved by the Institutional Animal Care and Use Committee (IACUC) at The University of Alabama in Huntsville (protocol no. 2023.R03.Niemiller. Subterranean).

Efforts were made to minimize stress, injury, and mortality during capture and processing. Individuals captured for morphological and genetic analyses were handled briefly, maintained in clean, aerated cave water during processing, and either preserved as voucher specimens or released at the site of capture following tissue sampling, as permitted. The number of individuals collected was minimized and limited to that necessary to document diagnostic morphological characters and obtain genetic material for phylogenetic analyses. Surveys and collections were conducted in a manner designed to minimize disturbance to cave habitats and associated fauna, including avoidance of unnecessary traversal through aquatic habitats and strict adherence to biosecurity protocols to reduce the risk of pathogen introduction or transfer among cave systems.

Data availability

All data generated or analysed in this study are included in this published article and/or its supplementary materials. All sequences used in the phylogenetic analyses are available in GenBank, with accession numbers listed in Supplementary Data 1. CT Scans are available through MorphoSource (Media 000828138, 000828143, and 000830304).

Received: 19 February 2026; Accepted: 18 May 2026

Published online: 06 July 2026

References

1. Rothschild, L. J. & Mancinelli, R. L. Life in extreme environments. *Nature* **409**, 1092–1101 (2001).
2. Fišer, C., Pipan, T. & Culver, D. C. Vertical extent of groundwater metazoans: An ecological and evolutionary perspective. *BioScience* **64**, 971–979 (2014).
3. Rampelotto, P. H. Extremophiles and extreme environments. *Life* **3**, 482–485 (2013).
4. Tobler, M. et al. Extreme environments and the origins of biodiversity: Adaptation and speciation in sulphide spring fishes. *Mol. Ecol.* **27**, 843–859 (2018).
5. Merino, N. et al. Living at the extremes: Extremophiles and the limits of life in a planetary context. *Front. Microbiol.* **10**, 780 (2019).
6. Shu, W. S. & Huang, L. N. Microbial diversity in extreme environments. *Nat. Rev. Microbiol.* **20**, 219–235 (2022).
7. Bickford, D. et al. Cryptic species as a window on diversity and conservation. *Trends Ecol. Evol.* **22**, 148–155 (2007).
8. Mora, C. et al. How many species are there on earth and in the ocean? *PLoS Biol.* **9**, e1001127 (2011).
9. Niemiller, M. L., Near, T. J. & Fitzpatrick, B. M. Delimiting species using multilocus data: Diagnosing cryptic diversity in the southern cavefish, *Typhlichthys subterraneus* (Teleostei: Amblyopsidae). *Evolution* **66**, 846–866 (2012).
10. Stork, N. E. How many species of insects and other terrestrial arthropods are there on earth? *Annu. Rev. Entomol.* **63**, 31–45 (2018).
11. Culver, D. C. & Pipan, T. *The Biology of Caves and Other Subterranean Habitats* 2nd edn (Oxford Univ. Press, 2019).
12. Priede, I. G. *Deep-sea Fishes: Biology, Diversity, Ecology and Fisheries* (Cambridge Univ. Press, 2017).
13. Kolora, S. R. R. et al. Origins and evolution of extreme life span in Pacific Ocean rockfishes. *Science* **374**, 842–847 (2021).

14. Clarke, A. & Johnston, I. A. Evolution and adaptive radiation of Antarctic fishes. *Trends Ecol. Evol.* **11**, 212–218 (1996).
15. Sandel, M. W. et al. Sink or swim: Phylogenomic analysis of Baikal sculpins reveals multiple transformations to pelagic, bathybenthic, and lotic ecomorphologies. *Mol. Phy. & Evol.* **25**, 108451 (2025).
16. Tobler, M. et al. Evolution in extreme environments: Replicated phenotypic differentiation in livebearing fish inhabiting sulfidic springs. *Evolution* **65**, 2213–2228 (2011).
17. Poulson, T. L. Cave adaptation in amblyopsid fishes. *Am. Midl. Nat.* **70**, 257–290 (1963).
18. Soares, D. & Niemiller, M. L. Sensory adaptations of fishes to subterranean environments. *BioScience* **63**, 274–283 (2013).
19. Niemiller, M. L. & Soares, D. Cave environments. In *Extremophile Fishes* (Springer, 2014).
20. Niemiller, M. L. et al. Cavefishes. In *Encyclopedia of Caves* (Academic Press, 2019).
21. Hart, P. B. et al. Ancient climate changes and relaxed selection shape cave colonization in North American cavefishes. *Proc. R. Soc. B.* **292**, 20242516 (2025).
22. Poulson, T. L. & White, W. B. The cave environment. *Science* **165**, 971–981 (1969).
23. Culver, D. C. *Cave Life: Evolution and Ecology* (Harvard Univ. Press, 1982).
24. Borowsky, R. Cavefishes. *Curr. Biol.* **28**, R60–R64 (2018).
25. Bai, X. et al. Global diversity patterns and threats of cave fish. *Glob. Ecol. Biogeogr.* **34**, e70160 (2025).
26. Strecker, U. et al. Parallel speciation in *Astyanax* cave fish (Teleostei) in Northern Mexico. *Mol. Phylogenet. Evol.* **62**, 62–70 (2012).
27. Niemiller, M. L. et al. Evidence for repeated loss of selective constraint in rhodopsin of amblyopsid cavefishes (Teleostei: Amblyopsidae). *Evolution* **67**, 732–748 (2013).
28. Flórez, J. S. et al. Repeated colonization of caves leads to phenotypic convergence in catfishes (Siluriformes: *Trichomycterus*) at a small geographical scale. *Zool. J. Linn. Soc.* **193**, 772–788 (2021).
29. Brownstein, C. D. et al. Convergent evolution in amblyopsid cavefishes and the age of eastern North American subterranean ecosystems. *Mol. Biol. Evol.* **42**, msaf185 (2025).
30. Niemiller, M. L. & Poulson, T. L. Subterranean fishes of North America: Amblyopsidae. In *Biology of Subterranean Fishes* (Science Publishers, 2010).
31. Zhao, Y. H., Gozlan, R. E. & Zhang, C. G. Out of sight out of mind: current knowledge of Chinese cave fishes. *J. Fish. Biol.* **79**, 1545–1562 (2011).
32. Hart, P. B. et al. Conservation implications for the world's most widely distributed cavefish species complex based on population genomics (*Typhlichthys*, Percopsiformes). *Conserv. Genet.* **25**, 165–177 (2024).
33. Woods, L. P. & Inger, R. F. The cave, spring, and swamp fishes of the family Amblyopsidae of central and eastern United States. *Am. Midl. Nat.* **58**, 232–256 (1957).
34. Adams, G. L., Burr, B. M. & Warren, M. L. Amblyopsidae: cavefishes. In *Freshwater Fishes of North America*, Vol. 2: *Characidae to Poeciliidae* (Johns Hopkins Univ., 2020).
35. Eigenmann, C. H. The eyes of the blind vertebrates of North America. I. The eyes of the Amblyopsidae. *Archiv für Entwicklungsmechanik der Organismen*. **8**, 545–617 (1899).
36. Poulson, T. L. Morphological and physiological correlates of evolutionary reduction of metabolic rate among amblyopsid cave fishes. *Environ. Biol. Fishes.* **62**, 239–249 (2001).
37. Armbruster, J. W., Niemiller, M. L. & Hart, P. B. Morphological evolution of cave-, spring-, and swampfishes of the Amblyopsidae. *Copeia* **104**, 763–777 (2016).
38. Hart, P. B. et al. Cave-adapted evolution in the North American amblyopsid fishes inferred using phylogenomics and geometric morphometrics. *Evolution* **74**, 936–949 (2020).
39. Dillman, C. B. et al. Regressive progression, progressive regression or neither? Phylogeny and evolution of the Percopsiformes (Teleostei, Paracanthopterygii). *Zool. Scr.* **40**, 45–60 (2011).
40. Niemiller, M. L. et al. Effects of climatic and geological processes during the Pleistocene on the evolutionary history of the northern cavefish, *Amblyopsis spelaea* (Teleostei: Amblyopsidae). *Evolution* **67**, 1011–1025 (2013).
41. Cucalón, R. V. et al. Population genomics and mitochondrial DNA reveal cryptic diversity in North American spring cavefishes (Amblyopsidae, *Forbesichthys*). *Conserv. Genet.* **25**, 1283–1301 (2024).
42. Rheams, K. F., Moser, P. H. & McGregor, S. W. Geologic, hydrologic, and biologic investigations in Arrowwood, Bobcat, Matthews, and Shelta caves and selected caves, Madison County, Alabama. *Geological Survey of Alabama* (1992).
43. Rheams, K. F., Moser, P. H. & McGregor, S. W. Hydrogeologic and biologic factors related to the occurrence of the Alabama cave shrimp (*Palaemonias alabamiae*), Madison County, Alabama. *Geol. Surv. Ala Bull.* **161**, 1–147 (1994).
44. McGregor, S. W. et al. Biological, geological, and hydrological investigations in Bobcat, Matthews, and Shelta caves and other selected caves in north Alabama. *Geol. Surv. Ala Bull.* **166**, 1–198 (1997).
45. Clements, A. C. Hydrological and geochemical analyses of the karstic Bobcat Cave watershed: a case study at Redstone Arsenal, northern Alabama. MSc thesis, Auburn Univ. (2017).
46. McGregor, S. W. & O'Neil, P. E. Investigations of the source and fate of ground water in the vicinity of Bobcat Cave, Redstone Arsenal, Alabama and initiation of a management plan for Bobcat Cave. *Geological Survey of Alabama* (1996).
47. Bearden, R. A., McGregor, S. W., West, D. A., Ponta, G. M. & Hastert, G. A. *Water quality and biological monitoring in Bobcat and Matthews caves, Redstone Arsenal, Alabama, 1990–2022* Geological Survey of Alabama Open-File Rep, **2204** (2022).
48. Niemiller, M. L. et al. Discovery of a new population of the federally endangered Alabama cave shrimp, *Palaemonias alabamiae* Smalley, 1961, in northern Alabama. *Subterr. Biol.* **32**, 43–59 (2019).
49. Niemiller, M. L. et al. A noteworthy range extension of the federally endangered Alabama Cave Shrimp (*Palaemonias alabamiae*) south of the Tennessee River in northern Alabama. *Southeast. Nat.* **24**, N47–N58 (2025).
50. Dooley, K. E. Phylogenetics of cave crayfish in the genus *Cambarus* and using environmental DNA to detect populations of *Cambarus jonesi* and *Cambarus speleocoopi*. MSc thesis Univ. of Alabama in Huntsville (2021).
51. McGregor, S. W. & O'Neil, P. E. Water quality and biological monitoring in Bobcat and Matthews caves, Redstone Arsenal, Alabama, 1990–2001. Geological Survey of Alabama (2001).
52. McGregor, S. W. & O'Neil, P. E. Water quality and biological monitoring in Bobcat and Matthews caves, Redstone Arsenal, Alabama, 1990–2004. Geological Survey of Alabama (2004).
53. McGregor, S. W. et al. Biological, geological, and hydrological investigations in Bobcat, Matthews, and Shelta caves and other selected caves in north Alabama. Geological Survey of Alabama (1994).
54. United States Fish & Wildlife Service (USFWS). *Alabama Cave Shrimp (Palaemonias alabamiae) Status Review: Summary and Evaluation* (USFWS, South Atlantic-Gulf Region, Alabama Ecological Services Field Office, 2023).
55. Cooper, J. E. & Cooper, M. R. Observations on the biology of the endangered stygobiotic shrimp *Palaemonias alabamiae*, with notes on *P. ganteri* (Decapoda: Atyidae). *Subterr. Biol.* **8**, 9–20 (2011).
56. Espinasa, L. et al. A new genus and species of nicoletiid silverfish (Insecta, Zygentoma, Nicoletiidae) from caves of northern Alabama, USA. *Subterr. Biol.* **49**, 1–17 (2024).
57. Hending, D. Cryptic species conservation: A review. *Biol. Rev.* **100**, 258–274 (2025).
58. Kuhajda, B. R. & Mayden, R. L. Status of the federally endangered Alabama cavefish, *Speoplatyrhinus poulsoni* (Amblyopsidae), in key cave and surrounding caves, Alabama. *Environ. Biol. Fishes.* **62**, 215–222 (2001).
59. Jeffery, W. R. Regressive evolution in *Astyanax* cavefish. *Annu. Rev. Genet.* **43**, 25–47 (2009).
60. Ma, L., Zhao, Y. & Yang, J. X. Cavefish of China. In *Encyclopedia of Caves* (Academic Press, 2019).
61. Soares, D. & Niemiller, M. L. Extreme adaptation in caves. *Anat. Rec.* **303**, 15–23 (2020).

62. Chippindale, P. T., Price, A. H., Wiens, J. J. & Hillis, D. M. Phylogenetic relationships and systematic revision of central Texas hemidactyliine plethodontid salamanders. *Herpetol Monogr.* **14**, 1–80 (2000).
63. Tovar, R. U. et al. Parallel sensory compensation following independent subterranean colonization by groundwater salamanders. *Proc. Natl. Acad. Sci. U.S.A.* **122**, e2504850122 (2025).
64. Phillips, J. G., Fenolio, D. B., Emel, S. L. & Bonett, R. M. Hydrologic and geologic history of the Ozark Plateau drive phylogenomic patterns in a cave-obligate salamander. *J. Biogeogr.* **44**, 2463–2474 (2017).
65. Herbert, T. D. et al. Late Miocene global cooling and the rise of modern ecosystems. *Nat. Geosci.* **9**, 843–847 (2016).
66. Zhang, R. et al. Vegetation feedbacks accelerated the late Miocene climate transition. *Sci. Adv.* **11**, eads4268 (2025).
67. Li, Z. & He, S. Relaxed purifying selection of rhodopsin gene within a Chinese endemic cavefish genus *Simocyclocheilus* (Pisces: Cypriniformes). *Hydrobiologia* **624**, 139–149 (2009).
68. Hinaux, H. et al. *De novo* sequencing of *Astyanax mexicanus* surface fish and Pachón cavefish transcriptomes reveals enrichment of mutations in cavefish putative eye genes. *PLoS ONE* **8** (2013).
69. Zhao, Q. et al. Novel genome sequence of Chinese cavefish (*Triplophysa rosa*) reveals pervasive relaxation of natural selection in cavefish genomes. *Mol. Ecol.* **31**, 5831–5845 (2022).
70. Arcila, D. et al. Transcriptomic analysis of the Brazilian blind characid, *Stygichthys typhlops*, reveals convergent selection with *Astyanax mexicanus* and other cavefishes. *Front. Ecol. Evol.* 1–11 (2023).
71. Montero-Mendieta, S. et al. Doubled genomes, divergent fates: Genomic insights into diversification in an allotetraploid cavefish. *Mol. Ecol.* **34** (2025).
72. Niemiller, M. L. et al. Doomed before they are described? The need for conservation assessments of cryptic species complexes using an amblyopsid cavefish (Amblyopsidae: *Typhlichthys*) as a case study. *Biodivers. Conserv.* **22**, 1799–1820 (2013).
73. Niemiller, M. L. & Taylor, S. J. Protecting cave life. In *Encyclopedia of Caves* (Academic Press, 2019).
74. Niemiller, M. L. et al. Out of sight and out of mind? The conservation status of subterranean biodiversity in the United States and Canada. *Biodivers. Conserv.* **34**, 2851–2882 (2025).
75. Shu, S. S., Jiang, W. S., Whitten, T., Yang, J. X. & Chen, X. Y. Drought and China's cave species. *Science* **340**, 272 (2013).
76. Mammola, S. et al. Climate change going deep: The effects of global climatic alterations on cave ecosystems. *Anthropocene Rev.* **6**, 98–116 (2019).
77. Cooper, J. & Kuehne, E. (ed, R.) *Speoplatyrhinus poulsoni*, a new genus and species of subterranean fish. *Copeia* **1974** 486–493 (1974).
78. Chakrabarty, P., Prejean, J. A. & Niemiller, M. L. The Hoosier cavefish, a new and endangered species (Amblyopsidae, *Amblyopsis*) from the caves of southern Indiana. *Zookeys* **412**, 41–57 (2014).
79. Kikinis, R., Pieper, S. D. & Vosburgh, K. G. 3D Slicer: A platform for subject-specific image analysis, visualization, and clinical support. In *Intraoperative Imaging and Image-Guided Therapy* (Springer, 2014).
80. Kocher, T. D. et al. Evolution of NADH dehydrogenase subunit 2 in east African cichlid fish. *Mol. Phylogenet. Evol.* **4**, 420–432 (1995).
81. Niemiller, M. L. et al. First definitive record of a stygobiotic fish (Percopsiformes, Amblyopsidae, *Typhlichthys*) from the Appalachians karst region in the eastern United States. *Subterr. Biol.* **20**, 39–50 (2016).
82. Fitzgibbon, J. et al. The rhodopsin-encoding gene of bony fish lacks introns. *Gene* **164**, 273–277 (1995).
83. Alley, Z. D., Fast, K. M. & Sandel, M. W. The complete mitochondrial genomes of *Notropis chlorocephalus* and *Notropis chiliticus*. *Mitochondrial DNA B* **10**, 139–143 (2025).
84. Edgar, R. C. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res.* **32**, 1792–1797 (2004).
85. Kumar, S. et al. MEGA 12: molecular evolutionary genetics analysis version 12 for adaptive and green computing. *Mol. Biol. Evol.* **41**, msae263 (2024).
86. Darriba, D. et al. jModelTest 2: More models, new heuristics and parallel computing. *Nat. Methods* **9**, 772 (2012).
87. Guindon, S. et al. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Syst. Biol.* **59**, 307–321 (2010).
88. Tamura, K. et al. Estimating divergence times in large molecular phylogenies. *Proc. Natl. Acad. Sci. USA* **109**, 1933–19338.
89. Faber-Langendoen, D. et al. *NatureServe Conservation Status Assessments: Methodology for Assigning Ranks* (NatureServe, 2012).
90. Master, L. et al. *NatureServe conservation assessments: factors for assessing extinction risk* (NatureServe, 2009).
91. Akçakaya, H. R., Ferson, S. & Root, W. R. *RAMAS Red List: Threatened species classifications under uncertainty*, v3.0 (Applied Biomathematics, 2007).
92. International Union for the Conservation of Nature (IUCN). *Guidelines for Using the IUCN Red List Categories and Criteria*, v16. <https://www.iucnredlist.org/documents/RedListGuidelines.pdf> (2024).
93. Bachman, S. et al. Supporting Red List threat assessments with GeoCAT: Geospatial conservation assessment tool. *ZooKeys* **150**, 117–126 (2011).
94. Salafsky, N. et al. A standard lexicon for biodiversity conservation: Unified classifications of threats and actions. *Conserv. Biol.* **22**, 897–911 (2008).

Acknowledgements

JWA wishes to thank Ramsis Farag for help with CT scanning *Demogorgonichthys*. OMB thanks the Friday Harbor Laboratories for support and assistance with CT scans. Its contents are solely the responsibility of the authors and do not necessarily represent the views of the Southeast Climate Adaptation Science Center or the USGS. This manuscript is submitted for publication with the understanding that the United States Government is authorized to reproduce and distribute reprints for Governmental purposes.

Author contributions

M.L.N. conceived and designed the study. M.L.N., E.C.M., C.L.A., A.H., and M.C., conducted cave biological surveys, obtained tissues, and collected and photographed specimens. M.W.S., J.S., and C.I.Q. generated genetic and mitogenome datasets. M.L.N., M.W.S., and K.D.K.N. performed molecular data analysis. J.W.A., O.B., and P.B.H. generated morphological datasets and conducted morphological data analysis. M.L.N., P.B.H., and J.W.A. drafted the manuscript. All authors reviewed, revised, and approved the final version of the manuscript.

Funding

This project was partially funded by the National Science Foundation (award no. 2047939 to M.L.N.). The project described in this publication also was partially supported by grant no. G22AC00270 from the U.S. Geological Survey (to M.L.N.) and grant no. G23AC00533 (to M.W.S.) and USDA-NIFA accession number 7009241 (to M.W.S.). P.B.H. was partially supported by the Alabama Water Institute Equipment Grant fund, CONSERVE

Network (University of Alabama), and the Center for Freshwater Studies (University of Alabama, Department of Biological Sciences).

Declarations

Competing interests

The authors declare no competing interests.

Additional information

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1038/s41598-026-54315-4>.

Correspondence and requests for materials should be addressed to M.L.N. or P.B.H.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by-nc-nd/4.0/>.

© The Author(s) 2026