

Doomed before they are described? The need for conservation assessments of cryptic species complexes using an amblyopsid cavefish (*Amblyopsidae*: *Typhlichthys*) as a case study

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Abstract The delimitation of cryptic species and lineages is a common finding of phylogenetic studies. Species previously considered to be of low conservation priority might actually be comprised of multiple lineages with substantially smaller geographic ranges and smaller populations that are of much greater conservation concern and that require different conservation strategies. Cryptic biodiversity is an especially common finding in phylogenetic studies of subterranean fauna; however, most cryptic lineages remain undescribed and have not been subjected to conservation assessments. As many subterranean species are of high conservation concern, the conservation assessment of cryptic lineages is important for developing effective conservation and management strategies. In particular, some lineages might be in need of immediate conservation action even before formal taxonomic description. Here we explore this issue by conducting IUCN Red List and NatureServe conservation assessments on recently discovered cryptic lineages of the southern cavefish (*Typhlichthys subterraneus*) species complex. We ascertained threats associated with extinction risk, identified priority lineages and populations for immediate conservation efforts, and identified knowledge gaps to expedite the development of

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conservation and management strategies before formal taxonomic description. Most cryptic lineages are at an elevated risk of extinction, including one lineage classified as “Critically Endangered.” We identified ten threats impacting cavefish lineages that vary in both scope and severity, including groundwater pollution, hydrological changes from impoundments, and over-collection. Our threat assessments and recommendations can be used by stakeholders to prioritize effective and appropriate management initiatives aiding in the conservation of these lineages even before they are formally recognized.

Keywords Amblyopsidae · Cave · Climate change · Cryptic lineages · Conservation status · Endangered species · Extinction · Groundwater · IUCN · Linnean shortfall · NatureServe · Over-collection · Pollution · Red List · Subterranean · Threat assessment

Introduction

A significant challenge facing the conservation and management of biodiversity is our limited understanding of the evolution and ecology behind species diversity as well as its origins, distribution, and maintenance through time (Beheregaray and Caccone 2007). Advances in the generation and analysis of molecular data have greatly improved our knowledge of geographic patterns of biodiversity. In particular, phylogenetic studies have revealed considerable cryptic diversity in many taxonomic groups, geographic regions, and habitat types (e.g., Bickford et al. 2007; Pfenninger and Schwenk 2007; Beheregaray and Caccone 2007). Multiple morphologically similar species or lineages that are genetically distinct but classified as a single species with a broader distribution have been discovered in recent years, with the uncovering of cryptic diversity in subterranean organisms being especially common (Verovnik et al. 2003; Finston et al. 2007; Trontelj et al. 2009; Niemiller et al. 2012). Strong selective pressures result in similar morphologies via convergent or parallel evolution in related groups (Culver et al. 1995; Wiens et al. 2003; Culver and Pipan 2009) and the long-term stability of subterranean habitats and limited connectivity among cave systems are thought to promote high endemism (Gibert and Deharveng 2002; Verovnik et al. 2003; Finston et al. 2007).

The discovery of cryptic species has important conservation implications for several reasons. Accurate assessment of regional species richness and endemism is essential for

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identifying biodiversity hotspots and guiding the design of preserves (Myers et al. 2000; Bickford et al. 2007; Beheregaray and Caccone 2007; Bernardo 2011; Funk et al. 2012). One of the most important implications involves assessing the conservation status of individual species. Understanding the distribution of a species is of paramount importance in conservation assessment. A species previously considered to have a wide distribution and to be of low conservation concern might actually be comprised of multiple cryptic species with substantially smaller geographic ranges and fewer individuals that are of greater conservation concern (Bickford et al. 2007; Funk et al. 2012; Niemiller et al. 2012). Different cryptic species might have varying levels of threats, requiring different conservation strategies.

Though studies uncovering cryptic diversity have improved our understanding of subterranean biodiversity and endemism in several regions, in general few cryptic lineages have been formally described, i.e., the Linnean shortfall (Brown and Lomolino 1998). Moreover, these lineages most often are not subjected to conservation assessment. A reluctance to formally describe well-supported genetic lineages in the absence of morphological discontinuities is not limited to subterranean taxa (reviewed in Bernardo 2011). Although it should be of little consequence to stakeholders whether an entity is classified as a species, subspecies, evolutionary significant unit (ESU), or local population for conservation and management purposes, in reality taxonomic epithets still weigh heavily in funding decisions, public perception, and legislation to conserve biodiversity (Isaac et al. 2004; Beheregaray and Caccone 2007). Recognition of cryptic taxa results in a higher proportion of species meeting threatened or endangered conservation status because of reduction in average geographical range and population size (Agapow et al. 2004; Isaac et al. 2004). This is especially important for cryptic species complexes in nominal species that are already of conservation concern, as threatened and endangered species might be comprised of multiple lineages at even greater risk of extinction (Bickford et al. 2007). In such instances, conservation assessments are warranted even before taxa are formally recognized.

The southern cavefish, *Typhlichthys subterraneus* (family Amblyopsidae), as previously understood, had the largest documented distribution of any subterranean fish in the world, spanning more than 140,000 km² and over 5° of latitude (Proudlove 2006; Niemiller and Poulson 2010) throughout the Interior Plateau and Ozark Highlands of the eastern United States. Although widely distributed, *T. subterraneus* is a species of conservation concern, as it was assessed as “Vulnerable” in 1996 by the International Union for Conservation of Nature (IUCN) (World Conservation Monitoring Centre 1996) but ranked as “Apparently Secure” (G4) by NatureServe (NatureServe 2013). This species is also of conservation concern at the state level throughout its distribution (reviewed by Niemiller and Poulson 2010), including Alabama, Arkansas, Georgia, Kentucky, Missouri, and Tennessee.

However, several authors have hypothesized that *T. subterraneus* is a cryptic species complex because of its large distribution across several distinct hydrological basins (e.g., Swofford 1982; Barr and Holsinger 1985; Holsinger 2000; Niemiller and Poulson 2010). Genetic analyses of six loci from 60 populations across the distribution by Niemiller et al. (2012) revealed at least ten cryptic lineages within nominal *T. subterraneus* (Fig. 1), many limited to separate watersheds (and presumably separate aquifers) (Fig. 2) and should likely be recognized as distinct conservation units. Here we refer to these lineages as the *Typhlichthys* species complex. The most recent common ancestor of these lineages dates to the Pliocene/early Pleistocene (3.5–2.1 Mya; Niemiller et al. 2013a). Niemiller et al. (2012) refrained from describing these lineages as distinct species until additional study was conducted, with the exception of resurrecting *T. eigenmanni* for Ozark Highland

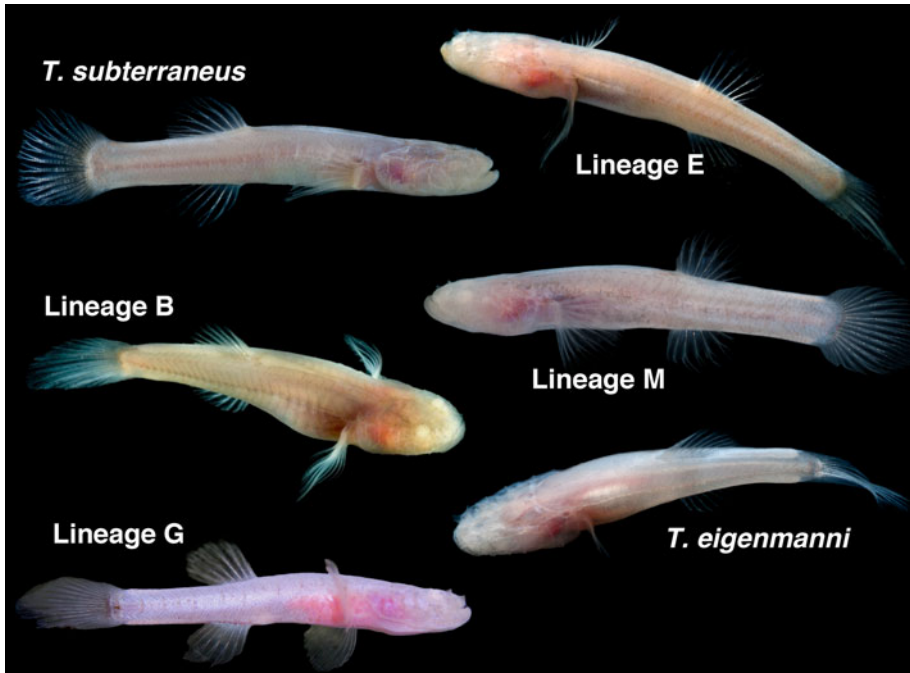


Fig. 1 At least ten lineages comprise the southern cavefish (*Typhlichthys subterraneus*) species complex

populations that are disjunct from the rest of the distribution of *T. subterraneus*, as this name was available as a subjective synonym (Parenti 2006).

Populations of *T. subterraneus* and *T. eigenmanni* face a number of threats, such as habitat degradation, groundwater pollution, hydrological manipulations, and over-collection (Proudlove 2006; Niemiller and Poulson 2010). The potential impacts of these threats vary with respect to magnitude and spatial distribution throughout the ranges of both nominal species; however, newly defined cryptic lineages likely differ in vulnerability to these threats. Given increasing anthropogenic threats impacting cavefish populations, an assessment of the conservation status of these cryptic lineages is critically needed. Accordingly, the objectives of the current study were to assess the status of each lineage identified in Niemiller et al. (2012) to identify priority lineages, populations, and habitats for immediate conservation efforts, and to identify gaps in knowledge of each lineage required for accurate conservation assessment. Specifically, we sought to: (1) determine the conservation status of each cryptic lineage; (2) identify lineages of greatest conservation concern; (3) ascertain threats and factors associated with extinction risk; and (4) make recommendations for official conservation status designations. Our goal is to prioritize these cryptic lineages for conservation action through IUCN Red List and NatureServe conservation status assessments, even before they are formally described, to expedite the development of conservation and management strategies. In addition, we discuss potential and existing threats facing cavefish and other groundwater organisms, including anthropogenic climate change.

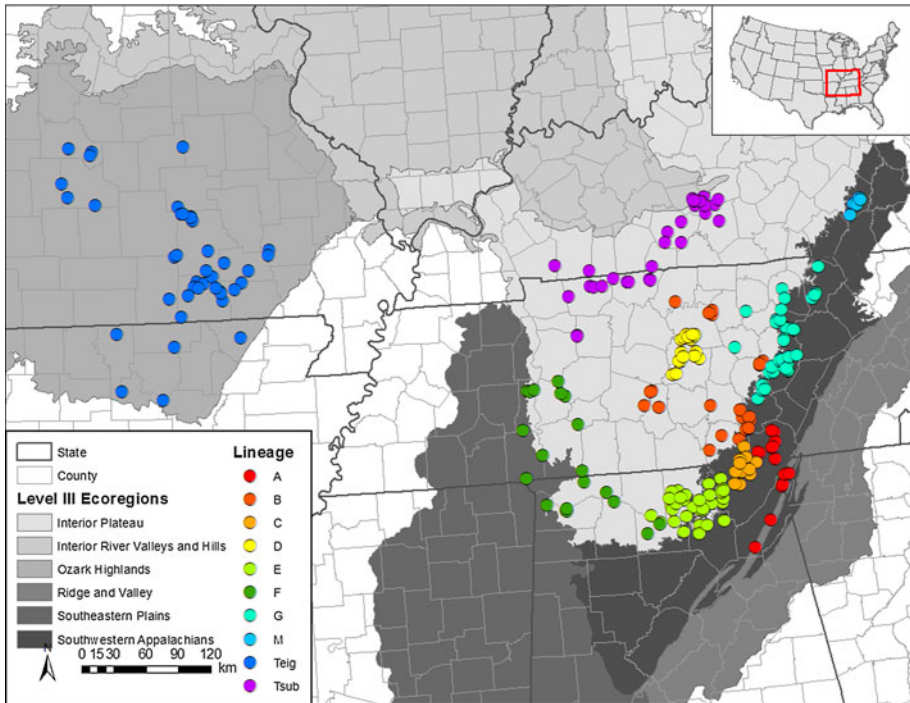


Fig. 2 The distribution of the southern cavefish species complex based on 240 documented occurrences includes six ecoregions across six states in the Interior Highlands of the United States. Cryptic lineages are color-coded. (Color figure online)

Methods

Sources of information

Much of the data on distribution and abundance of the *Typhlichthys* species complex were derived from direct fieldwork by the authors and their colleagues. We also compiled distributional and survey data from several other sources: (1) the Natural Heritage Program databases maintained by the Alabama Natural Heritage Program (Environmental Institute at Auburn University), Arkansas Natural Heritage Commission, Georgia Department of Natural Resources, Kentucky State Nature Preserves Commission, Missouri Department of Conservation, and Tennessee Department of Environment and Conservation; (2) primary and gray literature, including journal articles, technical reports, books, and caving organization newsletters and reports; and (3) accessions in museum collections. We also included incomplete survey counts, casual observations, and collection events in lieu of complete survey data when a qualified professional familiar with subterranean fishes made such observations. This included observations compiled by speleological societies (Kentucky Speleological Society, Missouri Speleological Survey, Tennessee Cave Survey, and Greater Cincinnati Grotto), state and federal wildlife biologists (Dr. Rick Toomey and Rick Olson), and cave biologists (Dr. Thomas Barr, Dr. John Cooper and Dr. Thomas Poulson).

Assignment of populations to lineages

Because only 63 of the 242 documented populations of *Typhlichthys* (Fig. 2) have been examined genetically to infer taxonomic affinities, we assigned unsampled populations to a specific lineage based on geographic proximity, hydrological basins, geological setting, and assemblages of other subterranean fauna to a genetically-sampled population. For example, a population was assigned to the same lineage as a genetically-sampled population if the cave system is located in close proximity (e.g., within 20 km) within the same hydrological subbasin or geological formation. For most populations this exercise was straightforward; however, a few populations could not be assigned with high confidence and are noted in Appendix A. The taxonomic affinities of some populations examined in north-central Alabama by Niemiller et al. (2012) are problematic, as the distributions of three lineages (B, E, and F) overlap in this region and there is evidence of a complex evolutionary history of isolation and secondary contact. While further study is warranted to better delineate and understand the barriers between lineages, we assigned all populations in this region to lineage E, which is the predominant lineage genetically sampled. The taxonomic assignment of some of these populations might change with more detailed genetic analyses, impacting in turn the conservation assessment of a given lineage.

NatureServe conservation assessment

NatureServe's system of assessing conservation status uses ten primary factors grouped into three main categories: rarity, trends, and threats (Master et al. 2009). Rarity factors include range extent, area of occupancy (AOO), number of occurrences, number of occurrences with good viability or ecological integrity, population size, and environmental specificity. Trend factors include both short-term and long-term trends in population size, extent of occurrence (EOO), AOO, number of occurrences, and viability or ecological integrity of occurrences. Finally, threat factors include threat impact and intrinsic vulnerability to threats. Other information is often used in addition to the ten conservation status factors to assess conservation status, including the number of protected or managed occurrences, rescue effect, and other considerations. NatureServe conservation global status assessments for each lineage were calculated using default points and weights with the NatureServe Rank Calculator worksheet available in Microsoft Excel (Faber-Langendoen et al. 2009). All *Typhlichthys* lineages are restricted to aquatic subterranean habitats within karst and cave-bearing rocks. Therefore, we considered all lineages to have a 'very narrow' environmental specificity.

Extent of occurrence and area of occupancy

Both NatureServe and IUCN Red List assessments use two different measurements of geographic range size: extent of occurrence (EOO; referred to as range extent by NatureServe) and area of occupancy (AOO). EOO is a geographic measure of the spatial spread of a species' range, whereas AOO is a measure of the area occupied by a species within its EOO. Both measures have been calculated in a variety of ways, which can result in dramatically different estimates (reviewed in Gaston and Fuller 2009). We calculated EOO and AOO using the web-based program GeoCAT (Bachman et al. 2011). EOO was calculated as a convex hull, which is the smallest polygon that contains all the sites of occurrence and no interval angles exceeding 180°. The exception was the EOO of nominal *T. subterraneus* that has a range in two disjunct areas (Interior Plateau and Ozark

Highlands; Niemiller and Poulson 2010). In this case, the total EOO was calculated as the sum of the polygons for each group of occurrences rather than the total EOO, because the area between these two regions would artificially increase the EOO. We followed NatureServe (Faber-Langendoen et al. 2009) and IUCN (2010) guidelines and used a grid size of 2 km (4 km²) to estimate AOO. Although we calculated (and present) AOO, we relied on EOO as a measure of geographic extent in conservation assessments due to the difficulty in accurately estimating potential AOO for organisms living in subterranean habitats and aquifers where biological surveys often cannot be reliably performed.

Number and viability of occurrences

We treated each observation of cavefish within a cave system as a single occurrence, with the exception of extensive cave systems (e.g., Mammoth Cave in Kentucky) that have distinct subterranean streams and encompass a large area (defined as >100 km²). An occurrence was deemed unviable if aquatic habitat showed evidence of degradation (e.g., gross evidence of groundwater pollution, sewage or septic system contamination, siltation from impoundments) and cavefish were not observed during the most recent survey. Information on the quality of habitat and existing threats were lacking for some occurrences. These occurrences were not included in calculations of the number of viable occurrences.

Population size

Accurate estimates of population size are rare for many species and are extremely difficult for subterranean fishes. Because of the inaccessibility of, and difficulty associated with surveying aquatic subterranean habitats, direct population censuses for amblyopsid cavefishes are limited to human-accessible portions of habitat. Estimates of population size by the few studies that have attempted mark-recapture methodologies are of low confidence, as cavefish have been documented to migrate to and from areas inaccessible to humans, violating the assumptions of many mark-recapture methods (Means and Johnson 1995; Pearson and Boston 1995; Brown and Johnson 2001). Consequently, we used a plausible range of values to estimate population size, using the average number of individuals observed for the most recent reliable survey as a proxy for population size. We multiplied these estimates by a factor of 1.8 as a low estimate of population size, based on data from the few mark-recapture studies that have been conducted on *Typhlichthys* and other amblyopsid cavefish populations (Poulson 1963; Pearson and Boston 1995; Niemiller and Poulson 2010), and by a factor of 15 as a high estimate. Populations for which cavefish have been documented but numbers of individuals have not been reported were assigned a value of 1. In addition, we assigned the number of individuals at Meramec Spring, Phelps Co., Missouri, to 5 based on the most recent dive survey in 1999 (Novinger, pers. comm.).

Trends

The degree of change in EOO, number of occurrences, and the percentage of occurrence with good viability was estimated over long-term and short-term time scales. Trends in population size over time were not used in conservation status assessment because of uncertainty in population size estimates and lack of historical data for the majority of occurrences. Long-term trends were considered from the year of first discovery of a lineage

to the present day, whereas short-term trends were considered over the past three generations or 45 years, assuming a generation time of 15 years (Niemiller and Poulson 2010).

Anthropogenic threats

The scope, severity, impact, and timing of specific threats that are either observed, inferred, or suspected to impact cavefish lineages were evaluated using the IUCN-Conservation Measures Partnership Classification of Threats (Salafsky et al. 2008) following the threat assessment process in Master et al. (2009). Scope, severity, and impact values employed followed Master et al. (2009).

Protected and managed occurrences

Although the number of protected or managed faunal occurrences is no longer used as a status factor in the NatureServe conservation assessment, this supplemental information is of interest in conservation assessment and management. We determined the ownership of each cavefish occurrence site (public versus private) and whether such properties were managed for conservation of groundwater and karst resources.

IUCN Red List assessment

To determine the appropriate Red List classification for *Typhlichthys* lineages, we compiled all available information with reference to each of the five criteria. A species may be classified as critically endangered (CR), endangered (EN), or vulnerable (VU) on the IUCN Red List if it meets specific conditions under any one of these five criteria (IUCN 2001): (A) past, present, or projected reduction in population size over three generations; (B) small geographic range in combination with fragmentation, population decline or fluctuations; (C) small population size in combination with decline or fluctuations; (D) very small population or very restricted distribution; and (E) a quantitative analysis of extinction risk. Species should be assessed against all criteria when possible to confirm that the highest possible threat classification is obtained (IUCN 2001). Criteria for threat classification under categories A, C, and E require evidence of declining trends in population size. Unfortunately, our estimates of historical trends in population sizes are low in confidence or lacking altogether for most cavefish lineages. Consequently, our assessments primarily focused on criteria under categories B and D.

Dealing with uncertainty

Uncertainty in values of assessment criteria is an important consideration when assessing conservation status, as how uncertainty is accounted for can strongly influence the assessment of extinction risk (Akcakaya et al. 2000; IUCN 2001; Gillespie et al. 2011). NatureServe accounts for uncertainty by allowing a range of ranks to show the degree of uncertainty in a conservation status when available information does not permit a single status rank (Master et al. 2009). The IUCN Red List assessment also deals with uncertainty by allowing a plausible range of values to be used to evaluate criteria (IUCN 2001, 2010; Mace et al. 2008). For both assessments, we adopted a moderate dispute tolerance considering the most likely plausible range of values for a criterion and excluding extreme or very unlikely values (Faber-Langendoen et al. 2009; IUCN 2010).

Results

Geographic extent

Compared to *T. subterraneus*, as previously understood which has an EOO of 106,668 km² and an AOO of 900 km², all ten cryptic lineages all had much smaller ranges (Table 1); however, there is substantial variation in the two measures of geographic extent among lineages. EOO averaged $6,806 \pm 9,221$ (mean \pm SD) km² and ranged from 30,737 km² for *T. eigenmanni* in the Ozark Highlands to as low as 38 km² for lineage M found in a small area of the Upper Cumberland River watershed in Pulaski Co., Kentucky. Two other lineages (C and D) had EOOs of less than 1,000 km². AOO was correlated with EOO ($r = 0.65$, $P = 0.04$). AOO averaged 90 ± 44 km² and ranged from 156 km² for *T. eigenmanni* to a low of 24 km² for lineage M.

Number and viability of occurrences

The *Typhlichthys* species complex includes at least 242 documented occurrence sites, whereas the number of occurrences averaged 24.2 ± 11.7 occurrences for cryptic lineages and ranged from 40 for *T. eigenmanni* to a low of 6 for lineage M (Table 1). All cryptic lineages except lineage M consisted of at least 10 occurrences. Of the 242 *Typhlichthys* occurrences with recent available data on quality of habitat, only eight (3.3 %) were deemed of poor viability at present. All cryptic lineages had >90 % of occurrences that were deemed viable except lineage A, which had 86.7 % of occurrences considered viable.

Population size

The estimated population size of the *Typhlichthys* species complex ranged 4,952–41,265. However, most cryptic lineages had small estimated population sizes (Table 1). In addition, surveys from most localities have yielded less than 5 individuals during any given survey (Fig. 3). Minimum population size estimates averaged 495 ± 402 individuals, with a minimum of 88 individuals for lineage M. Maximum population size estimates averaged $4,127 \pm 3,353$ individuals, with a maximum of 9,360 individuals for lineage G.

Trends

The EOO and AOO of the *Typhlichthys* species complex overall as well as individual cryptic lineages likely have not changed significantly since the species' original description in 1859. Number of occurrences has increased in the last 20 years, but this is undoubtedly an artifact of increased study and inventory efforts rather than reflecting an increase in geographic extent. Because of a lack of historical baseline data, inferences of trends in population size and quality of habitat are extremely limited, even in the short-term. A few populations have experienced documented declines over the last 40 years, such as Shelta Cave (lineage E) and Sloans Valley Cave (lineage M). In contrast, others have experienced recoveries after documented declines due to anthropogenic activities, such as Hidden River Cave (*T. subterraneus*). Quality of habitat is suspected to be declining due to several threats, particularly for lineages with distributions within agricultural regions (groundwater pollution) and near significant manmade impoundments (habitat degradation and alteration).

Table 1 Geographic information, NatureServe conservation criteria, and calculated rank for *Typhlichtylus subterraneus* sensu lato and ten cryptic lineages

	<i>Tsub</i> s.l.	A	B	C	D	E	F	G	<i>Tsub</i>	M	<i>Teig</i>
States	6	3 (AL, GA, TN)	1 (TN)	2 (AL, TN)	1 (TN)	1 (AL)	2 (AL, TN)	1 (TN)	2 (KY, TN)	1 (KY)	2 (AR, MO)
Counties	57	3	8	2	2	5	9	8	10	1	14
HUC8 watersheds	32	4	4	1	2	2	4	4	4	1	10
Level IV ecoregions	19	3	3	2	1	3	5	3	4	1	6
NatureServe criteria											
Rarity factors											
EOO (km ²)	106,668	1,863	9,923	382	524	2,318	9,312	4,875	8,086	38	3,0737
AOO (km ²)	900	56	84	44	68	132	76	128	128	24	156
Occurrences	242	15	27	11	17	34	22	37	33	6	40
Protected occurrences (%)	81 (33.5)	4 (26.7)	3 (11.1)	3 (27.2)	8 (47.1)	7 (20.6)	3 (13.6)	10 (27.0)	16 (48.5)	2 (33.3)	25 (62.5)
Population size	4,952–41,265	119–990	608–5,070	121–1,005	275–2,295	1,075–8,955	157–1,305	1,123–9,360	581–4,845	88–735	805–6,705
Threat factors											
Threat impact	M	M	HM	ML	H	H	H	HM	H	VHH	M
Calculated rank	G4	G3	G3G4	G2G3	G3	G3	G3	G3G4	G3	G3	G4

Threat impact categories are low (L), medium (M), high (H), and very high (VH)

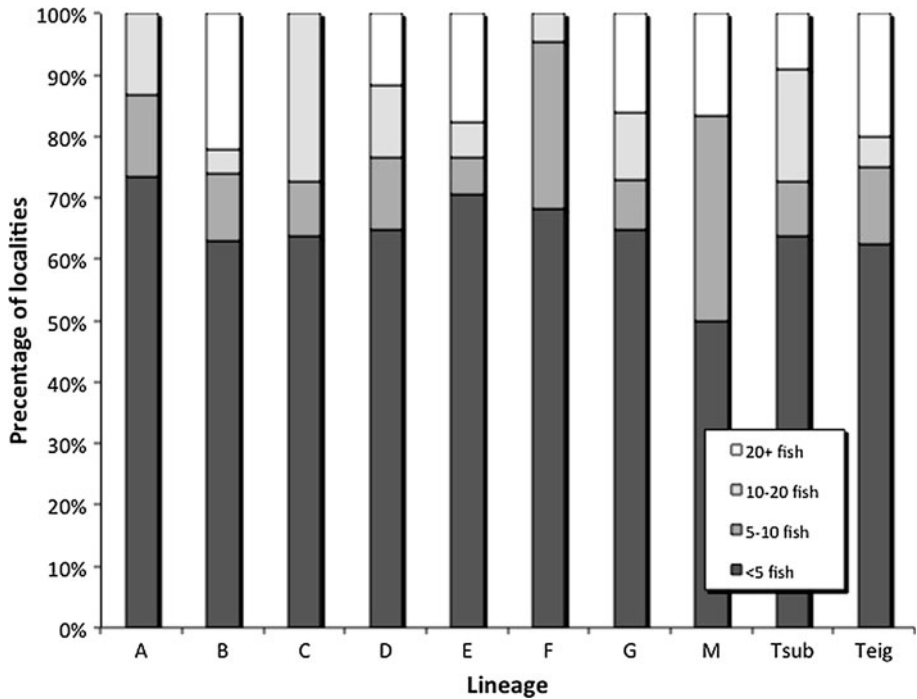


Fig. 3 Percentage of localities for each lineage in which the maximum abundance of southern cavefish during a single survey is <5 individuals, 5–10 individuals, 11–20 individuals, and >20 individuals. More than five individuals have been observed at only 34.7 % ($n = 84$) of all *Typhlichthys* localities

Threats

We identified ten threats documented or suspected to be affecting populations (and subsequently lineages) of *Typhlichthys* at the present or in the future (Table 2). However, these threats vary in overall impact among cryptic lineages and even among populations within lineages. Some threats, such as groundwater pollution, are large in scope and affect at least some populations of all cryptic lineages, whereas other threats are limited to just a few lineages or even populations, such as the commercial development of caves. Threats also vary in severity over geographical regions, and consequently among populations and cryptic lineages. The most significant threats include pollution of groundwater habitats from agricultural and forestry effluents, household sewage and urban wastewater, catastrophic chemical spills along roadways and railroads, as well as habitat degradation and alteration due to the construction of dams. Scientific and amateur collections as well as recreational caving also impact all lineages. At least 551 individual cavefish have been collected and accessioned into university and museum collections (Table 3), based on a query of 52 collections on FishNet2 (<http://www.fishnet2.net>) and additional regional collections, including at least 111 cavefish from Shelta Cave, Madison Co., Alabama.

Protected and managed sites

Of the 242 documented occurrences of *Typhlichthys* lineages, the main entrances of 81 (33.5 %) of these occur on public lands or are managed for their cave and karst resources (Online

Table 2 Threats facing southern cavefish lineages following classification proposed by Salafsky et al. (2008)

Threat	<i>Tsub</i> <i>s.l.</i>	A	B	C	D	E	F	G	<i>Tsub</i>	M	<i>Teig</i>
Residential and commercial development											
Tourism and recreation areas	L		L						L		L
Energy production and mining											
Oil and gas drilling	L								ML		
Mining and quarrying	L		L		L			L	L	L	L
Biological resource use											
Hunting and collecting animals	L	ML	L	L	L	M	L	L	ML	L	L
Human intrusions and disturbance											
Recreational activities	L	L	L	L	L	L	L	L	L	L	L
Natural systems modifications											
Dams and water management/use	M	M	M	L	M	HM	HM	M	M	H	M
Pollution											
Household sewage and urban wastewater	ML	L	L	L	ML	ML	ML	L	ML	ML	ML
Industrial and military effluents	ML	L	L	L	ML	ML	ML	L	ML	L	ML
Agricultural and forestry effluents	M	M	HM	M	HM	HM	HM	HM	HM	M	M
Climate change											
Habitat shifting and alteration	HM	HM	HM	HM	HM	HM	HM	HM	HM	HM	HM

Threat impacts are low (L), medium (M), high (H), and very high (VH)

Appendix A). The percentage of protected and managed occurrences (Table 1) varied among cryptic lineages from a low of 11.1 % for lineage B to a high of 62.5 % for *T. eigenmanni*, where several occurrences occur within the boundaries of the Mark Twain National Forest (U.S. Forest Service) and the Ozark National Scenic Riverways (U.S. National Park Service).

NatureServe ranking

We assigned a NatureServe conservation global status rank of “Apparently Secure” (G4) to *T. subterraneus* sensu lato, in agreement with previous rank assessments. Seven of the ten cryptic lineages are considered threatened with a ranking of “Vulnerable” or higher (G1–G3), with lineage M at the highest risk of extinction (ranking of ‘Critically Imperiled/Imperiled’ [G1G2]). *T. eigenmanni* is the least threatened (G4), while other lineages B and G are marginally at risk of extinction (G3G4). Because of uncertainty in estimations of population size and severity and scope of threats, the status rank of two cryptic lineages spans two conservation status categories (lineages B and G; Table 1).

IUCN Red List classification

We classified *T. subterraneus* sensu lato as “Near Threatened,” as a Red List category could not be met for any one criterion. A “Vulnerable” classification under Criterion D2 is

Table 3 Accessions of *Typhlichthys* in university and museum collections

Lineage	No. collected	Localities	Collections	Top localities (no. collected)
A	16	5	AUM, GMNH, UAIC	Sells Cave (12)
B	69	11	AUM, CMN, FMNH, UAIC, UF, UL, USNM	Crystal Cave (27), Big Mouth Cave (8), Blowing Springs Cave (7)
C	16	5	AUM, YPM	Salt River Cave (6), Garner Spring Cave (6)
D	14	5	AUM, MCZ, UMMZ	Herring Cave (3), Pattons Cave (3)
E	159	10	AUM, CMN, INHS, KU, MMNS, TU, UAIC, UMMZ, USNM, YPM	Shelta Cave (111), Muddy Cave (19), Hering Cave (14)
F	23	9	AUM, MCZ, TU, UAIC, UMMZ, USNM	Well near Hines (5), McKinney Pit (4)
G	34	10	AUM, INHS, UMMZ, USNM, UTIC, YPM	Blind Fish Cave (12), Anderson Spring Cave (5), Bartlett Cave (4)
M	12	3	AUM, YPM	Sloans Valley Cave (6), Drowned Rat Cave (5)
<i>Tsub</i>	128	17	ANSP, AUM, CAS, CMN, FMNH, KU, MCZ, MOSU, SIUC, TU, UAIC, UF, UL, UMMZ, USNM, UTIC, YPM	Mammoth Cave (49), Hidden River Cave (29), L & N Railroad Cave (15)
<i>Teig</i>	80	23	ASUMZ, AUM, CU, MDC, OKMNH, SIUC, UAFC, UF, UMMZ, USNM	River Cave (20), Meramec Spring (12), Ozark Fisheries Spring (9)

Collections—*ANSP* Academy of Natural Sciences, *ASUMZ* Arkansas State University Museum of Zoology, *AUM* Auburn University Museum, *CAS* California Academy of Sciences, *CMN* Canadian Museum of Nature, *CU* Cornell University Museum of Vertebrates, *FMNH* Field Museum of Natural History, *UF* Florida Museum of Natural History, *GMNH* Georgia Museum of Natural History, *MCZ* Harvard Museum of Comparative Zoology, *INHS* Illinois Natural History Survey, *MMNS* Mississippi Museum of Natural Science, *MDC* Missouri Department of Conservation, *USNM* Smithsonian Institution National Museum of Natural History, *MOSU* Morehead State University, *OKMNH* Sam Noble Oklahoma Museum of Natural History, *SIUC* Southern Illinois University-Carbondale, *TU* Tulane University Museum of Natural History, *UAIC* University of Alabama Ichthyological Collection, *UAFC* University of Arkansas, *KU* University of Kansas Biodiversity Institute, *UL* University of Louisville, *UMMZ* University of Michigan Museum of Zoology, *UTIC* University of Tennessee Ichthyological Collection, *YPM* Yale Peabody Museum

not warranted. No evidence suggests that a future threat affecting the species range-wide may drive the taxon to a “Critically Endangered” status or to extinction in a short period of time, although a number of threats that differ in scope and severity affect populations across the range. Despite a lack of data to confidently estimate population sizes and infer trends over time, there is sufficient information for the geographic range of *Typhlichthys* lineages to infer a probable threat classification. Nine of the ten cryptic lineages can be classified as threatened under Criterion B1ab(iii): four lineages as “Vulnerable,” four lineages as “Endangered,” and one lineage as “Critically Endangered” (Table 4). Molecular evidence suggests that populations in these cryptic lineages are sufficiently isolated with little gene flow among populations (Niemiller et al. 2012) to be classified under Criterion B1a. These lineages also can be classified under Criterion B1b(iii) because of a continuing decline in the quality of habitat due to a number of threats. The only

Table 4 IUCN Red List criteria for southern cavefish lineages

IUCN Red List criteria	<i>Tsub s.l.</i>	A	B	C	D	E	F	G	<i>Tsub</i>	M	<i>Teig</i>
A. Population reduction	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD
B. Geographic range											
B1. EOO		VU	VU	EN	EN	EN	VU	EN	VU	CR	
B2. AOO	VU	EN	EN	EN	EN	EN	EN	EN	EN	EN	EN
(a) Severely fragmented	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	N
(b) No. of locations										VU	
(c) Continuing decline	Y(iii)	Y(iii)	Y(iii)	Y(iii)	Y(iii)	Y(iii)	Y(iii)	Y(iii)	Y(iii)	Y(iii)	Y(iii)
(d) Extreme fluctuations	N	N	N	N	N	N	N	N	N	N	N
C. Small population size and decline	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD
D. Very small or restricted population											
No. of mature individuals			DD	DD	DD	DD	DD	DD	DD	VU ^a	
E. Quantitative analysis	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD	DD
Red List category	NT	VU	VU	EN	EN	EN	VU	EN	VU	CR	NT
Applicable criteria		B1ab(iii)	B1ab(iii)	B1ab(iii)	B1ab(iii)	B1ab(iii)	B1ab(iii)	B1ab(iii)	B1ab(iii)	B1ab(iii)	B1ab(iii)

Classification ranks include critically endangered (CR), endangered (EN), vulnerable (VU), near threatened (NT), and data deficient (DD)

^a Can also be classified as vulnerable under VU D2

lineage not considered threatened is *T. eigenmanni*, which is classified as “Near Threatened.”

Discussion

Cryptic lineages are at greater risk of extinction

Few studies have conducted conservation assessment of undescribed genetic lineages. Compared to *T. subterraneus* sensu lato, cryptic lineages are at greater risk of extinction based on both IUCN Red List and NatureServe criteria. *Typhlichthys subterraneus* was last assessed under IUCN Red List criteria as “Vulnerable” (World Conservation Monitoring Centre 1996); however, we recommend a classification “Near Threatened” for the entire species complex. Our NatureServe assessment assigned the same “Apparently Secure” (G4) status rank as before (NatureServe 2013). Nine of the 10 cryptic lineages are considered threatened under IUCN Red List criteria with a ranking of “Vulnerable” or higher and seven lineages under NatureServe criteria with a ranking of “Vulnerable” or higher (G1–G3). Threatened status is primarily because of limited geographic range, severe fragmentation of populations, and decline in the extent and quality of habitat. *Typhlichthys* has a large geographical range with an EOO over 100,000 km², but only one cryptic lineage has an EOO >10,000 km² (*T. eigenmanni*) and three lineages have an EOO <1,000 km² (Table 1). However, morphological and molecular evidence suggest that even *T. eigenmanni* may be comprised of multiple ESUs (Niemiller et al. unpublished data). In most instances, lineages are endemic to a single or just a few hydrological subbasins (Table 1; Online Appendix A) making them more vulnerable to extinction from habitat degradation or contamination.

By implication, cryptic lineages have smaller ranges and populations sizes compared to the more widespread nominal species, which leads to significant conservation concerns. Small range sizes are strongly associated with increased extinction risk (Waldron 2010; Bernardo 2011), as it is more likely that all populations of a species with a small geographic range will simultaneously be at risk from a threat. Likewise, species with smaller ranges are at higher risk of extinction due to demographic and environmental stochasticity (e.g., Allee effect; reviewed in Allendorf and Luikart 2007). Like many cave organisms, their risk of extinction, particularly due to environmental change, is exacerbated due to limited dispersal ability. It has often been assumed that aquatic cave organisms have larger ranges than terrestrial cave organisms because of their presumed greater dispersal ability and habitat connectivity (Barr and Holsinger 1985; Culver et al. 2000; Lamoreux 2004). However, several molecular studies have demonstrated that geographical ranges of groundwater organisms also may be small and are comprised of several significantly fragmented sets of populations (e.g., Trontelj et al. 2009; Niemiller and Fitzpatrick 2008; Niemiller et al. 2012, 2013b).

Threats to cavefish populations

Synergistic factors threaten several *Typhlichthys* lineages. The most common threats to *Typhlichthys* lineages are pollution of groundwater habitats, hydromodifications (which includes loss or alteration of habitat from manmade impoundments and groundwater withdrawal), scientific and amateur collection, and habitat disturbance associated with recreational caving activities (Table 2). In particular, karst groundwater and aquifers are

fragile habitats that are extremely susceptible to contamination, as they are often characterized by low potential for auto-depuration and high probability of retention of contaminants (White 1988; Ford and Williams 2007). However, because of varying hydrological processes and local geological settings, the impacts of pollution are difficult to predict and to remediate (Palmer 2000). Cavefish and other aquatic subterranean life are threatened by several sources of pollution, including wastewater systems, sewage, urban runoff, agricultural runoff, industrial wastewater and contamination, catastrophic chemical spills, and solid waste disposal in sinkholes (Elliott 2000; Graening et al. 2010; Niemiller and Poulson 2010).

Groundwater pollution can be chronic, occurring over several decades. For example, groundwater contamination from sewage, creamery wastes, and wastewater from a chrome-plating factory over 40+ years led to the extirpation of *T. subterraneus* and other cave life from Hidden River Cave, Barren Co., Kentucky (Elliott 2000). However, the aquatic cave ecosystem, including cavefish, recovered by 1995 after new sewage treatment facilities were constructed in 1989 (Lewis 1996). Recovery was likely facilitated by recolonization of the cave stream from upstream tributaries minimally affected by pollution. At Shelta Cave in Huntsville, Alabama (lineage E), groundwater pollution associated with land development near the cave system is thought to have played a role in the decline of cavefish and several other aquatic species (Elliott 2000), including the possible extirpation of an endangered cave shrimp (Cooper and Cooper 2011). Both the suspected illegal injection of sulfur-rich brine wastes into leaky well casings associated with exploratory drilling for petroleum as well as natural brine and sulfur deposits have been proposed as a cause of the decline of *Typhlichthys* at Parker Cave, Barren Co., Kentucky (Pearson and Boston 1995).

Groundwater contamination can also be acute, on the order of hours or days, and dramatically impact biota (e.g., Ryan et al. 2013). Perhaps the best example of such an event is the liquid fertilizer pipeline break in November 1981 that spilled an estimated 80 000 L of liquid fertilizer into the recharge zone of Meramec Spring, Phelps Co., Missouri. Within 7 days, the spill traveled 21 km from the point source to the spring, causing a catastrophic drop in dissolved oxygen and killing thousands of aquatic cave organisms, including >1,000 *T. eigenmanni*, which were previously unknown in the drainage (Vandike 1982). Water quality returned to normal levels within 38 days; however, the long-term effects of the spill are unknown, as follow-up studies are lacking. While this event points to the need for baseline data and ongoing hydrological and biological monitoring, it also highlights our limited knowledge of the distribution and demography of groundwater fauna.

The construction of dams and impoundments has been implicated in the decline and extirpation of populations of cave biota, including cavefishes (Elliott 2000; Kuhajda and Mayden 2001; Proudlove 2006; Graening et al. 2010; Niemiller and Poulson 2010). Several large impoundments exist within the distributions of *Typhlichthys* lineages. Associated flooding and rise of the local water table can result in habitat loss and alteration of flow patterns leading to reduction of organic input driving subterranean ecosystems and increased siltation that cover important microhabitats and breeding sites (Niemiller and Poulson 2010). Although impoundments might be associated with documented declines of some cavefish populations (Elliott 2000), direct evidence is lacking. Several cave systems inhabited by the “Critically Endangered” lineage M are partially inundated by Lake Cumberland in Pulaski Co., Kentucky, yet cavefish persist in these cave systems. This suggests that environmental impacts associated with impoundments might not be as

significant for some aquatic cave species, but long-term studies are needed to ascertain more subtle impacts that may be detrimental to local populations.

Because of their uniqueness, *Typhlichthys* have been exploited for cave tourism and the aquarium trade. Likewise, over-collection for scientific studies is a concern (Elliott 2000; Niemiller and Poulson 2010). Over 550 specimens have been accessioned into museum collections, with the majority collected from three lineages: lineage E, *T. subterraneus*, and *T. eigenmanni* (Table 3). In particular, 52 % of cavefish accessioned into ichthyological collections are from just eight localities, including at least 111 individuals from Shelta Cave in Madison Co., Alabama (lineage E). Such collection pressure likely has contributed to the decline of this population and perhaps others. The extent of amateur collection is impossible to quantify, but cavefish were commonly sold as souvenirs during the boom in cave tourism at the end of the 19th century. The decline in *T. subterraneus* and *Amblyopsis spelaea* populations in Mammoth Cave (Edmonson Co., Kentucky) was attributed to such collection and sale (Eigenmann 1909; Bailey et al. 1933; Pearson and Boston 1995). Amblyopsids were also prized by aquarium enthusiasts in the twentieth century until the captive-bred Mexican cave characins [*Astyanax jordani* (Hubbs and Innes 1936)] flooded the market for such novelties. While over-collection can reduce or possibly extirpate local populations, collection of cavefish for scientific or commercial purposes probably is not a major threat for most lineages. Exploitation is hindered by the difficulty in collecting significant numbers of cavefish, as most populations represent sinks rather than sources given the low number of individuals observed (Fig. 3). In addition, with the exception of Shelta Cave, no evidence suggests that cavefish abundance has significantly decreased at most localities where significant scientific collections have occurred. However, scientific and amateur collection has severely impacted populations of a related cavefish species, the Ozark Cavefish (*Troglichthys rosae*), and this is the primary reason that USFWS listed this species as federally threatened (reviewed in Graening et al. 2010).

Although data are lacking, habitat disturbance caused by recreational cavers may pose a threat, as the activities of even the most cautious caver could have significant impacts on cavefish populations that reside in shallow streams. Increased cave visitation may alter breeding activities, disturb prey populations, stress individual fish by increasing their activity, or even result in death by trampling (Graening et al. 2010; Niemiller and Poulson 2010). However, potential impacts from recreational caving are likely minimal for cavefish populations that occur in deeper water (>2 m), as these habitats are infrequently visited by humans.

Potential effects of climate change

Climate change is expected to have significant impacts on the quality, levels, and sustainability of groundwater through alteration of the hydrological cycle (Dragoni and Sukhija 2008; Treidel et al. 2012). While research programs have begun to reveal how groundwater might respond to climate change in the past 10 years (Treidel et al. 2012), knowledge of how groundwater organisms will be affected by climate change is still in its infancy. Species can shift their ranges, adapt, or go extinct in response to climate change (Brook et al. 2008). Because of their unique habitat requirements and endemism, most cave organisms, *Typhlichthys* lineages included, may be quite vulnerable to the impacts of climate change because of their limited dispersal ability. Potential effects of climate change may directly alter abiotic conditions, such as water temperature, dissolved oxygen content, and rates of groundwater recharge, and exacerbate already existing threats, including groundwater pollution and extraction. In addition, climate change may result in

increased frequency of inhabitation and abundance of non-obligate species sensitive to changing surface climatic conditions. Such a scenario would not be unprecedented, as past climatic change has been hypothesized to be the driving force behind subterranean colonization and evolution of several temperate North American troglobitic species (Holsinger 2000; Niemiller et al. 2008). These species may prey on or outcompete resident cavefish populations. Novel pathogens or parasites also may be introduced from other species that shift their ranges and niches into caves in response to climate change.

Lineages at risk of extinction

Our conservation assessment indicates that nine of the ten cryptic lineages of *Typhlichthys* are at an elevated risk of extinction under IUCN Red List or NatureServe criteria (Table 4), and we found one lineage (M) that should be ranked as “Critically Endangered” under IUCN Red List and “Critically Imperiled/Imperiled” (G1G2) under NatureServe criteria. This lineage is of extremely limited geographic range, occurring in a single county (Pulaski Co., Kentucky) with an EOO of 38 km², an AOO of 24 km² and only six documented occurrence sites—two of which are ostensibly protected by land management practices. Furthermore, we found that this lineage is threatened by significant hydromodification (dams) and contaminated water discharge (Table 2). Most surveys of caves harboring this lineage have found fewer than five individuals (Online Appendix A). To prevent the imminent extinction of this lineage, conservation measures should be immediately employed.

Recommendations

Given the results of our conservation assessments and available data on threats to cavefishes, we offer several recommendations for further study and management. First, hydrological studies are needed to delineate groundwater recharge zones and flow patterns of subterranean streams containing cavefish populations, particularly for those lineages most threatened. While recharge basins have been delineated for several well-known systems [e.g., Mammoth Cave region (Quinlan and Ewers 1981; Quinlan 1982; Quinlan and Ray 1989) and Key Cave (Aley 1990)], the vast majority of localities are in need of study. With accurate data of hydrological boundaries in hand, vulnerability mapping can be conducted to better ascertain the risk of groundwater pollution and other threats to individual populations and aid in land management decisions (Aley et al. 2008). While the entrances to some cave systems are gated or otherwise controlled, affording some protection to cavefish populations from threats such as over-collection and habitat disturbance caused by recreational caving, the recharge zones of most cave systems are not. Second, we recommend more mark-recapture studies to better estimate population sizes rather than relying on relative abundance data. While many occurrence sites represent small windows into available cavefish habitat, several cave systems are amenable to such studies. Third, we recommend more empirical studies examining the effects of potential threats that we have identified on cavefishes and other cave life. Several threats are thought to negatively impact cavefish, yet few studies have been conducted that actually examine their potential effects. For example, several sources of groundwater pollution have been proposed as threats, but few studies have examined their impacts on cavefish populations. Likewise, anecdotal evidence of population declines exists for some threats, such as over-collection and recreational caving, but we are unaware of any studies that have examined these possible stressors empirically. Fourth, research is greatly needed to investigate the

potential direct and indirect effects of climate change not only on cavefishes but other cave- and groundwater-limited species. What are the impacts of climate change and human demand for groundwater resources at spatial scales relevant to most cave taxa (e.g., recharge zones of cave systems and geographical extent of local aquifers) and how might troglobitic species respond? To our knowledge, no studies have modeled shifts in the distributions of troglobites in response to climate change. Finally, before any spatial and temporal changes caused by anthropogenic stressors can be accurately documented, baseline data on local cavefish populations and environmental conditions must be established, particularly for lineages most at risk of extinction.

Conclusions

The conservation assessment of recently defined cryptic lineages is important in developing effective conservation and management strategies, and in particular for prioritizing taxa in need of immediate conservation action. Our conservation assessment showed that most undescribed cryptic lineages of *Typhlichthys* are at risk of extinction, including one lineage classified as “Critically Endangered.” We identified ten threats impacting *Typhlichthys* lineages that vary in scope and severity. Although several lineages are inherently threatened because of their limited ranges and isolation of populations, more empirical data are needed on the effects of presumed threats to cavefish lineages, including future impacts of climate change. In particular, research and conservation efforts should focus on lineages deemed most at risk of extinction. Given increasing threats facing cavefish populations, we hope that our study will not only stimulate future research in cavefish conservation, but that our threat assessments and recommendations will be used by stakeholders to prioritize effective and appropriate management initiatives aiding in the conservation of these lineages even before they are officially recognized. Furthermore, protection of these top predators should also encapsulate protection of other endemic and threatened cave fauna found in subterranean ecosystems via the umbrella, or flagship, species effect (reviewed by Barua 2011).

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