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Nontroglobitic fishes in Bruffey-Hills Creek Cave, West Virginia, and other caves worldwide

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Synopsis

Six species of fishes were tagged and released in September and November 1995 and on five dates between November 1998 and October 1999 inside Bruffey-Hills Creek Cave. Most of the tagged fishes were creek chub, *Semotilus atromaculatus*, and green sunfish, *Lepomis cyanellus*. The overall recapture rate was 2.6% as only three of 117 fishes were recaptured. Forty-nine days was the longest time of residence by two *L. cyanellus* and one *S. atromaculatus*, and movement of 83.4 m was observed only for the creek chub. Tag loss was confirmed as one factor in the low recapture rate. Nine species of fishes were collected in the cave, including *Phoxinus oreas* and *Pimephales promelas*, two species never collected in a cave. Most of the fishes were pigmented normally, but many creek chubs were depigmented and appeared white or pigmentless when first observed in the cave stream. Four species, *Phoxinus oreas*, *Pimephales promelas*, *Ameiurus nebulosus*, and *Lepomis macrochirus*, were collected for the first time in Bruffey-Hills Creek Cave and in the Bruffey and Hills creeks drainage. Although data from this study did not shed light on residence time of fishes in the cave, the consistent occurrence of epigean fishes in this cave was shown. A list of epigean fishes from caves worldwide was included along ,vith a discussion of aspects of the ecology of epigean fishes in caves and of evolution of troglobitic fishes.

Introduction

Epigean fishes inhabiting caves have been given little attention in the past by speleologists and cave biologists (Proudlove 1979, 1982, Chapman 1993). Poly & Boucher (1996) reviewed the occurrence of epigean fishes in caves and discussed the relevance of these fishes to cave ecosystems and to understanding evolution of troglobitic organisms. Information on the ecology of nontroglobitic fishes in caves remains scarce, and the present study was undertaken to help fill this void. Tagging and periodic monitoring of fishes would provide useful biological information on epigean fishes in caves. Tagging and recapturing of individual organisms in caves has not been attempted often and most previous studies have dealt with troglobitic organisms. Cooper (1975) used ink to mark three species of troglobitic crayfish in Shelta Cave, Alabama, then made periodic observations and noted movements of individual crayfish within the cave (Cooper & Cooper[1]). Hobbs (1978) performed a mark-recapture study of the crayfish, *Orconectes inermis inermis*, in Pless Cave, Indiana. Means & Johnson (1995) marked Ozark cavefish, *Amblyopsis rosae*, with alphanumeric tags to estimate population size and to document movement of fishes within a cave, and Trajano (1991, 1997) performed similar studies on *Pimelodella kronei*,

[1] Cooper, J.E. & M.R. Cooper. 1971. Studies of the aquatic ecology of Shelta Cave, Huntsville, Alabama. ASB Bull. 18: 30 (abstract). Cooper, J.E. & M.R. Cooper. 1976a. Marking crayfish for long-term ecological studies. ASB Bull. 23: 52 (abstract). Cooper, M.R. & J.E. Cooper. 1976b. Growth and longevity in cave crayfishes. ASB Bull. 23: 52 (abstract).

P. transitoria (an epigean species), and *Trichomycterus itacarambiensis* in Brazil. Bruffey-Hills Creek Cave was chosen as a study site because several species of fishes occur in the cave in fair abundance. Information is available on the vertebrate fauna of Bruffey-Hills Creek Cave (Garton et al. 1993, Poly & Boucher 1996), but the invertebrate fauna has not been examined (Holsinger et al. 1976). The goals of the study were to gather information on: (1) residence time of individual fishes in the cave, (2) movement of individuals throughout the cave, (3) growth of individuals living in the cave environment, (4) reproduction within the cave, and (5) temporal changes in pigmentation of individual fishes.

That a relationship exists between pigmentation and length of residence in a cave is suggested by the study of Rasquin (1947) and reports of depigmented fishes in caves (Hora 1924, Kosswig 1937, Proudlove 1979). Some fishes that were depigmented when captured in a cave were able to regain some pigment after several months of exposure to light (presumably a normal photoperiod, Banister 1984), whereas a 'white' fish that appeared depigmented regained a more normal coloration in 24 h (Proudlove 1979). Also, fishes held in aquaria often exhibit temporary pigment changes, appearing pallid after exposure to darkness (overnight), then appearing normal following exposure to light (personal observation). Such a temporary state of depigmentation was observed in some epigean fishes in a cave (Poly & Boucher 1996).

Methods

Fish surveys were conducted on 22 October 1994, 30 September 1995, 18 November 1995, 14 November 1998, 6 February 1999, 27 March 1999, 27 July 1999, 2 October 1999, and 29 December 1999 in Bruffey-Hills Creek Cave, Pocahontas County, West Virginia. Fishes were tagged beginning 30 September 1995 and ending 2 October 1999. The cave is approximately 4 km north of Droop, WV, and approximately 2 km southeast of Lobelia, WV, Little Levels Township, 38°07′21″ N, 80°16′42″ W. The spring indicated on the Droop WV USGS 7.5′ quadrangle map is actually the inflow and cave entrance; Bruffey-Hills Creek Cave contains 2156 m of surveyed passages (Storrick 1992). Fishes were collected with a battery-powered backpack electrofishing unit, except on 22 October 1994 when seines and dipnets were used. Distance sampled and distance of capture or observation were measured to 0.1 m with a hip chain. Distance sampled (m) on each date was (in chronological order): 479.0, 264.4, 305.2, 132.6, 136.0, 138.0, 129.2, 127.6, and 123.4 m. Because of an impassable logjam, less than 140 m of the cave could be sampled between November 1998 and December 1999. Each tagged fish was weighed to the nearest 0.1 g on an Acculab Pocket Pro 250-B portable electronic balance (a spring scale was used during 1995 – weights recorded to the nearest 1 g), then measured to the nearest 1 mm standard length (SL) and total length (TL) with dividers and a stainless steel ruler. Initial pigmentation when captured (pallid or 'normal') as well as any changes from pallid (light) to 'normal' pigmentation after exposure to light for several minutes were noted. Then fishes were tagged with visible implant tags utilizing a syringe style injector and precut, alphanumeric tags (Northwest Marine Technology, Inc.). Tags were inserted on the top of the head of cyprinids, on the lower jaw of centrarchids, and on the adipose eyelid of salmonids. Voucher specimens were preserved in 10% formalin and deposited in the Ohio State University Museum of Biological Diversity Fish Collection (OSUM), Southern Illinois University at Carbondale Fluid Vertebrate Collection (SIUC), and University of Tennessee, Knoxville (UT). Fish nomenclature follows Robins et al. (1991) and Eschmeyer (1998).

Following completion of the field work, 55 creek chubs obtained from Big and Little creeks, Union County, Illinois (16 January 2000), were tagged on 17 January 2000 and checked weekly for the presence of tags. Creek chubs were held in circular fiberglass tanks in a greenhouse and fed every other day with commercial pellets; small minnows were kept with the large creek chubs as additional forage. Smaller fishes (n = 50, SL = 41–82 mm, n = 48 measured) were kept in a separate tank from the larger chubs (n = 5, SL = 114–134 mm, n = 4 measured) to reduce loss of fishes through predation. The temperature varied from 1.5 to 19.7°C between 17 January and 5 March.

Results

One hundred and ninety-six individuals of nine fish species were collected between 1994 and 1999, and most of the fishes were creek chub and green sunfish (Table 1). One hundred and seventeen individuals of six species were tagged and released inside the cave: 90 *Semotilus atromaculatus*, four *Rhinichthys atratulus*, 20 *Lepomis cyanellus*, one *Lepomis macrochirus*, one *Ambloplites rupestris*, and one *Oncorhynchus mykiss*.

Table 1. Fish species, number collected, and distance of capture in Bruffey-Hills Creek Cave, West Virginia.

Date	Species (size range, catalog number)	Number	Distance in cave[1]
22 Oct 1994	*Semotilus atromaculatus* (26–146 mm, $n = 11$, OSUM 77874)	46[2]	4.8-476.0
	Lepomis cyanellus (61 & 69 mm, OSUM 77875)	4[2]	45.8–476.0
30 Sep 1995	*Semotilus atromaculatus* (39–167 mm, $n = 18$)	19	2.7–264.4
	Lepomis cyanellus (54–115 mm, $n = 14$)	14	2.7–198.5
	Ambloplites rupestris (68 & 104.6 mm, SIUC 24889)	2	2.7–187.6
	Rhinichthys atratulus (73 mm)	1	35.8
	Oncorhynchus mykiss (275 mm)	1	35.8
18 Nov 1995	*Semotilus atromaculatus* (53–159 mm, $n = 7$)	7	2.5–305.2
	Lepomis cyanellus (63–110 mm, $n = 6$)	6	34.7–84.8
14 Nov 1998	*Semotilus atromaculatus* (45–185 mm, $n = 27$)	27	2.9–132.6
	Lepomis cyanellus (141 mm)	1	110.5
	Lepomis macrochirus (85 mm)	1	38.8
	Rhinichthys atratulus (40 & 42 mm)	2	14.8
	Phoxinus oreas (42–53 mm, $n = 3$, SIUC 33140)	3	2.9
6 Feb 1999	*Semotilus atromaculatus* (43–95 mm, $n = 3$)	3	23.2–29.0
	Rhinichthys atratulus (51 mm)	1	50.0
	Phoxinus oreas (53 mm, UT 44.8611)	1	23.2
27 Mar 1999	*Semotilus atromaculatus* (32–49 mm, $n = 6$)	6	19.5–37.0
27 Jul 1999	*Semotilus atromaculatus* (38–150 mm, $n = 18$)	18	2.6–100.4
	Phoxinus oreas (34 & 58 mm, UT 44.8611)	2	2.9–29.4
	Ameiurus nebulosus (210 mm, UT 48.1006)	1	2.6
	Ambloplites rupestris (110 mm, UT 90.2636)	1	2.6
2 Oct 1999	*Semotilus atromaculatus* (44–96 mm, $n = 12$)	12[3]	2.4–91.7
	Lepomis cyanellus (25 & 75 mm)	2	2.4–69.8
	Pimephales promelas (53 mm, UT 44.8609)	1	29.0
	Rhinichthys atratulus (size not recorded)	1	15.1
29 Dec 1999	*Semotilus atromaculatus* (36–152 mm, $n = 6$, UT 44.8612)	6[4]	2.4–106.2
	Lepomis cyanellus (20–69 mm, $n = 7$, UT 90.2628)	7[4]	2.4–46.0

[1]A range of capture distances is given when numerous individuals were captured. Distance in meters from Bruffey Creek entrance; distance was measured at the center of a distinct pool or stream segment.
[2]Distance not recorded for all fishes.
[3]Two additional creek chubs were observed but could not be captured, and another large creek chub was found dead on the stream bank.
[4]Four other creek chubs and one centrarchid were observed but could not be captured.
Notes: Stream flow was elevated on 18 November 1995, 6 February 1999, and 27 March 1999. Sampling distance was greater on the first three dates, then was <140 m on all other dates due to the presence of a logjam. *Phoxinus oreas* from 6 February and 27 July were combined in one lot (UT 44.8611).

Two green sunfish and one creek chub were recaptured and inhabited the cave for at least 49 d (30 September to 18 November 1995). These fishes exhibited little or negative growth as determined from length and weight data (only minor differences observed). Movement was not observed for the two green sunfish as both were recaptured in the same pool (35.8 m). However, the creek chub was 83.4 m further inside the cave (180.9 m) from the initial point of capture (97.5 m).

The recaptured creek chub was initially recorded as lightly pigmented, reverting to a normal pigment pattern after exposure to light, whereas the same fish on 18 November 1995 was lightly pigmented but did not revert to a normal pigment pattern. Lightly pigmented creek chubs were captured in all years; many of these fishes had contracted melanophores because most became darker after exposure to light for several minutes. However, a few creek chubs remained lightly pigmented. Overall, of 85 creek chubs, 60 were pigmented normally when captured, whereas 10 were listed as lightly pigmented initially but returning to normal, and 15 remained lightly pigmented even after exposure to light.

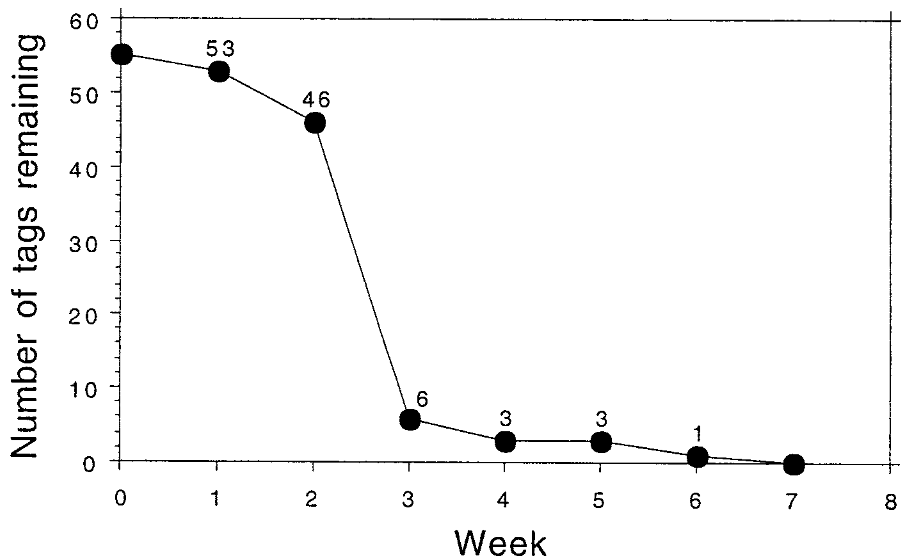

Figure 1. Number of creek chubs that retained alphanumeric implant tags at each weekly sample time (n = 55 creek chubs were tagged).

In the follow-up tagging study, most captive creek chubs lost tags within one month; the highest loss of tags occurred between the second and third weeks (Figure 1). Two of 55 creek chubs died but retained the tags, and a third moribund chub lost the tag; all were included as fishes that lost their tags. Moribund fishes were found between the second and third weeks and the fifth and sixth weeks. The longest retention time for a tag was between six and seven weeks, which is close to the 49 d residence time recorded for a creek chub and two green sunfishes.

Discussion

Phoxinus oreas and *Pimephales promelas* were captured in a cave and in the Bruffey-Hills Creek drainage for the first time (Hocutt et al. 1978, Stauffer et al. 1995, Poly & Boucher 1996). *Lepomis macrochirus* and *Ameiurus nebulosus* were not reported in this cave or in the basin previously. Brown bullheads have been collected rarely in the New River drainage of West Virginia and Virginia (Burkhead et al. 1980, Jenkins & Burkhead 1994, Stauffer et al. 1995), and Easton & Orth (1994) did not capture any brown bullheads during their survey. The only two records of brown bullhead in the Greenbrier River drainage have come from collections in caves (Poly & Boucher 1996, present study).

The low recapture rate of fishes during this study could be due to a number of factors. First, the epigean fishes might occur in the cave only for brief periods. High water during sampling and at other times between samples most likely affected collecting. The lowest abundance and diversity of fishes was obtained on dates when water levels were elevated (Table 1). A severe flood event had occurred (at least one) between September and November 1995 as evidenced by the scoured stream substrate and debris clinging to the cave walls nearly to the ceiling. The flood had not influenced the distribution of the two recaptured green sunfishes but might have contributed to the lack of recaptures. Another factor contributing to the low recapture rate was the logjam that limited the sampling area considerably. Tag loss was identified as a factor in lack of recaptures of tagged fishes, and an alternate method will be required for marking creek chubs and other cyprinids. Means & Johnson (1995) used *Phoxinus erythrogaster* (a cyprinid) and *Fundulus olivaceus* (a fundulid) as surrogates for testing visual implant tags; they found best tag retention in the caudal peduncle region, but they did not indicate results from placing tags elsewhere on the two surrogate species. No mention was made as to whether the tags could be seen well in the surrogate species when tags were in the caudal peduncle region, which has a cover of scales and pigmented epithelium in the surrogates. In this study, creek chubs were tagged on the top of the head because that region appeared to be the only position on the fish where tags could be read.

Limited information was gained on changes in pigmentation of fishes in Bruffey-Hills Creek Cave. The single creek chub that was recaptured initially became darker after exposure to light, whereas 49 d later the fish remained lightly pigmented; therefore, either the ability of this fish to rapidly expand melanophores had diminished, or the melanin content or number of melanophores might have decreased (Rasquin 1947). Rasquin (op. cit.) examined pigmentary changes of *Astyanax fasciatus* held in darkness, and a brief account of pigment loss in a crayfish was given in Kusek & Parker (1970).

No evidence of reproduction in the cave was found during the present study; tuberculate males of creek chubs have not been seen in this cave at any time. There is one report of a banded sculpin, *Cottus carolinae*, nesting just inside a cave (Craddock 1965), of a fully tuberculate male bluntnose minnow, *Pimephales notatus*, well inside a cave (Poly & Boucher 1996), and of a tuberculate male redeye chub, *Notropis harperi*, inside a cave (Brockman & Bortone 1977). Long-term residence of fishes in Bruffey-Hills Creek Cave was not demonstrated, although fishes were encountered regularly in the cave, and little information was obtained on movement of fishes within the cave.

Epigean fishes should be given greater consideration concerning their role(s) in the community of organisms within a cave, especially in cases that involve rare cave organisms. Even if epigean fishes cannot survive for extended periods within a cave, they might be capable of predation on troglophiles or troglobites during their short time of residence. Sculpins are presumed to be a likely potential predator of troglobites, but no case of such predation on troglobitic fishes or shrimps has been documented (Woods 1956, Lisowski 1983, Brown et al. 1994, Poly & Boucher 1996), although cases of predation on troglobitic and troglophilic crayfishes were noted by Brown et al. (1994). In addition, Relyea & Sutton (1974) found a troglobitic crayfish, *Procambarus lucifugus*, in the stomach of a yellow bullhead, *Ameiurus natalis*, in a Florida cave. Lee (1969) described instances of predation on cave-dwelling animals by an epigean species of frog, the bullfrog, *Rana catesbeiana*. Animals consumed by several bullfrogs included a cave salamander, *Gyrinophilus palleucus*, a troglobitic crayfish, *P. lucifugus*, and a bat, *Myotis austroriparius*. The frogs were well into the aphotic zones of the caves. Creek chubs could be potential predators in caves because even though they are active during the day, adults are known to feed and engage in various reproductive activities at night (Magnan & FitzGerald 1984, Maurakis et al. 1995); however, more light is available to the epigean fishes, even at night. Brown trout, *Salmo trutta*, living in British caves have been known to feed by 'grubbing' in the substrate for amphipods and insects (Proudlove 1979, 1982, Chapman 1993).

Occurrences of nontroglobitic fishes in caves are of interest also in that they represent various stages of evolution from accidental residents to occasional or seasonal residents (trogloxenes or troglophiles, e.g., Zhang 1986), which might lead to obligate troglobitic species. Studies on organisms in the early or intermediate intervals of cave-adapted existence were considered important for a better understanding of evolution of blind, depigmented troglobites (Dearolf 1956, Poly & Boucher 1996, Green & Romero 1997).

There are numerous reports of epigean species in caves (Appendix). Epigean fish species found in caves (or cave-like habitats) sometimes exhibit loss of pigment (Hora 1924, Kosswig 1937, Greenfield et al. 1982, Wüstemann 1990, Poly & Boucher 1996), reduced eye size (Burr et al.[2], Greenfield et al. 1982), and other structural differences (Paul et al.[3], Relyea & Sutton 1974) – convergent characters of hypogean species (Romero 1985b). A few examples of troglomorphic evolution in progress are found in some populations of *Astyanax fasciatus*, *Poecilia mexicana* (or *P. sphenops*), *Garra barreimiae*, and *Rhamdia* spp. (Norman 1926, Hubbs 1938, Gordon & Rosen 1962, Walters & Walters 1965, Greenfield et al. 1982, Banister 1984, Silfvergrip 1996, Weber & Wilkens 1998, Romero & Creswell 2000).

Neoteny (a form of paedomorphosis) appears to be a possible mechanism for troglomorphisms in fishes as discussed by Banister (1984), and he suggested that more experimental studies must be performed. The neotenic condition has been reported for a number of cave salamanders (Brandon 1971, references in Banister op. cit.), and Banister (op. cit.) provided examples of neoteny from fishes and a decapod crustacean. In a hypogean population of *Garra barreimiae* and

[2] Burr, B.M., M.L. Warren, Jr. & R.J. Paul. 1992. Cavernicolous sculpins of the *Cottus carolinae* species group from Mystery Cave, Missouri. 72nd Ann. Meeting Amer. Soc. Ich. Herp.,4–10 June 1992 (abstract).

[3] Paul, R.J., B.M. Burr & M.L. Warren, Jr. 1993. Cavernicolous sculpins of the *Cottus carolinae* species group from Perry County, Missouri: cave-life phenomena or speciation? 4th Ann. Argonne Symp. for Undergraduates in Science, Engineering and Mathematics, Argonne Natl Lab., 5–6 November 1993 (abstract).

in *Ancistrus cryptophthalmus*, eyes are visible externally in small individuals, then become covered in older fishes (Banister 1984, Reis 1987). However, in the salamander, *Typhlotriton spelaeus*, the eyes develop normally and are functional in the larvae, which inhabit springs and spring streams, but the cave-dwelling adults are blind due to tissue degeneration during and after metamorphosis (Stone 1964 cited in Brandon 1971). There could be many factors and processes involved in evolution of troglomorphisms (reviewed in Sket 1986). Experiments involving surface fishes deprived of light at early developmental stages and the converse, troglophilic or troglobitic species raised with light exposure, would provide information on the effects of light on development of eyes and other structures as well as pigment levels. Zeutzius & Rahmann (1980) found significantly fewer synapses per unit area in the cerebellum and optic tectum and lower density of synaptic vesicles in the optic tectum of rainbow trout raised without light from the larval period to 25 d. Similar studies carried out for longer time periods would help determine whether substantial troglomorphisms can develop within the lifespan of individual epigean fishes.

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Appendix. Nontroglobitic fish species reported from caves worldwide.

Species	States or countries
Order Petromyzontiformes	
Family Petromyzontidae	
Lampetra Bonnaterre sp.	AL[12]
Lampetra appendix (DeKay)	MO[57]
Order Amiiformes	
Family Amiidae	
Amia calva Linné	FL[62]
Order Anguilliformes	
Family Anguillidae	
Anguilla anguilla (Linné)	UK[40,54]
Anguilla rostrata (Lesueur)	FL[21,29,41,62]
Order Cypriniformes	
Family Cyprinidae	
Barbus binotatus Valenciennes	Sarawak[53]
Barbus hexastichus McClelland	India[30]

Appendix continued.

Species	States or countries
Barilius barna (Hamilton)	India[30]
Barilius bendelisis (Hamilton)	India[30]
Campostoma anomalum (Rafinesque)[(1)]	MN[36], MO[26], WV[7]
Clinostomus funduloides Girard	VA[8]
Cyprinella spiloptera (Cope)	KY[10], TN[11]
Cyprinus carpio Linné	FL[62], MO[24]
Danio aequipinnatus (McClelland)	India[30]
Garra barreimiae Fowler & Steinitz	Oman[56]
Hybognathus placitus Girard	TX[38]
Leuciscus cephalus Linné	Slovenia[47]
Luxilus cornutus (Mitchill)	MN[45]
Margariscus margarita Cope	WV[7]
Nematabramis everetti Boulenger	Sarawak[53]
Nocomis micropogon (Cope)	WV[7]
Notemigonus crysoleucas (Mitchill)	FL[62], MS[16]
Notropis atherinoides Rafinesque	KY[4,8,10]
Notropis harperi Fowler	FL[13,61,62,63]
Notropis photogenis (Cope)	WV[25]
Notropis volucellus (Cope)	WV[25]
Phoxinus erythrogaster (Rafinesque)[(1)]	MO[26]
Phoxinus oreas (Cope)	WV[72]
Pimephales notatus (Rafinesque)	IN[22], WV[25], unspecified[6]
Pimephales promelas Rafinesque	WV[72]
Psilorhynchus sucatio (Hamilton)	India[30]
Rasbora volzi Popta	Sarawak[53]
Rhinichthys atratulus (Hermann)	MN[36], TN[23], WV[5,8,25,72]
Rhinichthys cataractae (Valenciennes)	MN[36], WV[5,7,8]
R. cataractae × *N. micropogon*	WV[7]
Semotilus atromaculatus (Mitchill)	IL[24], KY[10], MN[36], MO[24], MS[16], TN[8], WV[7,25,72]
Varicorhinus macrolepis (Bleeker)	China[32]
Schizothoracinae	India[34]
Family Balitoridae	
Indoreonectes evezardi (Day)	India[73]
Nemacheilus sijuensis Menon	India[30,77]
Family Catostomidae	
Catostomus commersoni (Lacépède)	KY[10], MN[36], TN[2,8], WV[25]
Hypentelium nigricans (Lesueur)	TN[8], WV[7]
Ictiobus bubalus (Rafinesque)	AL[11]
Order Characiformes	
Family Lebiasinidae	
Piabucina erythrinoides Valenciennes	Venezuela[74]

Appendix continued.

Species	States or countries
Family Characidae	
Astyanax fasciatus (Cuvier)	México[66], Costa Rica[35]
Astyanax Baird & Girard sp.	Brazil[46]
Order Siluriformes	
Family Ictaluridae	
Ameiurus catus (Linné)	FL[62]
Ameiurus melas (Rafinesque)	MO[27], TX[37,38]
Ameiurus natalis (Lesueur)	AL[11], FL[13,21,29,61,62,65], MS[16]
Ameiurus nebulosus (Lesueur)	FL[21,62,65], WV[25,72]
Ameiurus serracanthus (Yerger & Relyea)	FL[62]
Ameiurus Rafinesque sp.	IL[24], KY[10], MO[24], OK[49]
Ictalurus lupus (Girard)	TX[23]
Ictalurus punctatus (Rafinesque)	FL[62,65], TX[9,37,38]
Noturus Rafinesque sp.	Unspecified[8]
Pylodictis olivaris (Rafinesque)	FL[62]
Family Bagridae	
Leiocassis poecilopterus (Valenciennes)[(6)]	Sarawak[53]
Family Siluridae	
Pterocryptis buccata Ng & Kottelat	Thailand[76]
Family Sisoridae	
Glyptothorax platypogon (Valenciennes)	Sarawak[53]
Family Clariidae	
Clarias Scopoli sp.	Sarawak[53]
Family Pimelodidae	
Imparfinis piperatus Eigenmann & Norris	Brazil[46]
Pimellodella chagresi odynea Schultz	Venezuela[74]
Pimellodella transitoria Miranda-Ribeiro	Brazil[46]
Rhamdia laticauda (Kner)[(5)]	México[59]
Rhamdia laticauda typhla Greenfield, Greenfield & Woods	Belize[69]
Rhamdia quelen (Quoy & Gaimard)	Brazil[46], México[6,59], Trinidad[58,59,78]
Family Trichomycteridae	
Trichomycterus banneaui maracaiboensis (Schultz)	Venezuela[74]
Trichomycterus emanueli (Schultz)	Venezuela[74]
Trichomycterus conradi (Eigenmann)	Venezuela[1,60]
Family Loricariidae	
Lasiancistrus maracaiboensis Schultz	Venezuela[74]
Loricariidae	Brazil[46]

Appendix continued.

Species	States or countries
Order Esociformes	
Family Esocidae	
Esox niger Lesueur	FL[62]
Order Salmoniformes	
Family Salmonidae	
Oncorhynchus mykiss (Walbaum)	WV[19,25,72], UK[40,54]
Salmo salar Linné	UK[54]
Salmo trutta Linné	Unspecified[47], UK[40,54]
Salvelinus fontinalis (Mitchill)	WV[19,25]
Order Percopsiformes	
Family Aphredoderidae	
Aphredoderus sayanus (Gilliams)	MS[16], FL[21,62,63]
Family Amblyopsidae	
Chologaster agassizi Putnam(2)	IL[71], KY[10], MO[70], TN[8]
Order Cyprinodontiformes	
Family Fundulidae	
Fundulus zebrinus Jordan & Gilbert(3)	TX[38]
Family Cyprinodontidae	
Cyprinodon rubrofluviatilis Fowler	TX[38]
Family Poeciliidae	
Brachyraphis rhabdophora (Regan)	Costa Rica[35]
Gambusia holbrooki Girard	FL[21,61]
Poecilia reticulata Peters	PR[55]
Poecilia sphenops (Cuvier & Valenciennes)(4)	México[50,64]
Xiphophorus Heckel sp.	Guatemela[52]
Family Hemirhamphidae	
Hemirhamphodon pogonognathus (Bleeker)	Sarawak[53]
Order Gasterosteiformes	
Family Gasterosteidae	
Gasterosteus aculeatus Linné	UK[54]
Order Synbranchiformes	
Family Mastacembelidae	
Mastacembelus maculatus Cuvier	Sarawak[53]
Order Scorpaeniformes	
Family Cottidae	
Cottus bairdi Girard	PA[8], WV[5,7,8,25]
Cottus carolinae (Gill)(7)	AL[23], AR[8,20], IL[24], IN[6,8,22], KY[4,23,43,75], MO[6,8,17,18,22,24], TN[2,3,22,23,44], VA[8,22,23,28], WV[15,25]
Cottus gobio Linné(8)	Germany[33], UK[39,40,54]
Cottus Linné sp.	TN[51], unspecified[67]
Order Perciformes	
Family Ambassidae	
Chanda nama Hamilton	India[30]

Appendix continued.

Species	States or countries
Family Moronidae	
Morone saxatilis (Walbaum)	FL[21,62]
Family Centrarchidae	
Ambloplites rupestris (Rafinesque)	MO[24], WV[25,72]
Centrarchus macropterus (Lacépède)	FL[62]
Lepomis auritus (Linné)	FL[62]
Lepomis cyanellus Rafinesque	AR[14], MO[24], TX[9,37,38], WV[25,72]
Lepomis gulosus (Cuvier)	MO[27], FL[62], TX[37,38]
Lepomis macrochirus Rafinesque	FL[21,62], MO[24,79], WV[25,72]
Lepomis marginatus (Holbrook)	MS[16]
Lepomis microlophus (Günther)	FL[62]
Lepomis punctatus (Valenciennes)	FL[62]
Lepomis Rafinesque sp.	KY[10]
Micropterus salmoides (Lacépède)	FL[62]
Pomoxis nigromaculatus (Lesueur)	FL[21,62]
Family Percidae	
Etheostoma blennioides Rafinesque	WV[7]
Etheostoma flabellare Rafinesque	MN[36]
Etheostoma nigrum Rafinesque	MN[36]
Etheostoma parvipinne Gilbert & Swain	MS[16]
Etheostoma zonale (Cope)	IN[22]
Percina nigrofasciata (Agassiz)	FL[62]
Family Sciaenidae	
Micropogonias undulatus (Linné)	FL[62]
Family Cichlidae	
Cichlidae	FL[62]
Family Eleotridae	
Eleotris pisonis (Gmelin)	Bahamas[68]
Family Gobiidae	
Gobiosoma bosc (Lacépède)	FL[62]
Family Channidae	
Channa orientalis Bloch & Schneider(9)	India[30]
Order Pleuronectiformes	
Family Achiridae	
Trinectes maculatus (Bloch & Schneider)	FL[62]
Unidentified fishes	KY[31,52,48], TN[51]

Authors of genera follow Eschmeyer (1998). (1)Reported as extremely emaciated and with empty intestinal tracts (Smith 1948). (2)Now called *Forbesichthys agassizi* by some authors. (3)Given as *Fundulus kansae* in source. (4)*Poecilia sphenops* or *P. mexicana*; taxonomy of *Poecilia* requires further study. (5)Includes *R. reddelli* Miller and *R. zongolicensis* Wilkens according to Silfvergrip (1996). (6)Given as *Leiocassis micropogon* in source. (7)Includes subspecies. (8)The record in Germany was from a cave-like habitat. (9)Given as *Ophiocephalus gachua* in source. Data from [1]Romero 1987, [2]Cope & Packard 1881, [3]Evermann & Hildebrand 1916, [4]Bailey 1933, [5]Reese 1934, [6]Hubbs 1938, [7]Frank

J. Schwartz unpublished, data collected in 1946 from Sinks of Gandy, [8]Dearolf 1956 (record of *Erimystax* sp. probably = *N. harperi*), [9]Jones & Hettler 1959, [10]Kuehne 1966, [11]Armstrong & Williams 1971, [12]Cooper & Iles 1971, [13]Relyea & Sutton 1974, [14]McDaniel & Gardner 1977, [15]Williams & Howell 1979, [16]Cliburn & Middleton 1983, [17]Pflieger[4], [18]Burr et al.[5], [19]Garton et al. 1993, [20]Brown et al. 1994, [21]Franz et al. 1994, [22]University of Michigan Museum of Zoology (UMMZ) records, [23]National Museum of Natural History (USNM) records, [24]Southern Illinois University at Carbondale (SIUC) records, [25]Poly & Boucher 1996, [26]Smith 1948, [27]Pembleton & Bake 1967, [28]Cornell University Ichthyology Collection (CU) records, [29]Streever 1995, [30]Hora 1924, [31]Tellkampf 1845, [32]Zhang 1986, [33]Wüstemann 1990, [34]Tehsin et al. 1988, [35]Romero 1984, 1985a, [36]Schmidt 1994, [37]Reddell 1967, [38]Reddell & Mitchell[6], [39]Proudlove 1979, [40]Proudlove 1982, [41]Pylka & Warren 1958, [42]Paul et al.[7], [43]Lisowski 1983, [44]Lawhon 1969, [45]Kusek & Parker 1970, [46]Trajano 1991, [47]Kosswig 1937, [48]Hobbs 1991, [49]Hobbs 1993 (bullhead catfish, presumably *Ameiurus* sp.), [50]Gordon & Rosen 1962, [51]Hay 1902, [52]Delamare-Deboutteville & Juberthie 1975, [53]Chapman 1985, [54]Chapman 1993, [55]Beck et al. 1976, [56]Banister 1984, [57]Hubbs & Trautman 1937, [58]Norman 1926, [59]Silfvergrip 1996, [60]Perez & Moodie 1993, [61]Marshall 1947, [62]Pruitt 1995, [63]Brockman & Bortone 1977, [64]Walters & Walters 1965, [65]Hale & Streever 1994, [66]Mitchell et al. 1977, [67]Woods 1956, [68]Yager & Williams 1988, [69]Greenfield et al. 1982, [70]McDonald & Pflieger 1979, [71]Weise 1957, [72]present study, [73]Pradhan & Biswas 1994, [74]Viloria et al. 1992, [75]Bryant et al. 1972, [76]Ng & Kottelat 1998, [77]Menon 1987, [78]Romero & Creswell 2000, [79]Brown & Todd 1987 (reported *L. macrochirus*, but all other sunfishes collected in this cave have been *L. cyanellus* (personal observation, K. Lister personal communication)). The record of *Ameiurus nebulosus* from a cave in Pennsylvania (based on Cope's '*Gronias nigrilabris*') was not included because the bullheads were collected from a surface stream rather than a cave (see Romero 2000).

[4] Pflieger, W.L. 1989. Aquatic community classification system for Missouri. Missouri Department of Conservation, Aquatic Series, No. 19. 70 pp.

[5] Burr, B.M., M.L. Warren, Jr & R.J. Paul. 1992. Cavernicolous sculpins of the *Cottus carolinae* species group from Mystery Cave, Missouri. 72nd Ann. Meeting Amer. Soc. Ich. Herp., 4–10 June 1992 (abstract).

[6] Reddell, J.R. & R.W. Mitchell. 1969. A checklist and annotated bibliography of the subterranean aquatic fauna of Texas. Texas Technological College, Water Resources Center, Lubbock, Special Report 24. 48 pp.

[7] Paul, R.J., B.M. Burr & M.L. Warren, Jr. 1993. Cavernicolous sculpins of the *Cottus carolinae* species group from Perry County, Missouri: cave-life phenomena or speciation? 4th Ann. Argonne Symp. for Undergraduates in Science, Engineering and Mathematics, Argonne Natl Lab., 5–6 November 1993 (abstract).