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A TROGLOMORPHIC SCULPIN (PISCES: COTTIDAE) POPULATION: GEOGRAPHY, MORPHOLOGY AND CONSERVATION STATUS

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In high latitudes, troglomorphic fish are absent despite the presence of caves. Glaciations during the Pleistocene may have prevented fish from colonizing this environment until very recent times. Here we present data on the northernmost cave adapted fish in the world, a troglomorphic sculpin (Cottus: Cottidae: Teleostei) from central Pennsylvania.

The characters normally used in recognizing troglomorphic fish, blindness and depigmentation, are not fully developed in this population. Nonetheless, these fish have a suite of modifications that readily identify them as cave-adapted: elongated pectoral fins, more numerous and enlarged cephalic lateralis pores, a broader head, increased subdermal fat reserves, and in the brain, size reduction of the tectum opticum.

Individuals from this newly discovered troglomorphic population have been found only in a single cave at the lower end of the Nippenose Valley. Because of the significance and uniqueness of this population, we recommend that the U.S. Fish and Wildlife Service consider this troglomorphic sculpin for listing under the U.S. Endangered Species Act.

Despite the presence of large karst areas and long river cave systems in latitudes above 40° N (e.g., Baichtal 1995) troglomorphic fish have not been found in these habitats. A total of 88 species of troglomorphic fishes, belonging to 19 families are known (Romero & Paulson 2001; Burr *et al*. 2001; Espinasa *et al*. 2001), but none has been reported from above 40° latitude. Europe has no described troglomorphic fish species, the northernmost species in Asia is *Nemacheilus starostini*, from a cave in Turkmenistan (ca. 37° 55' N), and in North America the northernmost is *Amblyopsis spelaea*, found in Kentucky and southern Indiana (37-39° N). The latter is the most northern troglomorphic fish species described thus far (Romero & Paulson 2001).

One factor that probably contributes to the restricted distribution of troglomorphic fish is the extent of polar ice sheet migration during the Pleistocene. At the peak of the last Wisconsinan glacial advance about 20 ka ago, ice sheets covered most of the northern hemisphere above 40-50° N (Flint 1971). Therefore, most northern caves were not available for colonization by fish, at least until 12 ka ago, when the Wisconsinan ended. Restrictions in available underground habitats, as well as limited time to evolve distinct troglomorphic traits such as blindness and depigmentation, apparently confined the presence of troglomorphic fish to below 40°.

The second most northern troglomorphic fish in North America (Salem Plateau, Missouri: 37° 35' - 37° 55' N) belongs to the *Cottus carolinae* species group (Burr *et al*. 2001). Although readily recognized as distinct from epigean fish by their body shape, reduction in pelvic fin ray number and enlarged cephalic lateralis pores (Burr *et al*. 2001), the "Grotto sculpins" are not fully eyeless or depigmented. They have small eyes (1-6% Standard Length vs. 6-10% SL in epigean samples) and significantly but not completely reduced pigmentation (Burr *et al*. 2001). Grotto sculpins are less troglomorphic than the cavernicolous amblyopsids, the archetypical North American hypogean fish group, which, with the exception of some localities for *Amblyopsis spelaea*, has a more southern distribution (32-39° N).

Here we present data on a previously unreported cave population of the *Cottus bairdi-cognatus* complex from a cave in central Pennsylvania (41° 9' N). This population represents the northern-most cave adapted fish in the world.

MATERIALS AND METHODS

Troglomorphic sculpins were collected from Eiswert #1 Cave, Nippenose Valley, Lycoming County, Pennsylvania (41° 9' 23.2" N, 77° 12' 21.1" W, 212 m msl; Stone 1953). Fifteen individuals were collected in 2002 for study on 10, 11, and 17 August; 6 September; and 23-24 November (4, 1, 3, 4, 2, & 1 specimens, respectively). Surface sculpins were collected on 10 August, 2002 from nearby Lochabar Spring of Antes Creek, Lycoming County, Pennsylvania, also at the mouth of the Nippenose Valley (41° 9' 28.6" N, 77° 13' 13.6" W, 200 m msl). Antes Creek is a 4 km long tributary of the West Branch of the Susquehanna River. All samples were collected by James C.D. Lewis (Resident Pennsylvania Fishing License number R 703557). Samples were fixed either in the field or kept alive in the laboratory for a week before fixing. Fin clips were preserved in 100% ethanol and the body in 10%

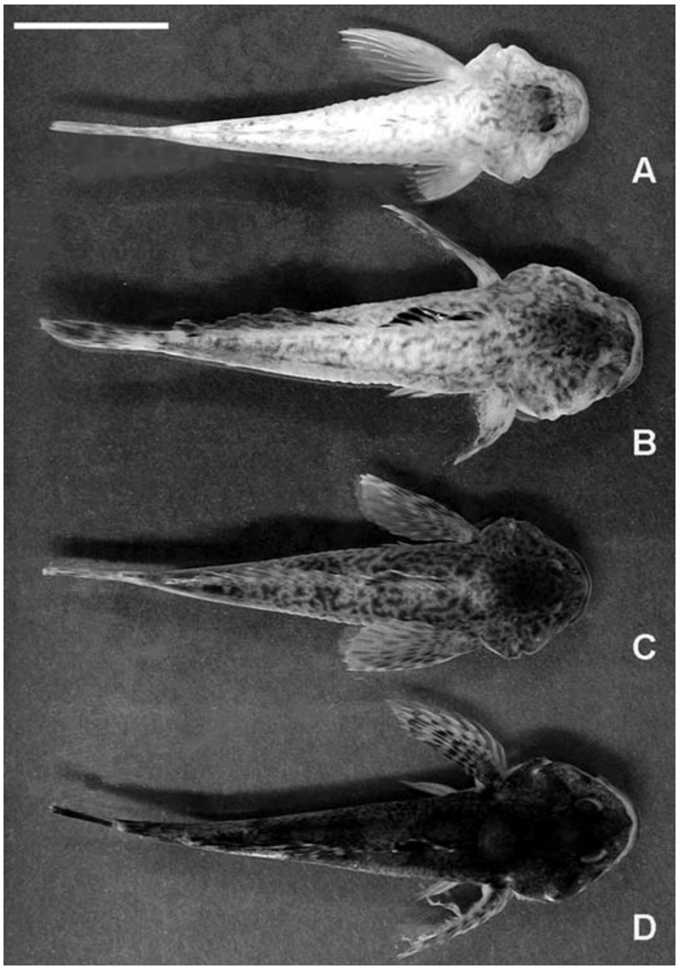


Figure 1. Dorsal view of Nippenose Valley troglomorphic (A-C) and epigean Antes Creek (D) sculpins showing patterns of coloration. A) Highly depigmented; B) Slightly depigmented; C) Pigmented; D) Highly pigmented. Scale bar: 2 cm.

formaldehyde. Specimens used in the analyses will be deposited in the Pennsylvania State University Fish Collection.

Eye and mandibular canal pore #3 lengths were measured in 15 cave and 15 surface fish using a binocular microscope fitted with an eyepiece micrometer. Standard length, pectoral fin length, head width, and head length from mouth to base of pectoral fins were measured with dial calipers to the nearest 0.1 mm.

Heads from two cave and two surface sculpins were dissected using a scalpel and forceps under a binocular microscope. Skin of the head and the top of the skull were removed to expose brain, eyes and optic nerves. Eyes were then fixed in 10% formaldehyde, embedded in paraffin, and sagitally sectioned in slices, which in turn were stained with hematoxilin-eosin.

Level of pigmentation and its response to light were studied in the field by exposing 15 live cave and 15 live surface individuals to direct sunlight for 10 min and assessing changes in color. Four levels of pigmentation were assigned (Fig. 1). A) highly depigmented: skin color white with some groups of melanophores forming scattered dark spots of about 1 mm each. Some spots can be found in body and fins, but mostly on head. B) slightly depigmented: skin color also whitish but with spots found throughout whole body forming patterns and weak bands. C) pigmented: skin color tan to olive. Spots throughout whole body forming patterns and bands. D) highly pigmented: skin color dark green or black. Spots barely visible against dark background.

A Mann-Whitney test was used to look for possible differences in levels of pigmentation, number of mandibular pores and number of rays in the fins. Possible differences in the size of the eye, head, pectoral fin and mandibular pore #3 were examined using linear regressions and *t*-tests.

Visual response was studied in the field or in the laboratory by directly focusing a presentation pointer red laser beam (Limate corporation. Class IIIa laser product. Max. output <5 mW, 630-680 nm) in the eye of 7 individuals and assessing evasion reactions.

RESULTS

STUDY AREA

The Nippenose Valley is a nearly closed, anticlinal karstic valley. Surface water from the valley goes underground into a cave system that emerges at the mouth of the valley at Lochabar Spring, the origin of Antes Creek. Only 445 m from this spring, also at the lower end of the valley, is Eiswert #1 Cave. The cave, with a total of 167 m of explored passage, has a small stream that, in dry months, percolates under the gravel, leaving only an isolated sump pool at each end of the cave (Fig. 2). With about only 10 m of altitude difference between the downstream sump in the cave and the resurgence, it is likely that only partial isolation is in effect between the cave and surface populations.

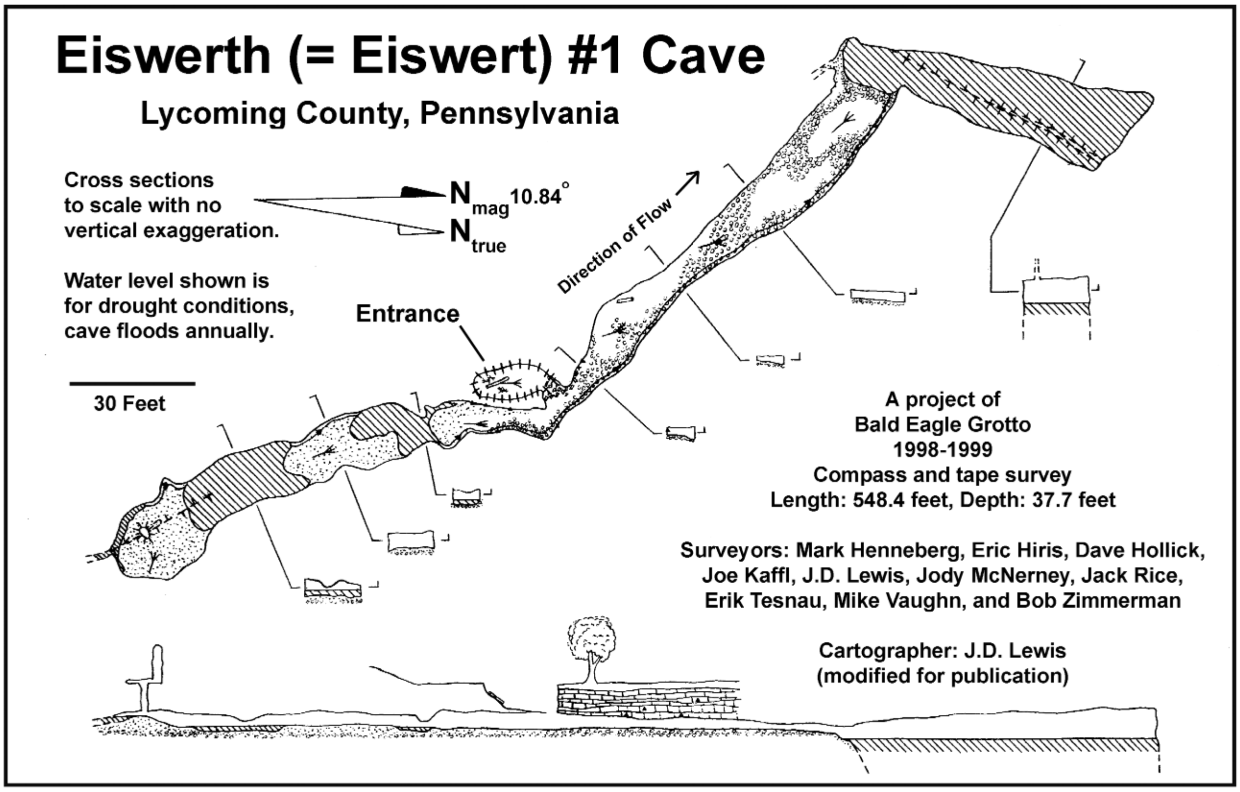
Apart from the sculpins, the cave stream fauna included white isopods (5 mm long), amphipods (15 mm long), crayfish, and an unidentified teleost fish. None was collected or further identified. The isopod and amphipod appear to be cave adapted, and the presence of eggs in the marsupium of the isopods indicated they were reproducing. The crayfish and teleost fish appear to be surface taxa.

Surface sculpins were abundant around the spring and throughout Antes Creek, where they were found mainly under rocks. The Antes Creek fish population is isolated from the West Branch of the Susquehanna River during most of the year because its water is restricted to the hyporheic zone in the gravel of the creek bed. Only at high water levels is there continuous water contact between Antes Creek and the West Branch of the Susquehanna River.

MORPHOLOGY

Mean standard length of the troglomorphic sculpins was 48.2 mm (n=15, SD=9.8, range=38.0-60.2 mm). All caverni-

Figure 2. Eiswert #1 Cave map.



colous samples, except for one individual, had comparatively smaller eyes than epigean fish from Antes Creek (Fig. 3a). Their eye length was on average 5.7% (±0.8 SD) the standard length (SL). In the smallest eyed individual, the eye was 4.5% of the SL. Troglomorphic fish had eyes about 25% smaller ($P<0.001$) when compared to similarly sized surface fish (7.4% ±0.8 of the SL). The eyes themselves are positioned differently. In surface fish the eyes protrude conspicuously on the top of the head, while in troglomorphic fish they are half-sunken into the head (Fig. 4). Despite having smaller eyes, lens size was not reduced in cavefish. Both in surface and cavefish, the lens were on average 1.3 mm (±0.3 SD) in diameter (Fig. 5).

Patterns and degree of pigmentation were variable in troglomorphic fish, but most individuals were conspicuously less pigmented than surface fish ($P<0.001$). Of the 15 troglomorphic fish studied, 3 were highly depigmented, 8 were slightly depigmented, and 4 were pigmented (Fig. 1). Of the 15 fish from Lochabar Spring, one was slightly depigmented, 12 were pigmented and 2 were highly pigmented. Reduction in eye size and pigmentation do not appear to be linked because pigmented fish could have highly reduced eyes and vice versa: Of the 4 pigmented cavernicolous fish, one of them had small eyes (4.8% of the SL), and of the 3 highly depigmented, one had big eyes (6.0% of SL). The other individuals had eyes roughly equal to the average in the Nippenose cave fish (5.7% ±0.8 SD of SL)

Histology of the eye from 2 cavernicolous individuals with small eyes (5.1% and 5.2% of SL) was performed. In these eyes, retina was present but its width was thinner. Cavefish

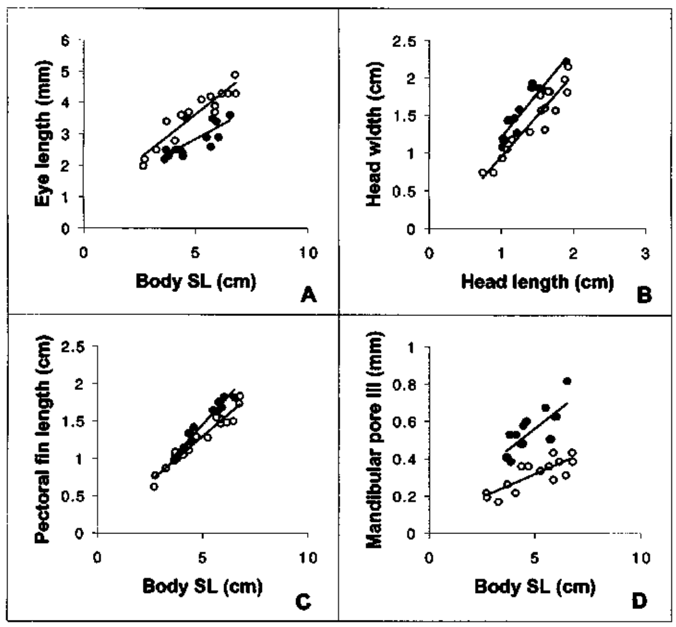


Figure 3. Morphometric comparisons between troglomorphic (closed circles, n=15) and epigean Antes Creek (open circles, n=15) sculpins. A) Eye length. B) Head width. C) Pectoral fin length. D) Mandibular pore III length. SL = Standard length. In C, linear regressions have significantly different slopes ($0.01<P<0.02$) and in A, B and D, linear regressions have significantly different elevations (all $P<0.001$).

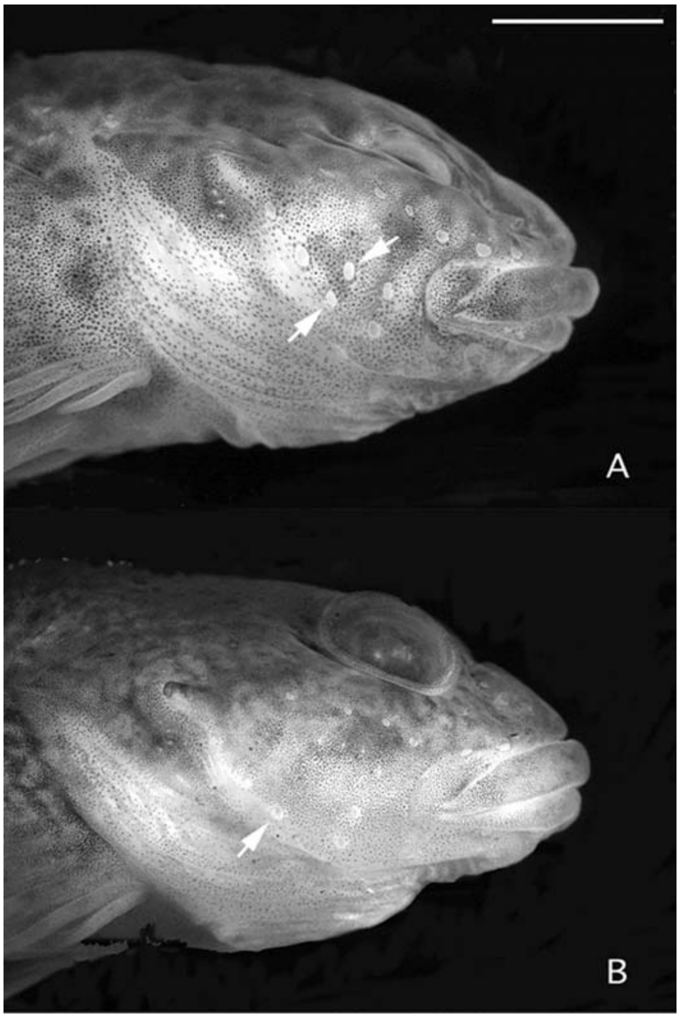


Figure 4. Lateral views of heads. Arrows point to mandibular pore VI (below) and the extra pore (above) found in most troglomorphic specimens. A) Pigmented troglomorphic sculpin; B) Pigmented epigean Antes Creek sculpin. Scale bar: 0.5 cm.

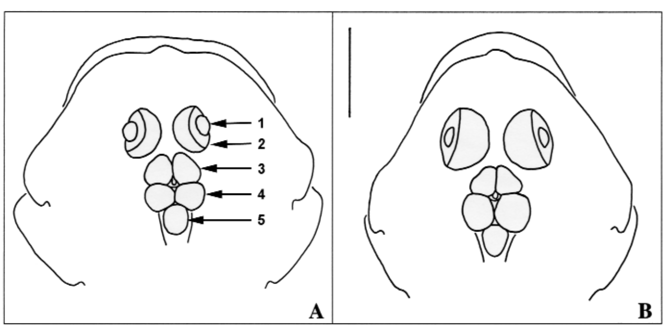


Figure 5. Drawing of dorsal views of heads showing the different eye size, tectum opticum and head width. A) Troglomorphic sculpin; B) Epigean Antes Creek sculpin. 1=Lens; 2= Eye; 3=Prosencephalon; 4= Mesencephalon (optic tectum); 5=Metencephalon. Scale bar: 0.5 cm.

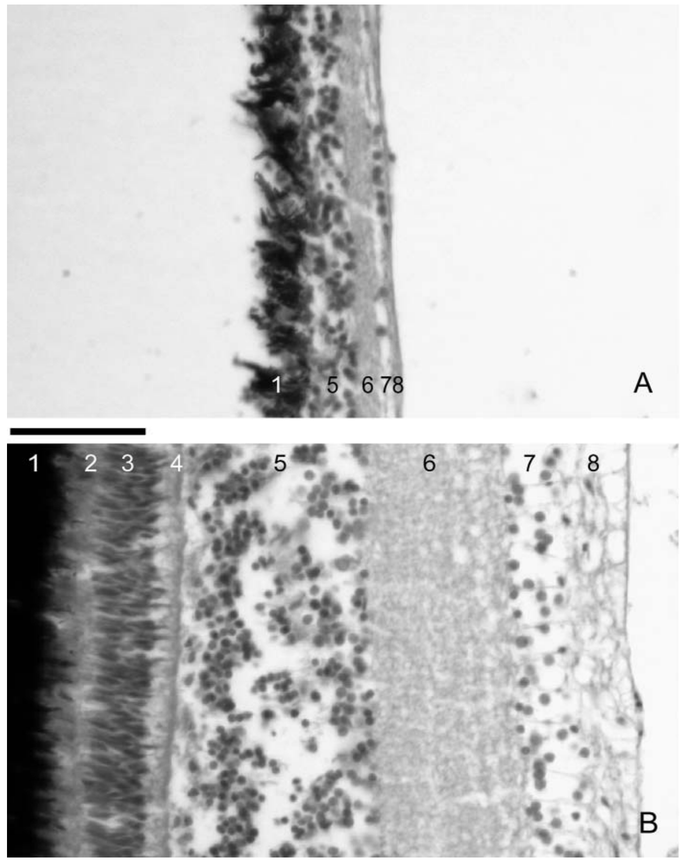


Figure 6. Histology of the retina. A) Troglomorphic sculpin; B) Epigean Antes Creek sculpin. Note absence of photoreceptors, outer nuclear layer, and outer plexiform layer in this troglomorphic individual. Other segments within the eye in this same individual had all retinal layers. 1=Pigmented epithelium; 2=Photoreceptors; 3=Outer nuclear layer; 4=Outer plexiform layer; 5=Inner nuclear layer; 6=Inner plexiform layer; 7=Ganglion cell layer; 8=Optic fiber layer. Scale bar for both images: 50 µm.

retinal thickness, measured at the widest point in the eye, were 150 and 170 µm. Two similarly-sized surface fish had retinas of 250 and 300 µm in width. Also, in one of the cavefish, almost half of the eye from one side of the optic nerve to the ciliary body showed a near absence of photoreceptor cells and outer nuclear layer (Fig. 6), and in this portion the thickness was only 50 µm. The other half of the eye had all the normal retinal layers. The optic nerve from the 2 cavernicolous individuals examined was 35% and 40% smaller in diameter compared to those of similar-sized surface fish, suggesting a reduced capacity to form connections to the brain.

Meristic features are presented in Table 1. Troglomorphic fish were not much different in their fin ray counts from Antes Creek surface fish and only slightly different from *C. cognatus* of Blockhouse Creek by averaging more rays in the first dorsal and pectoral fins. However, they were clearly different from *C. bairdi* and hybrids from Blockhouse Creek, particularly with respect to the first dorsal and pelvic fin ray counts (both $P<0.001$).

Table 1. Comparison of percent frequencies of fin-ray counts. Data for *Cottus cognatus*, *Cottus bairdi* and hybrids are taken from Strauss (1986) study of Blockhouse Creek fish.

	# of rays *	Troglomorphic (n=15)	Antes Creek (n=42)	Frequency *Cottus cognatus* (n=29)	Hybrids (n=8)	*Cottus bairdi* (n=42)
First dorsal fin						
	7	0.20	0.47	0.51	0.62	0.81
	8	0.73	0.42	0.48	0.37	0.19
	9	0.06	0.09			
Second dorsal fin						
	15	0.06		0.03		
	16	0.13	0.16	0.03		0.47
	17	0.66	0.69	0.65	0.37	0.45
	18	0.06	0.14	0.27	0.62	0.07
	19	0.06				
Pectoral fin						
	11.5			0.03		
	12			0.03		
	12.5		0.02			
	13	0.27	0.29	0.76		
	13.5	0.07	0.29			
	14	0.47	0.38	0.17		0.38
	14.5	0.13	0.02		0.13	0.05
	15	0.07			0.50	0.57
	15.5				0.13	
	16				0.25	
Pelvic fin						
	3	0.80	0.90	0.96		
	3.5	0.13	0.07	0.03	0.12	
	4	0.06	0.02		0.87	1.00
Anal fin						
	10			0.03		
	11	0.13	0.07	0.13		0.21
	12	0.62	0.57	0.58	0.50	0.66
	13	0.20	0.28	0.20	0.50	0.11
	14		0.07	0.03		

* values of .5 in # of rays indicates that the number of rays in the right and left fins is unequal, with one fin having one ray more than the other fin.

Bilateral asymmetry was high for both troglomorphic and Antes Creek surface fish. Right and left pectoral fins had different numbers of rays in 20% and 33% of the individuals respectively, while pelvic fin asymmetry was evident in 13% and 7% respectively (Table 1). This percentage of asymmetric individuals is an order of magnitude higher than for *C. bairdi* (pectoral 4.7%; pelvic 0.0%) and *C. cognatus* (pectoral 3.4%; pelvic 3.4%) from Blockhouse Creek, but is comparable to the bilateral asymmetry shown in hybrid individuals of that locality (pectoral 25.0%; pelvic 12.5%).

Head width measured both at the cheek level under the eyes or at its maximum width indicates that the troglomorphic fish head was about 20% wider ($P<0.001$) than in Antes Creek surface fish (Fig. 3b and 5). Pectoral fins in troglomorphic fish also differed ($0.01<P<0.02$) allometrically from Antes Creek. While small cave and surface fish had similarly sized pectoral fins, large cavefish individuals had 15% longer pectoral fins than similarly sized surface fish (Fig. 3c).

Cephalic lateralis pore size was a clear discriminating feature between troglomorphic fish and surface fish. All cave

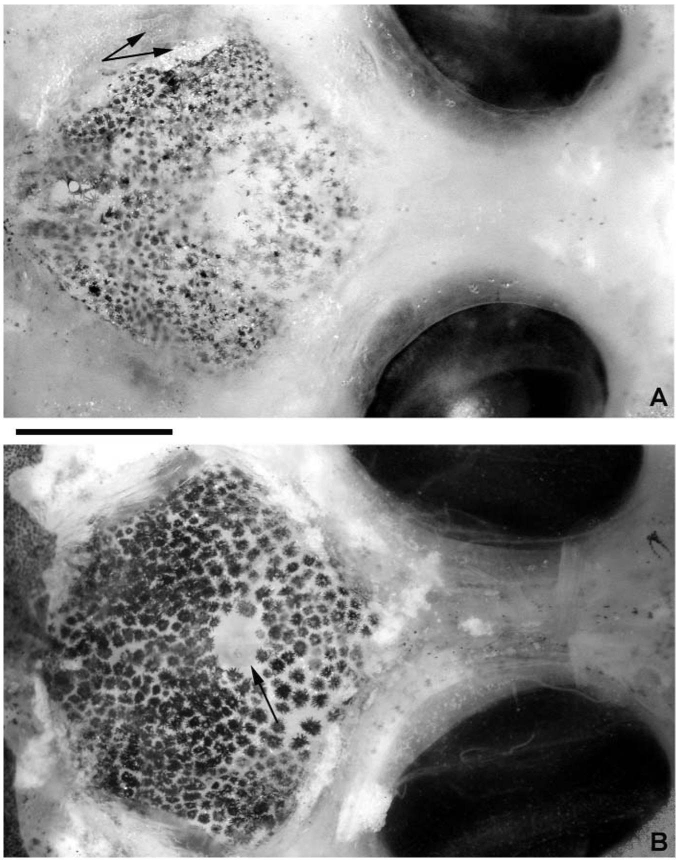


Figure 7. Dorsal view of head with skin removed. Melanophores in skull surround the pineal. A) Highly depigmented troglomorphic sculpin. Arrows point to subdermal fat layers. B) Pigmented epigean Antes Creek sculpin. Arrow points to pineal gland. Scale bar for both images: 2 mm.

individuals, including the most pigmented and large eyed individuals, had distinctly larger pore size than similarly sized surface fish ($P<0.001$). The mandibular pores of troglomorphic fish were readily visible with the unaided eye and, on average, were 1x-2x the size of those from epigean Antes Creek samples (Fig. 3d and 4). The number of pores also differed ($P<0.001$). In the mandibular canal above pore # 6 (counting from the chin backwards), 9 of 15 troglomorphic sculpins had an extra pore (Fig. 4). None of the 15 Antes Creek specimens had an extra pore.

Troglomorphic sculpins appear to have a thick subdermal layer of fat on the body. This was most clearly seen above the skull bones as a vacuolar matrix detachable from the skin and bone when dissecting (Fig 7a). A subdermal fat layer of this extent was not found in Antes Creek surface fish. Surface fish had abundant and orderly packed melanophores under the skin and above the skull, except for a window above the pineal gland (Fig 7b). Troglomorphic fish also showed these melanophores, but they were less abundant and disordered so that there are several uncovered sections and the window above the pineal was less obvious (Fig. 7a). The mesencephalon and prosencephalon also appeared to be different: troglomorphic fish brains had a comparatively smaller optic tectum than those of surface fish (Fig. 5). The mesencephalon in the two troglomorphic individuals examined was 1.01x and 1.03x the length of the prosencephalon while in similarly sized surface fish, mesencephalon was 1.20x and 1.40x the length of the prosencephalon.

RESPONSE TO LIGHT

Visual responses of the sculpins to the laser beam varied greatly among individuals. Two individuals evaded this stimulus instantly, 2 appeared never to notice it, and the remaining 3 were intermediate in their response, suggesting that vision capabilities are variable in the cavefish.

There were some changes in pigmentation levels after exposing live animals to direct sunlight for 10 minutes. Although pigment spots on troglomorphic sculpins were darker and more distinct, this general darkening was not intense enough to modify the level of pigmentation assigned originally to that individual while still in the darkness of the cave. After a week of living in the laboratory under illuminated conditions, a highly depigmented cave sculpin, although slightly darker, was still highly depigmented. Response to longer periods of light exposure could not be examined because fish failed to survive longer in the laboratory.

DISCUSSION

Two species of sculpins inhabit the West Branch of the Susquehanna River and its tributaries: mottled sculpin (*Cottus bairdi*) and slimy sculpin (*Cottus cognatus*). These cottids display extreme variation in form and color between and within populations (Strauss 1986). Both species can overlap in meristic and morphometric traits and are known to hybridize with each other (Strauss 1986). One locality where the two species hybridize, Blockhouse Creek, is in the same county as Eiswert #1 Cave.

At the Blockhouse Creek locality, the number of pelvic fin rays, 3 in *C. cognatus* and 4 in both *C. bairdi* and the hybrids, can differentiate the species (Strauss 1986). Most individuals in the cave and in Antes Creek had 3 pelvic fin rays (Table 1), which would place them as *C. cognatus*. But Grotto sculpins in Missouri from the *C. carolinae* species group show a reduction in pelvic fin ray number from 4+4 elements to often 4+3, or 3+3 (Burr *et al*. 2001). If a reduction in pelvic fin number is a trend for cavernicolous cottids, the Nippenose Valley troglomorphic fish could be *C. bairdi* with a reduced pelvic fin ray number.

Because of the occurance of 2 Cottus species in this area and the potential for fin ray number reduction (see discussion earlier), assignment of the troglomorphic sculpins and the Antes Creek sculpins to *Cottus cognatus*, *Cottus bairdi*, or a new taxon awaits more detailed assessments of characters and, perhaps, molecular studies. At this time, we restrict placement to the *Cotttus bairdi-cognatus* complex until further studies can be conducted.

Regardless of its final taxonomic position, the cave population has a unique set of morphological traits that permit their recognition as a distinct and unique population. We interpret these results as representative of losses and gains associated with cave habitation and evolution. Troglobitic characters found in other cavefish, such as elongated pectoral fins, more and enlarged cephalic lateralis pores, increased subdermal fat reserves, and modifications in the brain (Wilkens 1988) are also exhibited in these sculpins, but other characters, such as loss of pigmentation and blindness, are less developed.

Two contributing factors might explain why cave sculpins in the Nippenose Valley are not fully blind and depigmented. One could be that some genetic introgression with surface fish is still present, and second, that there has simply not been enough evolutionary time to fully regress these characters. These explanations are not mutually exclusive.

With a linear distance of 445 m between the cave and the spring, and only 10 m of altitude difference, it is unlikely that effective barriers prevent movement of sculpins between the surface and Eiswert #1 Cave. There is evidence suggesting that both the troglomorphic and Antes Creek fish populations are affected by hybridization. Bilateral asymmetry has been employed as a criterion of developmental instability on the assumption that coordination among loci within the genome protects the developing individual from developmental accidents (Felley 1980). Disruption of this coordination by hybridization with individuals having different coadapted gene complexes is expected to result in increased numbers of phenotypic variants and asymmetry (Strauss 1986). In the present study, the percentage of asymmetric individuals for both troglomorphic and Antes Creek fish is an order of magnitude higher than in *C. bairdi* and *C. cognatus* from Blockhouse Creek, but is comparable to the bilateral asymmetry shown in hybrid individuals of that locality. In this case, hybridization would not be between *C. bairdi* and *C. cognatus*, but instead, both Eiswert #1 Cave and Antes Creek populations could be an introgression gradient between an undiscovered troglobitic population that may exist deeper in the Nippenose karst and fish from the West Susquehanna River.

The other alternative, again not mutually exclusive, is that because of the northern location of the Nippenose karst, colonization of the cave environment could not be achieved until recent times due to Pleistocene glaciations. During the early Pleistocene (~850 ka BP), ice dammed the West Branch of the Susquehanna River at Williamsport, ~20 km east of the mouth of the Nippenose Valley, forming the 100 km long Glacial Lake Lesley (Ramage *et al*. 1998). At that time, the Nippenose Valley would have been 100-150 m under water. The lake may have existed for ~4000 years, but it is likely that it ended abruptly by ice dam failure at maximum volume (~100 km^3), causing a flood of catastrophic proportions, perhaps one of the largest glacial floods in the eastern United States (Ramage *et al*. 1998). Even if sculpins had entered caves before this period, they would not have survived the catastrophic and abrupt change of pressure and conditions in the underwater caves. Therefore, the current population presumably colonized the cave environment less than 850,000 years ago.

During the late Illinoian and the Wisconsinan (198-17 ka BP), the southern limits of glacial advance were only 26 and 34 km north respectively of the Nippenose Valley (Sevon & Fleeger 1999). Although not under glaciers or lakes, tundra conditions with tens to even hundreds of meters of thick permafrost would have prevented surface sculpins from inhabiting the area. If all cave water froze during those times, and thus prevented occupancy, the sculpins could have inhabited the cave for <17 ka. If, on the contrary, the groundwater flow system in the valley is deep enough to absorb significant geothermal heat, it may not have frozen. In this case, sculpins could have taken refuge in the cave during the severest cold conditions while other surface fish migrated south. As such, the above glacial periods could be the isolating events.

Conservation status

Burr *et al*. (2001) noted that troglomorphic sculpin populations might be threatened both by their restricted distributions and the potential for impacts of human activities. Grotto sculpins of the *Cottus carolinae* species group from Missouri have been designated as Candidates for the Endangered and Threatened Wildlife and Plants list of the U.S. Fish and Wildlife Service. The designation may come too late for one of the six caves they inhabit, considering that a mass mortality was recently observed in one of the caves (Burr *et al*. 2001), and subsequent visits to that cave in 2002 failed to document any living Grotto sculpins.

The case for the troglomorphic sculpins of the *Cottus bairdi-cognatus* species group of the Nippenose Valley, Pennsylvania, is equally, if not more, alarming. Restriction to a single site locality is a criteria used by the International Union for the Conservation of Nature and Natural Resources (IUCN) to be included within the Red List of threatened animals (IUCN/SSC 1999), because any single point threat has the potential to bring extinction to the whole species. The troglomorphic sculpins are known only to exist in a single cave of the Nippenose Valley.

Proudlove (2001) lists 5 types of threats that cavefish can face: Hydrological manipulation, habitat degradation, overexploitation, impacts of introduced aquatic animals, and finally, environmental pollution by eutrophication and contamination from factory farms, agricultural, and/or industrial runoff. All 5 should be considered towards preserving the Nippenose troglomorphic sculpins.

Due to the distinctness and significance of this population, we recommend that the U.S. Fish and Wildlife Service consider this troglomorphic sculpin for listing under the U.S. Endangered Species Act.

ACKNOWLEDGMENTS

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