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## ***Cottus specus*, a new troglomorphic species of sculpin (Cottidae) from southeastern Missouri**

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### **Abstract**

*Cottus specus*, a new species, is described from the karst regions of the Bois Brule drainage in eastern Missouri, USA. *Cottus specus* is distinguishable from all members of the genus *Cottus* using both genetic and morphological characters, including eye size and cephalic pore size. *Cottus specus* represents the first description of a cave species within *Cottus*. The addition of *C. specus* brings the total number of recognized species of *Cottus* to 33 in North American fresh waters.

**Key words:** sculpin, cavefish, *Cottus carolinae*

### **Introduction**

Adaptation of organisms to karst environments is a highly convergent event that has long fascinated biologists (Christiansen 1962; Poulson 1963) and is characterized by troglomorphy, defined as a lack of or reduction of eyes and body pigmentation, increased sensory organs to compensate for reduction of eyes, elongated appendages, and lower metabolic rates than epigeal (surface) relatives (Christiansen 1962). Cave colonization by fishes has occurred in approximately 100 species from 19 families, representing mostly freshwater fishes (Proudlove 2006).

*Cottus carolinae*, Banded Sculpin, occurs in both surface streams and springs in the eastern United States. Occasionally, *C. carolinae* has been reported in twilight or dark regions of cave systems and with the exception of the albino specimen report in Williams and Howell (1979), these populations do not appear to be more than accidentals or troglophiles (Burr *et al.* 2001). Several unique populations, previously designated as *C. carolinae*, have been found in the cave systems of Perry County, Missouri, and display morphological characteristics similar to other cave-adapted fish species including a reduction in eye size and pigmentation. These cave populations are described as a new species, *Cottus specus*, Grotto Sculpin, endemic to the Central Perryville and Mystery-Rimstone karst areas.

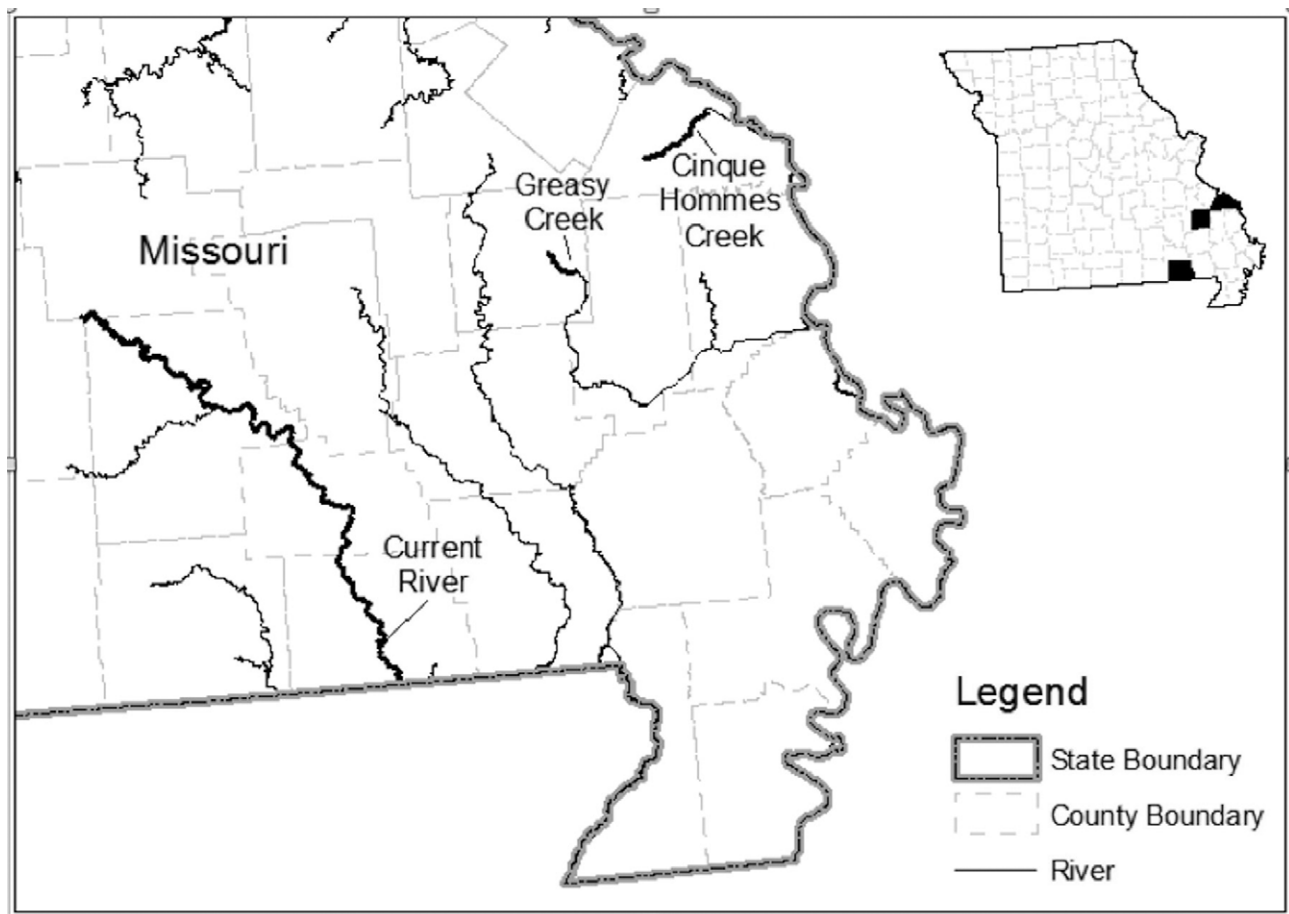
### **Methods**

Tissue samples for isolation and sequencing were collected from 153 individuals representing twelve sample localities (Table 1; Figs. 1 & 2) within the Cinque Hommes Creek drainage and two sample localities outside Perry County, Missouri, representing the Black River race of *C. carolinae* (Current River, 9 individuals) and Midlands race of *C. carolinae* (Greasy Creek, 7 individuals) (Kinziger *et al.* 2007). Fin clips or whole fish were collected from each sampling site. DNA was extracted from 1.0g caudal peduncle or fin clip using a standard phenol/chloroform protocol or cell lysis method (Hillis *et al.* 1996). A total of 747 bp of mtCR (mitochondrial control region) was amplified using CottusPro: 5'-TTCCACCTCTAACTACCCAAAGCTAG-3' and Caarh: 5'-AAGCACATTTTTCGCC-3'. Polymerase chain reaction (PCR) amplifications were optimized (Cobb *et al.* 1994) and performed using MJ Minicyclers. Twenty ml reactions consisted of 1–3ml (20–25ng DNA) DNA

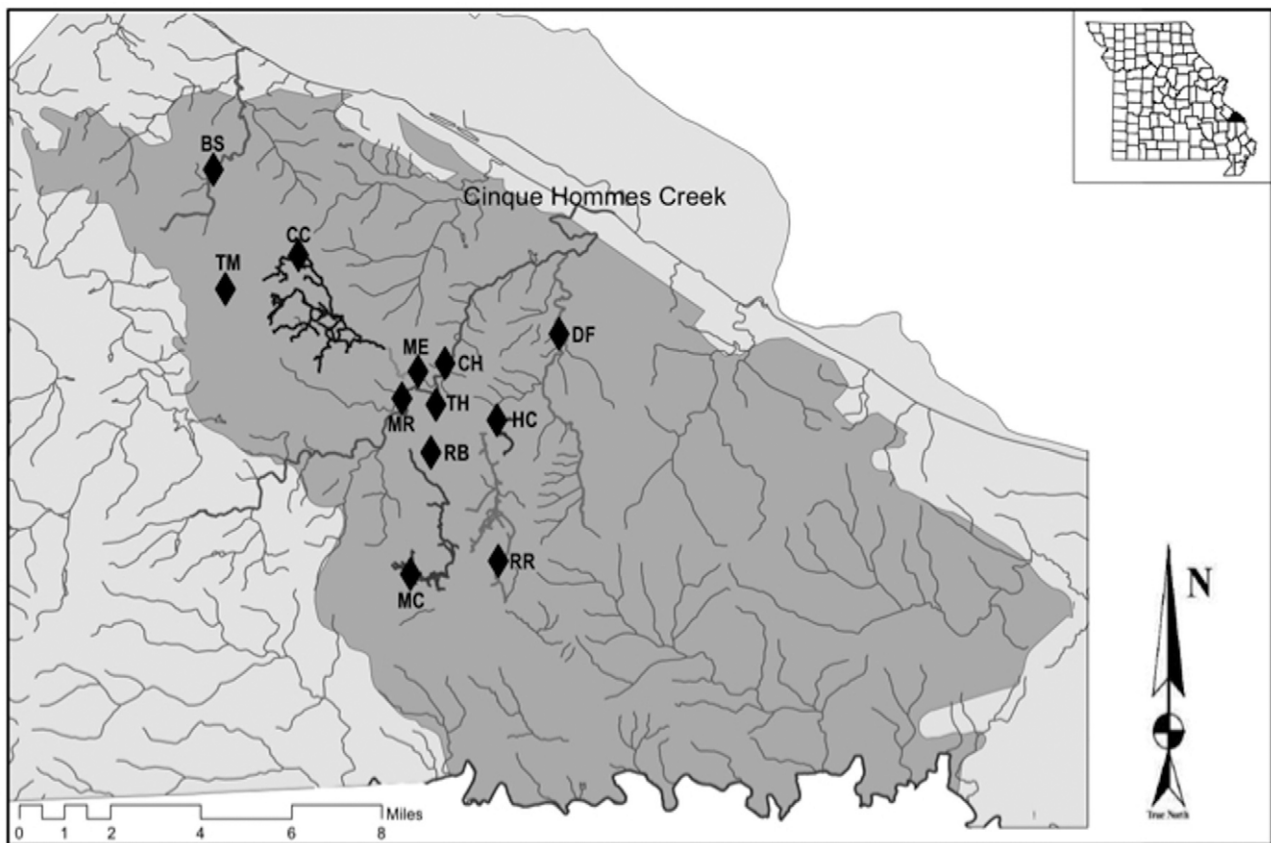
template, 1ml 10mM each primer, 7ml REDTaq DNA Polymerase (Thermo Scientific), and sterile water to a final volume of 20ml. PCR conditions consisted of an initial 95°C denaturation for 2 minutes, followed by 35 cycles of 95°C for 1 minute, 58°C for 1 minute, and 72°C for 1 minute, and a final 6-minute extension at 72°C.

**TABLE 1.** Geographic distribution of haplotypes found within sample localities, including 153 individuals. Grotto Sculpin = GR, Midlands race = MID, Black River race = BR.

County	Site	Abr.	GR1	GR2	GR3	GR4	GR5	GR6	GR7	MID1	MID2	BR1	BR2	N
Perry	Moore Cave	TM	5	8	0	0	0	0	0	0	0	0	0	13
Perry	Blue Spring	BS	11	5	0	0	0	0	0	0	0	0	0	16
Perry	Crevice Cave	CC	13	7	0	0	0	0	0	0	0	0	0	20
Perry	Cinque Hommes Cr.	CH	5	5	0	0	0	0	0	0	0	0	0	10
Perry	Mystery Cave	MC	0	0	17	0	0	0	0	0	0	0	0	17
Perry	Mystery Resurgence	MR	2	5	2	0	0	0	0	0	0	0	0	9
Perry	Dry Fork Creek	DF	1	1	5	0	0	0	0	0	0	0	0	7
Perry	Running Bull Cave	RB	0	0	11	8	0	0	0	0	0	0	0	19
Perry	Thunderhole Resurg.	TH	0	0	1	0	0	1	0	0	0	0	0	2
Perry	Rimstone R. Cave	RR	4	2	15	0	2	0	1	0	0	0	0	24
Madison	Greasy Creek	GC	0	0	0	0	0	0	0	0	0	2	5	7
Ripley	Current River	CR	0	0	0	0	0	0	0	2	7	0	0	9



**FIGURE 1.** Geographic distribution of five Ozark Highlands members of the banded sculpin complex adapted from Kinziger *et al.* (2007). *Cottus specus* is found in Cinque Hommes Creek and the unmapped surrounding caves; Midlands Race was sampled in Greasy Creek and Black River Race in the Current River.



**FIGURE 2.** Geographic distribution of sampling localities and drainage relationships in the Bois Brule drainage with caves overlaid. Abbreviations as in Table 1; HC (Hot Caverns) is part of Rimstone River Cave system, and ME (Mertz) is part of Crevice Cave system.

For nuclear samples, 420 bp of  $\alpha$ -enolase intron VIII were amplified using Enol-L: 5'-TGGACTTCAAATCCCCGATGATCCCAGC-3' and Enol-H: CCAGGCACCCCAGTCTACCTGGTCAAA-3' (Friesen 1997). Twenty ml reactions consisted of 1–3ml (20–25ng DNA) DNA template, 1ml 10mM each primer, 7ml REDTaq DNA Polymerase, and sterile water to 20ml. PCR conditions consisted of an initial 94°C denaturation for 2 minutes, followed by 30 cycles of 94°C for 30 seconds, 51–55°C for 30 seconds, and 72°C for 45 seconds, and a final 3-minute extension at 72°C.

Resulting reactions were purified using Exonuclease I and Shrimp Alkaline Phosphatase and cycle sequenced on an ABI 3130 Automated Sequencer using both primers for mtCR and Enol-L for  $\alpha$ -enolase. New, unique haplotypes were verified by sequencing several individual PCR products in forward and reverse directions. Sequences for haplotypes in this paper have been submitted to GenBank. Sequences were aligned and edited using Sequencher version 4.8. *Cottus bairdii* (Mottled Sculpin) was selected as the outgroup from GenBank (GQ290456.1). Phylogenetic analyses were performed using maximum likelihood (ML), maximum parsimony (MP), and a Bayesian optimality criterion.

ModelTest version 3.7 (Posada & Crandall 1998) was used to select the appropriate model of sequence evolution. ML searches were executed heuristically with 1,000 replications of a random stepwise addition of taxa and TBR branch swapping using HKY+G parameters (ti:tv = 1.7687;  $\alpha$  = 3.7892; Hasegawa *et al.* 1985), as specified by ModelTest. To evaluate node support, bootstrap analysis was performed with 1,000 pseudoreplicates of a heuristic search with 100 replicates of random stepwise addition and TBR branch swapping. Unweighted MP searches were conducted using a heuristic search with TBR branch swapping on initial trees, which were obtained by random stepwise addition of taxa for 1,000 replicates. Bootstrapping was performed heuristically with TBR branch swapping, random stepwise addition of taxa, and 1,000 pseudoreplicates. Bayesian analyses were conducted using MRBAYES version 3.1.2 (Huelsenbeck & Ronquist 2001).

Type material of the new species is deposited at Southern Illinois University Carbondale (SIUC). Other paratypes are deposited at the National Museum of Natural History (USNM). Morphological data were collected

from preserved specimens stored in 70% ethanol that were taken from field collections made from 2006–2009. Photographs of type material were taken in the laboratory on preserved specimens. Pelvic-fin ray number was determined for both the left and right side. Greatest length and width of the eye were taken on each specimen using dial calipers to the nearest 0.01 mm. Eye volume was estimated as a half sphere following Huber *et al.* (1997) using eye diameter. To determine potential asymmetry in eye size, measurements were made on both the left and right eye of a subset of individuals. In addition, greatest length of the dermal cornea was measured to the nearest 0.001 mm using an ocular micrometer. Eyes were embedded in paraffin and transverse sections were taken through the central retina. Lens diameter was quantified when present and not destroyed by the sectioning process. Morphological data were collected following Robins and Miller (1957) and non-significant relationships were summarized in Burr *et al.* (2001).

## Results

A single nuclear haplotype was found throughout the Bois Brule drainage (Mystery Cave, Running Bull Cave, Rimstone River Cave, Crevice Cave, Moore Cave, and Cinque Hommes Creek), a second in Greasy Creek (Midlands race), and a third in the Current River (Black River race) (designations based on Kinziger *et al.* 2007). A 2% sequence divergence was observed between Bois Brule and both the Current River and Greasy Creek. Results hereafter refer to mitochondrial sequence data.

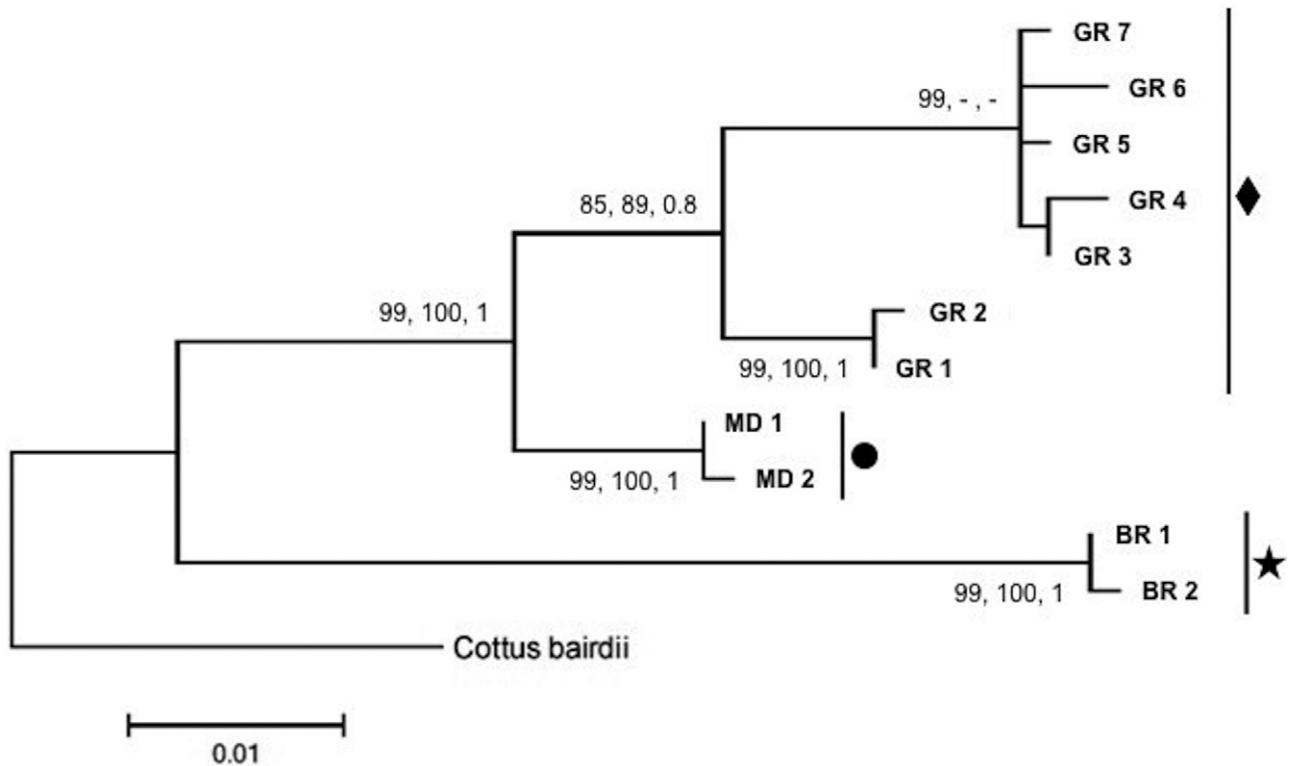
A total of 767 bp control region was analyzed after excluding primer sequences. Heuristic analyses found 86 variable characters, of which 58 were parsimony-informative in the total dataset, 71 variable characters (58 parsimony-informative) excluding the outgroup, and 25 variable characters (20 parsimony-informative) among Bois Brule haplotypes. Of the 14 total sample localities analyzed, eight contained unique haplotypes, and seven were restricted to the Bois Brule drainage.

Pairwise distance comparisons among all *Cottus* haplotypes ranged from 0.1 to 8.1%, and among Bois Brule drainage haplotypes 0.10 to 7.6%. Overall mean pairwise distance was calculated to be 4.1% including the outgroup and 3.7% excluding the outgroup. Fifty-eight sites, herein referred to as diagnostic sites were used to differentiate *C. specus* from yet unresolved *C. carolinae* races (Table 2).

**TABLE 2.** Parsimony informative sites used to separate *C. specus* from the Midlands and Black River races of *C. carolinae*.

	8	33	39	43	57	93	95	97	99	121	134	148	154	162	180	181	191	199	201	205
<i>C. carolinae</i> , Black River race	R	R	R	G	R	A	T	A	G	T	T	C	C	T	G	A	A	R	A	G
<i>C. carolinae</i> , Midlands race	-	G	T	A	-	T	C	-	-	A	C	A	T	G	A	G	G	-	T	C
<i>C. specus</i>	T	-	T	A	A	T	R	-	-	A	C	A	T	G	A	G	-	R	C	C
	206	207	208	209	212	213	214	224	225	228	234	235	246	247	270	289	292	409	429	439
<i>C. carolinae</i> , Black River race	T	C	C	A	G	A	A	G	G	T	A	G	C	G	T	T	T	T	G	G
<i>C. carolinae</i> , Midlands race	A	T	G	G	A	G	G	A	A	C	T	A	T	A	C	C	A	-	A	-
<i>C. specus</i>	R	T	G	G	A	G	G	A	A	C	T	A	T	A	C	C	A	C	A	A
	512	572	604	614	647	648	651	656	666	667	673	697	708	709	717	718	734	766		
<i>C. carolinae</i> , Black River race	C	T	T	T	T	T	A	T	T	A	C	C	C	C	C	-	G	T		
<i>C. carolinae</i> , Midlands race	T	-	G	C	A	C	G	A	G	G	-	A	A	A	T	C	A	G		
<i>C. specus</i>	R	C	-	C	A	-	G	A	G	G	T	A	A	A	T	C	A	-		

Each of the three phylogenetic methods employed recovered identical sets of major nodes (Fig. 3). Three major lineages were identified and well supported by all analyses (Fig. 3). Between the Bois Brule drainage and the Current River and Greasy Creek there was a 7.2% and 2.8% sequence divergence, respectively.



**FIGURE 3.** Relationships among 11 *Cottus* mtCR haplotypes and one outgroup (ML topology shown). Numbers on each branch correspond to bootstrap values based on 1,000 pseudoreplicates for MP and ML analyses, followed by posterior probabilities. Insignificant support placeholders are indicated by dashes. Lineages representing the Black River and Midlands races of banded sculpin are represented, as is the proposed *Cottus specus* lineage. Icons match those used in Kinzinger *et al.* 2007.

The first clade encompasses two haplotypes restricted to the Current River system and corresponds with the previously identified distribution of the Black River race sculpin. The second clade includes two haplotypes restricted to the Greasy Creek population, and corresponds with Midlands race sculpin (Kinzinger *et al.* 2007).

Considerable haplotype diversity was evident in the third clade, which includes exclusively Bois Brule drainage haplotypes (Table 1). The divergence of two distinct lineages within this clade is well supported. The first of these lineages represents caves and spring branches north and inclusive of Cinque Hommes Creek (GR3 – GR7), including Blue Spring, Tom Moore Cave, and Crevice Cave (Fig. 2). The second lineage encompasses haplotypes found primarily in caves and spring branches south of Cinque Hommes Creek (GR1 – GR2) at Running Bull Cave, Thunderhole Resurgence, Mystery Cave, and Rimstone River Cave. Several sample localities were found to contain haplotypes from both lineages, including Hot Caverns, Dry Fork Creek, and Mystery Resurgence, indicating that maternal gene flow exists between these lineages (Fig. 2).

### ***Cottus specus* Adams and Burr, new species**

Grotto Sculpin

Figure 4

Holotype. SIUC 88001, male, 80.2 mm SL, Mystery Cave, Perry County, Missouri 18 January 2012.

Paratypes. Missouri. Perry County. SIUC 88002 (2, 58.0–60.5 mm SL), USNM 409781 (2, 65.3–86.7 mm SL) taken with holotype.

Additional materials (nontypes).

Missouri: Perry County: SIUC 19279 (20, 66–103 mm SL) Cinque Hommes Creek, 1992. SIUC 18916 (3) Sculpin Stream, Mystery Cave, 21 April 1991. SIUC 19904 (3) Mystery Cave, 7 March 1992. SIUC 18923 (20,

52–80 mm SL), Mystery Cave, 1 October 1991. SIUC 21258 (4, 57–88 mm SL) Hot Caverns, 4 October 1992. SIUC 19839 (23, 51–95 mm SL) Flaming River Cave, 3 July 1992. SIUC 21260 (8, 34–56 mm SL) Running Bull Cave, 25 October 1992. SIUC 20916 (9, 31–80 mm SL) Tom Moore Cave, 25 October 1992.

GenBank accession numbers for haplotypes.

Haplotype GR1 (KC172892), Haplotype GR2 (KC172893), Haplotype MID1 (KC172894), Haplotype MID2 (KC172895), Haplotype BR1 (KC172896), Haplotype BR2 (KC172897).

A



B



C



**FIGURE 4.** *Cottus specus* (A) 80.2 mm SL, holotype, SIUC 88001; (B) male, 66.8 mm SL; (C) 74.5 mm SL showing variation in eye size and pigmentation.

**Diagnosis.** *Cottus specus* is diagnosed from all other described members of the genus *Cottus*, except *C. carolinae*, *Cottus chattahoochee*, *Cottus confusus*, *Cottus hubbsi*, and some populations of *Cottus beldingii* by the complete lateral line ending near the base of the caudal fin and dorsal fins not usually connected. *Cottus specus* only occurs geographically adjacent to *C. carolinae* and can be distinguished by a reduction in eye size: 1–5% SL in 98% of specimens (1–4% in 85% of specimens) versus eye size 5–9% of SL in 100% of specimens (Table 3). Using ANCOVA, only two individuals (<1%) overlapped in eye size between *C. specus* and *C. carolinae* when standard length was accounted for in the regression. *Cottus specus* exhibited a significant increase in cephalic lateralis pore size: 92% above 500 mm compared to 100% of *C. carolinae* between 200–500 mm; ANCOVA,  $P < 0.001$ ; Table 4.

**TABLE 3.** Frequency distribution of percent eye length of standard length in *Cottus carolinae* and *Cottus specus*. Apple Creek specimens were taken from Perry and Cape Girardeau counties in Missouri, and Big Creek specimens were collected from Union County, Illinois.

Species, System	Eye Length/SL									N	
	1	2	3	4	5	6	7	8	9		
Holotype, <i>C. specus</i>		1									
<i>Cottus carolinae</i>											
Apple Creek, MO					14	10					24
Big Creek, IL					5	35	15	6	1		62
<i>Cottus specus</i>											
Cinque Hommes	14	34	55	82	38	2					225
Crevice Cave			2	4	10	2					18
Rimstone River Cave	3		8	24	1						36
Mystery Cave	20	63	64	36	26	6					215
Running Bull Cave	26	26	13	7	1						73
Moore Cave			4	11	1						16

**TABLE 4.** Frequency distribution of cephalic lateralis pore 3 lengths in *Cottus carolinae* and *Cottus specus*. Apple Creek specimens were taken from Perry and Cape Girardeau counties in Missouri, Bay Creek from Pope County, Illinois, Collier Spring in Monroe County, Illinois, and Big Creek in Union County, Illinois.

Species, System	Pore Size								N	
	200	300	400	500	600	700	800	> 900		
<i>Cottus carolinae</i>										
Apple Creek, MO	5									5
Bay Creek, IL	1	3	1							5
Collier Spring, IL		2	6							8
Big Creek, IL	1	2	3							6
Midlands	4	2	7							13
<i>Cottus specus</i>										
Cinque Hommes		1	1	1	4	2	2	2		13
Crevice Cave			2		1	2	1	1		7
Rimstone River					1	2	3	4		10
Mystery Cave				1	1	1	2	4		9
Moore Cave			1		3	4	2			10

**Description.** *Cottus specus* is a moderate-sized species of the genus *Cottus*; the largest specimen examined is 104 mm SL. Typical body shape is illustrated in Figure 4. Frequency distribution of percent eye length as a

function of standard length, pelvic fins and lateral-line pore sizes are given in Tables 3–5. Additional information on morphology can be found in Burr *et al.* (2001).

Anal-fin rays 13, occasionally 12 or 14, dorsal-fin spines 7–8; dorsal-fin rays 16–18, usually 17–18; pectoral-fin rays 14–17, usually 16; pelvic-fin rays 3–4, with a high degree of asymmetry; branched caudal-fin rays 9; lateral-line pores 32–36, modally 35; infraorbital pores 8–9, modally 9; preoperculo-mandibular pores 10–12, modally 11, post-maxillary pore present; median chin pores 2, rarely 1; enlarged mandibular pores 3, 4 and 6 (Burr *et al.* 2001).

**TABLE 5.** Frequency distribution of pelvic-fin ray counts (4–4, 4–3, 3–3) in *Cottus carolinae* and *Cottus specus*.

Species, System	No. rays			N	% with 4-3 or 3-3 rays
	4–4	4–3	3–3		
Holotype, <i>C. specus</i>	1				
<i>Cottus carolinae</i>					
Big Creek, IL	40	0	0	40	0.00
Robins (1954)	1459	2	0	1461	0.14
<i>Cottus specus</i>					
Cinque Hommes	20	12	11	43	53.49
Crevice Cave	4	6	7	17	76.47
Rimstone River Cave	17	11	7	35	51.43
Mystery Cave	15	5	10	30	50.00
Running Bull Cave	10	9	3	22	54.54
Moore Cave	6	8	6	20	70.00

Several studies have found evidence of asymmetry in eye size in slightly to moderately microthalmic cave species (Wilkins 2001; Pouilly & Miranda 2003); however, we found no evidence of asymmetry in the eyes of *C. specus*. Eye volume, eye lens and dermal cornea of the eye were also significantly reduced in *C. specus* when compared to *C. carolinae*. Along with a decrease in eye size, mean lens diameter in *C. specus* was 50 to 70% smaller than mean lens diameter in *C. carolinae* samples. Twelve individual *C. specus* (of 31) were missing the lens in either one or both eyes.

A high degree of variability was seen in the general structure of the retina within cave populations. The overall size of the eye was markedly reduced in the cave populations along with a corresponding decrease in the size of the lens. Eye volume varied significantly among habitats (ANOVA,  $F_{5,71}$ ,  $P < 0.001$ ) with *C. specus* in caves exhibiting a significantly reduced eye size compared to *C. specus* in resurgence streams and *C. carolinae*. *Cottus specus* in resurgence streams had an intermediate eye volume that was significantly different from *C. carolinae*. No asymmetry was detected in eye length within any habitat type. Greatest length of the dermal cornea was also significantly reduced in *C. specus* compared to resurgence and surface sculpins (ANOVA,  $F_{2,71} = 14.82$ ,  $P < 0.0001$ ).

There is a high degree of variability in pigmentation in *C. specus* with some individuals lacking all apparent pigmentation in both the fins and on the body and others approaching the banding pattern of the dorsal saddles found in *C. carolinae*. Individuals with darker pigmentation are primarily found in the surface resurgence stream; however, high variability exists at every site examined.

**Etymology.** The specific epithet *specus* refers to a cave or cavern in reference to the primary habitat in caves. The common name, Grotto Sculpin, also refers to the cave habitat.

**Distribution.** *Cottus specus* is distributed in tributaries of the Bois Brule River drainage including Cinque Hommes Creek and Blue Spring (which is the resurgence stream for the Moore Cave system) and has never been collected in the Bois Brule mainstem (Fig. 2). The caves and the surface streams are direct tributaries of the Mississippi River through the Bois Brule River. *Cottus specus* can be found in cave streams as well as their resurgences, primarily over gravel, cobble, or bedrock bottom. *Cottus specus* is the only *Cottus* present throughout its distribution. It is not found in caves in nearby drainages (Burr *et al.* 2001).



**Reproduction.** We have found nests of *C. specus* in Perry County caves during December, but reproduction is thought to occur as late as February-March for sculpins residing in Cinque Hommes Creek as judged from the presence of yolk-sac larvae and a single nest (pers. obs.). In some years, there may be two nesting periods separated by one to two months within the cave systems. Nests, guarded by a single male, are found under cobble or pebble substrate. Eggs are adhesive, in a clump, and are attached to the underside of a rock. Males guarding rocks may have enlarged knobs on the dorsal fins (similar to those described for *Cottus* by Kinziger & Wood 2010) and have darker pigmentation on the lips and head (Fig 5).

A.



B.



**FIGURE 5.** Increased pigmentation on the lips and head (A) and presence of fin-knobs (B) on a male *Cottus specus* (69.8 mm SL).

**Conservation status.** We consider Cinque Hommes Creek sculpins (surface and resurgence) to be *C. specus* because they share similar morphological characteristics and haplotypes with both the northern and southern cave systems. High gene flow, possibly due to migration of nesting individuals upstream, between Cinque Hommes Creek and surrounding cave streams may have resulted in an intermediate population. It is also possible that sculpins colonized Cinque Hommes Creek from the northern caves after extirpation of the surface population (secondary colonization) or due to a lack of sculpins in Cinque Hommes Creek prior to colonization by *C. specus* (indicating initial colonization of the caves north of Cinque Hommes occurred through another route).

Presence of a troglomorphic species distinct from *C. carolinae* in Perry County may be the result of the extensive cave systems and relative isolation of related drainages due to the Mississippi River barrier, similar to *Gammarus minus* populations discussed in Culver and Wilkens (2000). Intermediate Cinque Hommes Creek sculpin may reflect the historical tendency of sculpin in Cinque Hommes Creek to move upstream into caves and springs to spawn, thus creating a high degree of interconnectedness of the respective populations.

*Cottus specus* is restricted to five cave systems and their corresponding resurgence streams in the Bois Brule drainage. Over the past fifteen years, two of the cave locations (Running Bull Cave and Mystery Cave) have experienced mass mortalities of unknown origin. Although both populations appear to have recovered from the perturbations, recent research provides evidence of pervasive and widespread contamination of *C. specus* habitat by a mixture of bioaccumulative organic contaminants (Fox *et al.* 2010). The presence of karst terrain throughout the range of *C. specus* exacerbates the concern. Direct pathways for contaminants into the cave systems through sinkholes could lead to future extirpations (Burr *et al.* 2001). Given the unique habitat requirements and morphological distinctiveness of *C. specus*, it is imperative that future studies further delineate drainage basins and potential sources of contaminants.

*Cottus specus* is typically found at abundances significantly lower than those reported for *C. carolinae* throughout their range and based on mark-recapture may be found at densities less than 0.06 individuals/m<sup>2</sup>, an order of magnitude lower than that of *C. carolinae* (Greenberg and Holtzman 1987, Burr *et al.* 2001). Low densities may limit the ability of populations to recover from anthropogenic disturbances.

**Discussion.** The addition of *C. specus* brings the total number of recognized species of *Cottus* to 33 in North American fresh waters (Kinziger & Wood 2010; Page & Burr 2011). The cave populations of the new species described here are more recognizable morphologically than most other species in the genus. Only two other reports of *Cottus* have been recorded to show troglomorphic traits in a cave environment (Williams & Howell 1979; Espinasa & Jeffery 2003). *Cottus specus* eye size was smaller than that reported for the troglomorphic sculpin from Pennsylvania, which had a mean eye size of 5.7% SL (Espinasa & Jeffery 2003). In addition, potential hybridization with a surface population may increase variation and asymmetry in the troglomorphic Sculpin from Pennsylvania compared to *Cottus specus*.

Species of *Cottus* are generally common to abundant in suitable habitat. Three species are protected in Canada as threatened, and the United States considers only one species, *Cottus paulus* (Pygmy Sculpin), as threatened (restricted to Coldwater Spring, Alabama), none as endangered. *Cottus echinatus* (Utah Lake Sculpin) is an extinct species that occurred in Utah Lake, Utah. *Cottus specus* is known only from 5 caves and one stream in a single county in Missouri. Its tiny range and known threats (chemical pollution of groundwater) make it one of the rarest and most imperiled sculpins in North America (Fox *et al.* 2010). On the basis of small geographic range, low population densities, and a multitude of threats to groundwater habitats, *C. specus* warrants formal protection at both state and federal levels.

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