



<http://dx.doi.org/10.11646/zootaxa.3609.5.4>

<http://zoobank.org/urn:lsid:zoobank.org:pub:2DB62225-474F-4F28-9C52-C8ED4A08B002>

***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