

***In-situ* observations of seven enigmatic cave loaches and one cave barbel from Guangxi, China, with notes on conservation status**

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South China's vast karst region is one of the largest contiguous limestone units in the world at over 620,000 km² (Huang et al. 2008). This area was recently selected as a United Nations Educational, Scientific, and Cultural Organization (UNESCO) World Heritage Site in 2007 because of its spectacular karst features and rich surface biodiversity. Not surprisingly, China boasts endemic and unique subterranean faunas, including the greatest number of subterranean fishes on the planet, with over 50 species exhibiting some degree of troglomorphy (Zhao et al. 2011). This assemblage of hypogean fishes constitutes roughly one third of the world's stygobitic ichthyofauna (Romero et al. 2009). Guangxi Province boasts the greatest diversity with 29 stygobitic species, followed by Yunnan and Guizhou with 14 and nine species, respectively (Zhao et al. 2011). Many of these taxa are threatened owing to anthropogenic influences (Romero et al. 2009; Zhao et al. 2009, 2011). Unfortunately, virtually nothing is known of the biology or ecology for the vast majority of China's hypogean fishes (Romero et al. 2009; Chen et al. 2010; Zhao et al. 2009, 2011).

All cavefishes in China belong to the order Cypriniformes, comprising three families. The most speciose family is the Cyprinidae and this note includes

observations of one species in the genus *Sinocyclocheilus*. Balitorid loaches dominate the biodiversity of subterranean loaches in southwest China. Of the three balitorid genera discussed in this note, loaches of the genus *Triplophysa* exhibit the greatest geographic coverage, ranging across the Qinghai-Tibet Plateau in both surface and subterranean waters (Zhao et al. 2011). Seventeen subterranean *Triplophysa* are known from southwestern China: six species from Yunnan, six from Guangxi, three species from Guizhou, one species from Chongqing, and one species from Hunan (Romero et al. 2009; Yang et al. 2011a, 2012; Zhao et al. 2011). All eight subterranean species of the genus *Oreonectes* occur in Guangxi. All but one of the described *Oreonectes* have been described in the past 30 years; over 50% of these species have been described in the past ten years (Zhao et al. 2011). Only two species of loaches in the Cobitidae are stygobites and both occur in southwestern China. The genus *Protocobitis* contains two groundwater inhabiting species endemic to Guangxi.

Because conservation assessments cannot be made without basic ecological and life history information, and because time is running short for so many of China's subterranean fishes, acquiring such data is paramount to conserve these species. Even basic information, such as color in life descriptions and clean color images of live specimens are not available for many species. We emphasize here that many of China's subterranean fishes face daunting conservation challenges (Zhao et al. 2011). For example, Proudlove (2006) lists five major threats to subterranean fishes of the world: (1) habitat degradation, (2) hydrological manipulations, (3) environmental pollution, (4) overexploitation, and (5) introduced alien species. We document at least four of the five threats here with this group of Chinese hypogean fishes; some of the species are dealing with all four issues at once in their limited ranges. For each balitorid loach and for one cyprinid species that we encountered during our fieldwork in China (April–May 2011), we include conservation status here to clarify the lack of general conservation assessments and the dire circumstances surrounding many of the species.

Blind Cave Loach, *Oreonectes anophthalmus* Zheng, 1981

The Blind Cave Loach is a member of the Balitoridae, subfamily Nemacheilinae. It is known only from the type locality, Taiji Cave, Wuming County, Guangxi Zhuang Autonomous Region, China. This species was described 30 years ago yet little information regarding the biology or ecology of the species is available. We observed three specimens swimming in midwater around the lone karst pool that is available to humans within the cave system. When attempting to net specimens, these fish are quick enough to enact successful maneuvers to evade capture. A physical description of this fish is provided in Romero et al. (2009), complete with a description of preserved specimens; however, in life, this species has greatly reduced pigment and the color over the entire body surface is pink (**Figure 1**). There are no eyes in adult

specimens nor are there vestiges of eyes. A series of adult male and female individuals that we measured (n=8, including the type specimen) ranged in standard length from 24.5 to 40.1 mm.

Conservation Status: No conservation plan has been devised for this species in spite of the fact that it is listed as “Rare” in China’s Red Data Book of Endangered Animals and “Vulnerable” in China’s Species Red List (Romero et al. (2009). The IUCN also lists the species as “Vulnerable D2” (Kottelat 1996a). We observed public and private collections in China with considerable numbers of preserved specimens. Over-collecting is listed as a threat to this species (Romero et al. 2009). The only small karst window and pool that is human accessible is in Qifeng Shan Hill (a park). The pool has had regular and repeated visitation and collection by private individuals and scientists, and there is little question that the species has suffered from collecting pressures. Proudlove (2006) indicated that the local government “is aware of this and is taking steps to prevent further losses.” We found no evidence of any protection by the local government. A conservation plan is in desperate need for this species, which should include a locked cave gate and controlled access.

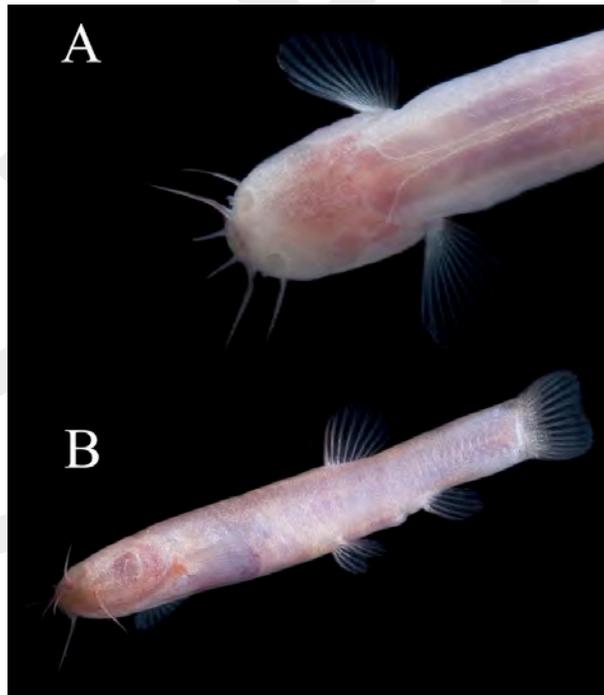


Figure 1. The Blind Cave Loach, *Oreonectes anophthalmus*, suffers from over-collection at the single locality where it has been found. The species has no discernible eyes (A) and greatly reduced pigment across its body (B). Photographs by D.B. Fenolio.

Luocheng Cave Loach, *Oreonectes luochengensis* Yang et al., 2011b

The Luocheng Cave Loach is a member of the Balitoridae, subfamily Nemacheilinae. This species is known only from the type-locality, which is an unnamed cave near Tianhe town, Luocheng County, Guangxi Zhuang Autonomous Region, China. The species is similar to *O. macrolepis* with greatly reduced pigmentation but may have partially functional eyes. A series of adult male and female individuals that we measured (n=5, including the type specimen) ranged in standard length from 66.8 to 73.9 mm.

We observed four live females that were gravid in March/April 2011 (**Figure 2**). Because of reduced pigmentation, eggs could be observed through the abdominal wall. This species is known only from the type-locality, so we did not sacrifice any individuals to obtain data on fecundity and reproductive condition. Instead, we took photographs of the abdomen and counted visible ova through the abdominal wall to minimally estimate fecundity in the species. Eggs were counted on the right side of the body only and by two independent observers (DBF and MLN) (**Table 1**). While we recognize that this approach is suboptimal and underestimates actual fecundity, our approach provides some data on the reproductive biology of a single-site endemic species of conservation concern.

Conservation Status: There is no conservation plan for this species in China and it has not been evaluated by the IUCN. Amateur collection has been documented as a potential threat to this species.

Table 1. Egg counts were estimated from four female *Oreonectes luochengensis* through their abdomens. Visible eggs were observed anterior to the pelvic fins; the ovaries extend well past the insertion of the pelvic fins but narrow as they approach the urogenital pore. Egg counts were made on the right side of the body and assume equal contribution of eggs by each ovary, without one side dominating egg production. Counts reported are the average between two observers.

Specimen	Egg Count on Right Side of Body
Female No1	54
Female No2	49
Female No3	61
Female No4	63

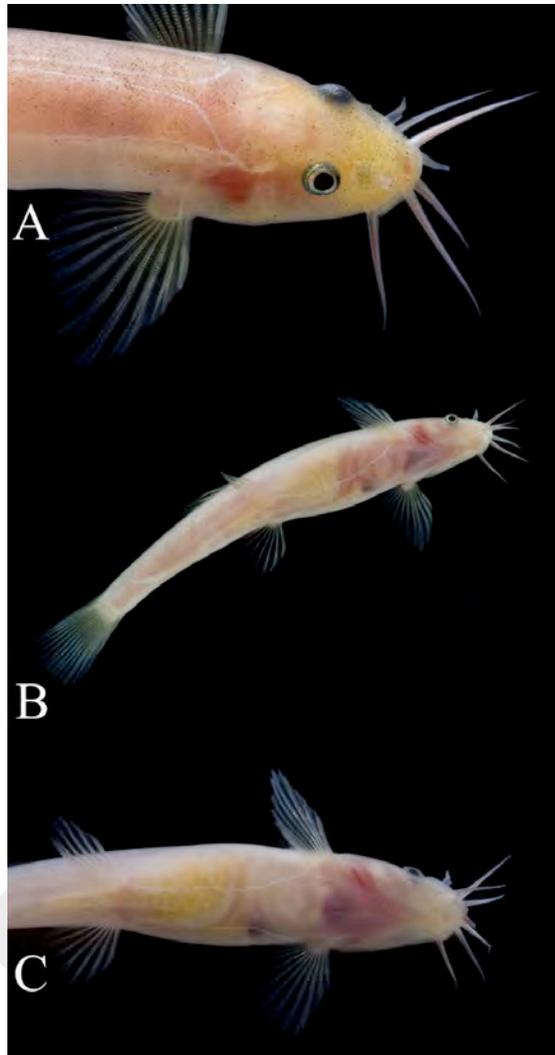


Figure 2. The Luocheng Cave Loach, *Oreonectes luochengensis*, has fully functional eyes (A) and little pigment. It is only known from a single locality. Developing eggs can be observed through the abdomens of females (B and C). Photographs by D.B. Fenolio.

Large-scaled Cave Loach, *Oreonectes macrolepis* Huang et al., 2009

The Large-scaled Cave Loach is a member of the Balitoridae, subfamily Nemacheilinae, and is known only from the type-locality: Palace of the Dragon God Cave, Dacai, Huanjiang County, Guangxi Zhuang Autonomous Region, China. The cave was formerly a commercial cave that had considerable human visitation. The mouth of the cave is now littered with garbage. We observed in excess of 25 specimens swimming in several small and shallow pools, which appeared to be connected by deeper, water filled conduits. The species is a strong midwater swimmer and is pelagic in nature. It does not behave like typical balitorid loaches that are more benthic, spending most of their time on the bottom

of aquatic habitats. The eyes and pigment are considerably reduced in this species (**Figure 3**). A series of adult male and female individuals that we measured (n=13, including the type specimen) ranged in standard length from 36.7 to 64.1 mm.

When we attempted to capture specimens of *O. macrolepis* from pools, three individuals swam into the soft silt and mud substrate and buried themselves. Burying behavior is exhibited by several epigean loaches but has only been reported from a single subterranean species in India, *Indoreonectes (=Nemacheilus) evezardi* (Day, 1872) (Biswas et al. 1990; Pati and Agrawal 2002). Multiple hypotheses have been proposed to explain burying behavior in *I. evezardi*: (1) as a light avoiding response, (2) as avoidance to extreme temperature variations, (3) as a predator avoidance response, (4) as a resting behavior, or (5) as foraging behavior for microorganisms in the sediment (Biswas et al. 1990; Pati and Agrawal 2002). Our observations suggest that burying behavior in *O. macrolepis* is elicited as an antipredator response. Two hypotheses could explain defensive burying behavior in *O. macrolepis*. First, the cave system might be home to an unknown extant (or could have been home to an extinct) larger predator that feeds on *O. macrolepis*. While we did not observe potential predators in the cave system, one possibility is a silurid catfish not uncommon to subterranean waters (*Pterocryptis cochinchinensis* (Valenciennes, 1840)) in the region. In this context, burying behavior in *O. macrolepis* could be explained as an escape response. Alternatively, burying behavior might also reflect retention of an ancestral behavior. The behavior may no longer have an adaptive value in subterranean habitats, but insufficient time has passed for mutations to accumulate in underlying genes to reduce or lose the behavior in *O. macrolepis*.

Conservation Status: There is no conservation plan for this species in China and it has not been evaluated by the IUCN. The cave that this fish inhabits has received considerable human visitation in the past. The potential for more human visitation is high and could impact the habitat and the species. Like many other subterranean fishes in China, this species is currently recognized as a single site endemic; a conservation plan needs to be implemented.

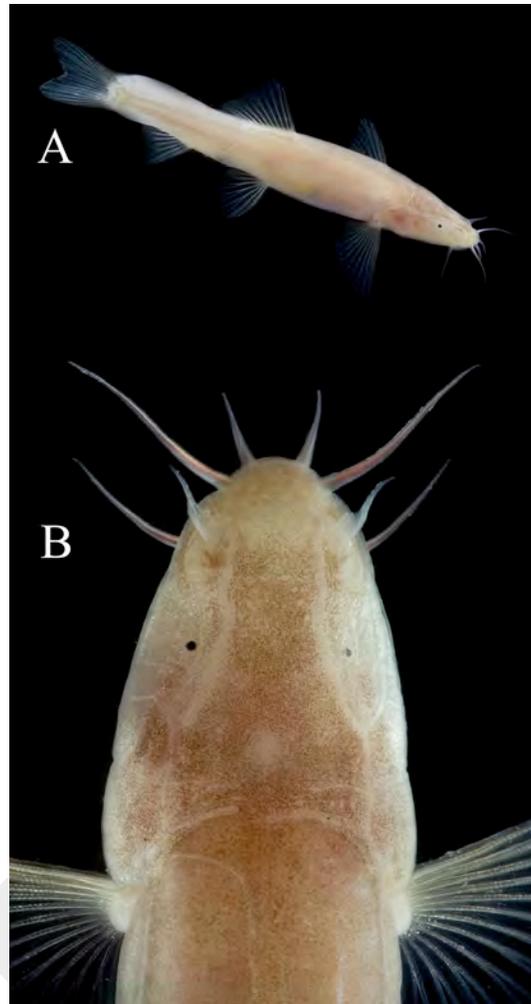


Figure 3. The Large Scale Mountain Cave Loach, *Oreonectes macrolepis*, is a strong mid-water swimmer that buries in cave sediments when threatened. The species has greatly reduced pigment (A) and reduced eyes (B). Photographs by D.B. Fenolio.

Small-eyed Cave Loach, *Oreonectes microphthalmus* Du et al., 2008

The Small-eyed Cave Loach is a member of the Balitoridae, subfamily Nemacheilinae. This species is only known from the type-locality: an unnamed cave in Du'an County, Guangxi Zhuang Autonomous Region, China. The species has vestigial eyes that can readily be seen in live animals (**Figure 4**); further, the vestiges of the eyes are more reduced than those in *O. translucens*. Color in life is brownish pink with a faint shade of purple to the tissue. The components of the lateral line system stand out against the pigmented skin. This species is a strong swimmer that can spend considerable periods of time in the water column but also rests on the bottom. A series of adult male and female individuals that we measured ($n=10$, including the type specimen) ranged in standard length from 31.0 to 48.7 mm.

Conservation Status: There is no conservation plan for this species in China and it has not been evaluated by the IUCN. Amateur collection has been noted as a threat to this species.

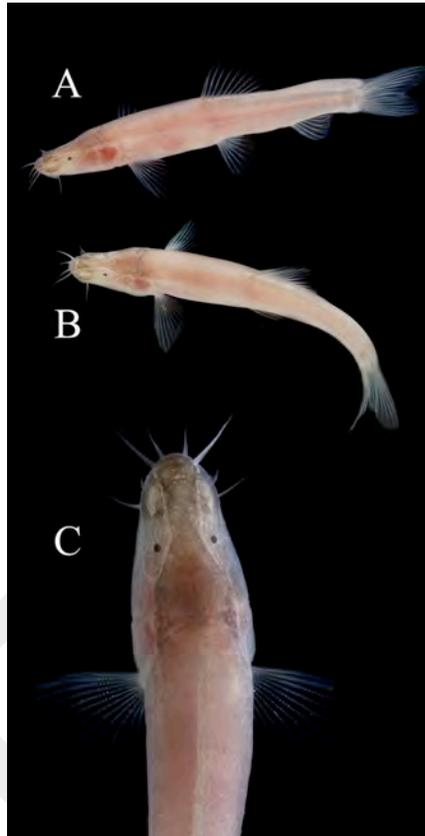


Figure 4. The Small-eyed Cave Loach, *Oreonectes microphthalmus*, has reduced pigment (A and B) and vestigial eyes (C). Photographs by D.B. Fenolio.

Tian’e Plateau Cave Loach, *Triplophysa tianeensis* Chen et al., 2004

The Tian’e Plateau Cave Loach is a member of the Balitoridae, subfamily Nemacheilinae, and is known only from the type-locality: Cave Number 8 near the Gandong Village, town of Bala, Tiane County, Guangxi Zhuang Autonomous Region, China. This species is benthic and spends most of its time on the substrate of cave streams. It also lives sympatrically with *Sinocyclocheilus furcodorsalis* (see below). A series of adult male and female individuals that we measured (n=15) ranged in standard length from 41.0 to 66.0 mm.

We examined 21 live specimens and noted significant variation in the degree of visible pigment expressed in each fish. Visible pigment in this species is expressed as patches of melanophores (**Figure 5**). Ten specimens (48%) had no

discernible pigmentation. Five specimens (24%) had 10% or less of their bodies covered with visible pigmentation patches. Five specimens (24%) had 11-25% of their bodies covered with visible pigmentation. Only one specimen (4%) had over 25% of its body covered with visible pigmentation. We also noticed considerable variation in the presence or absence of eyespots (**Figure 6**). Twelve specimens (57%) exhibited very faint or no discernible eyespots. Nine specimens (43%) had clearly discernible eyespots.

Conservation Status: There is no conservation plan for this species in China and it has not been evaluated by the IUCN. Amateur collection has been noted as a threat to this species.

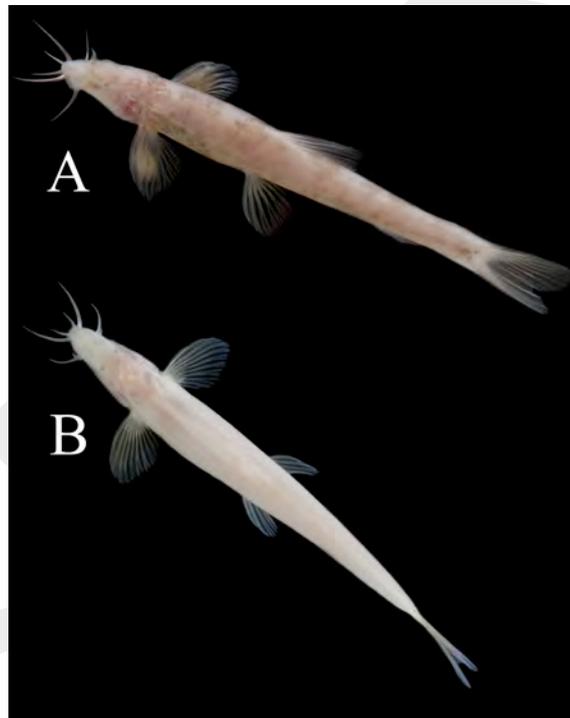


Figure 5. The Tian'e Plateau Cave Loach, *Triplophysa tianeensis*, exhibits considerable variation in the amount of pigment expressed between individuals in the same population. Some individuals have weak pigment patches covering roughly half of their bodies (A), whereas other individuals have few, if any, melanophores (B). Photographs by D.B. Fenolio.



Figure 6. The Tian'e Plateau Cave Loach, *Triplophysa tianeensis*, also exhibits variation in the regression of eyespots between individuals in the same population. Some individuals have visible eyespots (A), whereas other individuals have no apparent eyespots (B). Photographs by D.B. Fenolio.

Xia'ao Blind Cave Loach, *Protocobitis typhlops* Yang et al., 1994

The Xia'ao Blind Cave Loach is a member of the Cobitidae and is known only from the type-locality: a cave at 210 meters above sea level near Xia'ao Town, Du'An County, Guangxi Zhuang Autonomous Region, China. The cave system that this loach inhabits is unique in that it is home to four stygobitic fish species. *Protocobitis typhlops* lives sympatricly with *Sinocyclocheilus macrophthalmus* Zhang and Zhao, 2001, *Oreonectes translucens* Zhang et al., 2006 and another undescribed *Oreonectes*. A series of adult male and female individuals that we measured (n=8) ranged in standard length from 39.0 to 55.0 mm.

Nothing is known regarding the behavior of this species. Here we report on the movement and foraging behavior of *P. typhlops*. We performed focal observations of four live individuals for an hour from 1:00 to 2:00 PM local time. We observed *P. typhlops* on the bottom of cave pools spending most of its time on the mud and silt substrate. The pectoral and pelvic fins of this species are modified for movement on the substrate (**Figure 7**). The fins form a structure that looks like a small claw and the "crawling" ability of the fish is obvious with its efficient movement over sediment (they look like lizards crawling over a rock). We observed the fish foraging in the sediments for what we assumed are microcrustaceans and other microinvertebrates. Quick thrusts of the mouth and anterior portion of the head down into the sediments were interpreted as attempts

to capture prey. Although this species occurs with three other stygobitic species, we did not observe any interactions among species.

Conservation Status: There is no conservation plan for this species in China. This species is listed as “Vulnerable D2” by IUCN because of excessive collecting (Kottelat 1996b). The type-locality of *P. typhlops* is located off of the side of a major road outside of the city of Du’An. It is in extreme jeopardy from contamination and considerable human activity. Runoff from the road flows down a series of stairs and into the cave pool. The cave is strewn with garbage and visited regularly for the harvest of freshwater by locals. The cave is also a “pump station.” Frequent changes of water level are another threat for the fish owing to excessive water extraction (**Figure 8**). Noise and vibration from vehicles and pumps may also be a threat.

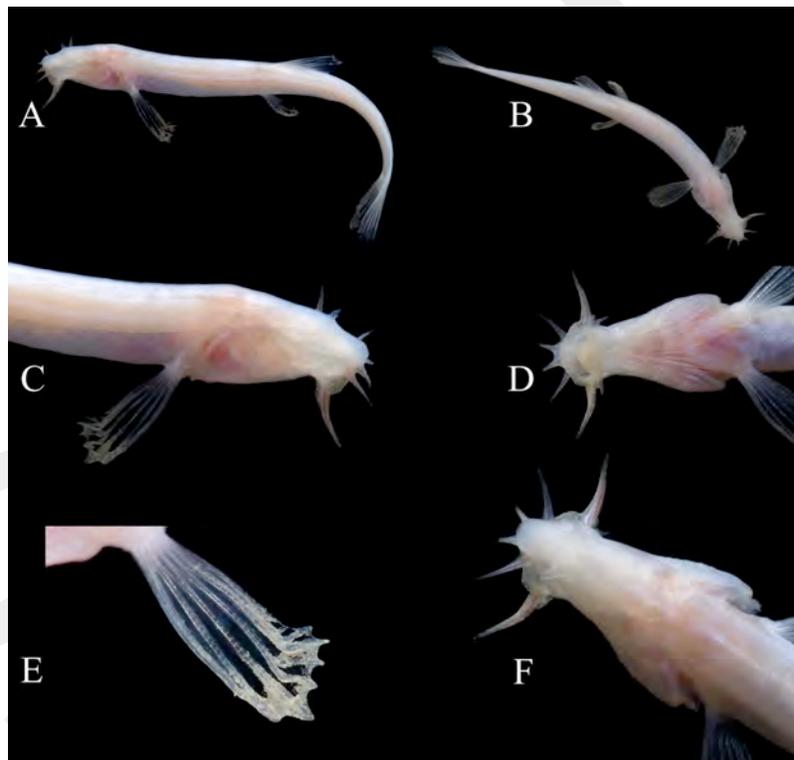


Figure 7. The Xia’ao Blind Cave Loach, *Protocobitis typhlops*, is listed by IUCN as “Vulnerable.” The species is strongly troglomorphic in having little pigment and completely degenerate eyes in adulthood (A–C). Note the cave sediments on the fins and mouth barbels of the fish (A–E) from crawling across cave pools. The ventrally oriented mouth of this species (D) and the entire anterior portion of the head (F) are thrust into cave sediments when foraging for food. This species crawls along the bottom of cave pools using well-developed pectoral (E) and pelvic fins (A and B). Photographs by D.B. Fenolio.



Figure 8. Overharvest of groundwater threatens stygobites around the world, Chinese cavefish included. This is the type-locality of another balitorid, *Heminoemacheilus hyalinus* Lan et al., 1996, a stygobitic fish that has not been seen for a number of years. The karst window depicted here is the only known window into the habitat and is heavily disturbed owing to water extraction.

Crossed Fork Back Golden Line Barbel, *Sinocyclocheilus furcodorsalis* Chen et al., 1997

The Crossed Fork Back Golden Line Barbel is a member of the Cyprinidae and is known from the type-locality and several more nearby caves: the type locality is a cave in Tiane County, Guangxi Zhuang Autonomous Region, China. The cave system that this barbel inhabits is special in that it is home to two stygobitic fish species, including *Triplophysa tianeensis* (see above).

Nothing is known regarding color variation or developmental ontogeny in this species. Here we report on the variation in juvenile color and development of the “horn” structure in *S. furcodorsalis*. We investigated differences between seven individuals, varying between smaller (and presumably younger individuals) to larger (and presumably older) specimens (39.1–109.4 mm standard length, including the type specimen). Juveniles vary between a white basal color with grey highlights to a solid pink color over the entire body surface (**Figure 9A and B**). All smaller individuals had partially translucent abdominal tissue such that the digestive tract is visible through the dermis. The structure that forms the “horn” at the anteriodorsal surface of the fish is less developed in smaller individuals, with the bifurcation of the structure barely visible and the orientation of the structure pointing forward. The largest individuals are solid pink across their body surface. The color of the abdomen is pink and not transparent. The “horn” is significantly more developed in larger specimens, with the bifurcation clearly visible and

curving slightly downward. Further, the thickening and swelling of the tissue along the entire anteriodorsal region is greater in larger specimens, extending all the way back to the dorsal fin and altering the angle at which that fin intersects the body. Further, the largest (and presumably oldest) specimen (**Figure 9C**) had the posterior portion of its body beyond the anal fin, curving downward. Our sample size for the largest specimens (n=1) prohibits us from indicating if this is common among larger individuals.

Conservation Status: There is no conservation plan for this species in China. This species has not yet been assessed by the IUCN, but Proudlove (2006) indicated that the status should be “Vulnerable”. The Crossed Fork Back Golden Line Barbel is from a geographically small range. We have documented heavy collection of this species for both scientific purposes and for the aquarium trade. A conservation plan and protected status is critically necessary for this species.

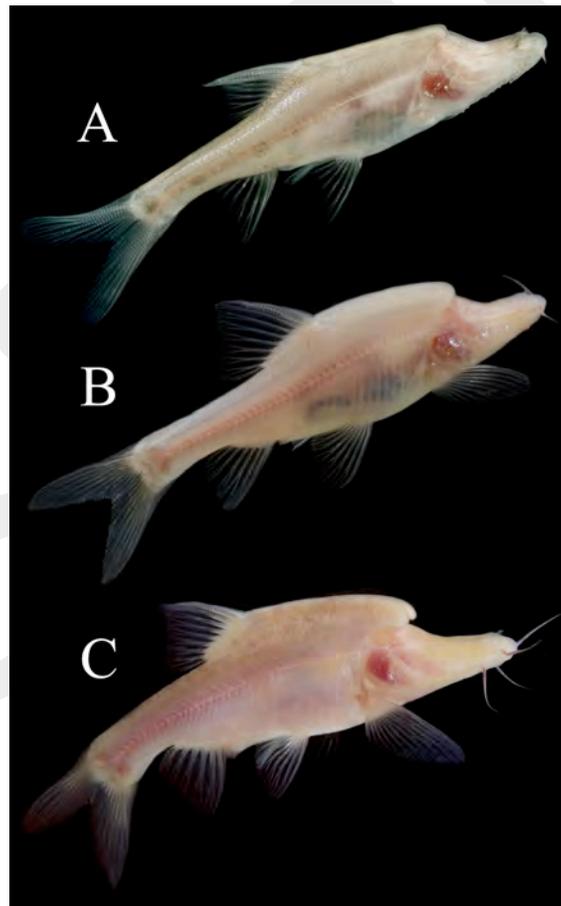


Figure 9. Variation in the color of juvenile Crossed Fork Back Golden Line Barbels, *Sinocyclocheilus furcodorsalis* (A and B), and physical differences between smaller individuals (A and B) and a larger specimen (C).

Acknowledgments

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