

Protection and exploration of the scientific potential of Chinese cavefish

The karst habitats of Southwest China contain the richest cavefish fauna in the world. However, many Chinese cavefish species are now at high risk of extinction. Chinese cavefish have evolved unusual traits, which are important for studying adaptive evolution and modeling human diseases. Here, we call on scientific research institutions to develop programs to conserve precious cavefish species and suggest international cooperation to promote research on cavefish in China.

The karst landscape of exposed and deeply dissected carbonate formations in Southwest China occupies approximately 2.4 million km² and harbors the richest diversity of cavefish species in the world (Ma et al., 2019). To date, 148 species of cavefish have been identified in this region (Supplementary Table S1), belonging to two teleost orders (Cypriniformes and Siluriformes) and four families (Cyprinidae, Cobitidae, Nemacheilidae, and Amblycipitidae), including 78 troglomorphic species (Ma et al., 2019). The golden-line barbel genus *Sinocyclocheilus* (Cypriniformes: Cyprinidae), endemic to the southwestern karst region bordering the Qinghai-Tibetan Plateau of China, contains more than half of all Chinese cavefish species, including a variety of surface and cave-dwelling fish with high phenotypic diversity (Figure 1). Initially established by Bingwen Fang in 1936 (Ma et al., 2019), the *Sinocyclocheilus* genus is primarily distributed in karst cave waters and surface rivers and lakes of the Yunnan-Guizhou Plateau and surrounding areas (including eastern Yunnan Province, central and southern Guizhou Province, and northwest Guangxi Zhuang Autonomous Region). *Sinocyclocheilus* cavefish live in caves or underground rivers for all or part of their life cycles. In total, 75 *Sinocyclocheilus* species have been documented (Jiang et al., 2019) along a relatively narrow geographic distribution range between 24–27 degrees north latitude in the Yunnan, Guizhou, and Guangxi provinces, encompassing nearly 80% in the Pearl River Basin (Zhao & Zhang, 2009). Consistently, recent studies have shown that most *Sinocyclocheilus* species reside in the karst regions of the Pearl River Basin, with a few species found in the lower reaches of the Jinsha River Basin, the confluence of the Jialing and Yangtze rivers, and the upper reaches of the Wujiang River (Lei, 2021).

While new Chinese cavefish species continue to be discovered, human activities and climate change are rapidly degrading their habitats, thus impacting population size and community structure (Zhao & Zhang, 2009). Accordingly,

many cavefish species are now at high risk of extinction due to groundwater pollution and extraction, high-impact tourism, water conservancy, overfishing, and alien species introduction (Zhao & Zhang, 2009). As most Chinese cavefish are regionally endemic, with small populations and ranges, sudden environmental disturbance can be catastrophic (Ma et al., 2019). In 2021, all members of *Sinocyclocheilus* were classified as Class II National Key Protected Species (National Forestry and Grassland Administration, 2021).

Caves are challenging habitats characterized by limited food resources, low oxygen levels, and continuous darkness (Zhao & Zhang, 2009). To survive in these unique environments, Chinese cavefish have evolved various features, including specialized jaws for feeding, additional taste buds and barbels, adipose storage, modified appendages, degenerated eyes, reduced (or lost) pigmentation, degenerated scales, reduced immune activity, lack of circadian rhythm, and degenerated hearing, as well as unusual traits such as humped backs and dorsal horns (Ma et al., 2019; Yang et al., 2016). Given these traits, cavefish are excellent models for studying the relationships among adaptation, evolution, development, and the environment. In addition, cavefish can serve as natural systems to study human health, including eye diseases such as cataracts and myopia, craniofacial abnormalities, albinism, and diabetes (Pennisi, 2016). The *Sinocyclocheilus* and classic Mexican tetra (*Astyanax mexicanus*) cavefish models have many traits in common and can be used for comparative studies in evolutionary developmental biology, neuroscience, behavior, and biomedicine. These two cavefish models also offer a unique opportunity to study convergent evolution. To date, research on Chinese cavefish has predominantly focused on systematics and evolutionary biology, genomics, ecology, physiology, and conservation biology, with an emphasis on the identification of new species, biogeography, laboratory rearing and breeding, morphology, phylogeny, and speciation (Ma et al., 2019).

In recent years, there has been a substantial increase in the use of Chinese cavefish in evolutionary developmental biology. Currently, five *Sinocyclocheilus* species have undergone whole-genome sequencing: i.e., *S. grahami* (Yang et al., 2016), *S. rhinoceros* (Yang et al., 2016), *S. anshuiensis* (Yang et al., 2016), *S. anophthalmus* (Li et al., 2021), and *S. maitianheensis* (Li et al., 2021). According to the *de novo* assemblies of the five genomes, each species is

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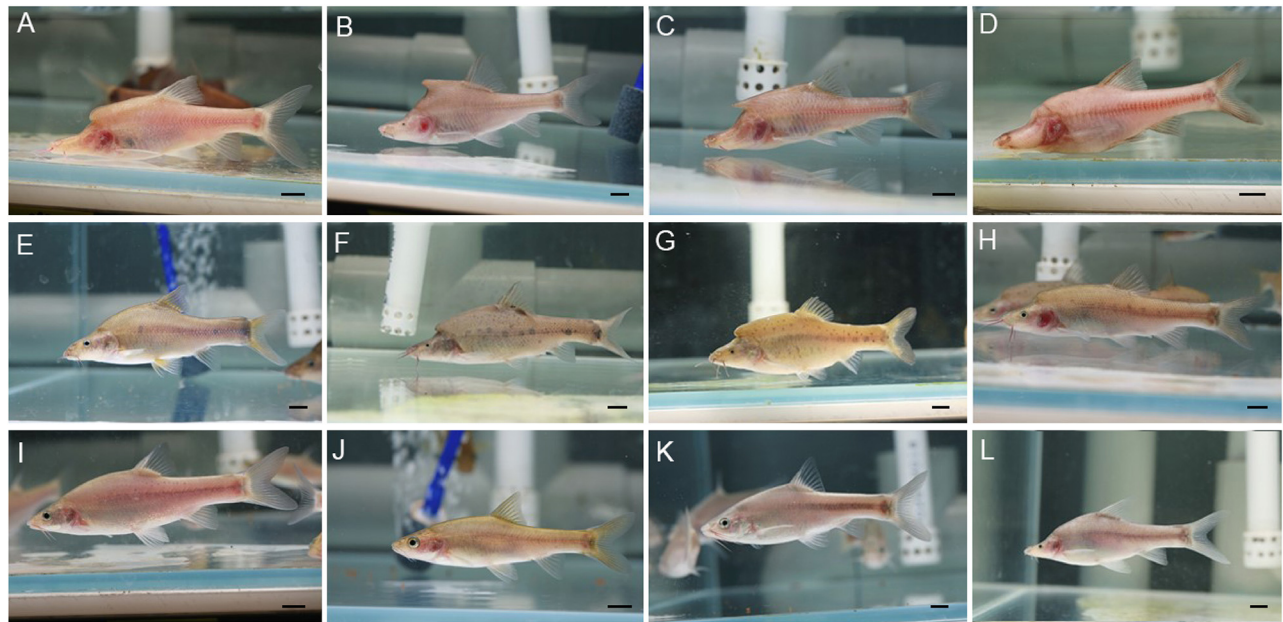


Figure 1 Live *Sinocyclocheilus* species housed in KIZ

A: *S. furcodorsalis*. B: *S. cyphotergous*. C: *S. rhinocerosus*. D: *S. tianlinensis*. E: *S. mashanensis*. F: *S. bicornutus*. G: *S. angularis*. H: *S. zhenfengensis*. I: *S. lingyunensis*. J: *S. guilinensis*. K: *S. huanjiangensis*. L: *S. microphthalmus*. Scale bar: 1 cm. Photos by Shu-Wei Liu.

tetraploid, thus supporting the tetraploid nature of the genus *Sinocyclocheilus*, with genome sizes ranging from 1.6 Gb to 1.9 Gb (Li et al., 2021; Yang et al., 2016), encoding approximately 40000 genes (Yang et al., 2016). *Sinocyclocheilus* evolution has been studied based on the mitochondrial cytochrome *b* (cyt *b*) gene (Jiang et al., 2019; Zhao & Zhang, 2009), the mitochondrial genome (Zhao & Zhang, 2009), and restriction site-associated DNA sequencing (RAD-seq) (Mao et al., 2022). These studies have revealed a relationship between population dynamics and speciation and the uplift of the Qinghai-Xizang (Tibet) Plateau as well as climate change (Yang et al., 2016).

Studies on phenotypic diversity in *Sinocyclocheilus* have revealed variations in eye degeneration (Yang et al., 2016), pigment loss (Yang et al., 2016), the immune system (Li et al., 2021), neuromast distribution (Yang et al., 2016), and lipid metabolism (Lam et al., 2022), thus providing a basis for exploring molecular adaptations to cave life. Genome-wide analyses of surface- and cave-dwelling *Sinocyclocheilus* species have identified candidate genes associated with cave-related traits, including loss of eyes, hearing, pigment, and diurnal rhythm, degeneration of scales, low fecundity, limited immune response, and enhancement of taste, thereby laying a solid foundation for future research (Yang et al., 2016). In addition, new research fields are also emerging for Chinese cavefish, such as bionics, which involves the use of artificial neural networks and 3D scanning technology to track fish movements (Lei, 2021). This system has successfully analyzed *Sinocyclocheilus* behavior, revealing the novel function of the dorsal horn in increasing drag force during swimming and enhancing perception in the lateral line system (Lei, 2021), thereby shedding light on this previously enigmatic structure.

The conservation of the unique Chinese cavefish species will require the cooperation of local communities and other stakeholders. The cavefish habitat is relatively closed, and species dispersal is very limited. Species distribution and differentiation patterns are mainly related to core distribution

area size, geological activity, and river network development (Lei, 2021). To ensure the preservation of Chinese cavefish species, emphasis must be placed on maintaining habitat quality and establishing effective protection measures to prevent the impact of human disturbance and other hazards on the karst, cave, and groundwater regions where these species reside (Ma et al., 2019). Systematic study of different natural cavefish habitats is also critical to provide scientific insights for effective conservation. Based on a recent population matrix model for cavefish, Lei (2021) suggested that cavefish living part (stygophiles) or all (stygobites) of their lives in caves exhibit different responses to environmental disturbances. Stygobites are most affected by adult survival rate, so protection and management efforts should emphasize the adult stage. In contrast, stygophiles are most affected by juvenile survival rate, so protection and management should focus on the juvenile stage (Lei, 2021). When habitat loss and fishing cannot be controlled simultaneously, habitat protection should be prioritized for the protection of stygophiles, whereas fishing bans should be prioritized for the protection of stygobites (Lei, 2021).

Chinese institutions also need to take strong measures to promote the scientific study of cavefish, focusing on topics such as artificial breeding and basic research. The Kunming Institute of Zoology (KIZ), Chinese Academy of Sciences, currently houses 40 live Chinese cavefish species (Figure 1) and has already sequenced several genomes. Kunming is situated in a critical zone for cave fauna conservation and hosted the World Biodiversity Conference (COP15) in October 2021. In 2020, the Cave Fish Development and Evolution Research Group was also established at KIZ. It is essential to encourage international exchange and cooperation to facilitate future research on Chinese cavefish. By strengthening cooperation with scientists studying other cavefish species worldwide, such as the active community of *Astyanax* cavefish researchers, we can leverage the distinct qualities of cavefish models and simultaneously promote the preservation of this unique and irreplaceable resource.

SUPPLEMENTARY DATA

Supplementary data to this article can be found online.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

L.M. conceived the article. L.M. and W.R.J. wrote the manuscript with the assistance of the co-authors. All authors read and approved the final version of the manuscript.

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