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Rapid Communication

First record of the non-native fish *Rhinogobius cliffordpopei* (Nichols, 1925) (Gobiiformes: Gobiidae) in Tibet, China

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Abstract

Nichols' common goby *Rhinogobius cliffordpopei* (Nichols, 1925) is a small-bodied benthic carnivorous fish, and native to central and southeast China. Due to the interregional commercial exchanges of aquaculture and aquatic trade, it has widely introduced numerous water bodies outside the original range in China, but has not been found in Tibet. During a fish survey in May 2022, we collected 22 individuals of *R. cliffordpopei*, including five juveniles, from four sites in the Yarlung Zangbo River in Tibet. This finding suggests that *R. cliffordpopei* has established wild populations in the Yarlung Zangbo. To protect the endemic species, government departments should strictly limit the release to reduce the potential risks of non-native species.

Key words: biological invasion, Tibetan Plateau, exotic fish, Yarlung Zangbo

Introduction

Biological invasions are increasingly serious threats to biodiversity and ecosystem function (Mack et al. 2000; Pimentel et al. 2000; Liu et al. 2021). Freshwater ecosystems are particularly vulnerable to the effects of biological invasions (Strayer 2010) and are also the most heavily invaded by non-native fishes (Gozlan et al. 2010). They are regarded to be potential threats to aquatic biodiversity worldwide (Clavero and García-Berthou 2005; Vitule et al. 2009). In China, numerous non-native fishes have been introduced via aquaculture and aquatic trade, and some have established populations in natural water bodies (Xiong et al. 2015).

Most fishes on the Tibetan Plateau belong to two groups of Cypriniformes: the tribe Schizothoracini (subfamily Schizothoracinae of Cyprinidae) and noemacheiline stone loaches in the genus *Triplophysa* (Nemacheilidae), and they are cold-water species adapted to rapid currents and high altitudes (He et al. 2020). The ecosystems of the Tibetan Plateau are often isolated and natural environments and climate are more serious and extreme that make species vulnerable to external changes, including the introduction of non-native species (Yu et al. 2012). Over the past 40 years, human activities, such as the irrational release of aquaculture fishes and aquaculture development, have resulted in colonization of non-native fish species. In some rivers (e.g., the upper Yellow River and Tarim River), non-native fishes have outnumbered native fish species (Tang and He 2015; He et al. 2020). However, few effective management rules exist to prevent the continued introduction of non-native fishes in Tibet (Zhu et al. 2023). Developing practical regulations is crucial for effective management of non-native species in China (Li et al. 2021).

Nichols' common goby *Rhinogobius cliffordpopei* (Nichols, 1925) is a small-bodied, benthic carnivorous fish native to central and southeast China. It prefers to the littoral zone of slow-moving rivers and streams with sandy or gravelly bottoms (Wu and Zhong 2008). It lies on the bottom of the water and swims intermittently, or swims against the current in the middle and upper water. The pelvic fins are shaped into a suction disc that allows the goby to attach itself to stones and await for opportunities to prey. As a carnivorous fish, it feeds mainly on large zooplankton and aquatic insects (Guo et al. 2014). In recent years, it has been unintentionally transmitted to the Liaohe River, the upper Yellow River, the upper Pearl River and most lakes in Yunnan through release and escape from aquaculture systems, aquatic trade, and accidental introductions as a trash fish (Du and Li 2001; Chen et al. 2021; Jiang et al. 2021). In this study, we report wild populations of *R. cliffordpopei* in natural waters on the Tibetan Plateau for the first time.

Materials and methods

A comprehensive fish survey was conducted in May 2022 in the middle and lower Yarlung Zangbo River of the Tibetan Plateau, involving 117 sites, including six lakes, four reservoirs, six wetlands, and two tributaries. The sampling sites belonged to the Dwc and Dwb categories according to the Köppen-Geiger climate classification (Kottek et al. 2006). The climate types are characterized by low temperature and dry winters. The average temperature in the warmest month is 10-15 °C, and the annual precipitation is approximately 400 mm (Lin and Wu 1981). Notably, the water temperature is lowest in January, close to 0 °C, and highest in July, approaching 16 °C.

Specimens were captured by dip nets (0.5 m diameter, mesh size 1 mm) and the total and standard length (nearest to 0.01 mm) and weight (nearest to 0.01 g) were measured. Then, the individuals were euthanized by an overdose of anesthesia. We clipped a small piece of fin tissue and stored it in 95% alcohol for subsequent molecular analysis. All specimens were stored in 95% ethanol for long-term preservation. Morphological measurements and sequencing (*COI*, Ward et al. 2005; *cyt b*, Sevilla et al. 2007) were conducted in the laboratory. Phylogenetic analysis based on mitochondrial *COI* gene was conducted using the maximum likelihood method. The sex of the specimens was determined by observing the gonads. The water environmental variables of the habitats were measured using the HACH 2100Q (USA, ©Hach Company) and YSI Pro20 (USA, ©YSI Incorporated).

Table 1. Latitude, longitude, altitude, and environmental variables of the sampling sites.

Site	Latitude (°N)	Longitude (°E)	Habitat type	Altitude (m)	Environmental parameters				
					Turbidity (NTU)	Water temperature (℃)	Dissolved oxygen (mg/L)	Conductively (us/cm)	pH
1	29.376116	90.831398	Marsh	3640	52.6	17.9	2.86	280.9	7.87
2	29.196527	94.099960	Stream	3879	8.1	15.9	4.82	181.3	8.49
3	29.312222	91.090650	Reservoir	5017	14.7	15.5	4.66	240.3	8.82
4	29.248386	91.694463	Marsh	5212	25.3	12.5	3.62	85.2	8.64

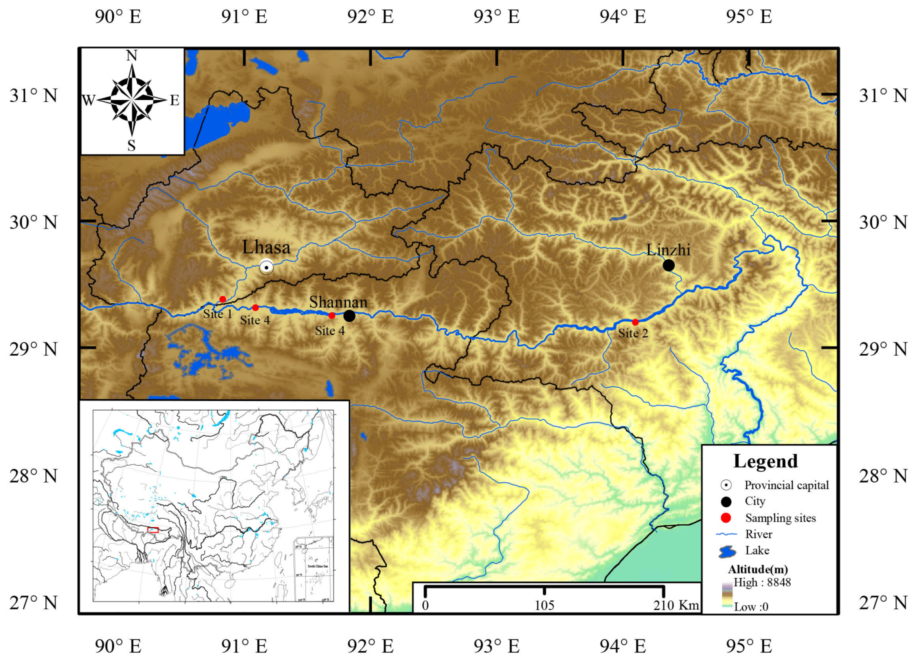

Figure 1. The location of the sampling sites.

Results

Rhinogobius cliffordpopei was found at four of 117 sampling sites (Table 1, Figures 1 and 2). A total of 22 individuals of *R. cliffordpopei* were captured, including six males, 11 females, and five juveniles. The specimens were small in size (TL < 50 mm; see Table 2), the total length, standard length and total weight of the specimens ranged from 23–47 mm, 18–39 mm and 0.17–1.28 g. The speciemens have two dorsal fins (Figure 3); no lateral line; the pelvic fins shaped into a suction disc; no predorsal scales; non-filamentous first dorsal fin in males; transverse sensory papillae on cheek; and more than 16 pectoral fin rays. These morphological characters are obviously different from that of the related species *Rhinogobius giurinus*. The sequencing results further validated the results of morphological analysis. The sequences of the specimens matched the published sequences of *R. cliffordpopei* (accession numbers MT413342 for *COI* and MK204744 for *cyt b* in the NCBI database), with 99.85% match for *COI* and 100% match for *cyt b*. Only two haplotypes were identified from twenty-two sequences. The maximum likelihood tree suggested that the two haplotypes were nested within the *R. cliffordpopei* clade (Figure 4), further supporting the taxon identification.

Figure 2. The habitat of the specimens. Photographs were taken by Jianshuo Qian.

Table 2. The morphometric characteristics of specimens.

Sex	Number	Total length (mm)		Standard length (mm)		Total weight (g)	
		Range	Mean	Range	Mean	Range	Mean
Male	6	32–47	39.83	28–38	32.79	0.36–1.28	0.69
Female	11	34–47	39.25	28–39	32.36	0.25–0.74	0.53
Juvenile	5	23–31	25.80	18–24	20.00	0.16–0.34	0.21

Discussion

The discovery of juvenile *R. cliffordpopei* strongly suggests that the species has established populations in natural water bodies in Tibet. High fecundity and preference for littoral habitats may have contributed to the establishment of the species on the Tibetan Plateau. In *R.* cliffordpopei, the average relative fecundity was 2,069 eggs g^{-1}, in contrasts to 1,580 in *R.* giurinus, the average egg diameter was 613 μm, in contrasts to 470 μm in *R.* giurinus (Guo et al. 2013). *Rhinogobius cliffordpopei* more frequently inhabits the littoral zone than the deep zone because of the abundance of macrozooplankton and aquatic insects (Guo et al. 2014). Thus, wetlands, as shallow water habitats, are more likely to be invaded by this species.

Rhinogobius cliffordpopei is *r*-selected, which may help establish populations in the wild (Sui et al. 2016). In the 1950-60s, the species was introduced to the Yunnan-Guizhou Plateau (southwestern China), where it rapidly dispersed

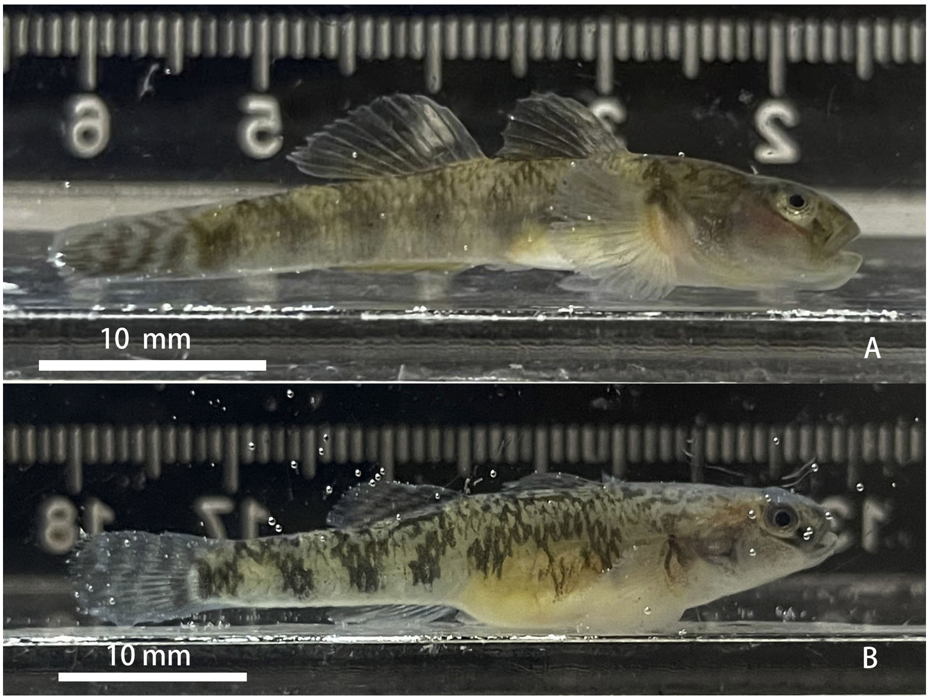

Figure 3. The photographs of *Rhinogobius cliffordpopei* individuals, A. male individual, B. pregnant female individual. Photographs were taken by Junhao Huang.

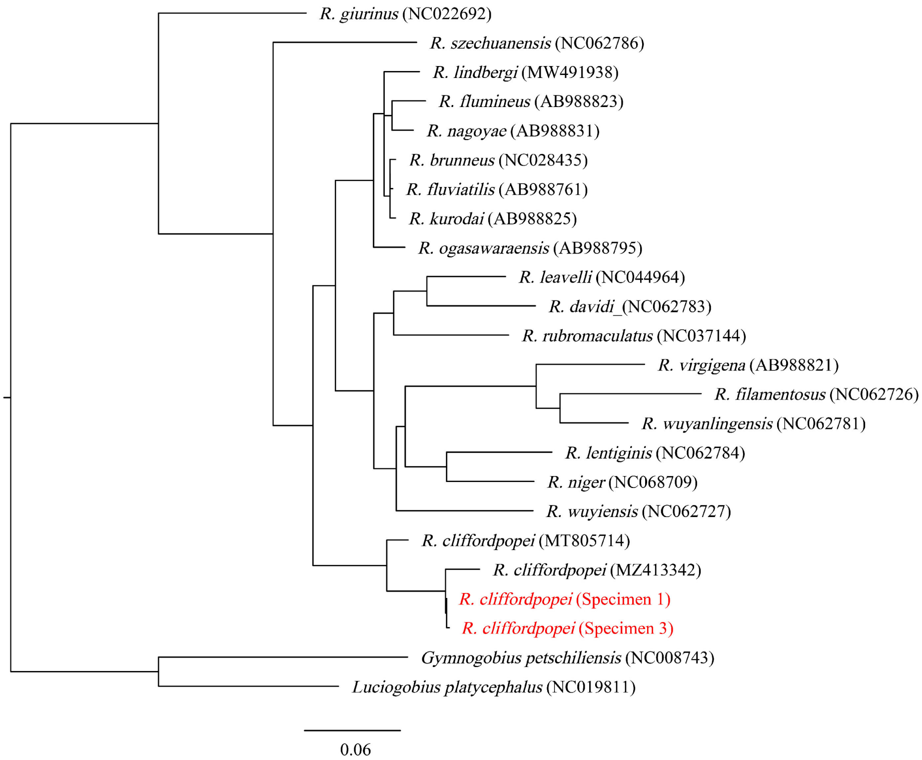

Figure 4. The maximum likelihood phylogeny based on the 553 bp of *COI* gene of the two specimens sequenced in this study and the sequences of 19 *Rhinogobius* species in GenBank were performed, with *Gymnogobius petschiliensis* and *Luciogobius platycephalus* as two outgroups. The specimens sequenced in this study are in red.

to most water bodies and became a dominant species in many lakes (Yuan et al. 2010; Tang et al. 2013). *Rhinogobius cliffordpopei* is the most common invasive species in upper Yellow River (Jiang et al. 2021) and has been found in upper Pearl River (Chen et al. 2021). The introduction of *R. cliffordpopei* into rivers and lakes outside its native range may negatively affect local freshwater ecosystems. A gut content analysis revealed that *R. cliffordpopei* feeds on fish eggs and larvae during the juvenile, sub-adult, and adult stages (Guo et al. 2014), which may lead to the decline of native fishes. Thus, *R. cliffordpopei* poses a risk to native fish populations in the local ecosystems.

Climate change and hydropower construction increase the invasion risk of non-native species, exacerbating their negative impacts on local ecosystems (Hellmann et al. 2008; Sun et al. 2020). Although *R. cliffordpopei* has a low-medium risk under current climatic conditions (Li et al. 2017), the potential risk is expected to increase under climate warming in the future in Yalung Zangbo River. A similar case occurred with the naked goby *Gobiosoma bosc,* which was introduced to Western Europe from North America (Dodd et al. 2022). The results of the model predictions also suggested that under the influence of climate change and hydropower construction, non-native fish species show substantial habitat expansion, while native species shift their distribution to tributaries and higher elevations (Sun et al. 2020).

Previous surveys have reported 15 non-native freshwater fish species on the Tibetan Plateau (Chen and Chen 2010; Fan et al. 2016; Ding et al. 2022). Recently, two more non-native fish species, tench (*Tinca tinca*) and mosquitofish (*Gambusia affinis*), have been identified in Tibet (Wang et al. 2023; Zhu et al. 2023). Most of non-native fishes in Tibet were released with aquaculture fishes. Some small-bodied fish released with aquaculture fishes, such as *R. cliffordpopei*, *Micropercops cinctus* and *Oryzias sinensis*, may go unnoticed but the invasion risk should not be underestimated. In fact, the proportion of the small-bodied trash fishes with strong adaptability and invasion potential is increasing among non-native fishes (Ding et al. 2014).

While previous scientific studies have focused on investigating the distributions and resources of species, more recent studies have begun to access the impacts and underlying mechanisms of invasive species on ecosystems (Gu et al. 2015; Yu et al. 2019). To reduce the threat of non-native species, it is necessary to develop strategies aimed at prevention, which is the most cost-effective way to manage future invasions (Liu et al. 2021). Predicting the potential distribution of non-native species is also essential for identifying high-risk areas and developing a comprehensive monitoring system to provide a basis for invasion management (Dodd et al. 2022).

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Author's contribution

Jianshuo Qian conducted field sampling work and wrote articles. Shaoqing Lin was responsible for species morphological identification, Xi Wang was responsible for editing and polishing the article, and Huanshan Wang was responsible for molecular sequencing and identification.

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