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Rapid Communication

First record of the non-native western mosquitofish, *Gambusia affinis* (Baird & Girard, 1853), in the Eastern Himalayas, China

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Abstract

The western mosquitofish, *Gambusia affinis* (Baird & Girard, 1853), from North America, is considered one of the 100 worst invasive species in the world. In October 2020, sixteen individuals of western mosquitofish were captured from the Eastern Himalayas of China, making this the first record of western mosquitofish in the region. Since the Eastern Himalayas is part of the Himalaya Biodiversity Hotspot, mosquitofish could represent a potentially significant threat to local biodiversity in the whole Himalaya or the Qinghai-Tibetan Plateau (QTP), including indigenous fishes. More monitoring and management of western mosquitofish should be conducted in the Eastern Himalayas to mitigate invasion risk and protect the native fish biodiversity from this exotic species.

Key words: invasive species, freshwater fish, Qinghai-Tibetan Plateau, invasion risk

Introduction

Freshwater fish represent one-fourth of vertebrates globally, including more than 18,000 species (Richard 2021). Freshwater fish communities are facing increasing anthropogenic pressures in aquatic ecosystems, which change their biodiversity and threaten the services they provide to human populations (Villéger et al. 2017; Su et al. 2021; Albert et al. 2021). During recent decades, freshwater fish invasions have caused unprecedented impacts on native fish biodiversity (Early et al. 2016; Liu et al. 2019). This problem has become a serious environmental issue, and is receiving more attention from scientists, government managers, and the public worldwide (Early et al. 2016; Su et al. 2021; Diagne et al. 2021).

China is a country with a mega-diverse freshwater fish fauna, with 1,651 species recorded (He et al. 2020). Recent studies have reported that a total of 439 freshwater fish species have been introduced to China (Xiong et al. 2015), contributing to homogenization of freshwater fish fauna across the country (Liu et al. 2017). The predicted risks of establishment of non-native

fish species in China suggest that the number of established invasive species and their impacts may increase over time (Liu et al. 2019; Luo et al. 2019).

The Himalayas are located in the southern margin of the Qinghai-Tibetan Plateau (QTP), known as the "Third Pole" (Qiu 2008) and the "Water Tower of Asia" (Immerzeel et al. 2010). The Eastern Himalayas harbours rich repositories of biodiversity; these include some of the world's most threatened and endemic species (Brooks et al. 2006). Because of disparities in habitat loss and protection (Hoekstra et al. 2005), the Eastern Himalayas is counted among "crisis ecoregions" within "biodiversity hotspots" (Brooks et al. 2006) and, the aquatic ecosystem there is very sensitive and vulnerable (Sharma et al. 2009). Indigenous fishes, mainly belonging to the family Schizothoracinae, Sisoridae and Nemacheilidae, are endemic to the Eastern Himalayas of China and are also very important fish germplasm resources (Wu and Wu 1992). Recently, many non-native fish species have been introduced and successfully established in the QTP and adjacent areas (Xiong et al. 2015; Sui et al. 2016; Li et al. 2017, 2019; Jia et al. 2019). Fish invasion has been reported to be a main reason for sharp declines in native fish stocks (Chen and Chen 2010; Liu et al. 2015; Ding et al. 2019).

The western mosquitofish *Gambusia affinis* (Baird & Girard, 1853) along with the eastern mosquitofish *Gambusia holbrooki* Girard, 1859, both native to North America, are the most widely distributed freshwater fishes in the world (Pyke 2005). Mosquitofish have been widely introduced as biological agents for mosquito control and have established populations on all continents except the Antarctica (Pyke 2008). Western mosquitofish has been included in the list of "100 of the World's Worst Invasive Alien Species" by the International Union for Conservation of Nature (IUCN) (Lowe et al. 2000). The western mosquitofish was introduced to mainland China in the 1920s (Cheng et al. 2018). In China, western mosquitofish not only widely enter plain areas, but also further spread to plateau areas (Chen 2013). The western mosquitofish is a potential threat to biodiversity, and has been categorized as having "high risk" of invasiveness in the middle reach of the Yarlung Tsangpo River, QTP, China (Sui et al. 2016; Li et al. 2017).

Materials and methods

Fish sampling was conducted in Mêdog and Zayü in the Eastern Himalayas in October 2019 and 2020 (Figure 1). The climate in the sampling area is mild and rainy. The annual average temperature in Mêdog is 16–18 °C and that in Zayü is 8–20 °C. The survey was conducted in both natural and artificial habitats, including in rivers, streams, lakes, and ponds. Fish were collected at each site using different sampling tools according to the different habitats. We surveyed a combined total of 64 sampling sites in October 2019 and October 2020 using drift gill nets (mesh size: 10, 30 and 50 mm; length: 25 m; depth: 1.2 m), circle dip nets (diameter: 0.5 m; mesh

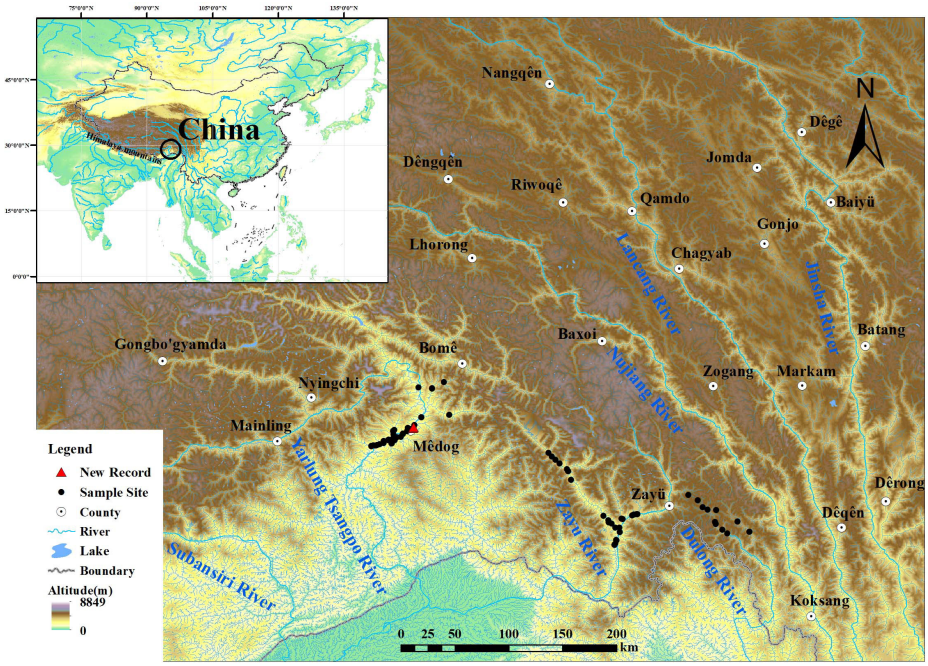

Figure 1. Map describing the sampling sites of the western mosquitofish *Gambusia affinis* from the Eastern Himalaya. The black circle on the top left-hand corner indicates the sampling location.

size: 4 mm; length: 6.5 m) that were placed in the river or lake from afternoon to the following morning (approximately 12 hr), and small backpack electrofishers that were mainly used in small, narrow streams (240 V, 50 Hz, 30 min).

All specimens were measured immediately after capture for total length (TL), standard length (SL), and body weight (W). A piece of fin or muscle tissue of the specimens was clipped and stored in 95% ethanol until DNA extraction, and the specimens were fixed in 5% formaldehyde for 24 hr and stored in 10% formaldehyde for long-term preservation. Fishes were identified to the species level according to dorsal and anal fin ray counts (Walters and Freeman 2000) and the Blast in the NCBI database (https://www.ncbi.nlm.nih.gov/) was used for the mitochondrial COI (Cytochrome oxidase subunit I) gene subsequent sequence similarity search and alignment. The phylogenetic analysis of sequence variation of the COI gene were conducted in MEGA X (Kumar et al. 2018) by using the Neighbor-Joining method (Saitou and Nei 1987). All specimens were deposited in the Museum of Hydrobiological Sciences, Institute of Hydrobiology, Chinese Academy of Sciences (MHBS, IHB, CAS).

Results

Both morphometric and molecular data confirmed that the mosquitofish individuals caught in this study are all *Gambusia affinis* (Figures 2, 3). Sixteen western mosquitofish individuals (three males and 13 females including 10 gravid individuals) were collected in Mêdog from the Eastern Himalayas (Lianhua Park: N29.325753; E95.332779; Yadong Farm: N29.330163; E95.337078) (Figure 1) and the morphometric features of the specimens are

Figure 2. Western mosquitofish *Gambusia affinis* collected from Mêdog, Eastern Himalayas (a: female; b: male) (the Museum of Hydrobiological Sciences, Institute of Hydrobiology, Chinese Academy of Sciences (MHBS, IHB, CAS)). Photo by Ren Zhu.

shown in Table 1. Total length (TL) ranged from 20 to 39 mm, standard length (SL) ranged from 16.5 to 32.5 mm and body weight (W) varied between 0.07 and 0.72 g. This collection is the first record of western mosquitofish in the Eastern Himalayas, China.

Discussion

Our study confirmed that the mosquitofish species in the Eastern Himalayas is *Gambusia affinis*, which is also known to have invaded other parts of China (Gao et al. 2017). Previous investigations of fish biodiversity in Tibet did not record the western mosquitofish (Chinese Academy of Sciences Integrated Expedition in Tibet 1964; Wu and Wu 1992; Tibet Autonomous Region Fisheries Bureau 1995; Jin and Ma 2020; Liu et al. 2021). Although the western mosquitofish has not been reported from the Eastern Himalayas of China prior to 2020, it had already invaded the adjacent area (most parts of Yunnan) (2019–2020 survey results; Li et al. 2017; Chen 2013). We infer therefore that western mosquitofish invaded the Eastern Himalayas relatively recently, likely introduced not much earlier than 2020. This species might establish a feral population in the Eastern Himalayas – especially given that specimens collected in this study already included gravid females. Western mosquitofish have a wide range of environmental tolerances in habitats where they occur and even survive under ice (Krumholz 1944). Nine species of fish were classified as being of high invasiveness risk in the middle reach of the Yarlung Tsangpo River,

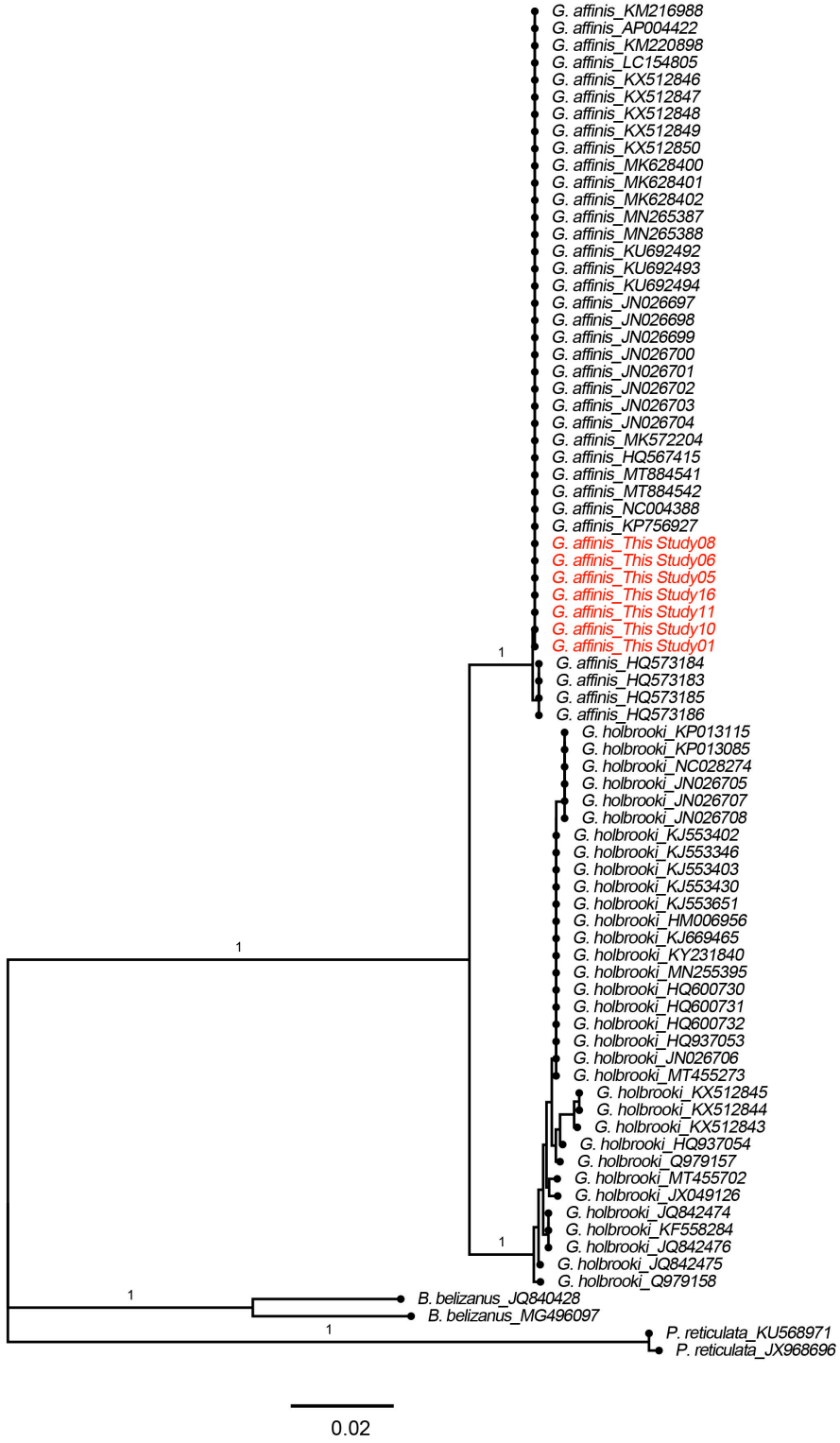

Figure 3. Molecular phylogenetic analysis by Neighbor-Joining method based on partial COI sequences of 620 bp length. The red-color font indicates individuals collected from this study and other sequence information is from NCBI database (https://www.ncbi.nlm.nih.gov/).

including the western mosquitofish (Sui et al. 2016; Li et al. 2017). With global warming and rapid economic development, western mosquitofish are likely to spread widely to other water systems in the whole Himalayas or the QTP due to more human activities, threatening further indigenous fishes.

Table 1. Features of the western mosquitofish *Gambusia affinis* collected in October 2020 from the Eastern Himalayas of China. All specimens are deposited in the Museum of Hydrobiological Sciences, Institute of Hydrobiology, Chinese Academy of Sciences (MHBS, IHB, CAS).

Sites	Specimen No.	TL(mm)	SL(mm)	TW(g)	Sex (♀ & ♂)
Lianhua Park	1	34.5	28.5	0.41	♀ (gravid)
	2	33.5	27.5	0.40	♀ (gravid)
	3	24.5	20.0	0.22	♀ (gravid)
	4	23.0	19.0	0.11	♂
	5	21.5	18.0	0.09	♀
	6	20.0	16.5	0.07	♀
Yadong Farm	7	37.0	30.0	0.54	♀ (gravid)
	8	37.0	30.0	0.56	♀ (gravid)
	9	39.0	32.5	0.72	♀ (gravid)
	10	34.5	28.5	0.39	♂
	11	35.0	29.5	0.49	♀ (gravid)
	12	34.5	29.0	0.36	♀ (gravid)
	13	32.5	27.0	0.33	♀ (gravid)
	14	33.5	27.5	0.36	♂
	15	30.5	25.5	0.29	♀ (gravid)
	16	26.0	21.0	0.18	♀

Abbreviations: TL: total length; SL: standard length; TW: total weight.

Since the 1970s, western mosquitofish have been introduced in many parts of China to kill mosquitoes. This strategy of encouraging the use of non-native mosquitofish to control *Aedes aegypti* (Linnaeus, 1762) by eating their larvae is misguided (Azevedo-Santos et al. 2016). The efficacy of targeted mosquito control by fish is questionable (Pyke 2008). In addition, there is potential for impact on non-target species. For example, field observations have shown that the western mosquitofish preys on eggs of native fish and on tadpoles in the central Yangtze River, China (Cheng et al. 2018). With many new toad species found in the Eastern Himalayas (Shi et al. 2020), this suggests that the western mosquitofish may cause significant negative impact on these amphibians (Pyke 2008; Cheng et al. 2018). Introducing non-native fishes (e.g., *Pseudorasbora parva* (Temminck & Schlegel, 1846)) into the aquatic environments of the QTP has been shown to negatively affect native biodiversity (Chen and Chen 2010; Ding et al. 2019; Wang et al. 2021). Despite growing awareness of the damage caused by non-native fish, China's existing policies and legislation still lag behind those already implemented in other countries, such as in Europe, the USA and Canada (Li et al. 2021). While the western mosquitofish was found in Mêdog in this study, it has not yet spread widely to other parts of the Himalayas. Mosquitos may threaten the health of humans and vertebrates; however, the use of non-native species to manage other non-native organisms should be carried out with caution. Therefore, we appeal to the local government, environmental protection organizations and fishermen to work together to mitigate the potential invasion risk of western mosquitofish.

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Author's contribution

ZW and RZ: investigation, data analysis and original draft; XL and JQ: methodology and software; XS: review, editing, project administration and funding acquisition. All authors contributed to the manuscript draft preparation and review.

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