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Research Article

New records and range expansions of invasive fish species in Georgia

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Abstract

Invasive species are a significant challenge for freshwater ecosystems on a global scale. They can cause serious ecological and economic damage. The aim of our research was to document the range expansion of freshwater invasive fishes in Georgia. Fish studies were conducted during the years from 2019 to 2022 using standard methodologies. A DNA barcoding approach was also employed to validate our findings. During the course of the study, invasive Sharpbelly – *Hemiculter leucisculus* from different locations of eastern Georgia has been detected, which is the first case of the existence of this species in the country. In addition, we document a significant range expansion of two other non-native species in Georgia. Amur goby, *Rhinogobius lindbergi,* was collected in western Georgia, which was previously known only from eastern Georgia, and the occurrence of mosquitofish, *Gambusia holbrooki,* was confirmed in east Georgia for the first time. It is expected that the rapid expansion of the range of the mentioned species may lead to a decrease in local and endemic species populations in Georgia. With the development of aquacultural activities in the country, these risks will also increase.

Key words: freshwater, illegal translocation, sharpbelly, Amur goby, mosquitofish, Caucasus

Introduction

Invasive species are one of the main threats to global biodiversity (Early et al. 2016; Leuven et al. 2017; Duenas et al. 2021). Their spread is often facilitated by humans, but they can expand their distribution independently within introduction areas (Hoffmann and Courchamp 2016; Eagderi and Moradi 2017; Mousavi-Sabet et al. 2019). These processes are common in inland waters, and many fish species became locally widespread and invasive after initial introduction (Robson et al. 2016; Leuven et al. 2017; Taybi et al. 2020). One of the most common vectors for fish introductions is aquaculture, where the target species are frequently accompanied by hitchhiker species. It is believed that the widespread introduction of many of the worst invasive species, such as *Pseudorasbora parva*, *Rhinogobius* spp., and *Carassius* spp., is because of unintentional introductions (Aparicio et al. 2012; Japoshvili et al. 2013; Ninua et al. 2013; Eagderi and Moradi 2017; Mousavi-Sabet et al. 2019). The second, equally acute problem is the translocation of new species

within a region or country, from one reservoir to another, by fishermen, which mostly happens illegally (personal observations in our study area, though hardly documented globally).

While spreading, invasive species are also accompanied by disease agents and parasites new to the invaded region (Molnár et al. 2019). At the end, the invasion of alien fish species into new areas may cause irreversible losses and degradation of local ecosystems, as well as huge economic costs (Arthington 1991; Cambray 2003; Ribeiro et al. 2009; Cuthbert et al. 2021). The aquatic invasion is challenging for the whole world; however, in developing countries, some peculiarities make the alien introductions even more dramatic. These include weakly developed legislation, the absence of control mechanisms, the absence of monitoring programs and early warning mechanisms, a low level of environmental education, and poverty (Perrings 2005; Kuljanishvili et al. 2021). Arguably, when developing countries are within biodiversity hotspots, the environmental and economic threats associated with alien species get more dramatic. The South Caucasus region and Georgia are part of the Caucasus biodiversity hotspot and are characterized by the abundance of water resources and the diversity of freshwater species (Myers et al. 2000; Ninua et al. 2013; Kuljanishvili et al. 2020; Epitashvili et al. 2020). At the same time, this biodiversity is under a multitude of threats, with one of the main ones being invasive species (Zazanashvili and Mallon 2009). According to recent studies, up to 119 freshwater fish species are distributed in the inland waters of the Southern Caucasus region, of which seven are considered alien and also seven are endemic (Kuljanishvili et al. 2020). Some species were introduced during the Soviet era for aquaculture purposes. Such introductions were often accompanied by the accidental entry and spread of small-sized alien fish in the region. This is for instance, how the invasive Prussian carp – *Carassius gibelio* (Bloch, 1782) and Stone moroko – *Pseudorasbora parva* (Temminck & Schlegel, 1846) are believed to have spread in Georgia during the 80s and 60s, respectively (Japoshvili et al. 2013; Ninua et al. 2013).

In the present work, we provide evidence for a new alien species – sharpbelly (*Hemiculter leucisculus* (Basilewsky, 1855)) for Georgian freshwaters and add to our knowledge of the distribution of oldly introduced invasive mosquitofish (*Gambusia holbrooki* Girard, 1859) and the recent invader Amur goby (*Rhinogobius lindbergi* Berg, 1933).

Materials and methods

Sample collection

Materials used in this study were collected during the various field campaigns in different parts of Georgia in 2019–2022 (Figure 1) within the framework of the Caucasus Barcode of Life (CaBOL) project (Thormann et al. 2019). Fish sampling has been conducted using the electro fishing device – EFGI 650

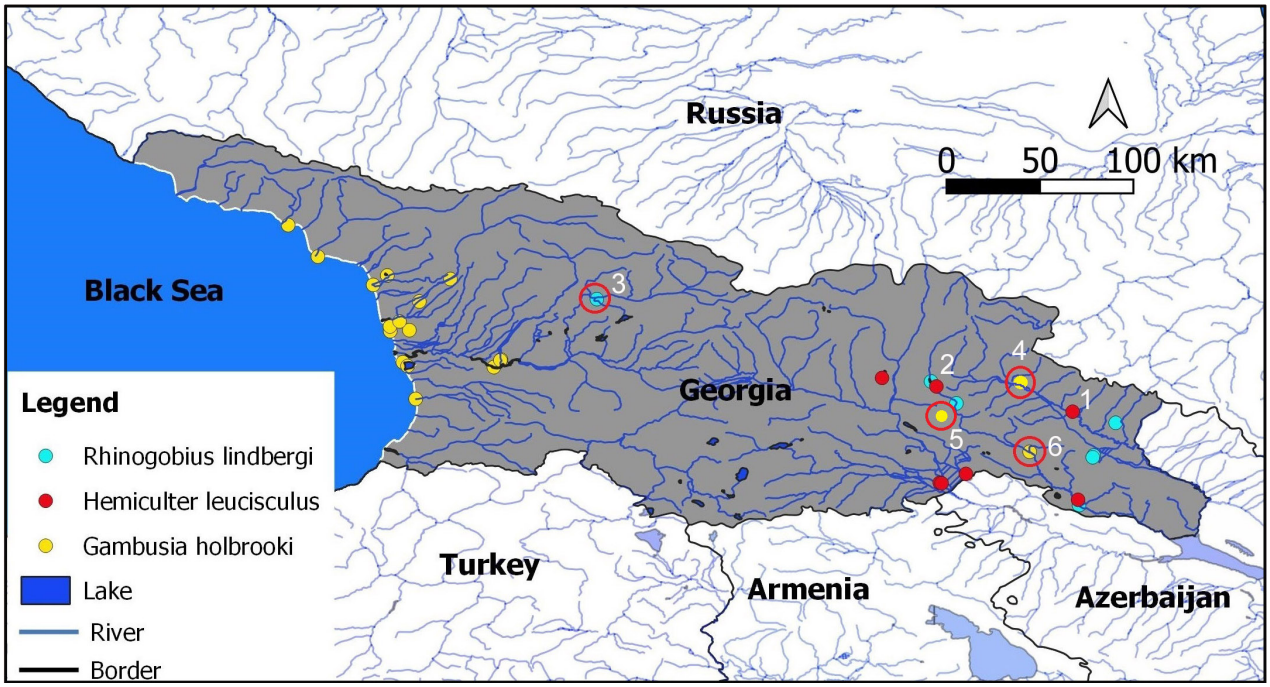

Figure 1. Map of Georgia with sample collection localities marked with colored dots. The red circles border new places where the *G. holbrooki* and *R. lindbergi* were observed for the first time. Particular localities mentioned in the main text are denoted with numbers: 1 – Alazani River (41.831858N; 45.866231E); 2 – Sioni Reservoir (41.9905N; 45.0128E); 3 – Shareula (Rioni basin) (42.470233N; 43.066942E); 4 – Shakriani village (42.001040N; 45.601422E); 5 – Ujarma village, 41.773537N; 45.143819E); 6 – right tributary of the Iori River (41.585057N; 45.586583E).

(http://www.electric-fishing.de/efgi6_e.html). In some cases, (mostly in lakes and deep channels), we also used fishing rods, a frame net (mesh size 3 mm), and a cast net (mesh size 10 mm) to obtain specimens. For species identification we used standard identification guides available for Caucasian fishes (Kottelat and Freyhof 2007; Ninua et al. 2013). Taxonomy is according to FishBase (Froese and Pauly 2022) and detailed information about sample collection localities and species is given in the supplementary table.

Sample processing

The fin clips of the obtained material were immediately fixed in 96% molecular grade ethanol for the subsequent genetic study. All specimens were initially identified to species level using standard morphological characters (e.g. Kottelat and Freyhof 2007) and vouchered in the fish collection of the Institute of Zoology of Ilia State University (Georgia). With morphological identification, we used a DNA barcoding approach based on mitochondrial cytochrome oxidase subunit I (COI) to validate species identity. For this reason, DNA extraction from fin clips, PCR (Polymerase Chain Reaction), and DNA sequencing were conducted within the framework of the CaBOL project using a standard barcoding pipeline (Epitashvili et al. 2020). All newly-generated DNA sequences were submitted to the Barcode of Life Data System (BOLD, http://v4.boldsystems.org/), where they were automatically assigned Barcode Index Numbers (BINs). They can be accessed via the public dataset "Freshwater Fishes of Georgia"

Figure 2. Vaucher speciemens of studied invasive alien species of Georgia. A –Sharpbelly (*Hemiculter leucisculus*) from the Alazani River, eastern Georgia; B – Amur goby (*Rhinogobius lindbergi*) from the Shareula River, Rioni Basin, western Georgia; C – Eastern mosquitofish (*Gambusia holbrooki*) from the small swamp, near Shakriani Village, Kakheti region, eastern Georgia. Scale bars: 1 cm. Photographs by G. Epitashvili

(project acronym: SCCBO). The sequences were further checked against the BOLD database and BOLD tools were used for the calculations of sequence divergence and relationships (Ratnasingham and Hebert 2007).

Results

Sharpbelly – *Hemiculter leucisculus* (Basilewsky, 1855)

In July 2020, during the field work on the Alazani River in eastern Georgia (41.831858N; 45.866231E), a single specimen, morphologically very close to *Hemicluter leucisculus* (Figure 2A) was collected. Later DNA barcoding confirmed that the specimen indeed belonged to *H. leucisculus*, and this was the first confirmed case of the occurrence of this fish in Georgia. During subsequent fieldwork in 2020–2022, we collected 35 additional specimens of *H. leucisculus* from 10 more locations (Figure 1; Supplementary material Table S1). Due to the high morphological similarity of *H. leucisculus* to Caspian Shamaya (*Alburnus chalcoides* (Güldenstädt, 1772)), reexamination of the recent collections kept in the Institute of Zoology of Ilia State University (Tbilisi, Georgia), resulted in the finding of another record of *H. leucisculus* collected in 2017 from the Sioni Reservoir (N41.9905; E45.0128) labeled as *A. chalcoides*.

From the collected material, a 658-bp-long COI barcode region was successfully generated for 12 specimens of *H. leucisculus* with no stop

codons or indels. The mean uncorrected *p*-distance within Georgian specimens was 0.33% (maximum – 0.66%). All the barcodes were assigned to a BIN BOLD: ACB5189, representing *H. leucisculus*. This BIN includes more than 600 specimens with a maximum within-BIN divergence (*p*-distance) of 3.83% and the assignment of Georgian specimens to the species is thus undoubted.

Amur goby – *Rhinogobius lindbergi* Berg, 1933

In July 2019, one specimen of an unknown goby (Figure 2B) was caught in Racha, Western Georgia, in the mountainous river Shareula (Rioni Basin) (42.470233N; 43.066942E). As DNA barcoding of the COI gene (BOLD process ID: SCCBO204-22, BIN BOLD: ACB4145) showed, this specimen belongs to the genus *Rhinogobius*. On the phylogenetic tree (not shown), this species was grouped with *R. lindbergi,* including the specimens previously collected from western Georgia. In addition, a single specimen of *R. lindbergi* was also observed in the Sioni Reservoir along with *Hemiculter leucisculus* (see above).

Eastern mosquitofish – *Gambusia holbrooki* Girard, 1859

A single specimen of *G. holbrooki* was obtained on July 23, 2019 in the Kakheti region, in a small swamp near Shakriani Village (42.001040N; 45.601422E). The specimen was caught with a kick net while sampling benthic macroinvertebrates. The second case of recording a single specimen of *G. holbrooki* (Figure 2C) occurred in December 2021, in the small right tributary of the Iori River (41.585057N; 45.586583E) and no subsequent sampling of the shoreline of the main river was successful. The third case of sampling this species happened in June 2022, during recreational fishing at Ujarma Lakes (Kakheti region, near Ujarma Village, 41.773537N; 45.143819E). In contrast to the previous two cases, we detected a large population of mosquitofish spread all over the lake. At the time of writing, there is evidence of the occurrence of *G. holbrooki* in three locations in eastern Georgia (Figure 1; Table S1).

Discussion

Hemiculter leucisculus is native to eastern Asia: China, North and South Korea, Hong Kong, Japan, and the Amur River Basin (Berg 1949; Dong et al. 2020). The species were introduced in the southern Caspian Sea Basin more than 30 years ago (Holčík and Razavi 1992) and then spread over the Iranian freshwater bodies. Most probably, the species invaded Azerbaijan territory from Iran as well (Mustafayev et al. 2015). Eastern Georgia and Azerbaijan both belong to the Caspian Sea Basin and share the Kura River. However, at the border of Georgia and Azerbaijan, there is the old and large Mingachevir Reservoir, which represents an insurmountable barrier

for migratory fish. Thus, the reservoir might have played a role in limiting the spread of *H. leucisculus* in eastern Georgia. On the other hand, the species managed to get over this barrier and appeared in Georgia during the first and most probable second decade of the 21st century. Nevertheless, in the period from 2010 to 2020, the species spread all over the eastern Georgian main water bodies. We can assume that species were introduced to Georgian freshwater by human facilitation and then expanded their range independently. On the other hand, unintentional and illegal introductions still take place, as indicated by their occurrence in the closed Bazaleti Lake. Another example is the Sioni Reservoir, which was built on the Iori River in 1963 for the Sioni hydropower plant (Demetrashvili 1972). It does not have a fish passage and represents an insurmountable barrier. Since *H. leucisculus* is not a commercially valuable species, its appearance in the Sioni Reservoir is most likely due to accidental translocation. Given the supposed distribution history of this species, we can assume that it will soon approach western Georgian inland waters – the Black Sea Basin.

Rhinogobius lindbergi is native to the Amur and Ussuri river basins in eastern Asia (Eagderi et al. 2018). It was only recently recorded as an alien species in the Southern Caucasus region (Eagderi et al. 2018; Kuljanishvili et al. 2020, 2021). The existence of this fish in Georgia was first confirmed through DNA barcoding using the mitochondrial COI by Japoshvili et al. (2020) and Epitashvili et al. (2020). It is noteworthy that all the individuals used in the above mentioned studies were from the same location which is situated in the easternmost part of Georgia, in the Kakheti region, near a small stream near Ozaani Village (41.548977N; 45.988441E). Therefore, it was believed that this location was the extreme north-western distribution margin of this goby (Japoshvili et al. 2020).

Our new finding of this species is the first observation in the Black Sea basin and represents a significant range expansion in the west (Figure 1). Interestingly, nearly at the same time, the DNA signature (mitochondrial Cyt-b gene) of this species was detected after an environmental DNA (eDNA) metabarcoding study (samples collected in 2018–2019) of the lower reach of the Rioni River (*own unpublished material*). Accordingly, it seems that *R. lindbergi* is already widespread in Georgia, including the Caspian and Black Sea basins, suggesting that the species' introduction events in Georgia are not recent, but relatively old. The small size of *R. lindbergi,* coupled with its high morphological similarity with other gobies, makes it difficult to easily detect the species. For this reason, species might escape notice if not studied deliberately. *Rhinogobius lindbergi* does not have any commercial value. It is not even used for recreational fishing. Thus, its spread to new river basins can only be attributed to its accidental introduction as a hitchhiker. After introduction, the species seems to have the ability to survive and spread easily throughout the basin.

Gambusia holbrooki is native to North America (Oscoz et al. 2008) and was first introduced into western Georgian lowland wetlands in 1925 for the mitigation of malaria disease (Elanidze 1983; Ninua et al. 2013). Since then, it has spread and established itself in most of the western Georgia lowland inland waters (Ninua et al. 2013). Nevertheless, until recent years, it was believed that *G. holbrooki* was distributed only in the Georgian Black Sea Basin. Elanidze (1983) noted that this species has also spread to eastern Georgia along the Alazani, Iori, and Mtkvari rivers. However, there was no hard evidence supporting this statement until 2019. From the three locations where *G. holbrooki* have been detected, Ujarma Lake harbors the most abundant population. Interestingly, during a year-old sampling in the same lake, we did not find any specimens of that species. After subsequent communication with the lake owner, it turned out that the species were deliberately released to control mosquito populations without any legal basis. This is one of the rare apparent cases of illegal translocation of non-native species in Georgia. In the other two locations (Shakriani Swamp and Iori River), the species is still very rare. Since there are artificial ponds near sampling locations, we assume that this fish might unintentionally be translocated to nearby localities and escape into the sampling areas. However, we could not deduce whether this happened from western Georgian populations or Azerbaijan. Even the source population for Ujarma Lake remains unknown. Based on available information, we can conclude that the species is regularly introduced into private waterbodies, intentionally and/or unintentionally (but illegally, as no legal basis for mosquitofish regulations exists in Georgia).

Summary

A recent review of alien and invasive species in Georgia and the South Caucasus by Kuljanishvili et al. (2021) indicated the lack of knowledge and research needs for invasive fish species in the region. In particular, except for mosquitofishes, most of the alien species seemed to have been introduced non-intentionally and illegally, while the exact pathways are hardly known. Neither in Georgia nor in any other South Caucasian country does legislation or a strategy dealing with alien species in general or aquatic aliens in particular exist. Similarly, the control mechanisms or mitigation measures for new introductions or local spread after introduction are strongly lacking. This is reflected in the current paper. The introduction and spread of *Hemicluter leucisculus* in Georgia as well as those of two other invasive species are currently out of control. On the other hand, this and other alien fish species must have a significant impact on both local fish communities and ecosystems as a whole (Coad and Hussain 2007; Neely et al. 2008; Macdonald and Tonkin 2008; Dong et al. 2020). As shown in recent summaries, invasive alien species cause huge economic losses worldwide (Haubrock et al. 2021). Such data is not available for Georgia, as no data

collection mechanisms related to expanses and losses related to alien species exist. However, we can easily assume that the economic loss is also significant in Georgia and could be even more dramatic given the low developmental stage of the country's economy.

The rapid expansion of alien species may lead to a decrease in local and endemic species populations in Georgia. Given the ever-intensifying aquaculture and recreational fisheries, the intensity of the introduction and establishment of alien and invasive fish in yet unoccupied areas is getting bigger. In their 2022 paper, while conducting risk screening of alien fish species, Mumladze et al. (2022) showed that all three species reported in the presented manuscript have great risks of further increasing their range within Georgia and becoming invasive throughout the region. Part of these expectations have already been met in a very short time. Unfortunately, the spread of alien species in Georgia's inland waters is continuing at an alarming rate, and no mitigation horizon is yet visible. It is therefore urgent to develop a country-scale strategy with regards to alien species and tighten the legislation in this regard. Aquaculture farms should be systematically monitored and illegal relocations restricted as much as possible.

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Authors' contribution

GE, BJ, LM – research conceptualization; LM – sample design and methodology; GE, BJ – investigation and data collection; GE, LM – data analysis and interpretation; GE – writing – original draft; LM – writing – final draft; BJ, GE – writing – review and editing.

Ethics and permits

Sampling and sample treatment was performed under a permission issued by Georgian ministry of Environmental Protection and Agriculture (#EPA2501/01, APA619; EPA2447/01, APA530).

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Supplementary material

The following supplementary material is available for this article:

Table S1. Occurrence of non-native fish taxa (*Hemicluter leucisculus*, *Gambusia holbrooki*, *Rhinogobius lindbergi*) in Georgia.

This material is available as part of online article from:
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