Taxonomic Revision of Freshwater Fishes of Georgia and the Assessment of Species Vulnerability

Giorgi Epitashvili

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Supervisors: Prof. Assoc. Dr. Bella Japoshvili and Prof. Assoc. Dr. Levan Mumladze

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Statement

As the author of this doctoral thesis, I confirm that this compilation thesis is my original work and does not include the materials of other authors that have already been published, accepted for publication or submitted for a degree, which have not been cited in an accepted manner.

Giorgi Epitashvili --

აბსტრაქტი

მტკნარი წყლის ეკოსისტემები და მისი ბიომრავალფეროვნება ღირებულ ბუნებრივ რესურსს წარმოადგენს ეკოლოგიური, ეკონომიკური, რეკრეაციული, კულტურული თუ სამეცნიერო/საგანმანათლებლო თვალსაზრისით. ამავდროულად, მათი დეგრადაცია ბევრად უფრო მასშტაბურია, ვიდრე ხმელეთზე არსებული ეკოსისტემების. მტკნარი წყლის თევზები ამ ეკოსისტემების ერთ-ერთი მთავარი შემადგენელი ნაწილია. დღეს მსოფლიოში 18 900 - მდე მტკნარი წყლის თევზის სახეობაა ცნობილი. მტკნარი წყლის თევზები ერთ-ერთ ყველაზე მოწყვლად ჯგუფად ითვლება ხერხემლიან ცხოველებში, რომლის მთავარი მიზეზი ადამიანის საქმიანობაა. მტკნარი წყლის ეკოსისტემებზე და მის ბინადარ თევზებზე მოქმედებს სხვადასხვა სახის ანთროპოგენური ფაქტორები: გარემოს დაბინძურება, წყალსატევების ფრაგმენტაცია/დეგრადაცია, არალეგალური და გადაჭარბებული თევზჭერა, უცხო/ინვაზიური სახეობების ინტროდუქცია/გავრცელება და სხვ. აღნიშნული პრობლემები განსაკუთრებით მოქმედებს ისეთ რეგიონებზე, რომლებიც გამორჩეულია ბიომრავალფეროვნებით და ენდემიზმის მაღალი დონით. კავკასიის რეგიონი და საქართველო სწორედ ერთ-ერთი ასეთი ბიომრავალფეროვნების ცხელი წერტილია. ჩვენი კვლევის დაწყებამდე არ არსებობდა განახლებული, გენეტიკური კვლევებით გამყარებული ინფორმაცია საქართველოში გავრცელებული თევზის სახეობების შესახებ. ჩემი სადოქტორო კვლევის ფარგლებში, შევისწავლე საქართველოს შიდა წყალსატევებში გავრცელებული თევზის სახეობები. კვლევის განმავლობაში გამოვიყენე მორფოლოგიური კვლევის სტანდარტული მეთოდები და აგრეთვე, მოლეკულურ-გენეტიკური მარკერები (დნმ-ზარკოდინგის მეთოდი), სახეობების იდენტიფიცირებისთვის.

ჩვენმა კვლევებმა აჩვენა, რომ ლიტერატურის მიხედვით ცნობილი სახეობების გარდა, საქართველოს წყალსატევებში ბინადრობს მეცნიერებისთვის ორი ახალი სახეობის თევზი - რიონის გოჭალა - *Oxynoemacheilus phasicus* sp. nov Freyhof, Kaya, Epitashvili & Geiger, 2021 და ალაზნის ღორჯო - *Ponticola alasanicus* sp. nov Epitashvili, Japoshvili & Mumladze, 2023.

კვლევის ფარგლებში ასევე დაფიქსირდა ახალი ინვაზიური სახეობების გავრცელება და უკვე არსებული ინვაზიური სახეობების მიერ არეალის გაფართოება საქართველოს ტერიტორიაზე.

სადოქტორო კვლევის განმავლობაში, სულ იდენტიფიცირებული იქნა 100 - მდე მტკნარი წყლის სახეობის თევზი საქართველოსთვის. უახლეს კვლევებზე და თანამედროვე ტაქსონომიურ ნომენკლატურაზე დაყრდნობით დაზუსტდა და განახლდა ყველა სახეობის სამეცნიერო სახელი. მოხდა ადგილობრივი თევზის

სახეობების კონსერვაციული სტატუსების განსაზღვრა IUCN - ის კრიტერიუმებზე დაყრდნობით, რაც სამომავლოდ მნიშვნელოვანი იქნება მოწყვლადი სახეობების კონსერვაციის და მენეჯმენტის გეგმის შემუშავებისთვის ეროვნულ დონეზე.

ძირითადი საძიებო სიტყვები: მტკნარი წყლის თევზები, სისტემატიკა, მორფოლოგია, გენეტიკა, დნმ ბარკოდინგი, კონსერვაცია.

Abstract

Freshwater ecosystems and their biodiversity represent a valuable natural resource from ecological, economic, recreational, cultural, and scientific/educational point of view. At the same time, their degradation is much more extensive than that of terrestrial ecosystems. Freshwater fish species are one of the main components of these ecosystems. Today, there are about 18, 900 known freshwater fish species in the world. At the same time, they are considered one of the most vulnerable groups of vertebrates, the main cause of which is human activity. Freshwater ecosystems and their resident fish are affected by various anthropogenic factors: environmental pollution, fragmentation/degradation of waterbodies, illegal or overfishing, introduction/spread of alien/invasive species, etc. These problems particularly affect the regions that are distinguished by their biodiversity and high levels of endemism.

The Caucasus region and Georgia are one such biodiversity hotspot. Prior to our research, there was no updated information, supported by genetic studies, about the fish species spread in Georgia. As part of my PhD research, I studied the fish species common in the inland waters of Georgia. During the study, I used standard morphological research methods as well as molecular-genetic markers (DNA-barcoding method) to identify species. Our research has shown that in addition to the species known in literature, two new fish species inhabit Georgian waters: Rioni loach - *Oxynoemacheilus phasicus* sp. nov Freyhof, Kaya, Epitashvili & Geiger, 2021 and Alazani goby - *Ponticola alasanicus* sp. nov Epitashvili, Japoshvili & Mumladze, 2023.

Also, within the framework of the study, the spread of new invasive species and the expansion of the area of existing invasive species within the territory of Georgia were observed. As part of the PhD research, a total of about 100 freshwater fish species were identified in Georgia. Based on the latest research and modern taxonomic nomenclature, the scientific names of all species have been clarified and updated. The conservation statuses of native fish species were determined based on IUCN criteria, which will be important in the future for the development of a conservation and management plan for vulnerable species at national level.

Keywords: Freshwater fishes, Systematics, Morphology, Genetics, DNA barcoding, Conservation.

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Table of Contents

Abbreviations

AOO - Area of Occupancy

BIN - Barcode Index Numbers

BOLD - Barcode of Life Data System

CaBOL - Caucasus Barcode of Life

COI - Cytochrome c Oxidase Subunit I

CR - Critically Endangered

DAAD - German Academic Exchange Service

DCI - Dendritic Connectivity Index

DD - Data Deficient

DNA - Deoxyribonucleic Acid

ED - Eye Diameter

EOO - Extent of Occurrence

EN - Endangered

EX - Extinct

EW - Extinct in Wild

HL - Head length

IUCN - International Union for Conservation of Nature

ISU - Ilia State University

K2P - Kimura 2-parameter

LC - Least Concern

LL - Lateral Line

MEGA - Molecular Evolutionary Genetics Analysis

MtDNA - Mitochondrial DNA

NE - Not Evaluated

NJ - Neighbour-Joining

NT - Near Threatened

PCR - Polymerase Chain Reaction

PD - Predorsal Distance

PoD - Postdorsal Distance

PrAD - Preanal Distance

RLG - Red List of Georgia

SL - Standard Length

SDM - Species Distribution Modeling

TL - Total Length

VU - Vulnerable

ZFMK - Zoologisches Forschungsmuseum Alexander Koenig

List of Publications:

1. Kuljanishvili, T., **Epitashvili**, G., Freyhof, J., Japoshvili, B., Kalous, L., Levin, B., Mustafayev, N., Ibrahimov, S., Pipoyan, S. & Mumladze, L. (2020). Checklist of the freshwater fishes of Armenia, Azerbaijan and Georgia. *Journal of Applied Ichthyology*, 36 (4): 501-514. https://doi.org/10.1111/jai.14038
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(Gobiiformes, Gobiidae) from the Alazani River Basin, Georgia. *Biodiversity Data Journal*, 11: e101095. https://doi.org/10.3897/BDJ.11.e101095

8. **Epitashvili**, G., Japoshvili, B. & Mumladze, L. (2023*b*). New records and range expansions of invasive fish species in Georgia. *BioInvasions Records*, 12 (4): 1111–1120. https://doi.org/10.3391/bir.2023.12.4.23

9. **Epitashvili**, G., Ramazashvili, G., Japoshvili, B. & Mumladze, L. (2025*a*). Distribution, community composition and vulnerability of fish species in the Algeti River Basin (Georgia). *Caucasiana*, 4: 45-58. https://doi.org/10.3897/caucasiana.4.e155494

10. **Epitashvili**, G., Japoshvili, B. & Mumladze, L. (2025*b*). Conservation profiles for native freshwater fish species of Georgia (Southern Caucasus region), compliant with the IUCN Red List of Threatened Species (in Prep)

11. Mumladze, L., **Epitashvili**, G. & Japoshvili, B. (2025). Fishes of the Caucasus ecoregion. In: Pullaiah, T., Mumladze, L., (eds). Caucasus Biodiversity Hot-Spot. Academic Press (in Press).

1. General Introduction

1.1. Literature review

Freshwater ecosystems and their biodiversity represent a valuable natural resource from an economic, cultural, recreational, scientific, and educational point of view (Strayer, 2010). At the same time, its degradation proceeds much faster than that of terrestrial ecosystems (Dudgeon *et al.* 2006). The degradation is influenced by many factors, the main cause of which is human activity (Dodds *et al.* 2013).

Fish occupy one of the main niches in freshwater habitats and, therefore, experience all the impacts that occur in the mentioned ecosystems. Also, fish are the most diverse group of vertebrates and include more than 37, 100 valid species globally, of which nearly 18, 900 inhabit freshwater ecosystems (Fricke *et al.* 2025). Despite this impressive diversity, many species are threatened with extinction due to overfishing, habitat modification/degradation, the spread of alien species, water pollution, etc. (Dudgeon *et al.* 2006; Collen *et al.* 2014). Nowadays, nearly a third of the known freshwater fish species are at risk of extinction globally ("The World's Forgotten Fishes", 2021). The situation is no less problematic in Europe, where almost 47% of freshwater fish species are under threat of extinction and, therefore, many of them are included in the European Red List (Freyhof & Brooks 2011). In general, regional and global red lists are used to assess the extinction risk of species based on quantitative criteria developed by the IUCN (Verreycken *et al.* 2014) and are an important mechanism for protecting vulnerable species.

The diversity of freshwater fishes is particularly high in the tropics, however, several regions in the Earth's temperate zone should be noted as a refugium and "hot spots" for rare and endemic species such as sturgeons. The Caucasus region, particularly Georgia, is a biodiversity hotspot characterized by the abundance of water resources and, therefore, diversity of freshwater species (Myers *et al.* 2000). Biogeographically, the Caucasian ichthyofauna belongs to the eastern Black Sea and western Caspian Sea basins. There are waterbodies with high ecological importance in the region, among which is the river Rioni, one of the last refugia and spawning rivers in the world for critically endangered sturgeons (Bacalbasa-Dobrovici & Holčík 2000; Kuljanishvili *et al.* 2020; Epitashvili *et al.* 2020).

Fish studies in Georgia have a long history starting from the 18th century. Vakhushti Bagrationi, in his work "Description of the Kingdom of Georgia" provides information about Georgian rivers and the fish species found there (Elanidze, 1983). German naturalist Johann Anton Güldenstadt was one of the first who explored the main rivers of Georgia between 1768 and 1775. As a result of these studies, the following fish species were described: Mursa - *Luciobarbus mursa*, Bulatmai barbell - *L. capito*, Caspian shamaya - *Alburnus chalcoides* and Khramulya - *Capoeta capoeta* (Barach, 1941; Sharvashidze, 1960; Elanidze & Demetrashvili 1973; Elanidze, 1983; Ninua & Japoshvili 2008; Ninua *et al.* 2013). Subsequently, this process continued more intensively and several foreign researchers studied Georgian ichthyofauna. During the Soviet period, scientific research continued more actively, however, the main goal was to derive the maximum economic advantage from the inland waterbodies. For this purpose, some species (*Coregonus albula* (Linnaeus, 1758), *C. lavaretus* (Linnaeus, 1758), *C. peled* (Gmelin, 1789), *Hypophthalmichthys molitrix* (Valenciennes, 1844), *H. nobilis* (Richardson, 1845) and *Ctenopharyngodon idella* (Valenciennes, 1844)) were introduced and released in some lakes (e.g. in Paravani, Sagamo, Tabatskuri, Kumisi, Japana, etc). As a result of these processes, invasive species such as *Pseudorasbora parva* (Temminck & Schlegel, 1846) and *Carassius gibelio* (Bloch, 1782) spread and settled in Georgia accidentally (Ninua *et al.* 2013).

From the 20th century to the present, especially in the 1990s, strong environmental changes also occurred in Georgia, as well as in the whole South Caucasus region, as a result of increasing land consumption, deforestation, water pollution, illegal fishing, and road and hydropower plant constructions (Epitashvili *et al.* 2020; Japoshvili *et al.* 2021). Consequently, negative impacts on the Georgian freshwater ecosystems have likely occurred. Over the last decades, the diversity and composition of the fish species in Georgia has changed. After the collapse of the Soviet Union, there was almost a 20-year gap in fish systematic and diversity studies, so the taxonomy of many species needed to be clarified and updated. Until 2017, it was believed that 168 fish species were common in Georgia, of which 77 were marine, 30 anadromous, and 61 were freshwater species (Ninua & Japoshvili 2008; Ninua *et al.* 2013).

As mentioned above, another problem was taxonomic uncertainties in many species, which was mostly resolved within my PhD study (Kuljanishvili *et al.* 2020; Epitashvili *et al.* 2020). However, as molecular studies showed, some gaps still exist in the accurate knowledge of species diversity and distribution within the Georgian inland waters and a good example of this is the newly described species: *Oxynoemacheilus phasicus* sp. nov Freyhof, Kaya, Epitashvili & Geiger 2021, from the Rioni and Enguri river basins and *Ponticola alasanicus* sp. nov Epitashvili, Japoshvili & Mumladze 2023, from the Alazani River basin (Freyhof *et al.* 2021; Epitashvili *et al.* 2023*a*). Also, in recent years, new invasive species have appeared in Georgia (Kuljanishvili *et al.* 2021; Mumladze *et al.* 2022; Epitashvili *et al.* 2023*b*), which will also have a significant impact on the native fish populations. Therefore, it will be important to continue research in this direction as well.

In my PhD research, I used diverse field survey techinques to collect data; standard morphological and molecular (mtDNA) approaches to study systematics and taxonomy, diversity, distribution and composition of fish species in Georgian inland waters; finally tried to identify factors affecting species and defining their vulnerability.

2. Methodology

2.1. Sample collection

Part of the fish samples used for the genetic and morphological analyses had already been collected by the students and researchers of the Institute of Zoology (Ilia State University) starting from 2006. Until 2017, when I started working on my PhD research, about 40 fish species distributed in Georgia (both freshwater and marine species) were already stored in the mentioned collection. Most of them had not been studied genetically and a certain number required additional morphological analysis. Also, the systematics of many species did not correspond to modern studies and needed updating. During my research period, some additional samples have been collected for genetic and morphological analyses. The material has been collected from all the main rivers and reservoirs of Georgia, from a total of up to 1,000 sampling locations (Fig. 1). As part of the PhD survey, we studied some rivers and reservoirs in detail: e.g. Sioni, Zhinvali, Tbilisi, Dali, Tsalka, Algeti reservoirs; Paliastomi, Jandari, Paravani, Saghamo, Madatapa and other lakes in Javakheti upland; Paravani, Bughdasheni, Ktsia/Khrami, Algeti, Iori, Alazani, Mtkvari, Aragvi, Ksani, Liakhvi, Prone, Dzirula, Kvirila, Rioni, Tskhenistskali, Gubistskali, Tskaltsitela, Khobi, Enguri, Chorokhi, Kintrishi, Supsa, Natanebi rivers and their tributaries, also other small to medium-sized rivers, channels, lakes, and artificial reservoirs within the framework of which two master's theses were prepared and defended (Ramazashvili, 2022; Gabrichidze, 2022).

Within the framework of the research, we used different sampling techniques depending on the habitat type. For instance, in lakes and reservoirs, we used gill nets (length 80-100 meters) with different mesh sizes (18, 20, 24, 26 mm), also seine nets (length 30 meters), trap nets, and fishing rods. In the streams and rivers, we used fishing rods, cast nets, frame nets, and an electrofishing device - EFGI 650 (http://www.electric-fishing.de/index_e.html), which is a high-quality fishing instrument for small to medium-sized rivers and streams. Some of the fish specimens we caught were transported to the Ichthyology and Hydrobiology Department of the Institute of Zoology at Ilia State University (ISU) for morphological and genetic analyses and the rest of the specimens were returned to the water bodies (catch-and-release principle). After anesthesia with MS-222 of a subsample of the collected fish, fin-clips of some individuals were taken and stored in 96 % ethanol and specimens were fixed in 4 % formaldehyde or, alternatively, directly fixed in 96% ethanol. All specimens were identified to species level using standard morphological characters (e.g. Berg, 1949; Elanidze, 1983; Kottelat & Freyhof 2007) and tissue samples of selected specimens submitted to the DNA barcoding routines at Ilia State University. Part of the genetic research (DNA barcoding) was carried out at the Zoologisches Forschungsmuseum Alexander Koenig (Bonn, Germany), within the scope of the Rustaveli Foundation and DAAD joint scholarship for PhD students in 2019. The fish collection deposited in the same museum was also used for this study, of which some materials are from the Abkhazia region (Fig. 1).

The next stage of the genetic study was conducted under the project - Caucasus Barcode of Life - CaBOL (https://ggbc.eu/). My goal is to collect genetic data on as many fish species as possible to have a complete picture of the species diversity in the Georgian freshwaters. Based on the results obtained, it is planned to publish a new study on the second stage of the DNA barcoding of Georgian freshwater fish.

2.2. DNA barcoding

The genetic study was conducted with standard CaBOL protocol. In particular, genomic DNA was extracted from pectoral fin-clips using a BioSprint96 magnetic bead extractor (Qiagen, Hilden, Germany). PCRs, targeting the standard DNA barcode region *COI*, were carried out in 20 µl reaction volumes including 2 µl undiluted DNA template, 0.8 µl of each primer (10 pmol/µl; LCO1490-JJ: 5´-CHACWAAYCATAAAGATATYGG-3´ and HCO2198-JJ: 5´ AWACTTCVGGRTGVCCAAARAATCA-3´, (Astrin & Stüben 2008; Astrin *et al.* 2016)), 2 µl 'Q-Solution' and 10 µl 'Multiplex PCR Master Mix' (Qiagen, Hilden, Germany). Cycler temperatures for PCR reaction: thermal conditions included denaturation at 95°C for 1 min, followed by first cycle set (15 cycles): 94°C for 30 sec, annealing at 55°C for 1 min (–1°C per cycle) and extension at 72°C for 1:30 min. Second cycles set (25 cycles): 94°C for 35 sec, 45°C for 1 min, 72°C for 1:30 min, followed by 1cycle at 72°C for 3 min and final extension step at 72°C for 5 min. Purification of PCR products and bidirectional sequencing were conducted at either BGI (Hong Kong, China) or Macrogen Europe Laboratories using the amplification primers. Voucher specimens are kept in the ichthyological collections at ISU and ZFMK. Extracted genomic DNA is also deposited in the ISU and ZFMK Biobanks.

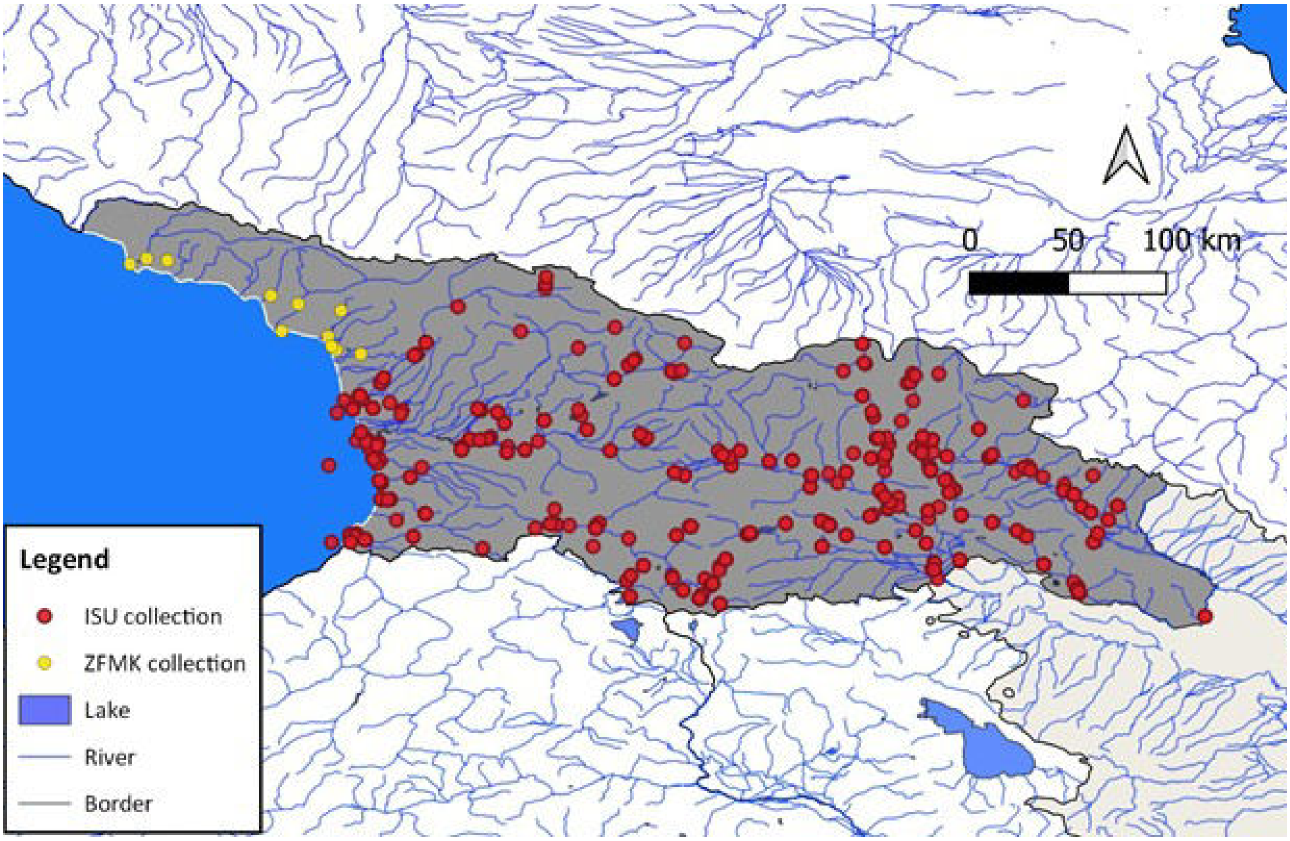

Figure 1. Map of Georgia. Sampling locations are marked with appropriate colors.

2.3. Molecular data analysis

Data processing and sequence assembly were performed using the Geneious Pro v.7 (Drummond *et al.* 2011) and the Muscle algorithm (Edgar, 2004) was used to align the DNA barcodes after screening for indels or stop codons (Epitashvili *et al.* 2020). All newly-generated DNA sequences with acceptable quality (with less than 1% ambiguous bases and free of stop codons) were submitted to the Barcode of Life Data System (BOLD, http://v4.boldsystems.org/), including relevant metadata, where they were automatically assigned Barcode Index Numbers (BINs). The sequences can be accessed via the public dataset: "Georgian Freshwater Fishes" (DS-GGBCPIS) in the BOLD Systems. Subsequently, we evaluated sequence divergence and relationships between species, based on uncorrected *p*-distance. A Neighbour-Joining tree based on K2P (Kimura 2-parameter model) distances with 1,000 bootstrap replicates was constructed to visualize the phylogenetic relationships among specimens (Fig. 2). Analyses were performed using MEGA 11 software (Tamura *et al.* 2021) and statistical tools provided by BOLD Systems (Ratnasingham & Hebert 2007). In total, we got 352 DNA barcodes for 50 species, 36 genera, and 15 families (Fig. 2) (Epitashvili *et al.* 2020). During the genetic research conducted in the CaBOL project, we obtained an additional 295 DNA barcodes for 55 species, 39 genera, and 15 families (unpublished data). Accordingly, we currently have DNA barcodes for 72 fish species distributed in Georgia.

2.4. Morphological analysis

A morphological study of the collected specimens was carried out in the Ichthyology and Hydrobiology laboratory of the Institute of Zoology at ISU and partly in the Zoologisches Forschungsmuseum Alexander Koenig in Bonn (ZFMK). After identification, each individual was assigned a corresponding number and stored in the laboratory collection with appropriate data, such as: place of extraction, date, sex, habitat type, etc. We used a character set commonly employed for the study of freshwater fishes. For counts and meristic and plastic measurements, we followed methodologies given by Berg, (1949), Kottelat & Freyhof (2007), and Armbruster, (2012). In particular, the following main parameters were measured: Total length (TL); Standard length (SL); Predorsal distance (PD); Postdorsal distance (PoD); Preanal distance (PrAD); Head length (HL); Eye diameter (ED); number of gill rakers; number of spines and soft rays in dorsal, anal, pectoral and pelvic fins, number of scales in lateral line (LL), etc. (for details see Appendix I). All measurements were made using a digital caliper with the precision of 0.1 mm. The last two branched rays articulating on a single pterygiophore in the dorsal (D) and anal (A) fins we noted as "1½".

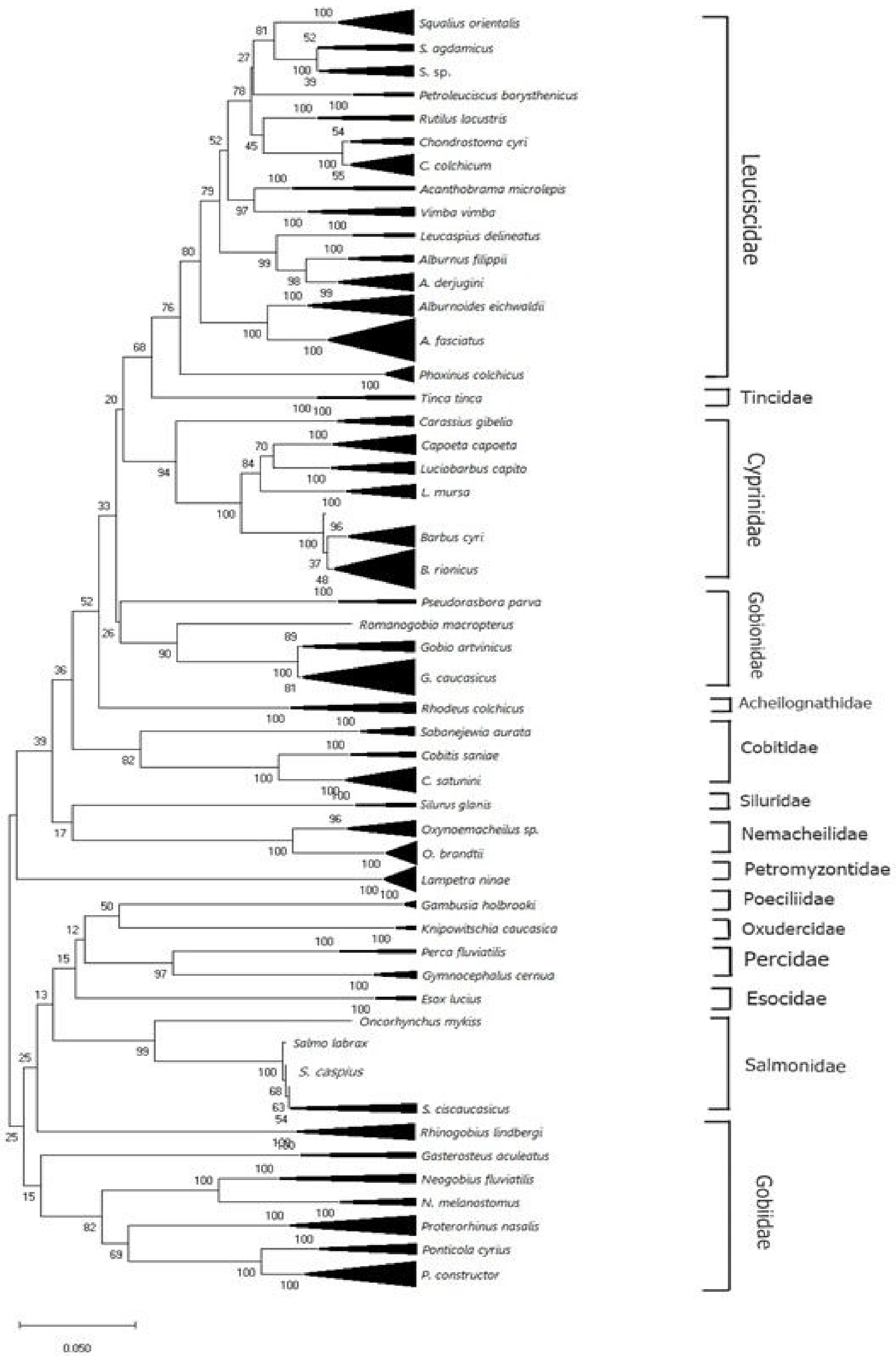

Figure 2. Compact Neighbour-Joining tree based on mitochondrial *COI* barcode region using K2P distance model with other default parameters provided by Mega 11 software. Numbers near nodes indicate bootstrap support values from 1,000 replicates. The analyses involved 352 *COI* nucleotide sequences of 50 fish species from Georgia (for details see Epitashvili *et al.* 2020).

2.5. Species vulnerability assessment (conservation profiles for native freshwater fish species of Georgia)

According to our data, currently, there are up to 100 freshwater fish species distributed in Georgia and most of them have not been evaluated by IUCN Red List criteria. Only 15 fish species have been assessed and included in the Georgian Red List and some of them with incorrect taxonomy (https://matsne.gov.ge/) (Appendix II). It should be noted that the national red list of Georgia was approved in 2006 and since then, conservation statuses for the fish species have not been updated. Currently, work is underway to update the Georgian Red List, and our research will be used to renew or grant conservation statuses at the legislative level to all native freshwater fishes (the article is ready for publication).

Another problem in the RLG was the inconsistency of conservation statuses with the IUCN Red List of globally threatened species, such as sturgeons. In addition to the above-mentioned species, some endemic and vulnerable varieties, such as *Luciobarbus capito* (Güldenstädt, 1773) (IUCN status – VU); *Anguilla anguilla* (Linnaeus, 1758) (IUCN status – CR); *Alosa immaculata* Bennett, 1835 (IUCN status – VU) and many others, still do not have conservation statuses at the national level.

This study aimed to assess the conservation profiles/statuses for all native freshwater fish species distributed in Georgia, according to the IUCN Red List criteria. Species data were collected from all bibliographies on Georgian fishes published until December 2024. We also used social networks (Facebook, YouTube, fishermen's forums and webpages), our own unpublished data and additional information collected from various projects. For all analyses, we used IUCN redlisting tools (the R package red). The R package red performs several spatial analyses based on either observed occurrences or estimated ranges for each species on which we have such data. The functions of the program also include: calculating the Extent of Occurrence (EOO), Area of Occupancy (AOO), mapping species ranges, species distribution modeling using different parameters, etc. (Cardoso, 2017; Cardoso *et al.* 2017).

The R package red includes algorithms to facilitate the assessment of extinction risk of species according to the IUCN Red List criteria (http://www.iucn.org).

From the data we possess (literary data and collection sites – e.g. coordinates for each species), we used two main criteria to assess species vulnerability: the area of occupancy (AOO) and the extent of occurrence (EOO). Accordingly, in order to determine conservation statuses for all native freshwater fishes, we relied on one of the five main criteria suggested by IUCN, namely, criterion B: Geographic range in the form of either B1 (extent of occurrence) and/or B2 (area of occupancy) (http://www.iucn.org).

Within this study, the EOO and AOO were calculated in the following ways: for endemic and range-restricted species, the full range of which is more or less known, these values were classified as observed; EOO was calculated as the minimum convex polygon covering all known or predicted sites for the species and AOO was calculated as the area of all known or predicted cells for the species. The resolution was 2x2km. When the EOO was smaller than the AOO, it was made equal as required by IUCN (Cardoso *et al.* 2017; IUCN Standards and Petitions Subcommittee 2022).

For widespread species or those for which we did not have confidence to know the full range, we performed species distribution modeling (SDM). Species distribution maps were prepared in Google Earth (Google Earth Pro, 2024).

3. Results & Discussion

3.1. Progress in pecies inventory

Within the framework of PhD research, several novelties and problems were identified regarding the systematics of Georgian freshwater fishes. Until 2017, it was considered that the Georgian freshwater ichthyofauna included up to 91 species. The taxonomic affiliation of many species was in question. Within the first critical checklist of freshwater fishes of the

Southern Caucasian countries (Kuljanishvili *et al.* 2020), we update the fish systematics to Georgia, based on the literary data and materials we had in our collections. In total, 119 freshwater fishes have been recorded to the South Caucasus region, from wich 96 species were recorded for Georgia. We also provided notes on several fish taxa, for which knowledge gaps were identified, and including additional argumentations with regard to distribution or taxonomy.

Despite this, the presence of some species included in the checklist required clarification. For this purpose, a genetic study of about 50 fish species from Georgia was conducted (Epitashvili *et al*. 2020). Within the framework of the mentioned study, our goal was to perform DNA barcoding of fish samples (pectoral fins) using mtDNA - Cytochrome c Oxidase Subunit I (*COI*) gene, to determine which species are prevalent in our waterbodies. A species that was not mentioned in any literary source, *Gymnocephalus cernua* (Linnaeus, 1758) was observed during the research. This fish was found in materials obtained from the Rioni River Delta and our study is the first evidence that the mentioned species has entered Georgia. In summary, it can be said that the first genetic study of Georgian freshwater ichthyofauna led to positive results and highlighted the advantages that DNA barcoding has in modern taxonomic studies.

However, our research shows that the number of reference barcodes available for Georgian fish species is still insufficient for implementing full-scale fish diversity monitoring programs. Indeed, the barcodes of nearly 30% of species are not available yet. This is mainly due to limited financial/human resources, which hinders a full exploration of the fish diversity in the country. Also, the materials in museum collections are in poor condition. For example, the largest fish collection kept in the Georgian National Museum has been damaged so badly (as a result of incorrect preservation) that it is no longer useful for genetic study. Thus, unresolved taxonomy in some groups (e.g. gobies – *Proterorhinus*, *Ponticola*, etc.) and insufficient barcode coverage are currently major gaps that need to be filled in the future. I hope this is indeed possible, given the relatively low number of species (compared to mega-diverse regions) and the existing progress in fish research in the region. The CaBOL project implemented in recent years by Ilia State University, as well as other similar projects that will be financed and implemented in the country over the next years, constitutes a chance to close some of these taxonomic uncertainties/gaps shortly.

Following the first stage of DNA barcoding, we have described two new fish species from Georgia. *Oxynoemacheilus phasicus* sp. nov and *Ponticola alasanicus* sp. nov were found in the Rioni and Alazani river basins respectively (Freyhof *et al.* 2021; Epitashvili *et al.* 2023*a*). Both species are endemic to Georgia, from where *O. phasicus* is spread between the Rioni and Enguri river basins, while the range of *P. alasanicus* is restricted only to the Alazani River basin.

These cases evidence that freshwater ecosystems of Georgia still require additional research. Among them, the use of genetic methods is especially important. We are still actively working in this regard. For example, in recent years, in collaboration with foreign colleagues, we have studied the phylogeography of the Kura barbell (*Barbus cyri*) De Filippi, 1865 in the Caucasus region (Gandlin *et al.* 2022), with using two molecular markers of mtDNA, the cytochrome *b* gene and the control region. By analyzing haplotype networks, the complex genetic structure of the population system of the Kura barbel was revealed.

Currently, we are working on the second stage of DNA barcoding. Within the framework of our research, we obtained additional 295 DNA barcodes for 55 species and now we have genetic data of at least 72 fish species from Georgia. My goal is to obtain some additional genetic material for the species for which such data are not available yet, in order to have a complete picture of Georgia's freshwater fish diversity.

3.2. An overview of the existing threats

Nowadays, the degradation of freshwater ecosystems and their biodiversity is proceeding at a rapid pace (Oberdorff, 2022). This is influenced by many factors, the main cause of which is human activity (Dodds *et al.* 2013). Fish are the most widespread group in freshwater ecosystems and, therefore, any changes in these ecosystems will affect them as well. In order to preserve the diversity of freshwater fishes globally, it is important to study biodiversity hotspots and less-studied water bodies worldwide, including Georgia.

Georgia, as part of the Caucasus and Irano-Anatolian biodiversity hotspots, is distinguished by its unique biodiversity and rich freshwater resources, which have been strongly impacted by anthropogenic pressure since the beginning of the 20th century until the present. During the Soviet time, presumably, large-scale industrial projects had a strong influence on the Georgian biodiversity and especially on the freshwater fish fauna. An example of this is the construction of the Mingachevir Dam in Azerbaijan, which acted as an insurmountable obstacle for anadromous species such as sturgeons, Caspian lamprey, and Caspian salmon (Kuljanishvili *et al.* 2020). As a result, these species (in particular, lampreys and sturgeons) lost the spawning areas in the whole upper Kura River basin. In addition, the following alien species were intentionally or accidentally introduced to Georgia between the 1920s and 1980s: *Gambusia holbrooki*, *Carassius gibelio* and *Pseudorasbora parva* (Japoshvili *et al.* 2013; Ninua *et al.* 2013; Kuljanishvili *et al.* 2020).

Although large industrial projects were halted with the collapse of the Soviet Union, the recent extensive development of small and medium-sized hydropower plants in Georgia will presumably have negative impacts on the local freshwater biodiversity (Japoshvili *et al.* 2021). Within the mentioned study, we explore the effects of existing and proposed HPP dams on the connectivity of rivers of Western Georgia and their potential consequences for the conservation of anadromous, and resident fish species. Using the Dendritic Connectivity Index (DCI) as an analytical lens, we found serial decreases in DCI values following different periods of hydropower development in the country.

The spread and range expansion of non-native species in Georgian waters, is also a significant challenge for the country's biodiversity. Regarding this issue, we assessed the potential risk of invasiveness of non-native species for the South Caucasus (Mumladze *et al.* 2022). In total, 32 non-native extant and horizon fish species were screened for their risk of invasiveness under current and predicted climate conditions for the region.

We documented the range expansion of freshwater invasive fishes in Georgia (Epitashvili *et al.* 2023*b*). A DNA barcoding approach was employed to validate our findings. The study detected invasive *Hemiculter leucisculus* from different locations in Eastern Georgia, which is the first case of the existence of this species in the country. In addition, we documented a significant range expansion of two other non-native species in Georgia. *Rhinogobius lindbergi* Berg, 1933 was collected in Western Georgia, which was previously observed only in Eastern Georgia, and the occurrence of *Gambusia holbrooki*, was confirmed in Eastern 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 the populations of local and endemic species in Georgia.

In addition to invasive species, other factors also affect Georgia's water bodies. For these reasons, some reservoirs are in a critical condition. For instance, the research we conducted in the Algeti River basin (Epitashvili *et al.* 2025*a*) revealed a concerning ongoing impact of anthropogenic factors on the Algeti River fish fauna and the whole ecosystem. The dam impedes fish migration, and pollution, particularly in the mid-downstream section, it has led to habitat degradation and a decline in native fish populations.

Illegal fishing, water pollution, and habitat modification are also altering the population dynamics and distribution of many native freshwater fish species in Georgia, including range-restricted, endemic, and especially, anadromous species. Given this situation, an intensive study and monitoring of fish populations is highly recommended for estimating population changes and species distribution, and for subsequent planning of conservation activities and mitigation of irreversible diversity loss. The fastest and perhaps most cost-effective tools in this regard will be the methods based on DNA barcoding (Kress *et al.* 2015).

3.3. Trends in fish conservation of Georgia

Currently, up to 100 freshwater fish species are known in Georgia and most of them are not evaluated by the IUCN Red List criteria. As mentioned above, only 15 fish species included in the Georgian Red List (RLG), have been assessed some of which were included with incorrect taxonomy (Appendix II). It is worth noting that the Georgian Red List was approved in 2006 and since then conservation statuses for the fish species have not been updated.

Within the presented study, we provide the first estimation of freshwater fish vulnerability in Georgia (Draft: Epitashvili *et al.* 2025*b*). The conservation statuses of 84 native fish species were assessed based on the IUCN Red List criteria. Out of 84 species evaluated, there is no reliable information on the range and population trends for 10 species (11.9%). Their conservation status was determined as Data Deficient (DD). Of the remaining species: 39 are numerous and widespread. Their status was defined as Least Concern (LC). One species, *Acipenser sturio* Linnaeus, 1758 is considered extinct at the national level, as it has not been observed in Georgia for the last decades. The remaining 34 species showed a restricted or fragmented range and small populations, which can be attributed to habitat destruction or degradation, and invasive species. Also, illegal fishing remains one of the main challenges for all native fish species in the country. Taking into account all the factors mentioned above, the conservation statuses of these species were determined as Near Threatened (NT), Vulnerable (VU), Endangered (EN), and Critically Endangered (CR) (Fig. 3).

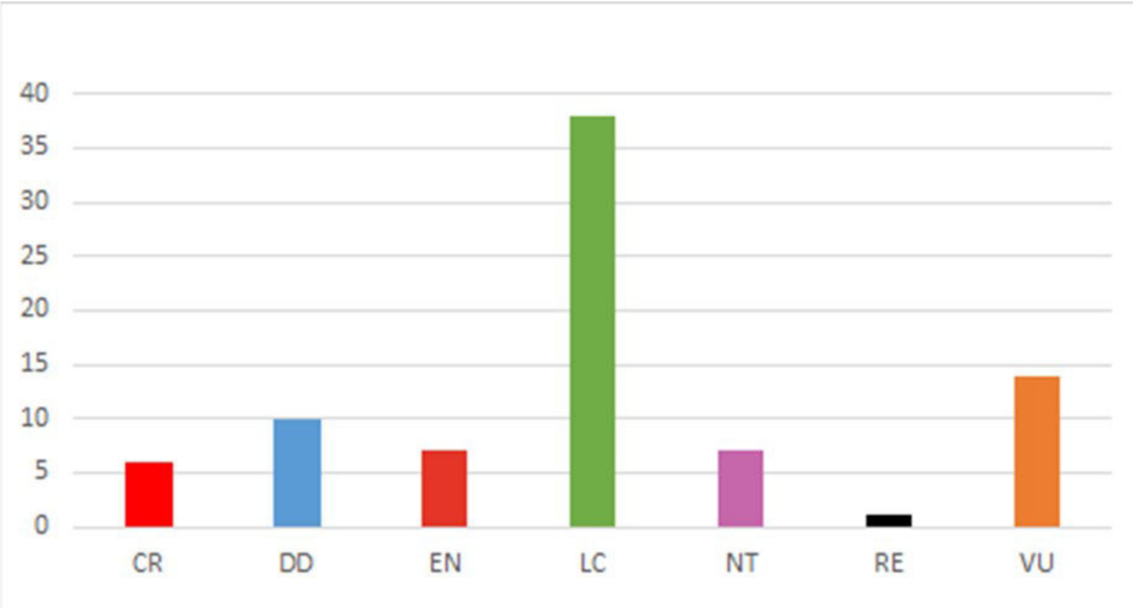

Figure 3. Visualization of conservation statuses of Georgian freshwater fish species

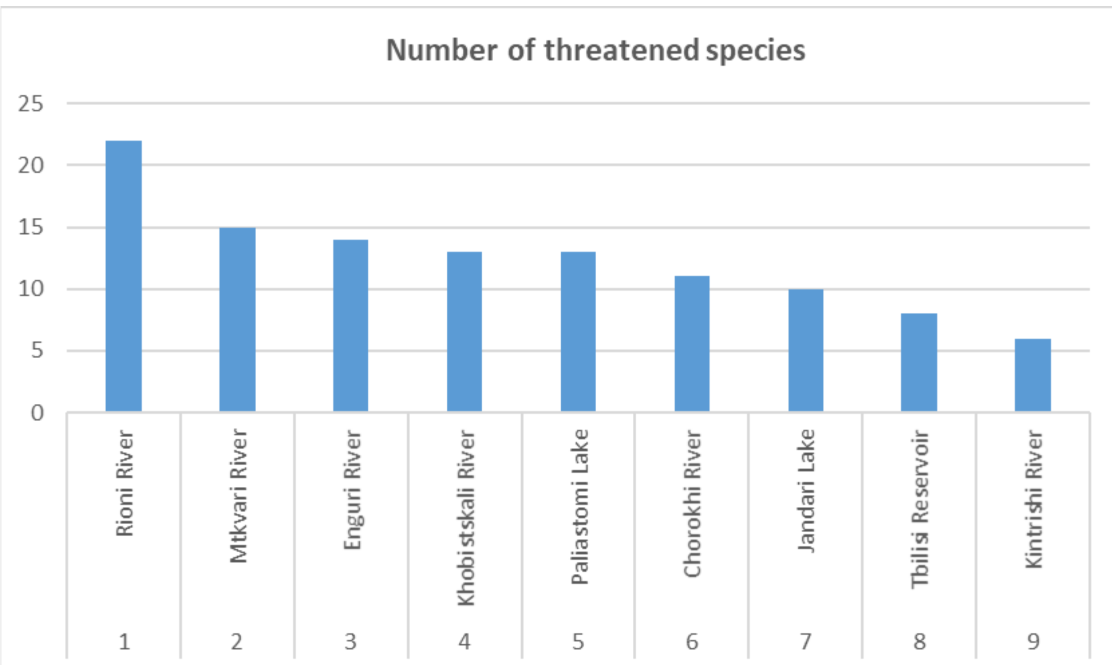

Figure 4. The main river basins and lakes/reservoirs of Georgia with approximate number of threatened fish species

We identified the most important habitats according to the number of vulnerable species, where the Rioni River occupies the first place with up to 22 threatened/vulnerable species (Fig. 4).

We also focused on the importance of the Rioni as a river with high ecological value in another paper, which is planned to be published this year (Submitted chapter: Mumladze *et al.* 2025). This work also highlights the importance of other habitats, such as transboundary Kura River (with its tributaries). Since these rivers are home to rare and endangered species, their protection is a priority, which in turn implies the conservation of the biodiversity present there.

Expanding the network of protected areas, dismantling the old dams, restoring original habitats, strengthening control over illegal fishing, also further monitoring and research of invasive, data deficient, and vulnerable species will be prioritized for further conservation measures.

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Appendices

Appendix I

Main meristic and plastic characters used to identify fish species as part of PhD research

Character in mm
Total body length (TL)
Standard body length (SL)
Head length (HL)
Eye diameter (ED)
Predorsal distance (PD)
Postdorsal distance (PoD)
Preanal distance (PrAD)
Number of spines and branched rays in anal fin
Number of spines and branched rays in dorsal fin
Number of spines and branched rays in pectoral fin
Number of spines and branched rays in pelvic fin
Number of spines and branched rays in caudal fin
Number of gill rakers
Number of scales in lateral line (LL)
In percent of standard length (SL)
Maximum body depth
Least depth of caudal peduncle
Minimum width of caudal peduncle
Length of dorsal fin base
Height of dorsal fin
Length of anal fin base
Height of anal fin
Length of pectoral fin
Length of pelvic fin
In percent of head length (HL)
Horizontal diameter of eye
Preorbital distance
Postorbital distance
Interorbital distance
Width of upper lip
Head depth at nape
Head width

Appendix II

National Red List of Georgian fishes. The species included in the old scientific names are marked in red

N	Species names in Georgian Red List	Valid scientific name	IUCN	Red List of Georgia (RLG)
1	*Huso huso* (Linnaeus, 1758)	*Huso huso* (Linnaeus, 1758)	CR	EN
2	*Acipenser sturio* (Linnaeus, 1758)	*Acipenser sturio* (Linnaeus, 1758)	CR	CR
3	*Acipenser nudiventris* Lovetsky, 1828	*Acipenser nudiventris* Lovetsky, 1828	CR	EN
4	*Acipenser stellatus* Pallas, 1771	*Acipenser stellatus* Pallas, 1771	CR	EN
5	*Acipenser gueldenstaedti* Brandt & Ratzeberg, 1833	*Acipenser gueldenstaedti* Brandt & Ratzeberg, 1833	CR	EN
6	*Acipenser persicus* Borodin, 1897	*Acipenser persicus* Borodin, 1897	CR	EN
7	**Salmo fario* Linnaeus, 1758	**(Salmo caspius; S. ciscaucasicus; S. rizeensis)*	LC	VU
8	*Salmo fario labrax* Pallas, 1814	*Salmo labrax* Pallas, 1814	LC	EN
9	*Rutilus frisii* (Nordmann, 1840)	*Rutilus frisii* (Nordmann, 1840)	LC	VU
10	*Varicorhinus sieboldi* (Steindachner, 1864)	*Capoeta sieboldii* (Steindachner, 1864)	LC	VU
11	*Sabanejewia aurata* (De Filippi, 1863)	*Sabanejewia aurata* (De Filippi, 1863)	LC	VU
12	*Neogobius fluviatilis* (Pallas, 1814)	*Neogobius fluviatilis* (Pallas, 1814)	LC	VU
13	*Alosa caspia paleostomi* Eichwald, 1838	*Alosa tanaica* (Grimm, 1901)	LC	VU
14	*Aspius aspius taeniatus* Berg, 1949	*Leuciscus aspius* (Linnaeus, 1758)	LC	VU
15	*Nemachilus angorae alasanicus* Elanidze, 1983	*Oxynoemacheilus lenkoranensis* (Abdurakhmanov, 1962)	DD	VU
IUCN - The categories are formulated as follows:				
EX – Extinct; **EW** – Extinct in Wild; **CR** – Critically Endangered; **EN** – Endangered; **VU** – Vulnerable; **NT** – Near Threatened; **LC** – Least Concern; **DD** – Data Deficient ; **NE** – Not Evaluated				

*Please note that all trout species were included in the national red list of Georgia as a single species - *Salmo trutta fario.*

Appendix III

Updated list of freshwater fish species in Georgia

N	Taxa	Common Name	Georgian Name
	Acheilognathidae		
1	*Rhodeus amarus* (Bloch, 1782)	European bitterling	ტაფელა
2	*Rhodeus colchicus* Bogutskaya & Komlev 2001	Georgian bitterling	კოლხური ტაფელა
	Acipenseridae		
3	*Acipenser gueldenstaedtii* Brandt & Ratzeburg 1833	Russian sturgeon	რუსული ზუთხი
4	*Acipenser nudiventris* Lovetsky, 1828	Fringebarbel sturgeon	ჯარღალა
5	*Acipenser persicus/colchicus* Marty, 1940	Colchic sturgeon	სპარსული/კოლხური ზუთხი
6	*Acipenser stellatus* Pallas, 1771	Starry sturgeon	ტარაღანა
7	*Acipenser sturio* Linnaeus, 1758	Atlantic sturgeon	ატლანტური ზუთხი
8	*Huso huso* (Linnaeus, 1758)	Beluga	სვია
	Anguillidae		
9	*Anguilla anguilla* (Linnaeus, 1758)	European eel	მდინარის გველთევზა
	Atherinidae		
10	*Atherina caspia* Eichwald, 1831	Big-scale sand smelt	შავი ზღვის ათერინა
	Cichlidae		
11	*Oreochromis niloticus* (Linnaeus, 1758)	Nile tilapia	ნილოსის ტილაპია
	Clupeidae		
12	*Alosa immaculata* Bennett, 1835	Pontic shad	პონტოური ქაშაყი
13	*Alosa maeotica* (Grimm, 1901)	Azov shad	აზოვის ზღვის ქაშაყი
14	*Alosa tanaica* (Grimm, 1901)	Black Sea shad	შავი ზღვის ქაშაყი
15	*Clupeonella cultriventris* (Nordmann, 1840)	Pontic sprat	შავი ზღვის სარდელი, ქარსალა
	Cobitidae		
16	*Cobitis saniae* Eagderi, Jouladeh-Roudbar, Jalili, Sayyadzadeh & Esmaeili, 2017	South Caucasian Spined loach	სამხრეთკავკასიური გველანა
17	*Cobitis satunini* Gladkov 1935	Colchic spined loach	კოლხური გველანა
18	*Sabanejewia aurata* (De Filippi, 1863)	Golden spined loach	წინააზიური/ ოქროსფერი გველანა
	Coregonidae		
19	*Coregonus albula* (Linnaeus, 1758)	Vendace	ევროპული ჭაფალა

20	*Coregonus* sp.		სიგი
	Cyprinidae		
21	*Barbus cyri* De Filippi, 1865	Kura barbel	მტკვრის წვერა
22	*Barbus rionicus* Kamensky, 1899	Colchic barbel	კოლხური წვერა
23	*Capoeta banarescui* Turan, Kottelat, Ekmekçi & Imamoglu, 2006	Banarescu's barb	მცირეაზიული/ ბანარესკუს ხრამული
24	*Capoeta capoeta* (Güldenstädt, 1773)	Khramulya	მტკვრის ხრამული
25	*Capoeta sieboldii* (Steindachner, 1864)	Colchic khramulya	კოლხური ხრამული
26	*Carassius gibelio* (Bloch, 1782)	Prussian carp	ჩვეულებრივი კარჩხანა
27	*Cyprinus carpio* Linnaeus, 1758	Common carp	ჩვეულებრივი/ სარკისებრი კობრი, გოჭა
28	*Ctenopharyngodon idella* (Valenciennes, 1844)	Grass carp	თეთრი ამური
29	*Hypophthalmichthys molitrix* (Valenciennes, 1844)	Silver carp	ჩვეულებრივი სქელშუბლა
30	*Hypophthalmichthys nobilis* (Richardson, 1845)	Bighead carp	ჭრელი სქელშუბლა
31	*Luciobarbus brachycephalus* (Kessler, 1872)	Aral barbel	არალის წვერა
32	*Luciobarbus capito* (Güldenstädt, 1773)	Bulatmai barbel	ჭანარი
33	*Luciobarbus mursa* (Güldenstädt, 1773)	Mursa	მურწა
	Esocidae		
34	*Esox lucius* Linnaeus, 1758	Northern pike	ქარიყლაპია/წერი
	Gasterosteidae		
35	*Gasterosteus aculeatus* Linnaeus, 1758	Three-spined stickleback	სამნემსა მახათა
	Gobiidae		
36	*Babka gymnotrachelus* (Kessler, 1857)	Racer goby	მდევარა ღორჯო
37	*Mesogobius batrachocephalus* (Pallas, 1814)	Knout goby	შოლტა ღორჯო
38	*Neogobius fluviatilis* (Pallas, 1814)	Monkey goby	მექვიშია ღორჯო
39	*Neogobius melanostomus* (Pallas, 1814)	Round goby	შავპირა ღორჯო
40	*Ponticola alasanicus* Epitashvili, Japoshvili & Mumladze 2023	Alazani goby	ალაზნის ღორჯო
41	*Ponticola constructor* (Nordmann, 1840)	Caucasian goby	კავკასიური მდინარის ღორჯო
42	*Ponticola cyrius* (Kessler, 1874)	Kura goby	მტკვრის ღორჯო
43	*Ponticola gorlap* (Iljin, 1949)	Caspian bighead goby	კასპიური ჭრელი ღორჯო
44	*Ponticola syrman* (Nordmann, 1840)	Syrman goby	ღორჯო შირმანი
45	*Proterorhinus nasalis* (De Filippi, 1863)	Eastern tubenose goby	აღმოსავლური მილცხვირა ღორჯო
	Gobionellidae		
46	*Knipowitschia caucasica* (Berg, 1916)	Caucasian	კავკასიური ჯუჯა

		dwarf goby	ღორჯო
47	*Knipowitschia longecaudata* (Kessler, 1877)	Longtail dwarf goby	გრძელკუდა ღორჯო
48	*Rhinogobius lindbergi* Berg, 1933	Amur goby	ამურის ღორჯო
	Gobionidae		
49	*Gobio artvinicus* Turan, Japoshvili, Aksu & Bektaş 2016	Artvin gudgeon	ართვინული ციმორი
50	*Gobio caucasicus* Kamensky, 1901	Colchic gudgeon	კავკასიური ციმორი
51	*Pseudorasbora parva* (Temminck & Schlegel, 1846)	Stone moroko	ფსევდორაზბორა
52	*Romanogobio macropterus* (Kamensky, 1901)	South caucasian gudgeon	სამხრეთკავკასიური ციმორი
	Leuciscidae		
53	*Abramis brama* (Linnaeus, 1758)	Freshwater bream	კაპარჭინა
54	*Acanthobrama microlepis* (De Filippi, 1863)	Blackbrow bleak	შავწარბა
55	*Alburnoides eichwaldii* (De Filippi, 1863)	Kura chub	მტკვრის მარდულა/სწრაფულა
56	*Alburnoides fasciatus* (Nordmann, 1840)	Transcaucasian spirlin	სამხრეთული მარდულა/ფრიტა
57	*Alburnus alburnus* (Linnaeus, 1758)	Bleak	თაღლითა/თეთრულა
58	*Alburnus chalcoides* (Güldenstädt, 1772)	Danube bleak	შამაია
59	*Alburnus derjugini* Berg, 1923	Georgian shemaya	ბათუმის შამაია
60	*Alburnus filippii* Kessler, 1877	Kura bleak	მტკვრის თაღლითა
61	*Alburnus hohenackeri* Kessler, 1877	North Caucasian bleak	ამიერკავკასიური თაღლითა
62	*Ballerus sapa* (Pallas, 1814)	White-eye bream	თეთრთვალა
63	*Blicca bjoerkna* (Linnaeus, 1758)	White bream	ჩვეულებრივი ბლიკა
64	*Chondrostoma colchicum* Derjugin, 1899	Colchic nase	კოლხური ტობი
65	*Chondrostoma cyri* Kessler, 1877	Kura nase	მტკვრის ტობი
66	*Leuciscus aspius* (Linnaeus, 1758)	Asp	ჩვეულებრივი ჭერეხი
67	*Leucaspius delineatus* Heckel, 1843	Belica	მზისებრი თაღლითა
68	*Petroleuciscus borysthenicus* (Kessler, 1859)	Dnieper chub	ჯუჯა ქაშაპი
69	*Phoxinus colchicus* Berg, 1910	Colchic minnow	კოლხური კვირჩხლა
70	*Rutilus frisii* (Nordmann, 1840)	Kutum	მორევის ნაფოტა/კუტუმი
71	*Rutilus lacustris* (Pallas 1814)	Roach	ნაფოტა

72	*Scardinius erythrophthalmus* (Linnaeus, 1758)	Rudd	ფარფლწითელა
73	*Squalius agdamicus* (Kamensky 1901)	Kura chub	მტკვრის ქაშაპი
74	*Squalius orientalis* Heckel, 1847	Oriental chub	აღმოსავლური ქაშაპი
75	*Vimba vimba* (Linnaeus, 1758)	Vimba bream	ვიმბა
	Moronidae		
76	*Dicentrarchus labrax* (Linnaeus, 1758)	European seabass	ლავრაკი/სიბასი
	Mugilidae		
77	*Chelon auratus* (Risso, 1810)	Golden grey mullet	სინგილი
78	*Chelon labrosus* (Risso, 1810)	Thicklip grey mullet	სქელტუჩა კეფალი
79	*Chelon ramada* (Risso, 1810)	Thinlip grey mullet	თხელტუჩა კეფალი
80	*Chelon saliens* (Risso, 1810)	Leaping mullet	მახვილცხვირა კეფალი
81	*Mugil cephalus* Linnaeus, 1758	Flathead grey mullet	ლობანი
82	*Planiliza haematocheilus* (Temminck & Schlegel, 1845)	So-iuy mullet	წითელთვალა კეფალი
	Nemacheilidae		
83	*Oxynoemacheilus brandtii* (Kessler, 1877)	Kura loach	მტკვრის გოჭალა
84	*Oxynoemacheilus cemali* Turan, Kaya, Kalayci, Bayçelebi & Aksu 2019	Coruh loach	ჭოროხის გოჭალა
85	*Oxynoemacheilus phasicus* Freyhof, Kaya, Epitashvili & Geiger 2021	Rioni loach	რიონის გოჭალა
	Percidae		
86	*Gymnocephalus cernua* (Linnaeus, 1758)	Ruffe	ევრაზიული ქორჭილა/ჩიქვი
87	*Perca fluviatilis* Linnaeus, 1758	European perch	მდინარის ქორჭილა
88	*Sander lucioperca* (Linnaeus, 1758)	Pike-perch	ფარგა
	Petromyzontidae		
89	*Lampetra ninae* (Naseka, Tuniyev & Renaud 2009)	Caucasian brook lamprey	კავკასიური სალამურა
	Poeciliidae		
90	*Gambusia holbrooki* Girard, 1859	Mosquitofish	გამბუზია
	Salmonidae		
91	*Oncorhynchus mykiss* (Walbaum, 1792)	Rainbow trout	ცისარტყელა კალმახი
92	*Salmo caspius* Kessler, 1877	Caspian trout	კასპიური მდინარის კალმახი
93	*Salmo ciscaucasicus* Kessler, 1877	Caspian salmon	თერგის კალმახი
94	*Salmo gegarkuni* Kessler, 1877	Sevan trout	იშხანი/სევანის კალმახი
95	*Salmo labrax* Pallas 1814	Black Sea salmon	შავი ზღვის ორაგული

96	*Salmo rizeensis* Turan, Kottelat & Engin 2010	Rize trout	რიზეს კალმახი
	Siluridae		
97	*Silurus glanis* Linnaeus, 1758	Wels catfish	ლოქო/ღლავი
	Syngnathidae		
98	*Syngnathus abaster* Risso, 1827	Black-striped pipefish	შავი ზღვის ლოყაფუნთუშა ნემსთევზა
	Tincidae		
99	*Tinca tinca* (Linnaeus, 1758)	Tench	გუწუ
	Xenocyprididae		
100	*Hemiculter leucisculus* (Basilewsky, 1855)	Sharpbelly	კორეული კარპი/ლანცეტა

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ORIGINAL ARTICLE

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Checklist of the freshwater fishes of Armenia, Azerbaijan and Georgia

Tatia Kuljanishvili[1] | Giorgi Epitashvili[2] | Jörg Freyhof[3] | Bella Japoshvili[2] | Lukáš Kalous[1] | Boris Levin[4,5] | Namig Mustafayev[6] | Shaig Ibrahimov[6] | Samvel Pipoyan[7] | Levan Mumladze[2]

[1]Faculty of Agrobiology, Food, and Natural Resources, Czech University of Life Sciences Prague, Prague, Czech Republic
[2]Institute of Zoology, Ilia State University, Tbilisi, Georgia
[3]Museum für Naturkunde, Leibniz Institute for Evolution and Biodiversity Science, Berlin, Germany
[4]Papanin Institute of Biology of Inland Waters, Russian Academy of Sciences, Yaroslavl Province, Russia
[5]Cherepovets State University, Cherepovets, Russia
[6]Institute of Zoology, National Academy of Sciences of Azerbaijan, Baku, Azerbaijan
[7]Armenian State Pedagogical University after Kh. Abovyan, Yerevan, Armenia

Correspondence
Tatia Kuljanishvili, Faculty of Agrobiology, Food, and Natural Resources, Czech University of Life Sciences Prague, Prague, Czech Republic.
Email: kuljanishvili.tatia@gmail.com

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Abstract

We present a critical checklist of freshwater fish species found so far in the countries of Armenia, Azerbaijan and Georgia. In total 119 freshwater fishes are recorded. There are 40, 86 and 96 species currently known for Armenia, Azerbaijan and Georgia respectively. From these 119 species, seven are endemic and seven species are alien. From the alien species, only three (*Carassius gibelio*, *Gambusia holbrooki* and *Pseudorasbora parva*) can be considered as widespread and invasive. There are four species (*Gasterosteus aculeatus*, *Gobio artvinicus*, *Perca fluviatilis* and *Salmo gegarkuni*) that are translocated within the region. Seven species are confirmed or recorded for the first time including *G. artvinicus* and *Oxynoemacheilus veyselorum* for Armenia, Azerbaijan and Georgia, *Capoeta kaput* and *Rhinogobius lindbergi* for Azerbaijan and Georgia, *Capoeta razii* for Azebaijan, *Oxynoemacheilus cemali* and *Squalius agdamicus* for Georgia. In this checklist, *Acipenser colchicus* is treated as a synonym of *Acipenser persicus*. Sand smelts of the Black and Caspian Sea basin are identified as *Atherina caspia* and *Clupeonella caspia* is treated as a synonym of *Clupeonella cultriventris*. *Coregonus sevanicus* is listed as *Coregonus* sp. until the situation of Sevan whitefish is better understood. *Capoeta sevangi* and *Capoeta ekmekciae* are synonyms of *Capoeta capoeta*. The fish often identified as *Capoeta capoeta gracilis* from rivers south of the Kura most likely belong to *C. razii*. The Black and Caspian Sea *Rutilus* populations are treated as conspecific, therefore *R. kutum* is a junior synonym of *R. frisii*. *Oxynoemacheilus veyseli* is valid as *O. veyselorum*. We list the alien *Rhinogobius* species as *R. lindbergi*, however the name is provisional and needs further confirmation. All *Squalius* species from the Kura River drainage are identified as *S. agdamicus*, however in the Aras, it is replaced by *S. turcicus*. *Squalius orientalis* is treated as a valid species restricted to the eastern Black Sea basin. The four forms of Lake Sevan trout (*Salmo ischchan*, *S. gegarkuni*, *S. danilewskii* and *S. aestivalis*) are treated as valid species, two of them (*S. ischchan* and *S. danilewskii*) are extinct. *Rutilus sojuchbulagi* from Azerbaijan is also extinct.

KEYWORDS

Caucasus biodiversity hotspot, distribution, freshwater fishes, taxonomy

1 | INTRODUCTION

Nowadays, freshwater biodiversity is under heavy anthropogenic pressure (such as overexploitation, environmental pollution, habitat degradation/loss) and therefore, knowledge and monitoring of freshwater species and ecosystems under threat should be a routine for conservation planning (Dudgeon et al., 2006). To this end, reliable and accurate biodiversity data can serve as an important tool in effective conservation management (Frankham, Briscoe, & Ballou, 2002; Mace, Possingham, & Leader-Williams, 2006). However, significant challenges are related to developing countries harbouring great biodiversity. Due to a lack of biodiversity data, these areas continue to suffer inability of proper systematic conservation planning. The example of such area is the South Caucasus region, the biodiversity of which remains poorly documented (Mumladze, Japoshvili, & Anderson, 2019).

Armenia, Azerbaijan and Georgia are located mostly in the South Caucasus region, which is a part of biodiversity hotspot encompassing the Colchis and Hyrcan plio-pleistocene refugia (Figure 1) (Mittermeier et al., 2004). Though less studied, the area is known with an incredible high diversity of landscapes, habitats, species as well as elevated levels of endemism. According to Abell et al. (2008), the South Caucasus freshwater fish fauna belongs to three freshwater eco-regions (Western Transcaucasian, Kura-South Caspian and Western Caspian drainages) and harbours many species endemic to these eco-regions. Furthermore, one of the rivers of the southern Caucasus (the Rioni) is acting as one of the world's last refuge for critically endangered sturgeon species (Bacalbaşa-Dobrovici & Holčík, 2000; Guchmanidze, 2012).

Strong environmental changes started from former Soviet times and continue to occur during the last decades in the South Caucasus because of modernization and industrial development (Davtyan, 2014). Hence, the freshwater ecosystems are particularly vulnerable due to increasing land and freshwater consumption, water pollution and hydropower development. Accordingly, knowledge of freshwater biodiversity and particularly current diversity and distribution of freshwater fishes have a great potential to serve as a tool for the prioritization of conservation efforts (Skelton, Cambray, Lombard, & Benn, 1995). In spite of the recent increase of biodiversity research in the region (Mumladze et al., 2019), the knowledge on freshwater fishes still remains significantly fragmented. Along with some taxonomic uncertainties, major gaps exist in the knowledge of species distributions within the region and there is no quantitative evaluation of anthropogenic pressures freshwater fish are facing today. The inadequacy of the data was the reason why Myers, Mittermeier, Mittermeier, da Fonseca, and Kent (2000) did not include Caucasian freshwater fishes for estimating the vertebrate diversity in the original designation of the biodiversity hotspots. The existing freshwater fish checklists of South Caucasian countries (Armenia, Azerbaijan, Georgia) are outdated considering the recent taxonomic/nomenclatorial changes and distributional updates. For instance, after the collapse of the Soviet Union, a first updated checklist of Armenian fishes was compiled in 1998–2002 (Gabrielyan, 2001; Pipoyan & Tigranyan, 1998, 2002; see also Levin & Rubenyan, 2010) and the last book regarding Armenian ichthyofauna was issued in 2012 (Pipoyan, 2012). The simple list of fishes of Azerbaijan by Ibrahimov and Mustafayev (2015) was issued in Azerbaijani language, showing no significant improvement in comparison to Abdurakhmanov (1962). Based on literature review, checklist of fishes of Georgia was compiled by Ninua and Japoshvili (2008) and reissued in 2013 (Ninua, Japoshvili, & Bochorishvili, 2013). Naseka (2010) provided the checklist of freshwater fishes for wider biogeographical regions in the Caucasus and offered ecoregional delineation of the Caucasus and surrounding. This later checklist, however, is hardly applicable for country-level fish diversity analyses as well as for conservational purposes.

Thus, mostly outdated information and/or data deficiencies in terms of taxonomy, distribution and conservation status of freshwater fishes of the South Caucasian countries have resulted a necessity to assemble most up to date checklist of South Caucasian countries, which is aimed to serve as a baseline for future investigation and conservation of fish biodiversity in the region.

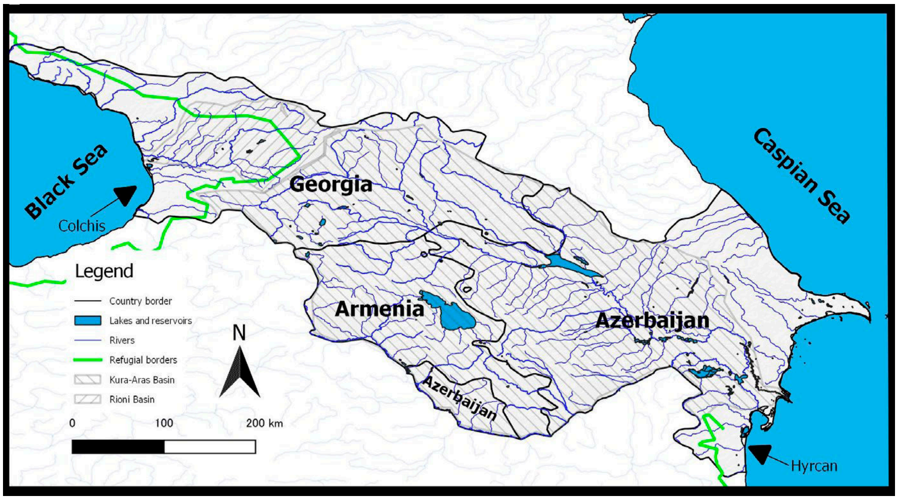

FIGURE 1 Map of freshwaters of Armenia, Azerbaijan and Georgia. Patterned areas show the main river drainages (the Kura and Rioni) encompassing almost 80% of the region. Green lines denote borders of two Caucaso-Anatolian refugia (Hyrcan and Colchis)

2 | MATERIALS AND METHODS

The study area covers the territories of Armenia, Azerbaijan and Georgia, the largest part of which is located south from the Great Caucasus Range, between the Black and Caspian Seas (Figure 1). The Eastern Black Sea basin is represented by a number of independent river systems over the territory of Georgia, one of them shared (the Coruh/Chorokhi system) with Turkey. There are other, smaller rivers including the Enguri, Supsa, Kintrishi, Coruh. The Rioni River is the largest among the eastern Black Sea tributaries in the area by means of basin extent and annual discharge (Figure 1). Almost all the South-Western Caspian Sea basin is represented by the Kura-Aras drainage discharging into the Caspian Sea from the territory of Georgia, Armenia, Azerbaijan and Turkey. Nearly 5% of the study area (north-eastern part of Azerbaijan and northern part of Georgia) is located in Northern Caucasus. This includes some headwater streams of the Terek River and other streams which originate in Georgia and discharges to the north-eastern part of Caspian Sea and some mountain streams (such as the rivers Samur, Mukhtadirchay, Gusarchay, Agchay, Garachay, Velvelechay, Gilgilchay, Atachay, Sumgaitchay, Pirsagat) of Azerbaijan.

To compile the regional checklist of fishes, we critically reviewed previously published country scale species lists (Gabrielyan, 2001; Ibrahimov & Mustafayev, 2015; Levin & Rubenyan, 2010; Ninua & Japoshvili, 2008; Pipoyan, 2012) and the literature published since then (see the references cited in the discussion section). In addition, species diversity was revised based on scattered morphological and molecular analysis done by the authors, allowing to propose changes for certain species distribution or taxonomy.

The checklist is presented in the form of a table (Table 1) which is ordered by families and genera according to the classification by Nsselson, Grande, and Wilson (2016) with the updates provided by Betancur-R et al. (2017) and the Eschmeyer's Catalogue of Fishes (Fricke, Eschmeyer & Van der Laan, 2020). Alien and Endemic species are indicated in the table. Table also provides information about species, that were not mentioned before for Armenia, Azerbaijan and Georgia. Additional notes about distribution and taxonomy for certain species are also provided for several taxa in the discussion section.

3 | RESULTS

In total, 119 species, belonging to 26 families were recognised for the South Caucasian countries (Table 1). From these, 40 species are recorded from Armenia, 86 - from Azerbaijan, and 96 – from Georgia. This list also includes few north Caucasian species (*Barbus ciscaucasicus*, *Oxynoemacheilus merga*, *Romanogobio ciscaucasicus*, *Salmo ciscaucasicus*), mostly from Azerbaijani rivers north of Baku. Seven species (6% of the whole ichthyofauna) are endemic on a country scale level.

The region has also experienced introductions of non-native fishes. Currently there are seven (6% of all freshwater fish fauna) alien species introduced intentionally or accidentally (Table 1). From this species *Carassius gibelio*, *Pseudorasbora parva* and *Gambusia holbrooki* are considered as invasive species posing a serious threat to the native fauna and freshwater ecosystems (see also the following section). Unfortunately, there are no management strategies for any invasive species in any South Caucasian countries. There are four species which are translocated within the region: introduction of *Gasterosteus aculeatus* in the Caspian Sea basin is attributed to the opening of the Volga-Don channel in 1984 (Bogutskaya, Kijashko, Naseka, & Orlova, 2013; Musayev et al.,2004); *Gobio artvinicus* has been introduced to the Caspian Sea basin; *Perca fluviatilis* was translocated by fishermen from the Black Sea basin to the Kura River drainage; *Salmo gegarkuni* is endemic for Lake Sevan, however, it is introduced in lakes and reservoirs of Georgia and Azerbaijan.

Seven species are mentioned for the first time for the countries. These are *G. artvinicus* and *Oxynoemacheilus veyselorum* for Armenia, Azerbaijan and Georgia, *Capoeta kaput* and *Rhinogobius lindbergi* for Azerbaijan and Georgia, *Capoeta razii* for Azebaijan and *Oxynoemacheilus cemali* and *Squalius agdamicus* for Georgia.

4 | DISCUSSION

In the following, we are giving notes on several fish taxa, for which knowledge gaps are currently identified and provide additional argumentations with regards of distribution or taxonomy.

4.1 | Acheilognathidae

Based on morphological characters, *Rhodeus colchicus* was described from the River Natanebi in the Georgian Black Sea basin (Bogutskaya & Komlev, 2001) and the species is widespread from the North Caucasus to the south, not reaching Turkey. Molecular studies (Bartáková et al., 2019; Bohlen, Šlechtová, Bogutskaya, & Freyhof, 2006) revealed that *R. colchicus* is nested within *Rhodeus amarus*. In their phylogenetic analysis, Bartáková et al. (2019) found six different molecular groups of bitterlings in the western Palearctic, *R. colchicus* representing one of these. The phylogenetic structure of these six clades is poorly supported in the analysis by Bartáková et al. (2019) and it cannot be excluded, that *R. colchicus* might be the sister group of all other western Palearctic bitterling clades. Similar results have been obtained earlier by the study done by Bohlen et al. (2006). Geiger et al. (2014) suggest that lineages of European bitterlings are very closely related and might be better considered as a separate populations rather than species. However, as the consensus of species status of *R. colchicus* is not yet reached, we keep *R. colchicus* as a separate species in the presented list.

4.2 | Acipenseridae

The actual distribution of sturgeons in the study area is not well understood. *Acipenser sturio* and *A. nudiventris* have vanished from the Caucasus since no records for decades exists. Even whether other

TABLE 1 Checklist of freshwater fishes of Armenia, Azerbaijan and Georgia

Taxa	Alien	Endemic	Armenia	Azerbaijan	Georgia
Acheilognathidae					
Rhodeus amarus (Bloch, 1782)			x	x	x
Rhodeus colchicus Bogutskaya & Komlev 2001					x
Acipenseridae					
Acipenser gueldenstaedtii Brandt & Ratzeburg 1833				x	x
Acipenser nudiventris Lovetsky, 1828				x	x
Acipenser persicus Borodin, 1897				x	x
Acipenser stellatus Pallas, 1771				x	x
Acipenser sturio Linnaeus, 1758					x
Huso huso (Linnaeus, 1758)				x	x
Anguillidae					
Anguilla anguilla (Linnaeus, 1758)				x	x
Atherinidae					
Atherina caspia Eichwald 1831				x	x
Clupeidae					
Alosa caspia (Eichwald, 1838)				x	
Alosa immaculata Bennett, 1835					x
Alosa kessleri (Grimm, 1887)				x	
Alosa maeotica (Grimm, 1901)					x
Alosa tanaica (Grimm, 1901)					x
Clupeonella cultriventris (Nordmann, 1840)				x	x
Cobitidae					
Cobitis amphilekta Vasil'eva & Vasil'ev, 2012				x	
Cobitis derzhavini Vasil'eva, Solovyeva, Levin, & Vasil'ev, 2020		x		x	
Cobitis saniae Eagderi, Jouladeh-Roudbar, Jalili, Sayyadzadeh & Esmaeili, 2017			x	x	x
Cobitis satunini Gladkov 1935					x
Sabanejewia aurata (De Filippi, 1863)			x	x	x
Sabanejewia caspia (Eichwald, 1838)				x	
Coregonidae					
Coregonus albula (Linnaeus, 1758)	x				x
Coregonus sp.	x		x		x
Cyprinidae					
Barbus ciscaucasicus Kessler, 1877				x	x
Barbus cyri De Filippi, 1865			x	x	x
Barbus rionicus Kamensky, 1899					x
Capoeta banarescui Turan, Kottelat, Ekmekçi & Imamoglu, s2006					x
Capoeta capoeta (Güldenstädt, 1773)			x	x	x
Capoeta kaput Levin, Prokofiev & Roubenyan 2019			x	x*	x*
Capoeta razii Jouladeh-Roudbar, Eagderi, Ghanavi & Doadrio, 2017				x*	
Capoeta sieboldii (Steindachner, 1864)					x
Carassius gibelio (Bloch, 1782)	x		x	x	x
Cyprinus carpio Linnaeus, 1758			x	x	x
Luciobarbus brachycephalus (Kessler, 1872)				x	x
Luciobarbus capito (Güldenstädt, 1773)			x	x	x
Luciobarbus mursa (Güldenstädt, 1773)			x	x	x

(Continues)

TABLE 1 (Continued)

Taxa	Alien	Endemic	Armenia	Azerbaijan	Georgia
Esocidae					
Esox lucius Linnaeus, 1758				x	x
Gasterosteidae					
Gasterosteus aculeatus Linnaeus, 1758				(x)	x
Pungitius platygaster (Kessler, 1859)				x	
Gobiidae					
Babka gymnotrachelus (Kessler, 1857)				x	x
Mesogobius batrachocephalus (Pallas, 1814)					x
Neogobius fluviatilis (Pallas, 1814)			x	x	x
Neogobius melanostomus (Pallas, 1814)				x	x
Neogobius pallasi (Berg, 1916)				x	
Ponticola constructor (Nordmann, 1840)					x
Ponticola cyrius (Kessler, 1874)			x	x	x
Ponticola gorlap (Iljin, 1949)				x	x
Ponticola syrman (Nordmann, 1840)				x	x
Proterorhinus nasalis (De Filippi, 1863)				x	x
Gobionidae					
Gobio artvinicus Turan, Japoshvili, Aksu & Bektaş 2016			(x)*	(x)*	(x)*
Gobio caucasicus Kamensky, 1901					x
Pseudorasbora parva (Temminck & Schlegel, 1846)	x		x	x	x
Romanogobio ciscaucasicus (Berg, 1932)				x	
Romanogobio macropterus (Kamensky, 1901)			x	x	x
Leuciscidae					
Abramis brama (Linnaeus, 1758)			x	x	x
Acanthobrama microlepis (De Filippi, 1863)			x	x	x
Alburnoides eichwaldii (De Filippi, 1863)			x	x	x
Alburnoides fasciatus (Nordmann, 1840)					x
Alburnoides samiii Mousavi-Sabet, Vatandoust & Doadrio, 2016				x	
Alburnus alburnus (Linnaeus, 1758)				x	x
Alburnus chalcoides (Güldenstädt, 1772)				x	x
Alburnus derjugini Berg, 1923					x
Alburnus filippii Kessler, 1877			x	x	x
Alburnus hohenackeri Kessler, 1877			x	x	x
Ballerus sapa (Pallas, 1814)				x	x
Blicca bjoerkna (Linnaeus, 1758)			x	x	x
Chondrostoma colchicum Derjugin, 1899					x
Chondrostoma cyri Kessler, 1877			x	x	x
Chondrostoma oxyrhynchum Kessler 1877				x	
Leuciscus aspius (Linnaeus, 1758)			x	x	x
Leucaspius delineatus Heckel, 1843			x	x	x
Pelecus cultratus (Linnaeus, 1758)				x	
Petroleuciscus borysthenicus (Kessler, 1859)					x
Phoxinus colchicus Berg, 1910					x
Rutilus atropatenus Derjavin, 1937		x		x	
Rutilus frisii (Nordmann, 1840)				x	x

(Continues)

TABLE 1 (Continued)

Taxa	Alien	Endemic	Armenia	Azerbaijan	Georgia
Rutilus lacustris (Pallas 1814)			x	x	x
Rutilus sojuchbulagi Abdurakhmanov 1950		x		x	
Scardinius erythrophthalmus (Linnaeus, 1758)				x	x
Squalius agdamicus (Kamensky 1901)				x	x*
Squalius orientalis Heckel, 1847					x
Squalius turcicus De Filippi, 1865			x	x	x
Vimba vimba (Linnaeus, 1758)				x	x
Lotidae					
Lota lota (Linnaeus, 1758)				x	
Moronidae					
Dicentrarchus labrax (Linnaeus, 1758)					x
Mugilidae					
Chelon auratus (Risso, 1810)				x	x
Chelon labrosus (Risso, 1827)					x
Chelon ramada (Risso, 1827)					x
Chelon saliens (Risso, 1810)				x	x
Mugil cephalus Linnaeus, 1758					x
Nemacheilidae					
Oxynoemacheilus bergianus (Derjavin, 1934)			x	x	x
Oxynoemacheilus brandtii (Kessler, 1877)			x	x	x
Oxynoemacheilus cemali Turan, Kaya, Kalayci, Bayçelebi & Aksu 2019					x*
Oxynoemacheilus lenkoranensis (Abdurakhmanov, 1962)				x	
Oxynoemacheilus merga (Krynicki, 1840)				x	
Oxynoemacheilus veyselorum Cicek, Eagderi & Sungur, 2018			x*	x*	x*
Oxudercidae					
Knipowitschia caucasica (Berg, 1916)			x	x	x
Knipowitschia longecaudata (Kessler, 1877)				x	x
Rhinogobius lindbergi Berg, 1933	x			x*	x*
Percidae					
Perca fluviatilis Linnaeus, 1758			(x)	x	x
Sander lucioperca (Linnaeus, 1758)			x	x	x
Petromyzontidae					
Caspiomyzon wagneri (Kessler, 1870)				x	
Lampetra ninae (Naseka, Tuniyev & Renaud 2009)					x
Poeciliidae					
Gambusia holbrooki Girard, 1859	x		x	x	x
Salmonidae					
Salmo aestivalis Kessler 1877		x	x		
Salmo caspius Kessler, 1877			x	x	x
Salmo ciscaucasicus Kessler, 1877				x	x
Salmo coruhensis Turan, Kottelat & Engin 2010					x
Salmo danilewskii Gulelmi 1888		x	x		
Salmo gegarkuni Kessler, 1877		x	x	(x)	(x)
Salmo ischchan Kessler 1877		x	x		
Salmo labrax Pallas 1814					x

(Continues)

TABLE 1 (Continued)

Taxa	Alien	Endemic	Armenia	Azerbaijan	Georgia
Salmo rizeensis Turan, Kottelat & Engin 2010					x
Siluridae					
Silurus glanis Linnaeus, 1758			x	x	x
Syngnathidae					
Syngnathus abaster Risso, 1827					x
Syngnathus caspius Eichwald 1831				x	
Tincidae					
Tinca tinca (Linnaeus, 1758)				x	x
Xenocyprididae					
Hemiculter leucisculus (Basilewsky, 1855)	x			x	
Total	7	7	40	86	96

Note: A cross – x, indicates the occurrence of the species in corresponding column; a cross in parenthesis – (x), indicates species translocated within the region, an asterisk – *, indicates the species that were not mentioned before for the countries.

sturgeon species still spawn regularly in the Kura and Rioni rivers, is doubtful. *Acipenser colchicus* was considered the Black Sea population of the Persian sturgeon (*Acipenser persicus*) by Marti (1940) or even the eastern Black Sea population of the Russian sturgeon *A. gueldenstaedti* by Berg (1949). It has been accepted as a valid and the Black Sea endemic species by Kottelat and Freyhof (2007). Following Marti (1940) we identify *A. colchicus* as the Black Sea population of *A. persicus* until the separate species status is confirmed.

Acipenser ruthenus has been recorded from the Kura River drainage in Azerbaijan and Berg (1949) mentions occasional findings from the western Caspian coast and the lower Kura River. However, there is no record or indication that there had ever been an established population of this species in the area. It is suspected, that sterlets found in the Kura mouth were migrants from the Volga population. The anadromous sterlet population in the Volga has vanished in the 20th century (Kottelat & Freyhof, 2007) and more recent records of sterlets are very likely to have come from fish farms. It should be mentioned that, apart from anadromous populations, there are resident populations within the Volga drainage, for instance the Oka and Sura Rivers populations (Ivancheva & Ivanchev, 2008; Shilin, 2001).

4.3 | Anguillidae

Anguilla anguilla is occasionally found in the Caspian Sea basin, for example in the Aras River in Armenia in 2015 (Pipoyan, 2015). As this species only spawns in the Atlantic Ocean, all records in the Caspian Sea basin must originate from stocking or from individuals which reach the Caspian Sea basin through canals.

4.4 | Atherinidae

Atherina caspia is widespread in the Black and Caspian Seas, where it is often identified as *A. boyeri* or *A. pontica* (see Vasil'eva, 2017). Naseka and Bogutskaya (2009), based on Tarasov (2001), treat *A. caspia* as a separate species. Own (unpublished) molecular and morphological studies support the Black and Caspian Sea populations as a distinct species from the Mediterranean *A. boyeri*, but fail to distinguish *A. pontica* (the Black Sea) from *A. caspia* (the Caspian Sea). *A. caspica* has been earlier treated as a valid species by Naseka and Bogutskaya (2009), while *A. pontica* was not. Here we also treat *A. pontica* as a synonym of *A. caspia*.

4.5 | Clupeidae

The diversity of shads (*Alosa*) from the Black and Caspian Seas is poorly understood and early authors follow Berg (1949) listing 15 species and forms from the Caspian Sea only. The number of species has not been reviewed comprehensively in recent years and we treat the species accepted by Kottelat and Freyhof (2007) as valid awaiting a sound taxonomic review especially of the Caspian marine species. *Alosa curensis* is sometimes mentioned as a valid species from Azerbaijan. This very poorly known shad was only found once by Suvorov (1907) and Berg (1949) mentioned that it is only known from the types collected from Kizilagach Bay (Qizilagac Bay) in Azerbaijan. *Alosa curensis* has teeth on the jaws, 31–43 gill rakers, and the gill rakers length exceed the length of the gill filaments. With this combination of characters, *A. curensis* keys out as *A. sphaerocephala* with the identification key given by Berg (1949) and we cannot exclude that it might be a synonym of this marine species. Since no records of *A. curensis* exist from freshwaters, we therefore excluded it from the current checklist.

Clupeonella cultriventris is widespread along coasts of the Black and Caspian Seas, entering lower reaches of rivers. Own (unpublished) molecular and morphological studies fail to distinguish the Caspian population (*C. caspia*) from the Black Sea population (*C. cultriventris*). Hoestlandt (1991) followed by Kottelat and Freyhof (2007) separate these two tyulkas largely based on the length of paired fins, a character we found to be very much overlapping in own materials examined. Therefore, we treat *C. caspia* as a synonym of *C. cultriventris*.

Clupeonella tscharchalensis is described from Lake Chelkar (47°50′N 59°36′E) in Kazakhstan. It is considered to be a freshwater species inhabiting rivers of the northern Caspian Sea basin (Hoestlandt, 1991) and to be invasive in the Don River. Tyulkas analysed from the Don are identified as *C. cultriventris* by own (unpublished) molecular and morphological data. Until tyulkas from Lake Chelkar are further studied, we treat *C. tscharchalensis* as a valid species having less gill-rakers than *C. cultriventris*.

4.6 | Coregonidae

Coregonus albula was introduced to Tabatskuri and Paravani Lakes (southern Georgia) from the Volkhov Hatchery at Ladoga Lake in 1930s (Barach, 1941). The local populations are still present and commercially valuable in Georgia though the abundance is decreasing due to the unavailability of local hatcheries (Kuljanishvili, Mumladze, Kalous, & Japoshvili, 2018).

The taxonomic status of the introduced Caucasian whitefish remains unsolved. Based on literature (Barach, 1940; Dadikyan, 1964; Mailyan, 1957) two species were introduced to Lake Sevan in the 1920s: *Coregonus ludoga* from Lake Ladoga and *Coregonus maraenoides* from Lake Chudskoe (both in Northern European Russia). These two species naturalised and hybridised in the lake (Mailyan, 1957) and an intermediate phenotype was subsequently described as a *Coregonus lavaretus sevanicus* by Dadikyan (1986). *Coregonus sevanicus* might represent a valid species of hybrid origin. However, due to scarcity of recent data we cannot confirm or reject any taxonomic hypothesis related to Caucasian whitefishes. Thus, we list only *Coregonus* sp. in the checklist. According to Elanidze (1983) in 1930-ies *C. ludoga* was also introduced from Volkhov hatchery to Lake Tabatskuri in Georgia through the species was not recorded for at least the last 50 years. As there are no recent records of any *Coregonus* from Lake Tabatskuri, we suspect that the population might be extirpated.

4.7 | Cyprinidae

There are three species of *Barbus* distributed in the region. *Barbus ciscaucasicus* in northern parts of Georgia and Azerbaijan, *B. cyri* in the Kura and Aras drainage and *B. rionicus* in the Black Sea basin in the west Georgia. The recent study done by Levin, Gandlin, et al. (2019) has shown that *Barbus goktschaicus* does not differ from *B. cyri* genetically and they both are distributed in the Kura-Aras system. Therefore, *B. goktschaicus* is synonymised with *B. cyri*.

Capoeta capoeta is widespread in the Kura and Aras drainages. Occurs in Lake Sevan and in Lake Urmia basin. *Capoeta sevangi* from Lake Sevan is treated as a valid species by Gabrielyan (2001), Ninua and Japoshvili (2008) and Ninua et al. (2013) without presenting any evidence. Levin, Prokofiev, and Roubenyan (2019) identify the *Capoeta* from Lake Sevan as *C. capoeta*, and we follow Levin, Gandlin, et al. (2019) treating *C. sevangi* as a synonym of *C. capoeta*, also supported by our own (unpublished) molecular data.

Capoeta ekmekciae is considered to be endemic to the Eastern Black Sea basins from the Rioni River south to the Coruh. The molecular sequences that were analysed by Bektas et al. (2017) suggest that this species is very closely related to *C. capoeta*. After checking the morphological characters given by Turan, Kottelat, Gülsün Kirankaya, and Engin (2006) to distinguish this species from *C. capoeta*, we found them largely overlapping. As we could not find other distinguishing characters between *C. capoeta* and *C. ekmekciae* in our own materials, we treat *C. ekmekciae* as a synonym of *C. capoeta*.

Capoeta gracilis was described from the area of Isfahan in Iran, this name is not available for the *Capoeta* species in the Caspian Sea basin. Despite, the fish from rivers south of the Kura was often identified as *Capoeta capoeta gracilis*. This fish most likely belong to *C. razii* that was described from the eastern Caspian Sea basin in Iran by Jouladeh-Roudbar, Eagderi, Ghanavi, and Doadrio (2017). Jouladeh-Roudbar et al. (2017) already reported this species to be very widespread in the rivers of the Iranian Caspian coast north-west to the Choobar River, close to the Azerbaijan border.

Carassius gibelio is widely introduced all over the study area. It appeared in the late 1970-ies in Armenia (Pipoyan & Rukhkyan, 1998), followed by record from Georgia in the middle 1980th by Daraselia (1985) (as *C. carassius*). Currently *C. gibelio* is the most widespread and abundant invasive species which distribution ranges from lowland waterbodies as high as mountain lakes that are up to 2,100 m above sea level (Bogutskaya et al., 2013; Gabrielyan, 2001; Japoshvili, Mumladze, & Küçük, 2013; Japoshvili, Mumladze, & Murvanidze, 2017; Kuljanishvili, Japoshvili, Mumladze, & Kalous, 2018; Oganesyan & Smolej, 1985; Pipoyan, 1993, 2012; Pipoyan & Rukhkyan, 1998). While *C. gibelio* and *C. auratus* can be distinguished by molecular characters (Rylková, Kalous, Bohlen, Lamatsch, & Petrtýl, 2013), there is no agreed set of morphological characters to separate both species and we cannot exclude that many records of *C. gibelio* actually refer to *C. auratus*. It should also be noted, that *Carassius langsdorfi* as an additional, superficially very similar species (Vetešník, Papoušek, Halačka, Lusková, & Mendel, 2007) is also expected to occur in the area.

4.8 | Gobiidae

Ponticola iranicus had been described by Vasil'eva, Mousavi-Sabet, and Vasil'ev (2015) from the Gisum and Sefid Rivers in Iran. As the fauna of the Sefid River is relatively similar to the fauna of coastal rivers of the Azerbaijani coast and the Aras, it is expected to find this species here in the future.

4.9 | Gobionidae

Gobio artvinicus was described from the lower Coruh River in Turkey and is expected to occur also in the Georgian part of the same river. The species is widespread in the Georgian Black Sea basin, where it is at least confirmed to the north to the Rioni River. Gudgeons are alien in the Kura and Aras River drainage. Gudgeons discovered in the Metsamor River (Armenia) in 1998 and identified as *Gobio gobio* by Pipoyan (1998) might also belong to *G. artvinicus*. The fish found in the Tashir and Debed rivers (The Kura river drainage) in Armenia are also morphologically similar to *G. artvinicus.* Own (unpublished) sequence data identify the *Gobio* species alien in the Kura in Georgia and Azerbaijan as *G. artvinicus* and the species might be widespread also in the Aras.

The species identity of other *Gobio* species in the region remains unclear. *Gobio caucasicus* was described as *Gobio lepidolaemus* var. *caucasica* by Kamensky 1901 based on a syntype series from three rivers: the Rioni in the Georgian Black Sea basin, the Podkumok in northern Dagestan, and the Sulak in southern Dagestan, both in the Caspian Sea basin. Furthermore, *Gobio holurus* was described from the Terek River drainage, also in the Caspian Caucasus. Kottelat and Freyhof (2007) treat *G. holurus* as a valid species and Turan, Japoshvili, Aksu, and Bektaş, (2016) treat *G. caucasicus* as a valid species. Our own (unpublished) molecular data suggest that at least two species (excluding *G. artvinicus*) might be involved in the Caucasian gudgeon complex and both seem to occur in the Black Sea as well as in the Caspian Sea basin in northern Caucasus and both are closely related, if not conspecific with adjacent species as *Gobio kubanicus*, *G. krymensis* and *G. brevicirris*. Further study is needed to unambiguously resolve the Caucasian gudgeon complex in the future.

Pseudorasbora parva was accidentally introduced together with Chinese carps fry (e.g. *Hypophthalmichthys nobilis*, *H. molitrix* or *Ctenopharyngodon idella*) from Asia followed the establishment of viable populations throughout the region (Bogutskaya et al., 2013; Gabrielyan, 2001; Naseka & Bogutskaya, 2009; Ninua & Japoshvili, 2008; Pipoyan, 2012; Pipoyan & Arakelyan, 2015). The species is still expanding its range in the study area and recently it was discovered in closed mountain lake Kartsakhi (N41.216 E43.231) in the Javakheti highland (Southern Georgia) (own unpublished data).

4.10 | Leuciscidae

Alburnoides samii was found by Levin, Simonov, Matveyev, et al. (2018) in the Lenkoran River in Azerbaijan. Future studies might show that some *Alburnoides* populations in rivers and streams south of the Kura and Aras drainages belong to this species.

Alburnus chalcoides is widespread in Azerbaijan and Georgian part of the Kura basin. It is also expected to occur in the Armenia (the Aras River) but has not yet been recorded here.

Rutilus caspicus from the Caspian Sea basin, *R. heckelii* from the Black Sea basin, and *R. shelkovnikovi* from the Metsamor River in the Ararat Valley (Armenia) were synonymised with *R. lacustris* based on their mtDNA identity (Levin et al., 2017). This opinion is followed here.

Rutilus kutum is treated as a valid species by Bogutskaya and Iliadou (2006: 294) and following authors without a detailed discussion. The gene trees presented by Kotlík et al. (2008) showed clear geographical structure between the Black and Caspian Sea populations but the two populations were not reciprocally monophyletic at any of the three loci studied and there had been considerable gene flow suggested from the Caspian population into the Black Sea population. Own (unpublished) molecular data (COI) fail to distinguish the Caspian *R. kutum* from the Black Sea *Rutilus frisii*. We were unable to find evidence why both species should be separated despite that they are frequently listed in un-commented checklists. Therefore, we treat both populations as conspecific, *R. kutum* being a junior synonym of *R. frisii*.

Rutilus sojuchbulagi was known only from the Akstafa Region in Azerbaijan where it has not been found despite of intensive fieldwork and specific research during the last ten years. Therefore, we assume that this species has gone extinct. *R. atropatenus* and *R. sojuchbulagi* from Azerbaijan were initially described as members of the genus *Rutilus* (Abdurakhmanov, 1950; Derzhavin, 1937) but later placed in *Pseudophoxinus* by Bogutskaya, Küçük, and Atalay (2006) without detailed discussion or arguments. Saç, Özuluğ, Geiger, and Freyhof (2019) place both species back to *Rutilus* based on molecular COI sequence data confirming the original generic placement (Abdurakhmanov, 1950; Derzhavin, 1937) Future research based on nuclear molecular data might again challenge this placement.

Squalius agdamicus was described from a small river called the Kuyra at Agdam town, not reaching the Kura River (Berg, 1949). Doadrio and Caramona (2006) treat this species as valid and our own (unpublished) data strongly suggest that it is widespread all over the Kura River drainage, but there is no indication, that it also occur in the Aras, where it is replaced by *Squalius turcicus*.

Squalius orientalis is treated as a valid species restricted to the eastern Black Sea basin (Khaefi, Esmaeili, Sayyadzadeh, Geiger, & Freyhof, 2016) and identifications are largely based on the COI gene of *Squalius* from the Ashe River in Russia, south to the Coruh River in Turkey. Its distribution range might be much larger and this molecular lineage is very closely related to a molecular lineage that is widely distributed in the northern Black Sea basin as well as in the Baltic Sea basin west to the Elbe River in the Czech Republic and Germany, where it is identified as *S. cephalus*.

Squalius turcicus was described from the upper Aras River in Turkey. Özulug and Freyhof (2011) suggested that this might be the species distributed in the southern Caspian Sea basin. Turan, Kottelat, and Doğan (2013) treat *S. turcicus* as a distinct species from *S. orientalis*. Also Khaefi et al. (2016) treat *S. turcicus* as a valid species. Accordingly, all previous notes on *Squalius* species from Aras river is treated as *S. turcicus*. As of now, there is no comprehensive distribution data available for *S. turcicus* and *S. agdamicus* in the Caspian Sea drainage and they might frequently live in sympatry.

Apparently, more data is needed to better understand the distribution of these two species.

4.11 | Lotidae

Only single individual of *Lota lota* was caught in 1920 in the Kura River which was considered as an accidental migrant from the northern Caspian basin species (Abdurakhmanov, 1962; Derzhavin, 1949; Musayev et al., 2004). However, *L. lota* also occurs in Iran and might have spread from this area to the north.

4.12 | Nemacheilidae

Oxynoemacheilus cemali was described from the Coruh River drainage in north-eastern Turkey by Turan, Kaya, Kalayci, Bayçelebi, and Aksu (2019). The species is expected to occur in the Georgian part of the river also.

Oxynoemacheilus merga is distributed in northern Caucasian rivers (the Kuma, Terek, Sulak) which flow to the Caspian Sea. Although it has not been recorded in Georgia yet, we assume that the fish could be distributed in the European part of the country.

Oxynoemacheilus veyseli was described from the upper Aras River in Turkey by Çiçek, Eagderi, and Sungur (2018). The species name is incorrect and must be replaced by *O. veyselorum*, since this species was named after two men, the father and son of the first author (see Çiçek et al., 2018). Loaches usually identified as *O. angorae* in the Kura and Aras Rivers (Abdurakhmanov, 1962; Barach, 1940, 1941; Berg, 1949; Dadikyan, 1986; Elanidze, 1983; Gabrielyan, 2001; Levin & Rubenyan, 2010; Ninua & Japoshvili, 2008; Pipoyan, 2012; Pipoyan & Tigranyan, 1998) might largely belong to *O. veyselorum*.

Overall, the systematics of *Oxynoemacheilus* species in the study area is still poorly understood and the taxonomic status of species such as *O. angorae alasanicus*, *O. bergi*, *O. brandti gibbusnazus*, and *O. lenkoranensis* need to be revisited.

4.13 | Oxudercidae

Betancur-R et al. (2017) recognised Gobionellidae as a junior synonym for Oxudercidae. Here we follow Betancur-R et al. (2017) and update the family name for the species which were before placed in Gobionellidae as Oxudercidae.

Knipowitschia bergi, sometimes considered as *Hyrcanogobius bergi*, has not been found in the last decades and new field data is encouraged to test, if this species might actually be extirpated in the area.

The *Rhinogobius* species, considered invasive in the Caspian Sea basin, seems to be now widespread also in the Caspian part of the study area (Japoshvili et al., 2020) and is expected to enter the Black Sea basin soon. There had been several studies aiming to identify this species, which was treated as *R. cheni* (Vasil'eva & Kuga, 2008), *R. similis* (Esmaeili et al., 2014), and *R. lindbergi* (Esmaeili, Sayyadzadeh, Eagderi, & Abbasi, 2018; Sadeghi, Esmaeili, Zarei, Esmaeili, & Abbasi, 2019). The name of this species in our list is provisional and needs further confirmation.

4.14 | Salmonidae

The diversity of Caucasian trouts is very poorly understood, what is related to the very recent evolutionary history of all species. Actually, trouts of the northern Caspian Caucasus are identified as *S. ciscaucasicus*, described from the Keyranchay River. Those of the southern Caucasus are identified as *S. caspius*, described from the Kura in Azerbaijan. Ninua, Tarkhnishvili, and Gvazava, (2018) support this view and suggest using *S. caspius* for trouts from the rivers flowing into the Caspian Sea south of the Caucasus and *S. ciscaucasicus* for that found in the Terek River and other rivers flowing into the Caspian north of the Caucasus. This system might be oversimplified and more research will for sure challenge our understanding of the species diversity of Caspian trouts. The Black Sea trouts were usually identified as *S. labrax* until Turan, Kottelat, and Engin (2009) showed that within the Coruh River drainage, two forms coexist, which they named *S. rizeensis* and *S. coruhensis*, the former purely riverine and the latter anadromous. Ninua et al. (2018) suggested that the mitochondrial haplogroup of *S. rizeensis* was never found in fish caught in the Black Sea. Ninua et al. (2018) support *S. rizeensis* and *S. coruhensis* as own species different from *S. labrax* but indicate some hybridisation between *S. coruhensis* and *S. labrax* in the Georgian Caucasus, a situation expected to happen in two anadromous, migratory species at their distribution border area.

The trouts of the *Salmo ischchan* complex are endemic to Lake Sevan (Armenia). Four "forms" of Sevan trouts are generally recognised (*S. ischchan*, *S. gegarkuni*, *S. danilewskii* and *S. aestivalis*) (Barach, 1940; Dadikyan, 1986; Gabrielyan, 2001; Levin, Simonov, Rastorguev, et al., 2018; Pipoyan, 2012). These have been interpreted as subspecies, ecological forms or life-history types. The available evidence strongly suggests that they had been species following the Evolutionary Species Concept (see Kottelat, 1997 for definitions). The four trout species were very closely related and might have been the result of sympatric speciation events happening after the last glacial times (<10,000 years ago). While Sevan trout diversity has been poorly studied, this very interesting example of intra-lacustrine trout speciation has already been lost. The abundance of all Sevan trouts drastically declined in the twentieth century due to overfishing and loss of spawning grounds due to water diversion and a critical 18.5-m drop of the lake water level finally leading to the extinction of two of the endemic species. *S. ischchan* and *S. danilewskii* are considered to be extinct since the 1980-ies (Levin & Rubenyan, 2010; Pipoyan, 2012). The two other species, *S. gegarkuni* and *S. aestivalis* are believed to still exist but they are largely or completely dependent on artificial reproduction and stocking.

4.15 | Syngnathidae

According to Berg (1949) there was *Syngnathus nigrolineatus* distributed in the Black Sea (Lake Paliastomi, the Rioni delta, Lake Nuriegel in Batumi, riverlets in Batumi region) and subspecies *S. n. caspius* in whole Caspian Sea and downstream of the Kura River. Naseka and Bogutskaya (2009) elevated *S. n. caspius* to the species level (*S. caspius*) mainly based on the arguments provided in Tarasov (2001). Here we follow Naseka and Bogutskaya (2009) treating *S. caspius* as a valid species from the Caspian Sea basin.

4.16 | Xenocyprididae

Hemiculter leucisculus is fast expanding its range into the southern Caspian Sea basin in Iran (Zareian, Esmaeili, Zamanian Nejad, & Vatandoust, 2015) and was recently recorded from Azerbaijan by Mustafayev, Ibrahimov, and Levin (2015). Its invasion of the Black Sea basin is only a question of time.

5 | CONCLUSION

During the period of the Soviet Union, large-scale industrial projects have taken place in all the republics of the Southern Caucasus. This resulted in strong impacts on freshwater biodiversity of the whole region (Freyhof et al., 2015). For example, construction of the Mingachevir Dam in Azerbaijan blocked the migration path for Caspian lamprey, shads and sturgeons. As a result, a significant part of the spawning areas (which included the middle and upper part of the Kura and Alazani Rivers in Georgia) for many species has been lost. During the same period, alien species were introduced intentionally or accidentally in the region such as for instance Prussian carp (*C. gibelio*) and topmouth gudgeon (*P. parva*) (Gabrielyan, 2001; Ibrahimov & Mustafayev, 2015; Ninua & Japoshvili, 2008; Pipoyan & Arakelyan, 2015, 2018). Although the pace of large industrial developments slowed down after the collapse of the Soviet Union, the pressure on freshwater species did not weaken. Extensive poaching and the recent explosion of hydropower plant developments in the region take freshwater biodiversity to the even higher treats. Most notably, population dynamics of range-restricted species, community composition, effects of alien species and intensity of threats throughout the region remains poorly known (Freyhof et al., 2015; Katouzian et al., 2016; Mumladze et al., 2019). Recent molecular-genetic studies (as discussed in previous section) have revealed several new species as well as taxonomic rearrangement in freshwater fish fauna of South Caucasus. However, yet less than half of the taxa were studied with the aid of molecular genetics methods. Unfortunately, we could not resolve all the issues regarding the freshwater fish fauna in Armenia, Azerbaijan and Georgia within our knowledge. Therefore, we point out the need of further morphological and genetic studies to address the questions that evolved within this study. In addition, we urge enhanced investigation of freshwater fish communities in the South Caucasian countries to ensure species conservation and mitigate biodiversity loss.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests with the present study.

ORCID

Tatia Kuljanishvili https://orcid.org/0000-0003-3085-4042
Levan Mumladze https://orcid.org/0000-0002-2172-6973

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Research Article

Towards retrieving the Promethean treasure: a first molecular assessment of the freshwater fish diversity of Georgia

Giorgi Epitashvili[‡], Matthias Geiger[§], Jonas J Astrin[§], Fabian Herder[§], Bella Japoshvili[‡], Levan Mumladze[‡]

‡ Institute of Zoology, Ilia State University, Tbilisi, Georgia
§ Zoological Research Museum A. Koenig, Bonn, Germany

Corresponding author: Levan Mumladze (lmumladze@gmail.com)

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Abstract

In this study, we provide a first estimation of the molecular diversity of the freshwater fishes of Georgia. In addition to field collections, we integrated DNA barcode data obtained from recent works and public databases (BOLD and NCBI GenBank). Currently, the DNA barcode reference library for freshwater fishes of Georgia comprises 352 DNA barcodes for 50 species, 36 genera and 15 families (52% of total Georgian freshwater fish diversity), from which 162 DNA barcodes belonging to 41 species were newly generated as part of this study. A total of 22 species are reported from the Caspian Sea basin and 31 from the Black Sea basin. Amongst the studied taxa, seven species were found with large interspecific divergences (> 2%) while 11 species were found to share DNA barcodes within our dataset. In the course of the study, we found the first evidence of the existence of *Gymnocephalus cernua* (Linnaeus, 1758) and also confirm the second occurrence of invasive *Rhinogobius lindbergi* (Berg, 1933) in Georgia. Based on the evaluation of currently-available barcode data for Georgian fishes, we highlighted major gaps and research needs to further progress DNA-based biodiversity studies in Georgia. Though this study lays a solid base for DNA, based biodiversity assessment and monitoring

approaches, further efforts within the recently started CaBOL (Caucasus Barcode Of Life) project are needed to obtain reference data for the species still lacking DNA barcodes.

Keywords

DNA barcoding, COI, molecular assessment, Caucasus

Introduction

Around 15000 freshwater fish species are known worldwide (Tedesco et al. 2017). Unfortunately, many species are under threat of extinction due to heavy anthropogenic pressures, such as over-exploitation, pollution, habitat degradation and loss or invasive species (Dudgeon et al. 2007, Collen et al. 2013). Therefore, monitoring of freshwater ecosystems and their species is important for conservation planning (Dudgeon et al. 2007). The Caucasus region (and Georgia as a part thereof) is a biodiversity hotspot (Myers et al. 2000) and characterised by high diversity of landscape types and ecosystems, as well as high levels of endemism approaching up to 90% in some taxa (e.g. gastropods (Walther et al. 2014, Grego et al. 2020), millipedes (Kokhia and Golovatch 2018) etc). This is due to long-term and uninterrupted development of biodiversity in the Great and Lesser Caucasus, parts of which are suggested as plio-pleisotecene refugial areas (thus Promethean treasure) (Tarkhnishvili et al. 2012). At the same time, diversity of freshwater organisms in the Caucasus region remains poorly studied, including fishes and their distribution (Kuljanishvili et al. 2020, Mumladze et al. 2020). Unfortunately, poaching and habitat destruction (in situ gravel mining, hydropower plant constructions) threaten the endangered biota with extinction (Kuljanishvili et al. 2020, Freyhof et al. 2015). The situation is even more critical as some habitats in Georgia, such as the Rioni River, are acting as the world's last refuge and spawning areas for critically-endangered sturgeons (*Acipenser sturio*, *A. gueldenstaedtii*, *A. nudiventri*, *A. persicus*, *A. stellatus* and *Huso huso*) (Bacalbaa-Dobrovici and Holčík 2000, Guchmanidze 2012, Kuljanishvili et al. 2020).

Broadly, the Georgian ichthyofauna can be divided into the eastern Black Sea and Caspian Sea basins. According to Abell et al. (2008), Naseka (2010), within the political borders of Georgia, however, three biogeographic regions exist: A) Black Sea basin, which is separated from east Georgia by the Likhi and Meskheti ridges and covering the whole territory of west Georgia; B) Kura basin and C) Terek basin in the northern part of the country, which is separated from the Kura basin by the Greater Caucasus Mountain Range (Fig. 1).

Since the late eighteenth century, industrial and economic developments have led to severe environmental changes in the whole Caucasus region (Davtyan 2014, Freyhof et al. 2015), similar to other parts of the world (Smith et al. 1999). Since freshwater ecosystems are particularly sensitive and vulnerable to alterations in (gravel mining, hydropower plants) and adjacent to (land use) the water body (Allan and Flecker 1993), negative impacts on the ichthyofauna have likely occurred, but have never been scientifically evaluated. This is also due to a general data deficiency concerning the whole freshwater realm of the region (Mumladze et al. 2020). Along with taxonomic uncertainties in some species, major gaps still exist in the accurate knowledge of species' distribution ranges within the Georgian inland waters, while no local conservation assessments exist for any freshwater fish species (Kuljanishvili et al. 2020). The highly-threatened group of sturgeons (*Acipenser* spp.), for example, have never been officially assessed in Georgia and only the international IUCN status is known. The Red List data for Georgian fishes are inherited from the Soviet time and thus outdated in many aspects. A new regional assessment, based on the IUCN system, is not yet available. Thus, the extent and magnitude of past disturbance or ongoing threats (such as habitat degradation, poaching, pollution and invasive species) to Georgian freshwater fishes remains largely unknown.

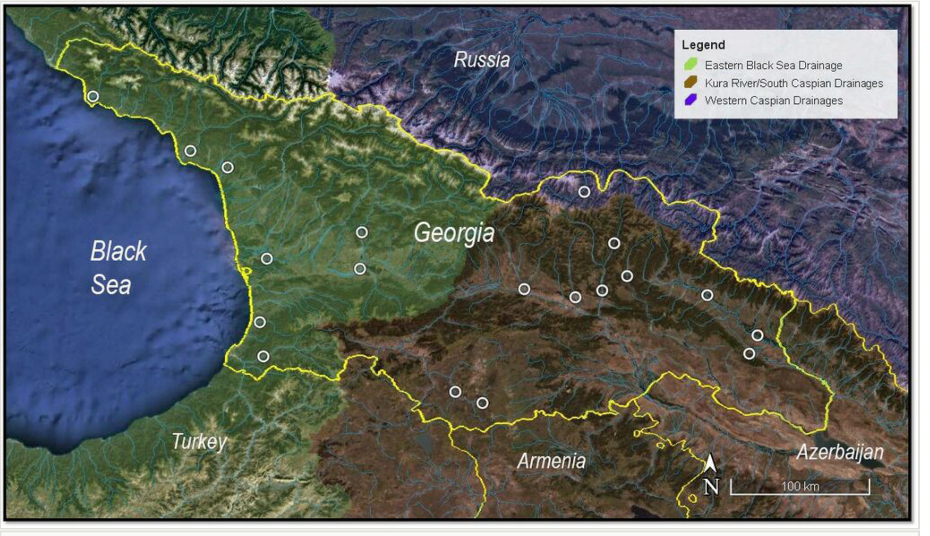

Figure 1. doi

Map of Georgia (based on Google Earth Pro 7.3), showing main watersheds and river basins (delimited by shaded colours) and annotated with sampling localities (white circles).

Along with traditional faunistic assessments, molecular genetic tools (such as DNA barcoding) have emerged as an important aid to deal with uncertainties related to taxonomy, species boundaries or cryptic diversity and have helped to enable innovative and efficient ways of biomonitoring (Hebert et al. 2003, Waugh 2007Hajibabaei 2012, Leese et al. 2018). DNA barcoding is a method to identify species via short, standardised and easily-obtainable DNA fragments (Hebert et al. 2003, Bhattacharya et al. 2015, Lakra et al. 2015, Bingpeng et al. 2018). This method is not only limited to identifying specimens, but can also help to screen unrecognised species diversity (Barman et al. 2018).

The important step for DNA barcoding to be useful in biodiversity study/monitoring, is to develop a DNA barcode library for a particular taxa or area. The successful completion of this step, however, requires the integration of traditional taxonomic expert knowledge and DNA technology. While traditional taxonomic expertise (not only in ichthyology), based on academic training, has been largely neglected in Georgia, as well as in many other countries — part of the phenomenon known as the 'taxonomic impediment' (e.g. Giangrande 2003) — DNA-based technologies for biodiversity inventories and research are gathering momentum in Georgia and can still provide new insights into freshwater fish diversity (Japoshvili et al. 2013, Ninua et al. 2018, Levin et al. 2018, Levin et al. 2019, Japoshvili et al. 2020). As a result, new, yet not fully exploited DNA-based information on Georgian fish diversity and their distribution has accumulated in recent years. Therefore, the aim of our work was to contribute to the development of a DNA barcode reference library for Georgian freshwater fishes and to summarise the current state of knowledge. We thus establish the starting point for DNA-based biodiversity evaluation and monitoring efforts of freshwater fishes of the Southern Caucasus region. The presented data will further aid in identifying taxonomically-interesting cases that need to be solved in the future.

Materials and methods

Data collection and DNA barcoding

In July 2018 and July 2019, concerted collecting activities (BioBlitzes) were organised by the Ilia State University - ISU (Georgia) and the Zoologisches Forschungsmuseum Alexander Koenig - ZFMK (Germany) in the Kintrishi areas in Western Georgia (N41.76 E42.02) and in the Kazbegi region in Northern Georgia (N42.65 E44.64), respectively (Thormann et al. 2019). During these events, fish sampling campaigns (permissions: #5615/01, #21/824 and #3875 – 2018/2019 issued by Ministry of Environmental Protection and Agriculture of Georgia) have been conducted in the Kintrishi and Terek River basins via electrofishing (device EFGI-650, http://www.electric-fishing.de) and frame net. After anaesthesia with MS-222 of a subsample of the collected fishes, a fin-clip was taken and stored in 99.9% molecular grade ethanol and specimens were fixed in 5-7% formaldehyde or, alternatively, specimens were directly fixed in 99% molecular grade ethanol. In addition, material collected in different areas (Fig. 1) prior to the above-mentioned activities was included (see Suppl. material 1). All specimens were identified to species level using standard morphological characters (e.g. Kottelat and Freyhof (2007)) and tissue samples of selected specimens submitted to DNA barcoding routines at ZFMK.

Genomic DNA was extracted from sub-samples using a BioSprint96 magnetic bead extractor (Qiagen, Hilden, Germany). PCRs, targeting the standard DNA barcode region COI, were carried out in 20 µl reaction volumes including 2 µl undiluted DNA template, 0.8 µl of each primer (10 pmol/µl; LCO1490-JJ: 5´-CHACWAAYCATAAAGATATYGG-3´ and HCO2198-JJ: 5´ AWACTTCVGGRTGVCCAAARAATCA-3´, (Astrin and Stüben 2008)), 2 µl 'Q-Solution' and 10 µl 'Multiplex PCR Master Mix' (Qiagen, Hilden, Germany). Thermal cycling was performed on GeneAmp PCR System 2700 machines (Applied Biosystems, Foster City, CA, USA) as follows: hot start Taq activation: 15 min at 95°C; first cycle set (15 repeats): 35 s denaturation at 94°C, 90 s annealing at 55°C (−0.2°C/cycle, 'touch down') and 90 s extension at 72°C. Second cycle set (25 repeats): 35 s denaturation at 94°C, 90 s annealing at 50°C and 90 s extension at 72°C; final elongation 10 min at 72°C. Purification of PCR products and bidirectional sequencing was conducted at either BGI (Hong Kong, China) or Macrogen Europe Laboratories using the amplification primers. Voucher specimens are kept in the Ichthyological collections at ISU and ZFMK. Extracted genomic DNA is deposited in the ZFMK Biobank.

Data processing

Data processing and sequence assembly was done with the software Geneious Pro v.7 (Drummond et al. 2011) and the Muscle algorithm (Edgar 2004) was used to align the DNA barcodes after manually screening for indels or stop codons. All newly-generated DNA sequences with acceptable quality (with less than 1% ambiguous bases and free of stop codons) were submitted to the Barcode of Life Datasystem (BOLD, http://v4.boldsystems.org/), including relevant metadata where they were automatically assigned Barcode Index Numbers (BINs). They can be accessed via the public dataset "Georgian Freshwater Fishes" (DS-GGBCPIS).

In addition to the newly-generated DNA barcodes, we included all BOLD-deposited DNA barcodes that originated from Georgia. Sequences from the BOLD database were included in our dataset if the specimen metadata explicitly stated the origin of the sample and provided geo-referenced data (Suppl. material 1). Subsequently, the BOLD v4 tools evaluated sequence divergence and relationships between and within taxa, based on uncorrected *p*-distance. A Neighbour-Joining tree (based on K2P distances) with 1000 bootstrap replicates was constructed to investigate congruence between morphological identity and genetic relationships. Analyses were performed using MEGA X software (Kumar et al. 2018) and statistical tools provided by BOLD Systems (Ratnasingham and Hebert 2007).

Results and discussion

DNA barcodes of 352 individuals representing 50 species, 36 genera and 15 families (52% of Georgian freshwater fish diversity) are currently available for Georgian fishes. From these, 162 COI sequences were newly generated for this study, through the GGBC (Georgian-German Biodiversity Center) initiative, 153 were contributed through the FREDIE (Freshwater Diversity Distribution for Europe) project (https://www.fredie.eu/), 19 sequences stem from the "Russian Freshwater Fishes" project on BOLD and 18 DNA barcodes were mined from GenBank through BOLD. In the final dataset of all 352 barcodes, the length of the COI sequences was, on average, 648 base pairs (minimum 465 and maximum 658) including no stop codons, insertions or deletions. A total of 82 positions out of 658 (13%) were variable, from which 60 positions (9%) were diagnostically informative. On average, nucleotide base frequency (A-24.47%, C-27.67%, G-18.56%, T-29.29%) and GC content (46.24%) were well within the range known for fishes (see, for example, Bingpeng et al. (2018)). Distance summary statistics are provided in Table 1, showing significant changes of average *p*-distance amongst family, genus and species level.

Table 1.

Summary table of K2P genetic distances within the different taxonomic levels derived from 349 specimens analysed. The list of studied species is provided in Suppl. material 1

Label	N	Taxa	Comparisons	Min Dist (%)	Mean Dist (%)	Max Dist (%)	S.E.
Within Species	349	50	1835	0.00	0.33	2.46	0.01
Within Genera	253	13	1268	0.00	4.1	9.54	0.00
Within Families	295	5	21804	4.00	16.1	27.98	0.00

Amongst the 50 barcoded species, 26 were represented by more than four sequences in the dataset allowing intraspecific distance estimates (Fig. 2; Suppl. material 2). The highest number of barcodes per genus was reached for the genus *Alburnoides* with two species: *A. fasciatus* (24 barcodes) and *A. eichwaldii* (12 barcodes), followed by the genus *Barbus* represented also by two species: *B. rionicus* (23 barcodes) and *B. cyri* (12 barcodes). Most species however, are represented by less than 10 DNA barcodes. Two species, *Romanogobio macropterus* and *Oncorhynchus mykiss* are represented by a single DNA barcode. Summarised information on barcoded specimens for each species, intraspecific distances and nearest neighbours according to the 'Barcode Gap Analysis' tool available in BOLD Systems are provided in Suppl. material 2. The full Neighbour-Joining tree, including all specimens (Fig. 3), resolved almost all morphological species as unique clusters in congruence with morphological species identification. However, three of the genera (*Chondrostoma*, *Gobio, Salmo*) showed complicated sequence relationships where maximum within-species distances were larger than the minimum between-species distances amongst the congenerics (Suppl. material 2), with also low bootstrap support for species level clusters on the tree (Fig. 3).

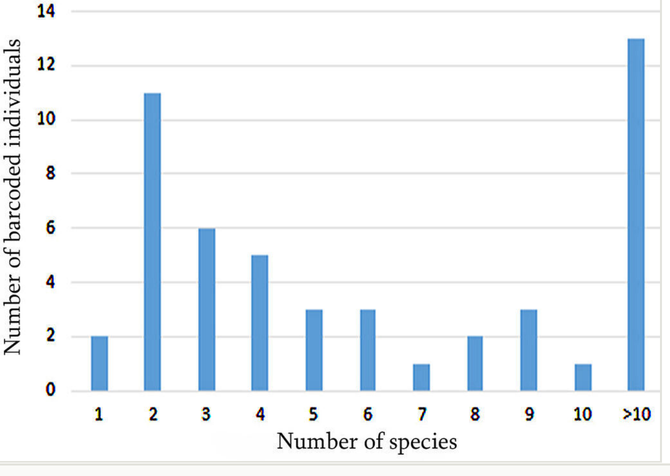

Figure 2. doi

Barcode frequency distribution for Georgian fish species in a BOLD System at the time of writing.

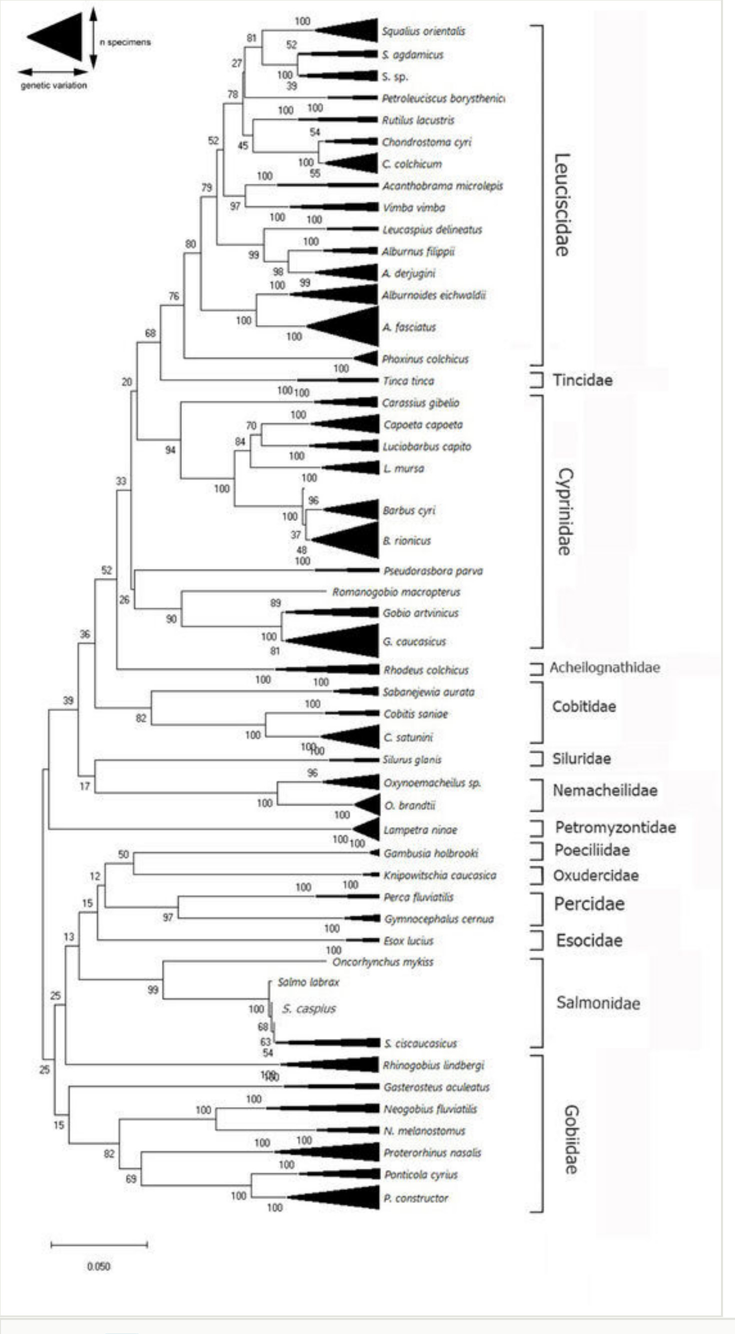

Figure 3. doi

Compact Neighbour-Joining tree based on mitochondrial COI barcode region using K2P distance model with other default parameters provided by MegaX software. Numbers near nodes indicate bootstrap support values from 1000 replicates. The analyses involved all 352 COI nucleotide sequences.

Due to small interspecific genetic distances (i.e. distance to nearest neighbour), several species were not predicted to be separate taxa by barcode gap analyses. For instance, nearest-neighbour distances for species belonging to *Salmo*, *Barbus*, *Capoeta*, *Chondrostoma*, *Gobio* and *Squalius*, showed < 2% divergence, indicating a possible need for re-evaluating species limits. As an example, all three specimens from Natanebi and Supsa rivers (Western Georgia, Black Sea Basin), which were identified as *Gobio artvinicus* (sequence IDs in BOLD: EUFWF3080-18; EUFWF3079-18; EUFWF4984-19) according to Turan et al. (2016), though are represented as a separate cluster in the COI cladogram Fig. 3, have near-zero divergence with the *G. caucasicus* (23 specimens from Kura River - Caspian Sea Basin). This might be considered as a result of geographic divergence between populations rather than attributing the populations to different species. Thus, additional integrative taxonomic investigation could help to solve the systematics of the genus *Gobio* in Georgia. Though the species of some of those genera (*Salmo*, *Barbus*, *Gobio*) were (re)evaluated as valid species relatively recently (Turan et al. 2016, Ninua et al. 2018, Aksu and Bektaş 2019, Levin et al. 2019, Kuljanishvili et al. 2020), increasing the sampling (adding both species-level taxa and barcodes per species) and geographic coverage is necessary to develop an effective barcode library.

Seven species (*Chondrostoma cyri*, *Rutilus lacustris*, *Alburnus derjugini*, *Phoxinus colchicus*, *Oxynoemacheilus* sp., *Neogobius fluviatilis* and *Proterorhinus nasalis*) (Fig. 4) showed maximum interspecific divergence larger than 2% (*p*-distance) pointing them out as interesting subjects for further in-depth studies. All these species await additional studies to clarify their taxonomic positions. The genera *Chondrostoma, Rutilus, Alburnus, Neogobius* and *Oxynoemacheilus* are diverse (i.e. more than two species according to Kuljanishvili et al. (2020)) in the Caucasus region for which unambiguous systematics, as well as detailed distribution of separate species or genetic profiling, is still lacking. For instance, Kuljanishvili et al. (2020) assume the occurrence of four species of *Rutilus* in the South Caucasus. However, a study, based on the Cytochrome b marker by Levin et al. (2017), suggests the possibility that only a single genetically-highly polymorphic *R. lacustris* occurs in the Caucasus region. Likewise, the systematics of Caucasian species and genera of Gobiidae and Nemacheilidae are amongst the most confusing, as already pointed out by Kuljanishvili et al. (2020). For these taxa, our COI barcode dataset is insufficient in order to draw meaningful conclusions; however, large intraspecific distances further indicate possible yet undescribed diversity within these taxa. For instance, regionally monospecific genera with high intraspecific genetic distances, such as *Phoxinus* and *Proterorhinus*, are interesting taxa that might be represented by genetically deeply-structured populations (if not cryptic species complexes) associated with Black vs Caspian Sea basins.

Loaches of the genus *Oxynoemacheilus* from the Black Sea basin have recently been studied including the description of a new species – *O. cemali* – from the Coruh river drainage (Turan et al. 2019). The Coruh enters the Black Sea near Batumi in Georgia about 70 km south of the Rioni River mouth. We therefore compared the DNA barcodes made available by Turan et al. (2019) to the newly-generated COI sequences, also to test whether there is molecular support for the Rioni loach belonging to *O. cemali*. The comparison showed that the Rioni *Oxynoemacheilus* (Fig. 4) is genetically closer to *O. brandtii* from the Kura River (7.5% min. K2P distance) than to the presumably also Georgian *O. cemali* (11.9% min. K2P distance). It is, therefore, highly unlikely that the Rioni loach belongs to one of the two species (Fig. 5). As the taxonomic status of *Oxynoemacheilus* species in the study area is only partially understood (e.g. *O. angorae alasanicus*, *O. bergi*, *O. brandti gibbusnazus* or *O. lenkoranensis* – see Kuljanishvili et al. (2020)), further studies with larger series of adult specimens are needed to address these issues and allow taxonomic sound examinations and conclusions.

Figure 4. doi

Sample of taxa studied in this article and their habitats. A - *Neogobius fluviatilis* (downstream of Kintrishi River); B - *Phoxinus colchicus* (Kintrishi River, close to river mouth); C - *Oxynoemacheilus* sp. (Lajanuri River - right tributary of Rioni river).

DNA barcoding confirmed for the first time an occurrence of *Gymnocephalus cernua* (Linnaeus, 1758) and contributed the second record of alien *Rhinogobius lindbergi* (Berg, 1933) in Georgia. The former species has never been considered to occur in the country (Elanidze 1983, Ninua and Japoshvili 2008, Kuljanishvili et al. 2020), but is abundant in the adjacent northern Caucasus area (Kottelat and Freyhof 2007). Possibly, the species currently extends its range to the Southern Caucasus, although reports from fishermen are lacking. As the species is a strong invader and has drastically expanded its range over the last decades (e.g. to the North American Great Lakes, where it possibly poses a threat to their endemic fish fauna (Gunderson et al. 1998, Newman 1999), monitoring its status in Georgia is recommended. *Rhinogobius lindbergi* was recently first reported from Georgia as an alien species (Japoshvili et al. 2020). Our data confirm the finding and further indicates the widespread distribution of this species in eastern Georgia as already supposed by Japoshvili et al. (2020). The likely introduction pathways or vectors for this species are currently unknown. The direct migration from southern Caspian rivers is probably impossible due to impermeable barriers at the Mingachevir reservoir. Accordingly, *R. lindbergi* have been introduced in eastern Georgian rivers by humans, most probably unintentionally.

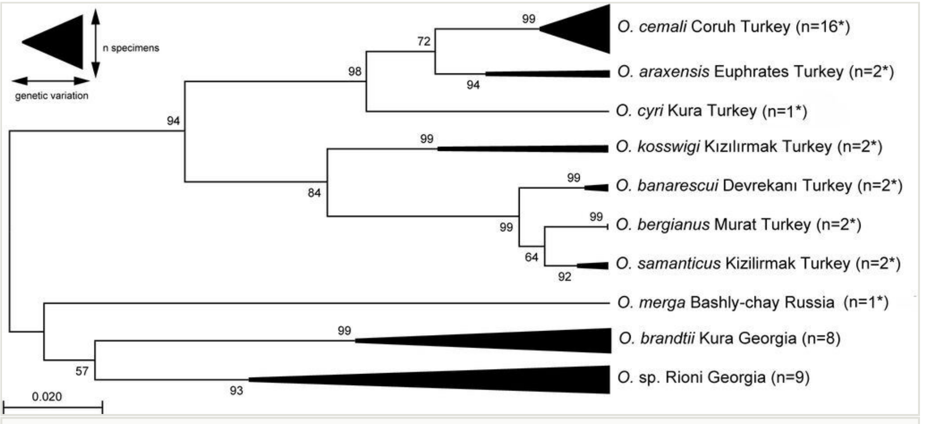

Figure 5. doi

Maximum Likelihood estimation of the phylogenetic relationships of *Oxynoemacheilus* loaches, based on the mitochondrial COI barcode region (Kimura 2-parameter model, discrete Gamma distribution for rate differences with three categories + G parameter = 0.0610). Nucleotide positions with less than 95% site coverage were eliminated, resulting in 637 analysed positions. Numbers near nodes indicate bootstrap support values from 1000 pseudoreplicates. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis includes 45 nucleotide sequences taken from Turan et al. (2019) (for asterisked species, NCBI GenBank accession numbers are given in Suppl. material 3) of this study.

Conclusions

Georgia, as part of the Caucasus and Irano-Anatolian biodiversity hotspots, is distinguished by its unique biodiversity and rich freshwater resources, which have been strongly impacted by anthropogenic pressure throughout the 20th century until the present. During the Soviet time, large-scale industrial projects presumably had a strong influence on the Georgian biodiversity and especially on the freshwater fauna. An example of this is the construction of the Mingachevir Dam in Azerbaijan which acted as an insurmountable obstacle for anadromous fishes such as sturgeons, Caspian lampreys and salmon (Kuljanishvili et al. 2020). As a result, these species (in particular, lampreys and sturgeons) lost the spawning areas in the whole upper Kura basin. In addition, from the 1930s to 1980s, alien species were introduced intentionally or accidentally to Georgia, such as gibel carp and topmouth gudgeon (Ninua and Japoshvili 2008, Japoshvili et al. 2013, Kuljanishvili et al. 2020). Although large industrial projects were halted with the collapse of the Soviet Union, the recent extensive development of small and medium-sized hydropower plants in Georgia will presumably have negative impacts on the local freshwater biodiversity. In addition, illegal fishing, range expansion of non-native species, water pollution and habitat modification will alter the population dynamics and distribution of most native freshwater fishes of Georgia, including rare, endemic and especially anadromous species. Given these expectations, intensive study and monitoring of fishes is highly recommended to estimate population changes and species distribution and for subsequent planning of conservation activities and mitigation of irreversible diversity loss. The fastest and perhaps most cost-effective tools in this regard will be methods based on DNA barcoding (Kress et al. 2015). However, our study shows that the number of reference barcodes available for Georgian fishes is not yet sufficient to implement full-scale fish diversity monitoring programmes. Indeed, the barcodes of nearly 50% of Georgian fish species are not yet available. This is mainly due to the limited financial/human resources to investigate the fish diversity on one hand and also due to poor museum collections available for Georgian fishes. For example, the largest fish collection kept in the Georgian National Museum has been damaged so badly (as a result of incorrect preservation) that it is no longer useful for genetic study. Thus unresolved taxonomy (such as *Oxynoemacheilus* or *Squalius*) and insufficient barcode coverage are currently major gaps that need to be filled in the near future. We hope this is indeed possible, given the relatively-low species number (compared to mega-diverse regions) and the existing progress in fish research in the region. The recently-initiated CaBOL project (Caucasus Barcode of Life) constitutes a chance to close some of these taxonomic gaps over the next few years.

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Author contributions

MFG, GE, BJ and LM developed conception and design of the study, collected material and analysed data. FH, JA and LM supported the research logistics. GE, LM and MFG drafted manuscript. All authors participated in revising the article and contributed with intellectual content. All authors gave final approval of the submitted version.

Conflicts of interest

The authors have declared no conflicts of interest.

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Supplementary materials

Suppl. material 1: Epitashvili et al. Supplementary Table S1. doi

Authors: Epitashvili G, Geiger M, Astrin J, Herder F, Japoshvili B, Mumladze L
Data type: Occurrences, Collection data
Brief description: Details on barcoded specimens from Georgia currently available in BOLD. Abbreviations used in the table: NA - information is not available; ISU - Ilia State University; FJF - Fischsammlung Jorg Freyhof; PC - Prior Collection i.e. before 2018-2019 bioblitz; RAS - Russian Academy of Sciences, Zoological Institute; ZFMK - Zoologisches Forschungsmuseum Alexander Koenig. Samples in bold face are mined from GenBank with BOLD ID same as GenBank accession number.
Download file (41.37 kb)

Suppl. material 2: Epitashvili et al. Supplementary Table S2. doi

Authors: Epitashvili G, Geiger M, Astrin J, Herder F, Japoshvili B, Mumladze L
Data type: Summarized data
Brief description: Taxonomic structure and BOLD summary data of barcoded Georgian fishes with mean and maximum intraspecific distances and nearest-neighbour statistics.
Download file (16.59 kb)

Suppl. material 3: GenBank accession numbers for the sequences used in Figure 5. doi

Authors: Epitashvili G, Geiger M, Astrin J, Herder F, Japoshvili B, Mumladze L
Data type: Genomic
Download file (9.43 kb)

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Oxynoemacheilus phasicus, a new nemacheilid loach from the eastern Black Sea basin with some remarks on other Caucasian *Oxynoemacheilus* (Teleostei: Nemacheilidae)

JÖRG FREYHOF[1], CÜNEYT KAYA[2], GIORGI EPITASHVILI[3] & MATTHIAS F. GEIGER[4]
[1]*Museum für Naturkunde, Leibniz Institute for Evolution and Biodiversity Science, 10115 Berlin, Germany.*
joerg.freyhof@mfn.berlin; https://orcid.org/0000-0002-7042-3127
[2]*Recep Tayyip Erdogan University, Faculty of Fisheries and Aquatic Sciences, 53100 Rize, Turkey.*
cnytkaya@yahoo.com; https://orcid.org/0000-0002-4531-798X
[3]*Institute of Zoology, Ilia State University, 0179, Tbilisi, Georgia.*
giorgi_epitashvili@iliauni.edu.ge; https://orcid.org/0000-0001-5921-5362
[4]*Zoological Research Museum Alexander Koenig, Leibniz Institute for Animal Biodiversity, Adenauerallee 160, 53113 Bonn, Germany.*
m.geiger@leibniz-zfmk.de; https://orcid.org/0000-0003-2403-0805

Abstract

Oxynoemacheilus phasicus, new species, is described from the Rioni and Enguri River drainages in Georgia. It is distinguished from other *Oxynoemacheilus* species in the *O. brandtii* group by a mottled or marbled flank pattern in adults, a slightly emarginate caudal fin, and a deep caudal peduncle. Molecular data suggest that the new species is characterized by a minimum K2P distance of 7.5% from *O. brandtii* from the Kura drainage in the mtDNA COI barcode region. *Oxynoemacheilus brandtii* and *O. elsae* are re-diagnosed. A very slender *Oxynoemacheilus* from the Aras drainage clusters as sister to *O. elsae* in our molecular analysis and not with *O. brandtii* from the Kura River. However, it is identified as *O. brandtii* as it is indistinguishable from this species in morphological characters.

Key words: Freshwater fish, Taxonomy, Cytochrome oxidase I, Middle East

Introduction

The nemacheilid loach genus *Oxynoemacheilus* is the largest freshwater fish genus of the Western Palearctic, with currently about 60 species recognised as valid (Kottelat 2012) in addition to about 20 undescribed candidate species (see Geiger *et al*. 2014 for some details). From the Caucasus, only *O. angorae*, *O. brandtii*, *O. merga* and *O. cyri* had been described in the 20th century (see Berg 1949 for details). Freyhof *et al.* (2012) have already mentioned that the diversity of *Oxynoemacheilus* in the Caucasus is still poorly understood and the taxonomic status of species such as *O. angorae alasanicus*, *O. bergi*, *O. brandtii gibbusnazus*, and *O. lenkoranensis* need to be revisited. The first attempt to resolve such challenges was made by Çiçek *et al.* (2018), who collected some loaches in the Kura and identified these as *Oxynoemacheilus bergi*. Çiçek *et al.* (2018) also described *O. veyselorum* from the Aras, a species previously identified as *O. angorae* or *O. araxensis;* and Eagderi *et al*. (2018) described *O. elsae* from Iranian Lake Urmia basin, previously identified as *O. brandtii*. Turan *et al*. (2019) recently described *O. cemali* from the Çoruh drainage in the eastern Black Sea basin (south of the Rioni River mouth). Despite these efforts, some uncertainty still exists about the loaches of the western Caspian Sea basin; therefore, additional work is needed to fully understand the diversity of Caucasian loaches.

Here we revisit the *Oxynoemacheilus brandtii* species group, which is a major phylogenetic entity in the large genus *Oxynoemacheilus,* with three known species (*O. brandtii*, *O. merga* and *O. elsae*) all endemic to the western Caspian Sea basin. An additional distinct mitochondrial lineage of this group was found recently in the Rioni River (eastern Black Sea basin) (Epitashvili *et al*. 2020). It has remained without further examination and it is the aim of this study to test whether this lineage from the Rioni is a valid (new) species, or if it can be identified as one of the known species of the Caspian basin.

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Materials and methods

After anaesthesia, fishes were fixed in 5% formaline and stored in 70% ethanol or directly fixed in 99% ethanol. Measurements were made with a dial caliper and recorded to 0.1 mm. All measurements were made point-to-point, never by projections. Methods for counts and measurements followed Kottelat & Freyhof (2007), definition of the suborbital groove and the adipose crest followed Freyhof *et al.* (2019) and nomenclature of head pores follows Kottelat (1990). Standard length (SL) was measured from the tip of the snout to the posterior extremity of the hypural complex. The length of the caudal peduncle was measured from behind the base of the posterior anal-fin ray to the posterior extremity of the hypural complex, at mid-height of the caudal-fin base. The last two branched rays articulating on a single pterygiophore in the dorsal and anal fins are counted as „1½". Simple dorsal- and anal-fin rays were not counted since they are deeply embedded. An equal variance *t*-test was applied to test the null hypothesis that the mean morphometric values of examined groups are not significantly different from each other. All morphometric values are standardised for percentage SL or percentage HL. For a better demonstration, we plotted significantly different morphometric characters against SL. The map (Fig. 6) was developed in QGIS, version 3.16 (QGIS Development Team, 2009).

Abbreviations used: SL, standard length; HL, head length; ML, Maximum Likelihood; mt, mitochondrial; K2P, Kimura 2-parameter. Collection codes: FSJF, Fischsammlung J. Freyhof, Berlin, Germany; FFR, Recep Tayyip Erdogan University Zoology Museum of the Faculty of Fisheries, Rize, Turkey; IMNRF, Ichthyological Museum of Natural Resources Faculty, University of Tehran, Iran; ZFMK-ICH, Zoological Research Museum Alexander Koenig, Ichthyology Collection, Bonn, Germany; HIDBb, Hydrobiology and Ichthyology Department of Institute of Zoology of ISU, Ilia State University, Tbilisi, Georgia.

DNA extraction, PCR and Sequencing. Genomic DNA was extracted from muscle tissue using Macherey & Nagel NucleoSpin® Tissue kits following the manufacturer's protocol on an Eppendorf EpMotion® pipetting-roboter with vacuum manifold. The standard vertebrate DNA barcode region of COI was amplified using a M13-tailed primer cocktail including FishF2_t1 (5'TGTAAAACGACGGCCAGTCGACTAATCATAAAGATATCGGCAC), FishR2_t1 5'CAGGAAA- CAGCTATGACACTTCAGGGTGACCGAAGAATCAGAA), VF2_t1 (5'TGTAAAACGACGGCCAGTCAAC- CAACCACAAAGACATTGGCAC) and FR1d_t1 (5'CAGGAAACAGCTATGACACCTCAGGGTGTCC- GAARAAYCARAA) (Ivanova *et al*. 2007). PCR were performed using Qiagen Multiplex ® taq as follows: 15min at 95°C; 10 cycles of 35s at 94°C, 90s at 52–49°C ('touch-down') and 90s at 72°C followed by 25 cycles of 35s at 94°C, 90s at 55°C and 90s at 72°C with final elongation for 10min at 72°C and hold at 10°C. Sequencing of the ExoSAP-IT (USB) purified PCR product in both directions was conducted at Macrogen Europe Laboratories with forward sequencing primer M13F (5'GTAAAACGACGGCCAGT) and reverse sequencing primer M13R-pUC (5'CAGGAAACAGCTATGAC).

Molecular Analysis. Molecular analysis was conducted with 29 newly-generated DNA barcodes in addition to 35 already published COI sequences from the Barcode of Life database (http://v4.boldsystems.org/) and NCBI GenBank (Table 1). We included as outgroup DNA barcodes of one individual each of *Seminemacheilus ahmeti* (MT077014), *Oxynoemacheilus bureschi* (KJ553753) and *O. pindus* (KJ554005). Data processing and sequence assembly was done with the software Geneious Pro (Biomatters 2013) and the Muscle algorithm (Edgar 2004) used to align the DNA barcodes after manually screening for unexpected indels or stop codons. The Species Delimitation Plugin (Masters *et al*. 2011) for Geneious Pro (Biomatters 2013) was used for summarizing measures of genetic K2P distances to provide readily comparable data with other studies using this standard DNA barcoding metric. The sequence evolution model test implemented in the MEGA X software (Kumar *et al*. 2018) was used to determine the most appropriate evolution model for the given data and to reconstruct the mitochondrial relationships between the studied taxa. The model with the lowest BIC scores (Bayesian Information Criterion) is considered to best describe the substitution pattern. According to the test the Tamura-Nei model (Tamura & Nei 1993) with discrete Gamma distribution best explained the multiple sequence alignment and was used to model the evolutionary rate differences among sites (3 categories (+G, parameter = 0.3477)). We, therefore, generated neighbour-joining (Saitou & Nei 1987), maximum parsimony (Swofford 2002, with PAUP4b) and maximum likelihood phylogenetic trees with 1000 bootstrap replicates in MEGA X (Kumar *et al*. 2018) to explore phylogenetic affinities of the mitochondrial lineages (all positions with less than 98% coverage removed, 557 bp were analysed in total).

TABLE 1. List of public COI sequences downloaded from NCBI GenBank or BOLD with information on drainage and country of origin.

Species	Drainage	Country	GenBank	Reference
Oxynoemacheilus angorae	Sakarya	Turkey	KU928279	Freyhof *et al*. (2016)
Oxynoemacheilus bergianus	Euphrates	Turkey	MK546450	Freyhof *et al*. (2019)
Oxynoemacheilus brandtii	Kura	Georgia	MW564439	Epitashvili *et al*. (2020)
Oxynoemacheilus brandtii	Kura	Georgia	MW564536	Epitashvili *et al*. (2020)
Oxynoemacheilus brandtii	Kura	Georgia	MW564263	Epitashvili *et al*. (2020)
Oxynoemacheilus brandtii	Kura	Georgia	MW564319	Epitashvili *et al*. (2020)
Oxynoemacheilus brandtii	Kura	Georgia	MW564289	Epitashvili *et al*. (2020)
Oxynoemacheilus brandtii	Kura	Georgia	MW564325	Epitashvili *et al*. (2020)
Oxynoemacheilus brandtii	Kura	Georgia	MW564456	Epitashvili *et al*. (2020)
Oxynoemacheilus brandtii	Kura	Georgia	MW564466	Epitashvili *et al*. (2020)
Oxynoemacheilus brandtii	Kura	Georgia	MW564529	Epitashvili *et al*. (2020)
Oxynoemacheilus brandtii	Kura	Georgia	MW564518	Epitashvili *et al*. (2020)
Oxynoemacheilus brandtii	Kura	Georgia	MW564312	Epitashvili *et al*. (2020)
Oxynoemacheilus brandtii	Aras	Turkey	KX833727	Sayyadzadeh *et al*. (2017)
Oxynoemacheilus brandtii	Kura	Turkey	KU928281	Freyhof *et al*. (2016)
Oxynoemacheilus bureschi	Nestos	Greece	KJ553753	Geiger et al. (2014)
Oxynoemacheilus cemali	Çoruh	Turkey	MH018875	Turan *et al*. (2019)
Oxynoemacheilus cemali	Çoruh	Turkey	MH018876	Turan *et al*. (2019)
Oxynoemacheilus evreni	Ceyhan	Turkey	KJ553678	Geiger *et al.* (2014)
Oxynoemacheilus galilaeus	Muzarib	Syria	KJ553821	Geiger *et al.* (2014)
Oxynoemacheilus merga	Bashly	Russia	KU928285	Freyhof *et al*. (2016)
Oxynoemacheilus persa	Kor	Iran	KP050538	Esmaeili *et al*. (2015)
Oxynoemacheilus phasicus	Rioni	Georgia	MW564259	Epitashvili *et al*. (2020)
Oxynoemacheilus phasicus	Rioni	Georgia	MW564373	Epitashvili *et al*. (2020)
Oxynoemacheilus phasicus	Rioni	Georgia	MW564302	Epitashvili *et al*. (2020)
Oxynoemacheilus phasicus	Rioni	Georgia	MW564481	Epitashvili *et al*. (2020)
Oxynoemacheilus phasicus	Rioni	Georgia	MW564442	Epitashvili *et al*. (2020)
Oxynoemacheilus phasicus	Rioni	Georgia	MW564381	Epitashvili *et al*. (2020)
Oxynoemacheilus phasicus	Rioni	Georgia	MW564371	Epitashvili *et al*. (2020)
Oxynoemacheilus phasicus	Rioni	Georgia	MW564435	Epitashvili *et al*. (2020)
Oxynoemacheilus phasicus	Rioni	Georgia	MW564307	Epitashvili *et al*. (2020)
Oxynoemacheilus pindus	Devolli	Albania	KJ554005	Geiger *et al.* (2014)
Oxynoemacheilus samanticus	Seyhan	Turkey	KJ553807	Geiger *et al.* (2014)
Oxynoemacheilus seyhanensis	Seyhan	Turkey	KJ553980	Geiger *et al.* (2014)
Oxynoemacheilus seyhanicola	Seyhan	Turkey	KJ553976	Geiger *et al.* (2014)
Seminemacheilus ahmeti	Soysalli	Turkey	MT077014	Yoğurtçuoğlu *et al*. (2020)

Results

The COI sequence data analyses (Fig. 1) verify the previous results and place our test group as a distinct lineage within a group of species with a wide distribution in the Caspian Sea basin. Here we recognise it as a new species in this group and name it *O. phasicus*. This group is defined here as the *O. brandtii* species group and our molecular data suggest that it includes *O. brandtii*, *O. elsae*, *O. merga*, and *O. phasicus*. The K2P distance between *O. phasicus* and *O. brandtii* from the Kura drainage, its closest relative in our dataset based on COI sequences, is 7.5%. All fish identified as *O. brandtii* from the Aras drainage cluster as sister group to *O. elsae* with 1.5% K2P minimum distance between the two groups.

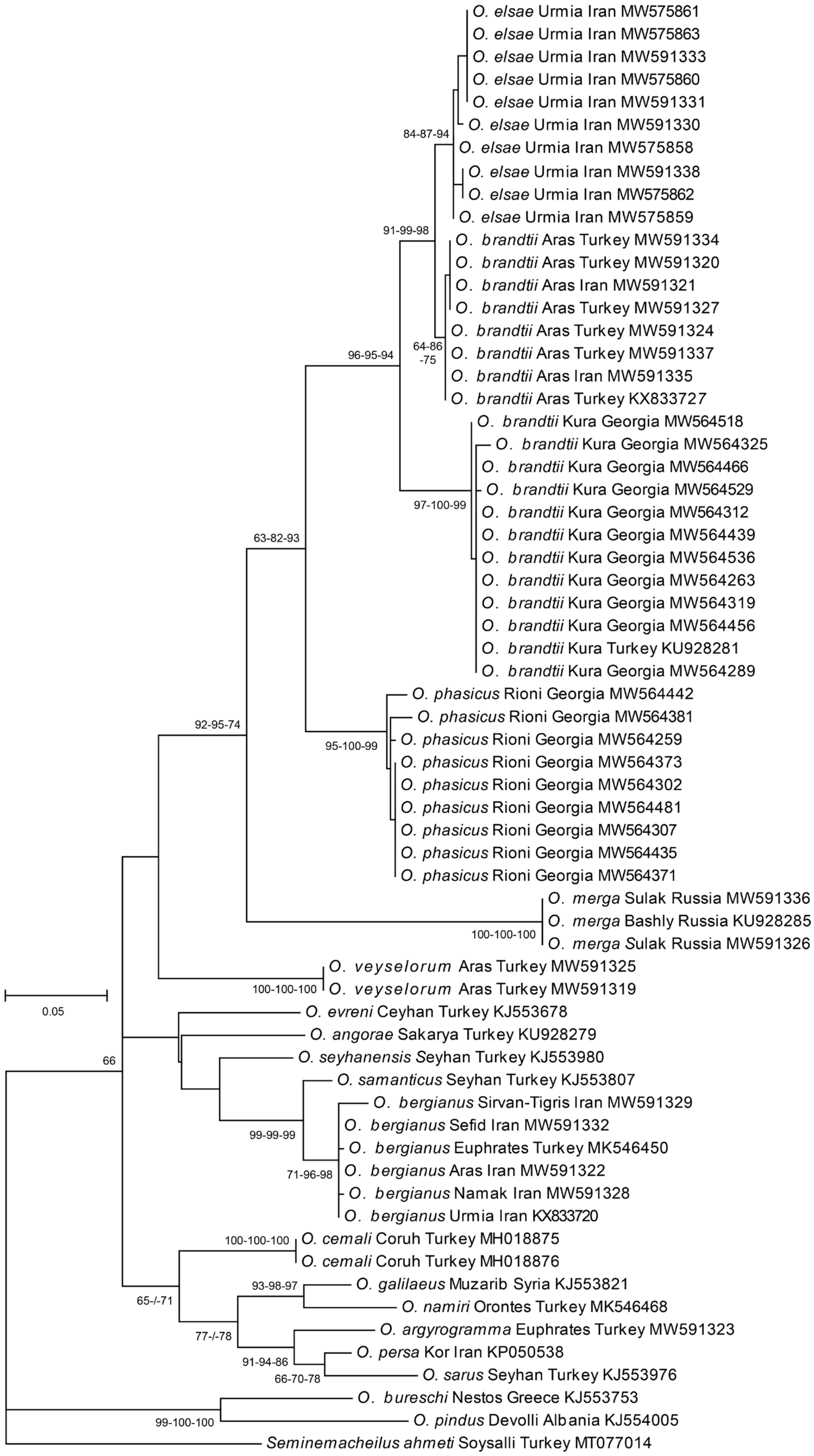

FIGURE 1. Maximum likelihood estimation of the phylogenetic relationships based on the mitochondrial DNA barcode region (Tamura-Nei model, discrete Gamma distribution for rate differences with 3 categories (+G, parameter = 0.3477). Nucleotide positions with less than 98% site coverage were eliminated, resulting in 557 analysed positions. Numbers of major nodes indicate bootstrap values from 1000 pseudoreplicates from the ML, NJ and MP methods. The tree is drawn to scale with branch lengths depicting number of substitutions per site.

***Oxynoemacheilus phasicus*, new species**
(Figs 2–5)

Holotype. ZFMK ICH-124180, 74 mm SL; Georgia: Dzirula River at road from Tbilisi to Kutaisi 1 km upstream from Khunevi, 42.0975 43.3900.

Paratypes. FSJF 3592, 1, 77 mm SL; same data as holotype.—FSJF 3576, 3, 50–59 mm SL; Georgia: Enguri River at Lia, 42.6617 41.9911.—FSJF 3615, 2, 44–54 mm SL; Georgia: Tskhenitskali River upstream of Samtredia, 42.1694 42.4314.—FSJF 4061, 2, 32–39 mm SL; Georgia: Gubistskali River east of Samtredia, upriver of confluence with Rioni and Rioni channel, 42.16251 42.41069.

Material used in molecular genetic analysis. ZFMK ICH-NB184; same data as holotype (GenBank accession number: MW564259, holotype or paratype, association unclear).—ZFMK ICH-106137-106138; Georgia: Swlana stream west of Jurukveti, 42.07829 41.91643 (GenBank accession numbers: MW564307, MW564435).—ZFMK ICH-106139-106140; Georgia: Gubistskali River east of Samtredia, upriver of confluence with Rioni and Rioni channel, 42.16251 42.41069. (GenBank accession numbers: MW564371, MW564381).—ZFMK ICH-NB190-NB191; Georgia: Tskhenitskali River upstream of Samtredia, 42.1694 42.4314 (GenBank accession numbers:MW564373, MW564302, MW564481).—ZFMK ICH-NB195; Georgia: Enguri River at Lia, 42.6617 41.9911 (GenBank accession number: MW564442).

Diagnosis. *Oxynoemacheilus phasicus* is distinguished from *O. cemali*, the only other species of *Oxynoemacheilus* known from the eastern Black Sea basin, by a slightly emarginate caudal fin (vs. deeply emarginate), pelvic fin not reaching to origin of genital papillae (vs. reaching to or beyond posterior tip of genital papillae), pelvic-fin origin below first branched dorsal-fin ray (vs. below second or third ray), a wide band in posterior half of caudal fin (vs. absent), and a mottled or marbled flank pattern (juveniles with bars on flank behind dorsal-fin base) (vs. usually with 9–15 narrow, irregularly shaped and spaced, dark-grey bars or vertically elongate blotches on flank posterior to dorsal-fin origin).

Oxynoemacheilus phasicus is distinguished from the other members of the *O. brandtii* species group (*O. brandtii*, *O. elsae*, and *O. merga)* by having a slightly emarginate caudal fin (vs. forked in *O. brandtii*, deeply emarginate in *O. elsae* and *O. merga*), pelvic fin not reaching to origin of genital papillae (vs. reaching to or beyond anus in *O. brandtii* and many *O. elsae* and *O. merga*), caudal peduncle 1.5–2.1 times longer than deep (vs. 2.5–3.2 in *O. brandtii*, 2.3–2.9 in *O. elsae*, 2.0–2.5 in *O. merga*), and a mottled or marbled flank pattern (vs. usually with irregularly shaped and spaced, dark-brown bars or vertically elongate blotches on flank posterior to dorsal-fin origin, much wider than interspaces, often fused into a plain brown pattern on flank in front of dorsal-fin base in *O. brandtii*; usually with irregularly shaped and spaced, rarely regularly shaped and spaced, dark-brown bars, often split in middle, or flank almost plain brown in *O. elsae*; with many, narrow, pale-brown bars, usually much more narrow than interspaces, often dissociated in vertically elongate blotches on flank and dorsal saddles, rarely mottled in *O. merga*).

Description. See Figures 2–5 for general appearance and Table 2 for morphometric data. Medium-sized and moderately slender species. Body deepest at about midline between nape and dorsal-fin origin. Body width greatest at pectoral-fin base, at cheek in largest individual. Section of head roundish, flattened on ventral surface, slightly convex in interorbital space, convex on snout. Snout blunt. Caudal peduncle compressed laterally, 1.5–2.1 times longer than deep. Pelvic axillary lobe present, its tip attached to flank. Pelvic-fin origin below first branched dorsal-fin ray. Anal-fin origin at about vertical of midline between dorsal and caudal-fin origins. Pectoral fin reaching to approximately 50–70% of distance from pectoral-fin origin to pelvic-fin origin. Pelvic fin not reaching anus; reaching slightly anterior to vertical of tip of last dorsal-fin ray; almost reaching anus in juveniles smaller than 40 mm SL. Anus about 30–70% of an eye diameter anterior to anal-fin origin. Anal fin not reaching caudal-fin base. No dorsal or ventral adipose crest on caudal peduncle. Largest known individual 77 mm SL.

Dorsal fin with 8½–9½ branched rays, outer margin straight or convex. Anal fin with 5½ branched rays, outer margin straight or slightly concave. Pectoral fin with 9–11 branched rays, outer margin straight. Pelvic fin with 7 branched rays, outer margin straight or slightly convex. Caudal fin slightly emarginate with 8+8 branched rays, moderately emarginate in juveniles smaller than 40 mm SL. Flank, back and abdomen densely covered by scales. Lateral line complete, terminating at caudal-fin base. Anterior nostril opening at end of a low, ovoid and flap-like tube. Posterior tip of anterior nostril overlapping in small individuals, not overlapping posterior nostril when folded backwards in large individuals. One central pore, two in one individual, and one lateral pore on each side of supratemporal head canal, 8–10 pores in anterior infraorbital canal, 3–4 pores in posterior infraorbital canal, 6–9 pores in supraorbital canal and 5–7 pores in mandibular canal. A short suborbital groove in male. Mouth small, arched. Lips thick without furrows, lower lip thicker than upper lip. A median interruption in lower lip. Upper lip with a small and short median incision in some individuals, absent in others. Processus dentiformis narrow, very small and rounded. Lower jaw rounded, without median notch. Barbels long; inner rostral barbel reaching to or beyond base of maxillary barbel, outer reaching beyond anterior margin of eye, to middle of eye in some individuals. Maxillary barbel reaching beyond vertical through middle of eye or to or beyond posterior margin of eye. Barbels very short in largest individuals.

TABLE 2. Morphometric data of *Oxynoemacheilus phasicus* (holotype ZFMK ICH-124180 and paratypes FSJF 3576, 3591, 3615, n=7). Holotype is included in calculations.

	holotype	**holotype & paratypes**			
		mean	min	max	SD
Standard length (mm)	74.1		44.4	77.0	
In percent of standard length					
Head length	22.3	22.7	22.1	23.8	0.6
Body depth at dorsal-fin origin	15.9	15.6	13.8	17.7	1.5
Body width at dorsal-fin origin	12.9	12.5	11.4	13.5	0.9
Predorsal length	49.8	50.0	48.8	51.0	0.7
Postdorsal length	39.9	40.1	39.1	41.0	0.8
Preanal length	74.1	74.0	72.2	76.1	1.4
Prepelvic length	50.9	50.7	49.9	51.4	0.6
Distance between pectoral- and pelvic-fin origins	29.0	29.0	28.5	29.4	0.3
Distance between pelvic- and anal-fin origins	23.4	23.5	22.5	25.5	1.0
Distance between vent and anal-fin origin	2.3	1.5	0.9	2.3	0.5
Depth of caudal peduncle	12.3	11.2	8.6	12.4	1.3
Length of caudal peduncle	19.3	19.0	18.1	20.3	0.9
Dorsal-fin depth	15.6	17.2	10.5	19.8	3.3
Anal-fin height	15.5	16.2	14.3	19.2	1.5
Pectoral-fin length	18.4	20.5	18.4	23.4	1.8
Pelvic-fin length	16.4	15.9	14.6	16.8	0.9
In percent of head length					
Head depth at eye	46	47	43	58	4.9
Snout length	43	42	39	45	1.6
Eye diameter	15	17	15	21	2.2
Postorbital distance	47	46	43	49	1.8
Maximum head width	70	68	61	75	4.3
Interorbital width	30	29	25	34	3.0
Length of inner rostral barbel	19	21	14	26	4.2
Length of outer rostral barbel	19	29	17	42	9.3
Length of maxillary barbel	23	28	16	33	6.3

Coloration. Body with yellowish or pale brown background and dark- or pale-brown pattern in live and preserved individuals. Preserved individuals with a wide inner-axial stripe, absent in life. Dorsal head and upper part of cheek brown, with a vermiculate pattern. Ventral surface of head yellowish without pattern. Flank densely mottled, without bars or blotches, marbled or with bars on flank behind dorsal-fin origin in individuals smaller than 50 mm SL, almost plain brown in some individuals. Flank pigmentation reaching down to horizontal of pectoral-fin base or to belly in some individuals. Back mottled, marbled or with 3–4 squarish or triangular predorsal blotches, confluent with flank pattern, one saddle at dorsal-fin origin, one or none in middle of dorsal-fin base and one at end of dorsal-fin base, and 3–6 saddles behind dorsal-fin base, all saddles wider than interspaces. A narrow, black bar at caudal-fin base. Posteriormost upper and lowermost caudal peduncle with a pale yellowish, unpigmented blotch. Dorsal and pectoral fins with small brown blotches on rays, forming 1–3 bands. Caudal fin with many small brown blotches on rays, forming 1–3 wide and bold bands, often band at second half of caudal fin very wide or caudal-fin rays almost completely brown. Pectoral, anal and pelvic fins with dark-brown blotches on rays; hyaline or with few blotches in individuals smaller than 60 mm SL.

FIGURE 2. *Oxynoemacheilus phasicus*, ZFMK ICH-124180, holotype, 74.1 mm SL; Georgia: Dzirula River.

Distribution. *Oxynoemacheilus phasicus* was collected from the Enguri and Rioni River drainages in Georgia. It has not been found in the Chorokhi / Çoruh River in Georgia or Turkey or in small rivers in-between despite intensive field work in that area.

Etymology. The species name is derived from *Phasis*, the Greek name of the Rioni. An adjective.

Remarks. *Nemacheilus bergi* was named by Gratzianov (1907) based on a description by Berg (1899) of two individuals (61 mm, 35 mm) from the Akstapha [Agstafa] River, a right tributary of the Kura in Azerbaijan. Berg (1899:73) wrote in his chapter on *Nemacheilus sp*.: „Die Schwanzflosse ist im Verhältnis zu N. Brandt schwach ausgeschnitten (bei dem Exemplar von 61 mm. Länge ist der mittelste Strahl der Schwanzflosse 8 mm. Und die seitlichen 11 mm lang)." [The caudal fin is slightly emarginate in comparison with *N. brandtii* (in the specimen of 61 mm the length of the central caudal-fin ray is 8 mm and of the lateral ones 11 mm)]. Also, Berg (1899:72–73) mentions that the pelvic fin reaches the anus, there is a bold bar at the caudal-fin base, a wide band in the middle of the caudal fin and incomplete bars on the flank. This species is further treated as a synonym of *O. angorae* by Berg (1949), who distinguished *O. angorae* from *O. bergianus* by the caudal peduncle being less than two times longer than deep in *O. angorae* vs. more in *O. bergianus*. Çiçek *et al*. (2018) treat *N. bergi* as a valid species in *Oxynoemacheilus* without giving further details. Soheil Eagderi (Tehran) sent JF a picture (Fig. 7) of one individual they identified as *O. bergi* collected in the Kura, very close to the inflow of the Agstafa. Measured from the picture, this individual has the caudal-peduncle depth two times in the caudal-peduncle length, the caudal fin is emarginate and the middle caudal-fin ray is 75% of the length of the longest principal ray in the lower caudal-fin lobe (72% in the description of Berg 1899, but unclear which lobe was measured). These two character states as well as its colour pattern distinguish this species clearly from *O. brandtii* (caudal peduncle 2.5–3.2 times longer than deep, caudal fin forked). Indeed the fish on Fig. 7 agree with the characters given for *N. bergi* by Berg (1899) and we support the view of Çiçek *et al.* (2018) treating *O. bergi* as a valid species.

FIGURE 3. *Oxynoemacheilus phasicus*, paratypes, from top, left to right: FSJF 4061, 32 mm SL; 39 mm SL; Georgia: Gubistskali River; FSJF 3615, 44 mm SL, 54 mm SL; Georgia: Tskhenitskali River; FSJF 3576, 59 mm SL; Georgia: Enguri River; FSJF 3592, 77 mm SL; Georgia: Dzirula River.

Oxynoemacheilus bergi is clearly distinguished from *O. phasicus* by possessing a deeply emarginate caudal fin (vs. slightly emarginate in *O. phasicus*), and the pelvic fin reaching the anus (vs. not reaching). Potentially *O. bergi* is conspecific with *O. bergianus*, described from the Sefid River in Iran and known to occur in the adjacent Aras, a major tributary of the Kura. Future research should clarify if *O. bergi* is different from *O. bergianus*. If both are conspecific, *O. bergianus* would become a junior synonym of *O. bergi*.

We also follow Çiçek *et al.* (2018) in treating *N. angorae alasanicus* as a synonym of *O. bergi* and *N. brandtii gibbusnazus* as a synonym of *O. brandtii.* Çiçek *et al.* (2018) did not examine any materials of *N. a. alasanicus* or *N. b. gibbusnazus*, both described by Elanidze (1983) from the upper Alazani in the Georgian Kura drainage. G. Epitashvili and L. Mumladze (Ilia State University, Tbilisi, Georgia) visited the upper Alazani though yet only *O. brandtii* was collected. Çiçek *et al.* (2018) give no arguments for their conclusion and did not analyse the description of both species. However, we also have no arguments to reject the hypothesis of Çiçek *et al.* (2018), as the descriptions are not very conclusive, without figures and we also did not examine enough materials by our own. Only four loaches from the type locality of both species were available for this study and these are identified as *O. brandtii*. It is likely that Elanidze (1983) had identified his materials by the key provided by Berg (1949) and correctly identified one species as *O. brandtii* and the second species, which has a deeper caudal peduncle and an emarginate (vs. furcate) fin, was identified as *O. angorae*. He then described new subspecies for both species, and thus *N. angorae alasanicus* might be identified as *O. bergi*.

FIGURE 4. *Oxynoemacheilus phasicus*, paratypes, from top, left to right: FSJF 4061, 32 mm SL; 39 mm SL; Georgia: Gubistskali River; FSJF 3615, 44 mm SL, 54 mm SL; Georgia: Tskhenitskali River; FSJF 3576, 59 mm SL; Georgia: Enguri River; FSJF 3592, 77 mm SL; Georgia: Dzirula River.

FIGURE 5. *Oxynoemacheilus phasicus*, paratypes, from top, left to right: FSJF 4061, 32 mm SL; 39 mm SL; Georgia: Gubistskali River; FSJF 3615, 44 mm SL, 54 mm SL; Georgia: Tskhenitskali River; FSJF 3576, 59 mm SL; Georgia: Enguri River; FSJF 3592, 77 mm SL; Georgia: Dzirula River.

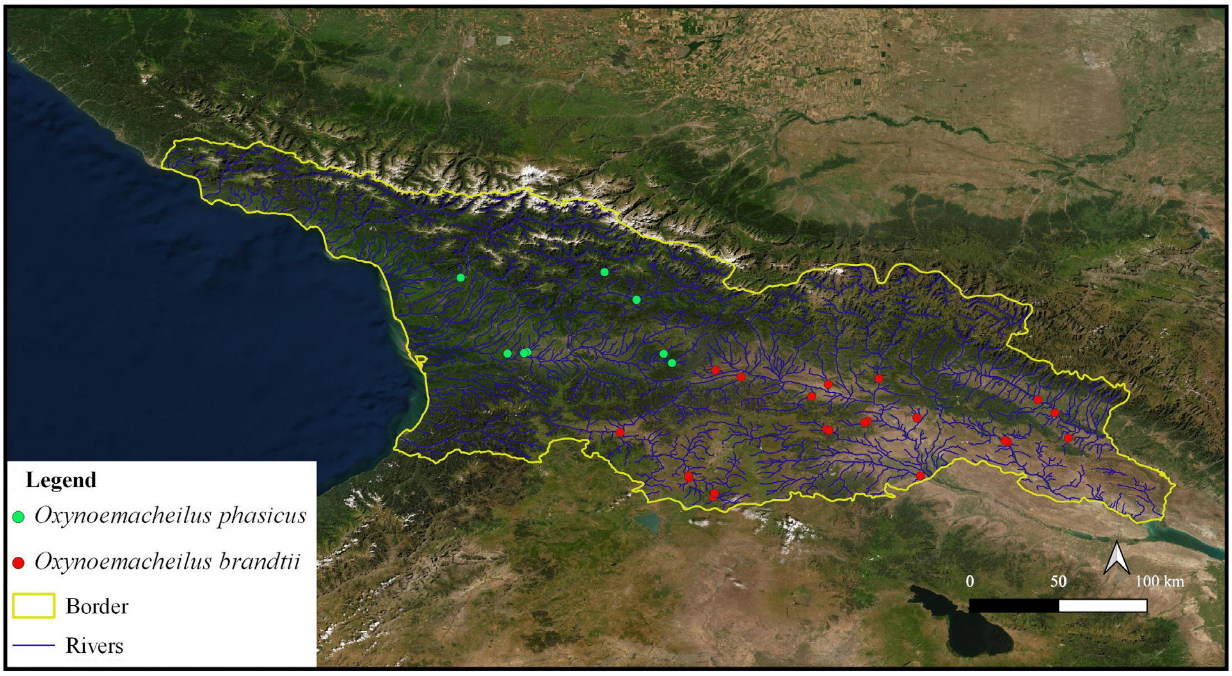

FIGURE 6. Records of *Oxynoemacheilus brandtii* and *O. phasicus* in Georgia based on site-scale records mentioned above.

FIGURE 7. *Oxynoemacheilus bergi*; IMNRF-1030-2, 50.4 mm SL; Azerbaijan: Kura River, 41°14'25''N 45°25'57''E. Photo: Soheil Eagderi

Eagderi *et al*. (2018) distinguish *O. elsae* (Fig. 9) from *O. brandtii* (Fig. 8) based on 10, non-overlapping morphometric characters (dorsal-fin base length, anal-fin base length, anal-fin depth, pelvic-fin length, caudal-fin length, caudal-peduncle width, snout length, eye diameter, interorbital distance, maxillary barbel length). They compared 20 *O. elsae* with five *O. brandtii* from the Kura drainage. We examined 20 individuals from each species and found all the 10 morphometric characters overlapping. In seven out of 10 characters no significant difference from equality of their mean values were detected. Only the means of the pelvic-fin length, length of the lower caudal-fin lobe, and the eye diameter were significantly different (Fig. 10). Also these morphometric characters largely overlap: pelvic-fin length 14–20, mean 18% SL in *O. brandtii* (vs. 14–17, mean 16 in *O. elsae*), length of lower caudal-fin lobe 20–27, mean 24% SL (vs. 19–23, mean 21), and the eye diameter 13–21, mean 17% HL (vs. 17–24, mean 20). The overlap in the pelvic-fin length is due to two individuals only and from 49 *O. brandtii* all except two individuals, have a pelvic-fin length 17–20 % SL, one has 14% SL and one has 15% SL. This character allows to distinguish *O. brandtii* from *O. elsae* if a larger number of individuals is available, while the other characters can just support their diagnosis. *Oxynoemacheilus elsae* is well distinguished from *O. brandtii* by the shape of the caudal fin, which is emarginate in *O. elsae* (vs. forked in *O. brandtii*) and by details in colour pattern. *Oxynoemacheilus elsae* usually possess a flank pattern of irregularly shaped and spaced, dark-brown bars, often split in middle, more narrow than interspaces, or flank almost plain brown (vs. flank with brown bars or vertically elongate blotches, much wider than interspaces, usually regularly set) and the caudal fin possess many small brown blotches on rays arranged in 3–6, narrow, pale-brown band (vs. caudal fin with 1–3, wide and very bold bands). However, the flank pattern is variable in both species and occasionally almost plain brown individuals or individuals with many, very narrow bars occur. *Oxynoemacheilus elsae* is separated from *O. brandtii* by a minimum K2P distance of 1.5% (Aras) and 4.9% (Kura) in the mtDNA COI barcode region (Fig. 1).

FIGURE 8. *Oxynoemacheilus brandtii*, Kura drainage, from top: Not preserved, about 70 mm SL; Georgia: Alazani River; FSJF 3430, 50 mm SL; 48 mm SL; Turkey: stream between Göle and Ardahan; Not preserved; about 65 mm SL; Kura River at Yalnızçam.

FIGURE 9. *Oxynoemacheilus elsae* FSJF 4109, from top: 55 mm SL; 60 mm SL; 53 mm SL; Iran: Zarrineh River.

Oxynoemacheilus from the Aras River (Fig. 11), a major tributary to the Kura, cluster as sister to *O. elsae* in our molecular analysis based on all three approaches (ML, NJ, MP). In all morphological characters distinguishing *O. elsae* from *O. brandtii*, the population from the Aras agrees with *O. brandtii* and not with *O. elsae* (Fig. 9) including the characteristic colour pattern of *O. brandtii*. These fish are significantly different from *O. elsae* in the pelvic-fin length mentioned above, but not different from *O. brandtii* in any of these or other characters examined. The obvious mismatch between morphological and molecular characters, which suggests that the fish from the Aras should be identified as *O. elsae* cannot be resolved here. It could be speculated that past river-captures might have resulted in an invasion of a mitochondrial haplotype of *O. elsae* from Urmia into the adjacent Aras. While they hybridised with native *O. brandtii,* their mitochondria might have performed better and gave the carriers an evolutionary benefit which spread in the Aras. If so, what cannot be excluded, we would find *O. brandtii* with mtDNA of *O. elsae*. The small molecular distance between *O. elsae* and *O. brandtii* from the Aras in their mtDNA indicates the time passed since the connection of the Aras with the Urmia basin. Further nuclear molecular research is needed in the future aiming to clarify the case. We suggest to identify the fish from the Aras as *O. brandtii* due to its morphological similarity to this species.

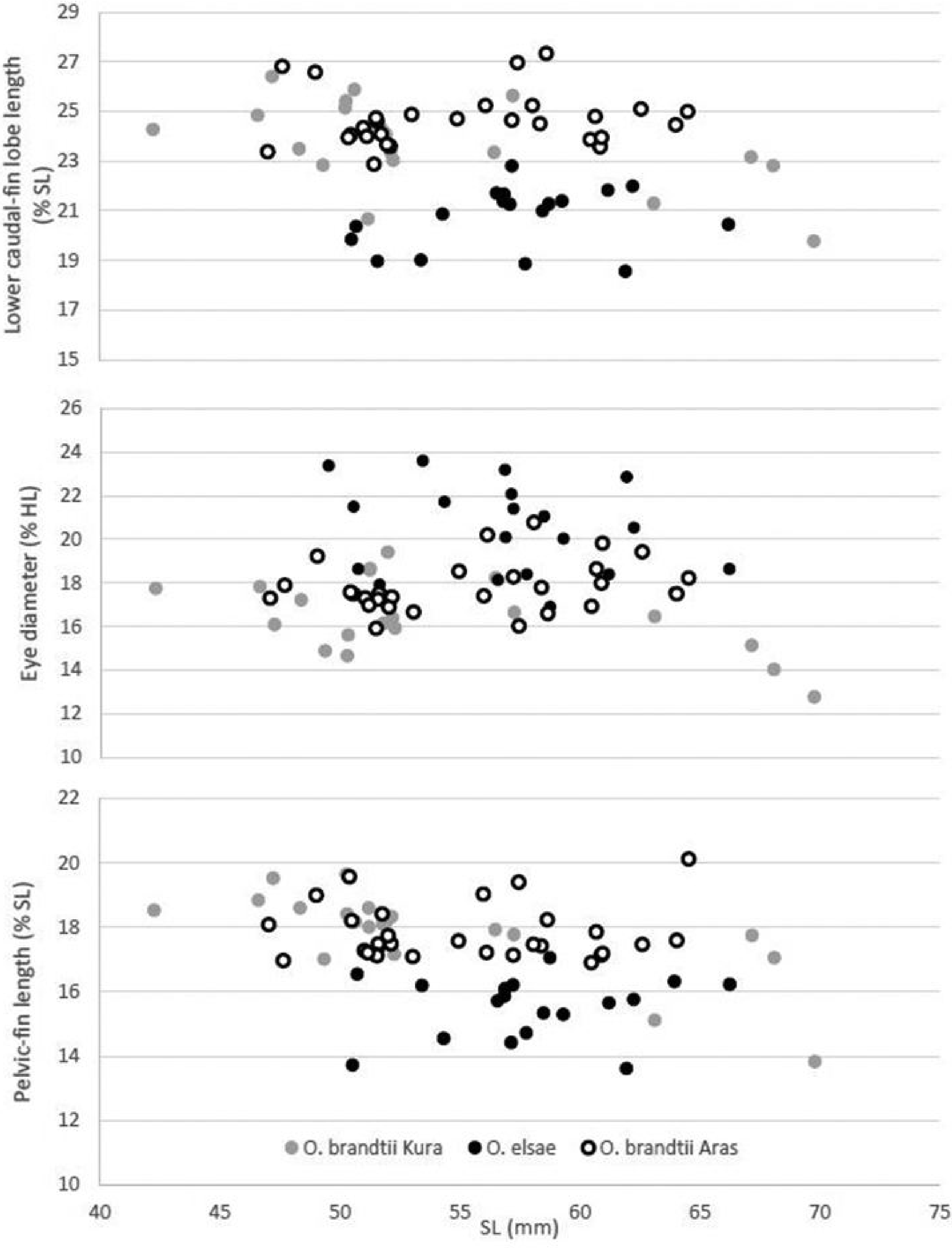

FIGURE 10. Morphometric characters of *O. brandtii* from Aras (FSJF 3441 n=9, FFR 1427 n=11, FFR 1351 n=9), and Kura (FSJF 3544 n=7, FSJF 3571 n=5, FSJF 3630 n=1, FSJF 3627 n=1, FSJF 4023 n=6) and *O. elsae* from Zarrineh (FSJF 2264 n=9, FSJF 4109 (n=11).

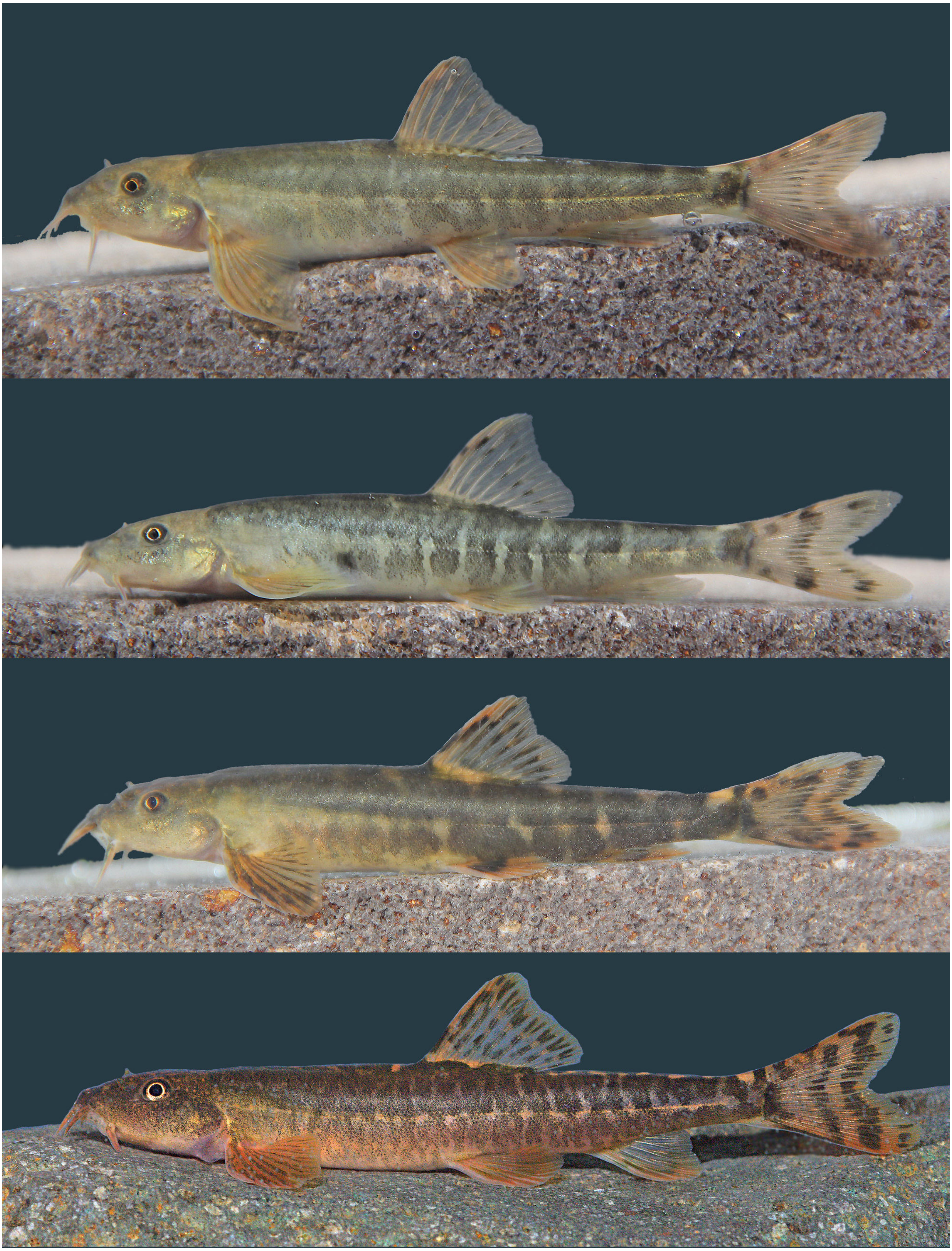

FIGURE 11. *Oxynoemacheilus brandtii*, Aras drainage, Turkey: from top: FFR 15549, 54 mm SL; 52 mm SL, stream Büyükçayır; FFR 15551, 54 mm SL, stream Fehmiharabesi; FSJF 3441, 65 mm SL; Turkey: stream Derinöz.

Comparative materials

Oxynoemacheilus brandtii FSJF 3430, 2, 50–52 mm SL; Turkey: stream between Göle and Ardahan, close to Yiğitkonağı, 40.9669 42.5867.—FSJF 3441, 9, 49–65 mm SL; Turkey: stream Derinöz at Digor, 40.3734 43.4258.—FSJF 3544, 4, 39–54 mm SL; Georgia: tributary of Iori River, 41.5861 45.5986.—FSJF 3553, 1, 20 mm SL; Georgia: Iori River at Kermugolo, 41.5747 45.6126.—FSJF 3571, 9, 37–70 mm SL; Georgia: Aragvi River at Tsiteltsopeli, 41.9936 44.7603.—FSJF 3607, 1, 21 mm SL; Georgia: Alazani River at road from Liliani to Gurdzhaani, 41.7672 45.9242.—FSJF 3627, 1, 47 mm SL; Georgia: Ptsa River at road from Tbilisi to Kutaisi, 42.0497 43.6789.—FSJF 3630, 21, 27–51 mm SL; Georgia: Khrami River at confluence with Kura, 41.3511 45.0344.—FSJF 4020, 6, 49–58 mm SL; Turkey: stream Kekeç at Kekeç, 40.4917 42.7436.—FSJF 4023, 4, 56–61 mm SL; Turkey: stream Bölükbaþ at Katranlı, 40.4747 42.7303.—FSJF 4110, 4, 42–53 mm SL; Georgia: Alazani south of Alvani, 42.0470 45.3653.—FFR 15549, 12, 44–62 mm SL; Turkey: stream Fehmiharabesi at Akçalar, 40.7725, 43.2950.—FFR 15551, 27, 45–64 mm SL; Turkey: stream Büyükçayır at Arpaçay, 40.8430, 43.3222.—FFR 1460, 1, 60 mm SL; Turkey: stream Hanak at Hanak, 41.2299, 42.8420.—FFR 1351, 22, 32–61 mm SL; Turkey: stream Kekeç at Selim, 40.4715, 42.8005.—HIDBb 1, 2, 53–55 mm SL; Georgia: Kura, 41.6398 43.0465.—HIDBb 2, 4, 50–56 mm SL; Georgia: stream Khrami, a right tributary of Kura, 41.2104 45.2040.—HIDBb 3, 1, 55 mm SL; Georgia: small tributary to Iori near Kazlari, 41.5826 45.5940.—HIDBb 5, 1, 52 mm SL; Georgia: stream Vere, a tributary to Kura, 41.7125 44.6853.—HIDBb 8, 5, 49–56 mm SL; Georgia: Bugdasheni in Javakheti highlands, 41.2133 43.6617.—HIDBb 9, 5, 48–53 mm SL; Georgia: Paravani in Javakheti highlands, 41.3364 43.5026.—HIDBb 10, 2, 46 mm SL; Georgia: stream Lekhura near Kaspi, 41.9558 44.4240.—HIDBb 11, 2, 49–55 mm SL; Georgia: stream Vere, 41.7027 44.6666.—HIDBb 13, 1, 46 mm SL; Georgia: Paravani in Javakheti highland, 41.3501 43.4990. —HIDBb 14, 8, 45–58 mm SL; Georgia: Bugdasheni in Javakheti highlands, 41.2356 43.6710.—HIDBb 16, 1, 40 mm SL; Georgia: Algeti River, 41.6603 44.4121.—HIDBb 17, 6, 45–57 mm SL; Georgia: Alazani near Kvareli bridge, 41.8540 45.8136.—HIDBb 18, 1, 39 mm SL; Georgia: Alazani channel, 41.6016 46.0138.—HIDBb 19, 3, 37–40 mm SL; Georgia: Tedzami, a right tributary of Kura, 41.8760 44.3144.—HIDBb 21, 10, 38–48 mm SL; Georgia: Algeti River, 41.6505 44.4289.—HIDBb 22, 1, 40 mm SL; Georgia: Dzama river near Kareli, 42.0049 43.8472.—HIDBb 23, 9, 28–36 mm SL; Georgia: Lochini near Lilo, 41.7354 45.0111.

Oxynoemacheilus cemali FSJF 3166, 30, 21–60 mm SL; Turkey: stream Tortum 1 km south of Tortum Lake, 40.5867 41.6089.—FSJF 3182, 2, 39–82 mm SL; Turkey: stream Uzunkavak 1 km south of Pehlivanlı, 40.4946 41.4894. See also Turan *et al*. (2019).

Oxynoemacheilus elsae FSJF 4109, 27, 32–61 mm SL; Iran: Zarrineh River north of Shain-Dezj, 36.6890 46.5394.—FSJF 2264, 31, 44–68 mm SL; Iran: Zarrineh River at Qosha, 37.0029 46.0989.

Oxynoemacheilus merga FSJF 1561, 15–46–59 mm SL; Russia: River Rubas at village Syrtych, 41.8438 48.2312.

Material used in molecular genetic analysis

Oxynoemacheilus argyrogramma FSJF DNA-1501, Turkey: a tributary to Merziman stream south of Yavuzeli, 37.2768 37.5324 (GenBank accession number: MW591323).

Oxynoemacheilus bergianus FSJF DNA-1688, Iran: Agh Chai, a tributary to Aras, 38.8586 45.1335 (GenBank accession number: MW591322).—FSJF DNA-1993, Iran: Kordan river near Karaj, 35.9530 50.8375 (GenBank accession number: MW591328).—FSJF DNA-2725, Iran: upper Quezel Owzan River at Nemehil (GenBank accession number: MW591332).—FSJF DNA-2317, Iran: Zarivar south of Qellace, 35.3509 46.2746 (GenBank accession number: MW591329).

Oxynoemacheilus brandtii FSJF DNA-2729, Iran: Aras west of Kordasht, 38.8675 46.2334 (GenBank accession number: MW591321).—FSJF DNA-2731, Iran: stream south of Jananlo, 39.0926 46.9201 (GenBank accession number: MW591335).—FSJF DNA-2399, Turkey: stream Derinöz at Digor, 40.3734 43.4258 (GenBank accession numbers: MW591320, MW591337).—ZFMK-ICH-TIS-66595 Turkey: stream Süngütasi west of Handere, 40.2757 42.4622 (GenBank accession number: MW591334).—ZFMK-ICH-TIS-66544 Turkey: Selim stream, 40.2223 43.2535 (GenBank accession numbers: MW591324, MW591327).

Oxynoemacheilus elsae FSJF DNA-2745 & DNA-2746, Iran: stream at Miriseh, 36.4986 45.5651 (GenBank accession numbers: MW591330, MW591331, MW591333, MW591338).—FSJF DNA-3059, Zarrineh River north of Shain-Dezj, 36.6890 46.5394 (GenBank accession numbers: MW575858, MW575859).—FSJF DNA-2002 & DNA-2311, Azerbaijan: Soufichai River north of Maraghan, at inflow to Alavian reservoir, 37.4581 46.2699 (GenBank accession numbers: MW575860, MW575861, MW575862, MW575863)

Oxynoemacheilus merga ZFMK-ICH-TIS-NB75, Russia: irrigation canal L'vovskiy, 43.3880 47.1511 (GenBank accession numbers: MW591326, MW591336).

Oxynoemacheilus namiri FSJF DNA-1384, Turkey: Afrin about 16 km west of Kilis, 36.81327 36.98375 (GenBank accession number: MK546468).

Oxynoemacheilus veyselorum ZFMK-ICH-TIS-66592 Turkey: stream Süngütasi west of Handere, 40.2757 42.46221 (GenBank accession number: MW591325).—ZFMK-ICH-TIS-464TK Turkey: Aras drainage, stream Güllü south of Bölükbasi, 40.48519 42.68240 (GenBank accession number: MW591319).

Site-scale records listed in this study and additional records from ISU collection shown in Fig. 6.

Oxynoemacheilus phasicus 42.6617 41.9911, 42.1694 42.4314, 42.1625 42.4107, 42.0975 43.3900, 42.1596 42.3011, 42.5151 43.1554, 42.1580 43.3349, 42.6983 42.9433.

Oxynoemacheilus brandtii 41.5861 45.5986, 41.5747 45.6126, 41.9936 44.7603, 41.7672 45.9242, 42.0497 43.6789, 41.3511 45.0344, 41.6398 43.0465, 41.5825 45.5940, 41.7125 44.6853, 41.2133 43.6617, 41.3364 43.5026, 41.9558 44.4240, 41.7027 44.6666, 41.3501 43.4990, 41.2356 43.6719, 41.6603 44.4121, 41.8540 45.8136, 41.6016 46.0138, 41.8760 44.3144, 41.6505 44.4289, 42.0049 43.8472, 41.7354 45.0110.

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Hydropower development in the Republic of Georgia and implications for freshwater biodiversity conservation

Bella Japoshvili [a], Thiago B.A. Couto [b], Levan Mumladze [a], Giorgi Epitashvili [a], Michael E. McClain [c,d], Clinton N. Jenkins [b,e], Elizabeth P. Anderson [b,*]

[a] Institute of Zoology, Ilia State University, 3/5 Cholokashvili ave, 0169 Tbilisi, Georgia
[b] Department of Earth & Environment and Institute of Environment, Florida International University, Miami, FL 33199, USA
[c] Department of Water Resources and Ecosystems, IHE Delft Institute for Water Education, Delft, the Netherlands
[d] Faculty of Civil Engineering and Geosciences, Delft University of Technology, Delft, the Netherlands
[e] Kimberly Green Latin American and Caribbean Center, Florida International University, Miami, FL 33199, USA

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Sturgeon

ABSTRACT

The Caucasus region is a meeting point for culture and nature, lying at the nexus of Europe, the Middle East, Central Asia, and Africa, and identified as one of 36 global biodiversity hotspots. The Republic of Georgia, the center of the Caucasus biodiversity hotspot, encompasses a geographically diverse landscape inhabited by a remarkable, endemic, and understudied flora and fauna under increasing threat from human activities. A wave of new and proposed dams for hydropower presents one of the most pressing challenges for freshwater biodiversity conservation in Georgia, a country where hydropower accounts for >90% of electricity. However, this situation remains largely unknown to the international scientific community and there is limited scientific information available about Georgia in the internationally indexed peer-reviewed literature. In this article, we describe the geography, politics, and freshwater biodiversity of rivers of Georgia, with a focus on fishes. We examine trends in hydropower development over the past century and identify four distinct periods: the pre-Soviet period (until 1921), the Soviet period (1921–1991), the 1990s immediately following Georgia's declaration of independence, and the 21st century. We explore the effects of existing and proposed dams on the connectivity of rivers of western Georgia and their potential consequences for conservation of diadromous, potamodromous, and resident fish. Using the Dendritic Connectivity Index (DCI) as an analytical lens, we found serial decreases in DCI values following different periods of hydropower development in the country. Finally, we offer four considerations for future research and conservation in light of ongoing hydropower development: i) expand biodiversity research and environmental monitoring, ii) assess and implement environmental flows for Georgian rivers, iii) implement strategic planning for new hydropower development, and iv) establish strict conservation areas for protection of endangered sturgeons.

1. Introduction

Few issues have defined the challenges for conservation of the world's freshwater fish fauna in the first part of the 21st century as significantly as the unprecedented boom in hydropower dam development currently underway in many countries. Whereas an earlier wave of hydropower dam building in the mid-20th century resulted in extensive river modification throughout North America and Western Europe, the current proliferation of hydropower dams has targeted rivers in countries with emerging economies (Zarfl et al., 2015; Couto and Olden, 2018). Geographically, this modern wave of hydropower development overlaps with places that harbor extraordinary freshwater fish species richness and endemism, including numerous migratory species, and extensive inland river fisheries that provide a major source of protein and income to riparian human populations (Winemiller et al., 2016; Anderson et al., 2018; Barbarossa et al., 2020). Indeed, the conservation challenge of the 21st century's rapid hydropower development for freshwater biodiversity has received substantial coverage in the scientific literature and popular media over the past decade. Nevertheless, most articles have documented the hydropower trends and related

* Corresponding author.
E-mail address: epanders@fiu.edu (E.P. Anderson).

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conservation challenges for tropical river basins such as the Amazon and Mekong (Ziv et al., 2012; Winemiller et al., 2016; Anderson et al., 2018).

This paper aims to increase knowledge of modern hydropower trends and their consequences for freshwater biodiversity beyond these already documented areas by offering a case study from a lesser-known area: the Republic of Georgia. Georgia, an important part of the Caucasus biodiversity hotspot (Myers et al., 2000), encompasses a geographically diverse landscape inhabited by a remarkable, endemic, and under-studied flora and fauna under increasing threat from human activities, including hydropower development. In the past two decades, Georgia has experienced a proliferation of hydropower dams (most <100 MW) that have added ~1000 MW in installed generation capacity. More than 100 proposed projects are in various stages of planning or licensing as of 2020. The same rivers in Georgia that are subjected to hydropower development harbor several fish species classified as critically endangered (CR) by the International Union for the Conservation of Nature (IUCN), including seven species of sturgeon. However, to our knowledge, no published studies to date have examined the effects of hydropower dams on these and other freshwater fishes in Georgia, and the conservation status of Georgia's freshwater fauna currently is not well understood.

Here, we examine trends in hydropower development in Georgia and their implications for rivers and their biodiversity. For context, we provide an overview of river geography, relevant political history, and freshwater biodiversity in Georgia, as many readers may be unfamiliar with this region given the paucity of published studies about Georgia in the natural resource conservation literature, particularly in English. We then examine historical and modern trends in hydropower development, highlighting their consequences for river connectivity and freshwater fish diversity in Georgia. Finally, we articulate a list of research needs for freshwater science and conservation in light of current hydropower development and its future trajectory.

2. Geography, politics, and rivers of Georgia

The Caucasus region has long been a meeting point for culture and nature, lying at the nexus of Europe, the Middle East, Central Asia, and Northern Africa. Within the larger Caucasus region, the Republic of Georgia covers roughly 70,000 km^2 and is home to approximately 3.7 million people (Fig. 1). Within this relatively small geographic area, Georgia is rich in freshwater resources, including an estimated 59,000 km of rivers. Most Georgian rivers flow either westward into the Black Sea or eastward through Azerbaijan and into the Caspian Sea; the Likhi ridge divides these two drainages. Major rivers of eastern Georgia include the Kura, Alazani, and Iori, all of which drain transnational basins shared with either Turkey or Azerbaijan, or both. Of these, the Kura River is the largest and longest fluvial system of the Caucasus ecoregion, flowing for ~1515 km over Turkey, Georgia, and Azerbaijan, with a substantial portion of its headwaters in Georgia and an estimated annual discharge of 18.1 km^3 (579 m^3/s). The Rioni River (estimated annual discharge of 12.7 km^3 (403 m^3/s), flowing ~327 km, is the principal system of western Georgia, its 13,500 km^2 basin entirely within national territory and spanning from the Caucasus mountains to the Black Sea. Although its basin is less than 10% of the Kura in land area, the Rioni's average annual discharge is nearly 70% that of the Kura River (Gigineishvili, 2000). In its upper drainage, the glacier-fed Rioni River flows through mountainous regions (Fig. 2a, b). Its lowland region is characterized by extensive swamps, marshes, and floodplain areas as it approaches the Black Sea coastline (Fig. 2c). Also draining western Georgia into the Black Sea are the Kodori, Enguri, Supsa and numerous smaller rivers. Given the country's mountainous topography, most rivers in Georgia are short (<25 km long), high gradient, turbulent systems.

Rivers in present-day Georgia reflect the country's complex history over the past century. From 1921 to 1991, Georgia was part of the Soviet Union. This period, referred to as the 'Soviet period' was marked by large scale industrial projects in Georgia and other former-Soviet republics of the southern Caucasus region, like Azerbaijan and Armenia (Kuljanishvili et al., 2020). For example, the Mingachevir Dam, built in Azerbaijan between 1945 and 1954, fragmented the Kura River and disconnected upstream areas of the Kura and Alazani basins within Georgia from the Caspian Sea. Other noteworthy industrial projects were the Vartsikhe Dam for hydropower, built between 1971 and 78 on the Rioni River, and the construction of an irrigation/high water protective dam and the largest hydropower dam on the Enguri River in the 1970e80. These dams resulted in the disconnection of the Rioni and Enguri basins into isolated parts.

Introduction of non-native species to Georgian freshwaters, both accidentally and intentionally for aquaculture, also occurred during the Soviet period, especially between 1960 and 70 (Kuljanishvili et al., 2021). For example, the most problematic invasive species, such as *Carassius gibelio* (Prussian carp), *Pseudorasbora parva* (topmouth gudgeon or stone moroko), and *Gambusia holbrooki* (mosquitofish), were all introduced in the 20th century and are now very widespread in the region. The Soviet period in Georgia was also a time of substantial research

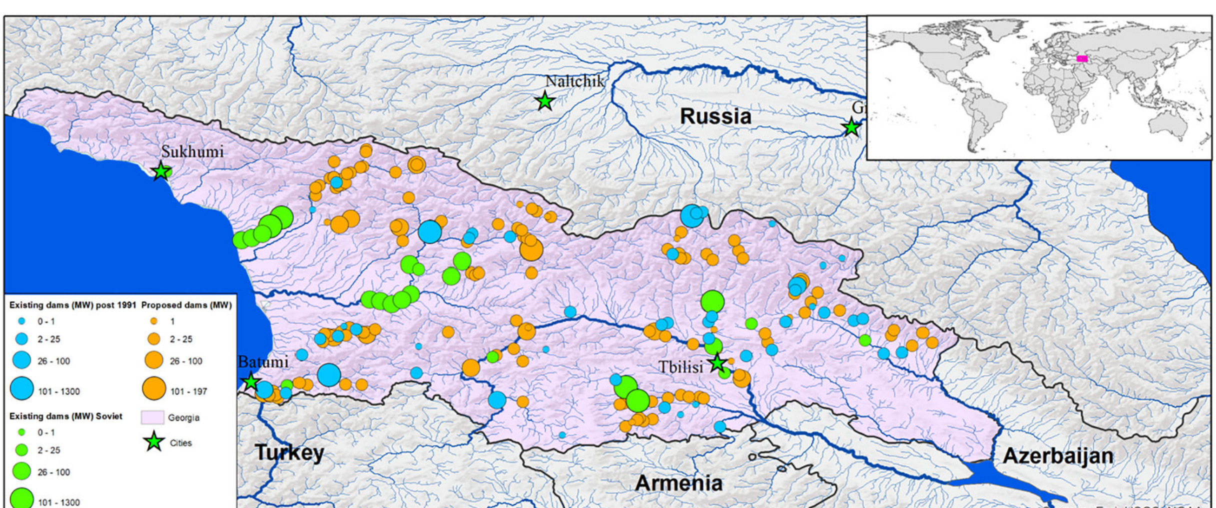

Fig. 1. The Republic of Georgia is located in the south Caucasus region. Existing hydropower dams are marked in green (built before 1991) and blue (built post 1992) to distinguish dams built during the Soviet period. Proposed dams are in orange. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

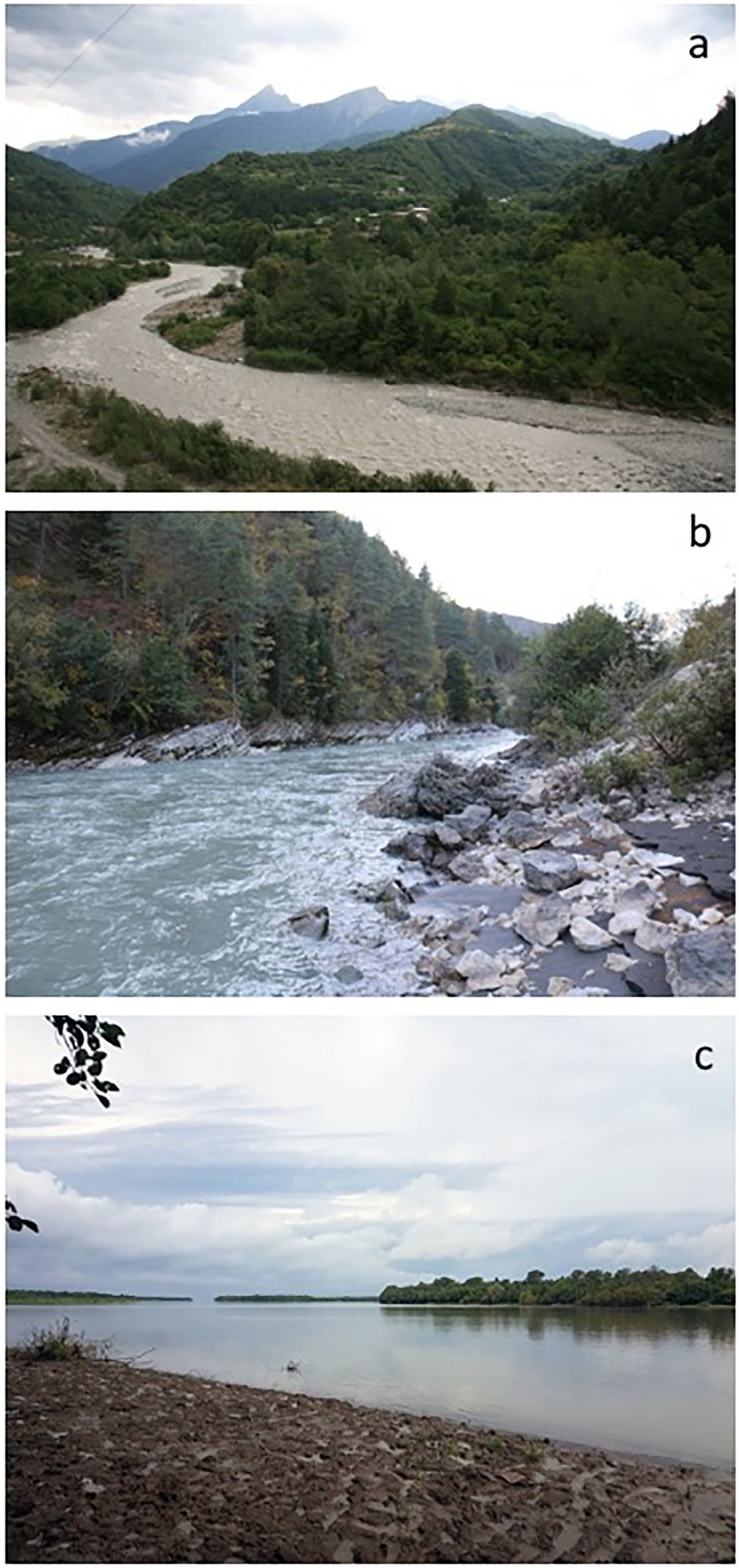

Fig. 2. The Rioni River is the largest fluvial system draining western Georgia, with its discharge into the Black Sea. Here shown are the (a) upper Rioni, near Glola village; (b) middle Rioni, 9 km upstream from Namokhvani village, near an area of ongoing construction of the Namokhvani hydropower plant; and (c) lower reaches of the Rioni River, near the city of Poti.

on freshwater systems and their fauna, particularly fishes, largely motivated by interest in aquaculture (Barach, 1941; Demetrashvili et al., 1977; Sharvashidze, 1982; Elanidze, 1983).

Georgia declared itself independent from the Soviet Union in 1991. In Georgia and throughout the Caucasus, the dissolution of the Soviet Union reportedly marked a shift in management of freshwater resources, such as fisheries, as fishing rights and regulations were suddenly divided among new independent states. In the Caspian Sea, for example, an increase in illegal harvest and trade of fish, particularly sturgeons, occurred immediately after Soviet disintegration, and continued throughout much of the 1990s (Pikitch et al., 2005).

3. Freshwater fishes of Georgia

Presently available information on freshwater biota in Georgia is fragmented and incomplete. A recent analysis of Georgia's freshwater biodiversity reviewed 300 published works between 1930 and 2012 that contained data on fishes and other freshwater species in Georgia and identified a few trends (Japoshvili, 2012). First, most published works are in Russian or Georgian, without English translations, and therefore not readily accessible to an international scientific audience. Second, ichthyological research in general peaked in the 1960s and was focused primarily on rivers during that time. It gradually declined over the remainder of the 20th century but has started to see a slight rise since the early 2000s. These trends are similar across countries of the southern Caucasus (Armenia, Azerbaijan, Georgia) (Kuljanishvili et al., 2020), but run contrary to those seen in other parts of the world, particularly the tropics, where ichthyological research, both in terms of alpha taxonomy and ecological studies, has increased markedly since the 1970s (Reis et al., 2016). Ichthyological research has also lagged behind other biodiversity studies in Georgia (Mumladze et al., 2020). Indeed, the first checklist of fishes of Georgia from the post-Soviet period was compiled by Ninua and Japoshvili (2008) only just over a decade ago and based mainly on literature review. Third, nearly all publications on freshwater fishes and other freshwater biota in Georgian waters report results of taxonomic, ecological, or fisheries-related studies. While there has been an uptick in application of molecular methods that has improved ichthyological knowledge since 2000, there have been only very limited attempts at any kind of ecological monitoring or long-term research. Finally, of all freshwater animals, fishes are the best studied taxa in Georgia. Fishes are the primary freshwater species listed in the Georgian Red List, part of the Georgian Biodiversity Database, which is freely available online (Georgian Biodiversity Database, 2021).

Acknowledging these limitations, current estimates report 96 freshwater fish species from Georgian freshwater bodies (Kuljanishvili et al., 2020). Within a larger biogeographic context, Georgian freshwater fishes represent an important vertebrate faunal component of the Caucasus biodiversity hotspot, from which 162 species of freshwater fishes and four species of lamprey—scaleless, jawless fishes that emerged roughly 280 million years ago—have been documented to date (Freyhof et al., 2020; Kuljanishvili et al., 2020). Of these, 51 fish species and one lamprey are endemic to the Caucasus (Freyhof et al., 2020). Abell et al. (2008) recognize three main freshwater eco-regions in the Caucasus: the Western Transcaucasian, Kura-South Caspian, and Western Caspian. Similar to other biodiversity hotspots in their original designations (Myers et al., 2000), freshwater fish diversity was not considered among the criteria used to assign the Caucasus (and Georgia as a part) as a biodiversity hotspot. However, recent studies have shown that the freshwater biota is very diverse in the Caucasus, potentially exceeding terrestrial ecosystems in rate of endemicity (including small range endemics) in many animal groups (Gabelashvili et al., 2018; Grego et al., 2020; Oláh et al., 2020).

Georgia and the larger Caucasus region once harbored a remarkable diversity of sturgeons (Order Acipenseriformes: Family Acipenseridae), but the current status of their populations in the region is unclear (Freyhof et al., 2020). Sturgeons are native to subtropical, temperate, and sub-Arctic freshwaters and coastal areas of Eurasia and North America. Of the four extant genera of sturgeons, two have been documented in the Caucasus region and in Georgia (*Huso* and *Acipenser*). Of the 26 sturgeon species recognized worldwide, seven are native to Georgia: Russian sturgeon (*A. gueldenstaedtii*), Persian sturgeon (*A. persicus*), Colchic sturgeon (*A. colchicus*), Fringebarbel or Ship sturgeon (*A. nudiventris*), Star sturgeon (*A. stellatus*), Atlantic (Baltic) sturgeon (*A. sturio*), and Beluga sturgeon (*Huso huso*) (CEPF, 2003; Pikitch et al., 2005; Ninua and Japoshvili, 2008; Fricke et al., 2020). However, there remain many ambiguities about the taxonomy of Black Sea sturgeons. All Caucasian sturgeon species, and nearly all sturgeons worldwide, are considered threatened or endangered (Table 1). Several characteristics of sturgeon's natural history make them especially vulnerable to hydropower development and to overfishing. Sturgeons are generally long-lived fishes, slow to grow and mature; female Fringebarbel sturgeon (*A. nudiventris*), for example, reach maturity at about 17 years and spawn once every three years (Vecsei et al. 2002). Ages of >100 years have been reported for Beluga sturgeon (*Huso huso*; Helfman et al., 1997). Commercial sturgeon species can reach lengths of several meters and weigh >100 kg. Life history and catch statistics for Caucasian species report large sizes for *A. gueldenstaedtii* (2.3 m max length; 100 kg max weight). *A. persicus* (2.4 m; 70 kg), *A. stellatus* (2.2 m; 80 kg). *Huso huso* is considered the world's largest freshwater fish (>6 m; 1300 kg; Pikitch et al., 2005). Finally, sturgeon spawn in freshwaters and most are diadromous; therefore, they depend on longitudinally connected riverine pathways that are unimpeded by dams to complete their life cycle. The Rioni River in Georgia is considered critical to global sturgeon conservation: it is thought to still harbor surviving populations of *A. persicus*, *A. stellatus*, *A. gueldenstaedtii*, and *H. huso* (Freyhof et al., 2020).

The Caucasian fish fauna includes other anadromous species, represented by shads (Clupeidae), trout (Salmonidae), and barbels (*Luciobarbus*) that are also at risk from hydropower development and from commercial and recreational fisheries (Freyhof et al., 2020; Kuljanishvili et al., 2020). Bulatmai barbell (*Luciobarbus capito*) for instance, is one of the largest (semi) anadromous fish that are restricted to Caspian Sea drainage. The Kura River basin is still the largest part of its distribution area. However, within the Kura River, the population of this species is strongly fragmented as a consequence of barriers presented by dams and local extinction in the near future is a major concern. Other species with migratory life history traits (such as for instance potamodromous species; see Table 1) that are not globally threatened and have never been evaluated locally are subjected to the same threats of habitat fragmentation and impeded movement because of existing and future dams on Georgian rivers.

4. Methods

We used a mixed methods approach to gather information about hydropower development and its implications for river connectivity and freshwater fish species persistence in Georgia. To understand historical and modern trends in hydropower development, we reviewed information on dams constructed, planned, or proposed in Georgia over the past century. Information on hydropower development from the Soviet period (19211–1991) and before was gathered from publicly available information on the web page of the Ministry of Economy and Sustainable Development of Georgia (http://energy.gov.ge) and technical reports (Kochladze, 2013). For information on hydropower development in Georgia since 1991, we requested and retrieved the most updated dataset of existing and proposed hydropower facilities from the United States Agency for International Development's (USAID) Energy Program of Georgia (https://ge.usembassy.gov). We used this dataset to classify dams as existing, planned, or proposed, and to plot the locations of all dams according to the most updated data available.

We used available data on dam locations and reported fish distributions to examine the consequences of dams for longitudinal river

Table 1
Fish species recorded in western Georgia (Black Sea drainages). Conservation status follows IUCN criteria – Critically Endangered (CR), Near Threatened (NT), Least Concern (LC), and Not Evaluated (NE) – and species that are endemic to the Caucasus region are marked with an asterisk (*). Average DCI estimates across the species' range (i.e., all basins occupied by the species) are provided for the present-day (existing dams by 2021) and projected-future scenarios (all planned and proposed dams). DCI estimates were adjusted according to the species migratory behavior: Diadromous (DCId) and potamodromous/residents (DCIp). Lower and upper ranges of DCI estimates are presented inside parenthesis, and average range-wide connectivity losses that exceed 30% are highlighted in bold (i.e., DCI < 70).

Species	Family	Common name	Conservation status	Migratory behavior	DCI present	DCI future
Rhodeus colchicus Bogutskaya & Komlev, 2001	Acheilognathidae	Georgian bitterling	LC*	Resident	93.4 (33–100)	90.2 (21–100)
Acipenser gueldenstaedtii Brandt & Ratzeburg, 1833	Acipenseridae	Danube sturgeon	CR	Diadromous	79.6 (33–100)	**67.3** (21–100)
Acipenser nudiventris Lovetsky, 1828	Acipenseridae	Ship sturgeon	CR	Diadromous	**48.9** (15–100)	**47**.0 (13–100)
Acipenser persicus Borodin, 1897	Acipenseridae	Persian sturgeon	CR	Diadromous	75.1 (33–100)	**51.8** (21–100)
Acipenser stellatus Pallas, 1771	Acipenseridae	Starry sturgeon	CR	Diadromous	76.7 (33–100)	**68.2** (21–100)
Acipenser sturio Linnaeus, 1758	Acipenseridae	Atlantic sturgeon	CR	Diadromous	**45.5** (33–58)	**25.1** (21–29)
Huso huso (Linnaeus, 1758)	Acipenseridae	Beluga sturgeon	CR	Diadromous	81.4 (15–100)	78.5 (13–100)
Anguilla anguilla (Linnaeus, 1758)	Anguillidae	European eel	CR	Diadromous	88.5 (15–100)	86 (13–100)
Cobitis satunini Gladkov, 1935	Cobitidae	Colchic spined loach	LC*	Resident	92.5 (33–100)	88.9 (21–100)
Barbus rionicus Kamensky, 1899	Cyprinidae	Rioni barbel	NE*	Resident	92.5 (33–100)	89.1 (21–100)
Capoeta banarescui Turan, Kottelat, Ekmekçi & Imamoglu, 2006	Cyprinidae	Banarescu's barb	LC*	Resident	**14.6** (15–15)	**13.4** (13–13)
Capoeta sieboldii (Steindachner, 1864)	Cyprinidae	Colchic khramulya	LC	Potamodromous	90.2 (34–100)	86.9 (29–100)
Esox lucius Linnaeus, 1758	Esocidae	Northern pike	LC	Resident	93.1 (33–100)	90 (21–100)
Babka gymnotrachelus (Kessler, 1857)	Gobiidae	Racer goby	LC	Diadromous	76.2 (33–100)	**64.9** (21–100)
Knipowitschia longecaudata (Kessler, 1877)	Gobiidae	Longtail dwarf goby	LC	Diadromous	73.1 (46–100)	**63.8** (28–100)
Neogobius fluviatilis (Pallas, 1814)	Gobiidae	Monkey goby	LC	Diadromous	78.4 (33–100)	**64.1** (21–100)
Ponticola constructor (Nordmann, 1840)	Gobiidae	Caucasian goby	LC	Potamodromous	87.5 (33–100)	80.7 (21–100)
Ponticola syrman (Nordmann, 1840)	Gobiidae	Syrman goby	LC	Diadromous	73.5 (58–100)	**61.2** (21–100)
Gobio artvinicus Turan, Japoshvili, Aksu & Bektaş, 2016	Gobionidae	Gudgeon	NE*	Resident	86.5 (46–100)	79.1 (28–100)
Gobio caucasicus Kamensky, 1901	Gobionidae	Colchic gudgeon	LC*	Resident	90.8 (33–100)	86.5 (21–100)
Abramis brama (Linnaeus, 1758)	Leuciscidae	Freshwater bream	LC	Potamodromous	76.2 (33–100)	**64.9** (21–100)
Alburnoides fasciatus (Nordmann, 1840)	Leuciscidae	Transcaucasian spirlin	LC*	Potamodromous	88.7 (33–100)	83.5 (21–100)
Alburnus alburnus (Linnaeus, 1758)	Leuciscidae	Common bleak	LC	Potamodromous	79.7 (33–100)	**67.4** (21–100)
Alburnus derjugini Berg, 1923	Leuciscidae	Georgian shemaya	LC	Resident	86.3 (15–100)	83.8 (13–100)
Petroleuciscus borysthenicus (Kessler, 1859)	Leuciscidae	Dnieper chub	LC	Resident	86.1 (33–100)	79.6 (21–100)
Phoxinus colchicus Berg, 1910	Leuciscidae	Minnow	LC*	Resident	88.5 (15–100)	86.0 (13–100)
Squalius orientalis Heckel, 1847	Leuciscidae	Oriental Chub	NE*	Potamodromous	87.0 (33–100)	79.4 (21–100)
Vimba vimba (Linnaeus, 1758)	Leuciscidae	Vimba bream	LC	Diadromous	79.8 (15–100)	75.5 (13–100)
Oxynoemacheilus cemali Turan, Kaya, Kalayci, Bayçelebi & Aksu, 2019	Nemacheilidae	Stone loach	NE	Resident	**67.1 (46–100)**	**60.9** (28–100)
Oxynoemacheilus veyselorum Cicek, Eagderi & Sungur, 2018	Nemacheilidae	Stone loach	NE*	Resident	80.4 (33–100)	**66.1** (21–100)
Perca fluviatilis Linnaeus, 1758	Percidae	European perch	LC	Diadromous	90.0 (15–100)	88.3 (13–100)
Sander lucioperca (Linnaeus, 1758)	Percidae	Pike-perch	LC	Potamodromous	84.4 (33–100)	73.0 (21–100)
Lampetra ninae Naseka, Tuniyev & Renaud, 2009*	Petromyzontidae	Western transcaucasian lamprey	NT*	Diadromous	92.9 (33–100)	89.5 (21–100)
Salmo labrax Pallas, 1814	Salmonidae	Black Sea salmon	LC	Diadromous	90.8 (33–100)	86 0.0 (21–100)
Salmo rizeensis Turan, Kottelat & Engin, 2010	Salmonidae	Rize trout	LC	Potamodromous		

(continued on next page)

Table 1 (*continued*)

Species	Family	Common name	Conservation status	Migratory behavior	DCI present	DCI future
Silurus glanis Linnaeus, 1758	Siluridae	Wels catfish	LC	Resident	87.7 (15–100) 89.3 (15–100)	85.5 (13–100) 87.4 (13–100)

connectivity and freshwater fishes in western Georgia as revealed by a commonly-applied metric, the Dendritic Connectivity Index (described below; Cote et al., 2009). Longitudinal connectivity refers to the connectedness of upstream and downstream habitats in a riverscape, and has been identified as a critical ecological component for the dispersal and migration of freshwater organisms (Ward, 1989; Haxton and Cano, 2016). Our analysis of connectivity focused on the rivers of western Georgia that drain into the Black Sea whose basins fall entirely or nearly entirely within the country. Distributional data on fish species occupying basins of western Georgia were assembled based on the most up to date faunistic literature (Epitashvili et al., 2020; Kuljanishvili et al., 2020, 2021). Life history traits were based on Kottelat and Freyhof (2007) and Froese and Pauly (2021).

We used HydroSHEDS and HydroBASINS global hydrographic mapping products (Lehner et al., 2008; Lehner and Grill, 2013) to delimit rivers and their basins across western Georgia (55 basins in total, each one containing a river network that drains to the Black Sea), and to plot the locations of existing, planned, and proposed hydropower dams. To improve reliability of results, we performed spatial edits on the dataset of hydropower dams to accurately match it to the river network, which included removal of duplicate points (e.g., from sequences of powerhouses over canals that share the same impoundment) and automatic snapping of dams within 300 m to the nearest river reach. We manually repositioned points for dams >300 m from the river network using visual reference to Google Earth imagery and the reservoir polygons from HydroLAKES dataset (Messager et al., 2016). Additionally, all dams larger than 50 MW were inspected manually to ensure their location in the river network followed visual references. These steps reduced our dataset from an initial 105 datapoints to 91 for the purpose of the analyses described below.

To examine losses in longitudinal riverine connectivity over space and time as a consequence of hydropower dam development, we applied the Dendritic Connectivity Index (DCI). The DCI is a widely-used metric of longitudinal river connectivity that estimates the probability that a fish can move between two randomly selected points in a river network (see Cote et al., 2009 for detailed methods and equations). Based on a review by Jumani et al. (2020), we identified the DCI as the most appropriate metric for our analysis due to its flexibility and adjustability to different fish life histories. The DCI ranges from 100 to 0, with the larger number indicating a completely connected river network with no barriers. We adopted two DCI equations in our analyses to approximate the connectivity estimates to the migratory behavior of the different fish species present in the region (Cote et al., 2009; Table 1). The DCIp provides connectivity estimates for potamodromous fish, referring to those migratory species that perform movements entirely within freshwaters. The DCId adjusts the index to be more suitable for diadromous fish, referring to those migratory species that move between fresh and saline water to complete their life cycle. Whereas the DCIp considers the connectivity of any combination of random locations across the entire river network, the DCId focuses on the connectivity between random locations of the network and the mainstem river mouth (i.e., where every diadromous fish should pass to access the saline waters of the Black Sea). Considering the reported inefficiency or total absence of fish passages in the region, we adopted 0.1 (10%) as a conservative estimate of barrier permeability in upstream and downstream directions for all dams. This estimate was made on the basis of professional judgement and following similar analysis in the literature that assume dams to be fully impassible or to have low permeabilities (Barbarossa et al., 2020; Couto et al., 2021). This barrier permeability parameter (p) reflects a two-way passibility for both DCI equations of this study. The spatial steps of the analyses were conducted in a GIS environment using the Barrier Analysis Tool (BAT), an extension to ArcMap (version 10.7) that takes barrier datasets and cuts up a river network into connected segments with unique fragment IDs (BAT, 2010). The spatial data was then imported into the software R (version 4.0.2), where DCI functions were applied to the spatial data following the codes provided by Couto et al. (2021).

Based on the start of operations of each existing hydropower dam in the dataset, we were able to calculate DCId and DCIp values for various scenarios during the period 1900 to 2021. An additional projected future scenario was created to accommodate planned and proposed dams. Basin-level estimates of river connectivity were assigned to each fish species according to their occurrence records and migratory behavior, with DCId being adjusted for diadromous species, and DCIp for potamodromous and residents. Although resident fish do not migrate, connectivity loss by dam building has been reported to limit dispersal, promote local extinctions, and affect genetic diversity in resident species, with DCIp being the most suitable metric to track changes in connectivity in this case (Perkin and Gido, 2012; Barbarossa et al., 2020). Based on the occurrence records of each species (see the Supplementary Information), basin-level estimates of species richness and average river connectivity at the species' range were estimated. To assess how improvements in dam permeability could affect DCI estimates, we ran a sensitivity analysis that simulated DCId and DCIp estimates for permeability values ranging from 0 (i.e., fully impassible) to 0.9 (i.e., 90% passible). All of these analyses were run with the software R (version 4.0.2).

5. Results

5.1. Trends in hydropower development in Georgia

Hydropower development trends in Georgia reveal four distinct periods: pre-Soviet (until 1921), Soviet (1921–1991), post-independence Georgia (1991-2010), and modern Georgia (2010–present). In the pre-Soviet period, the first small hydropower dam (0.1 MW) was built in 1898 on the Borjomula River by German engineers, followed by a number of small hydropower projects in different regions by 1922. This year, 1922, marked the starting date of construction of the first large hydropower project on the Kura River near Tbilisi, the Zahes Dam (36.8 MW), completed in 1927. Until the 1960s, construction of small hydropower projects (<1 MW capacity) was the priority, with an estimated 300 small projects constructed unsystematically around Georgia, mainly for providing electricity to local populations. However, centralization of electricity production and distribution in the 1960s triggered a wave of construction of much larger dams. Perhaps the most notable hydropower project of this time period was the Enguri Dam (1300 MW) on the Enguri River in western Georgia, whose construction began in 1961 and spanned nearly two decades. At the time and up until 2012, Enguri Dam was the world's largest concrete arch dam (272 m high), and it remains the largest industrial project in the Caucasus region. By the end of the Soviet period in 1991, there were approximately 30 medium and large hydropower dams in Georgia and an unknown number of smaller projects, with a total installed generation capacity of 2800 MW (Kochladze, 2013).

Since Georgia declared its independence in 1991, hydropower development has been on a varied trajectory. Post-independence, an economic recession halted growth in the hydropower sector during the 1990s; Georgia struggled to maintain existing infrastructure projects from the Soviet period and was unable to pursue new projects. Hydropower dams and other infrastructure in Georgia in the 1990s deteriorated significantly or was plagued by vandalism vandali at many levels, from theft of power to breaking and stealing of metal and other materials associated with infrastructure projects. Most of the country experienced chronic electricity blackouts during this period, receiving access to no or only a few hours of power a day. A series of economic and energy sector reforms established the Georgian National Energy and Water Supply Regulatory Commission (GNERC) in 1997 and resulted in deregulation and privatization, allowing for energy generators, distributors, customers, and exporters to enter into direct contracts (IHA, 2016). Practical outcomes of these reforms in the electricity sector appeared in the early 20th century, following the Rose Revolution in 2003, with increased international and private sector interest in the investment environment of Georgia.

In modern Georgia (2010–present), international and private investments have helped catalyze a proliferation of new hydropower development. During the last decade, several large hydropower projects were completed including Paravani Dam in 2014 (86 MW), Dariali Dam in 2016 (108 MW), and Shuakhevi Dam in 2018 (178 MW) along with a high number of small to medium size projects. Presently, hydropower

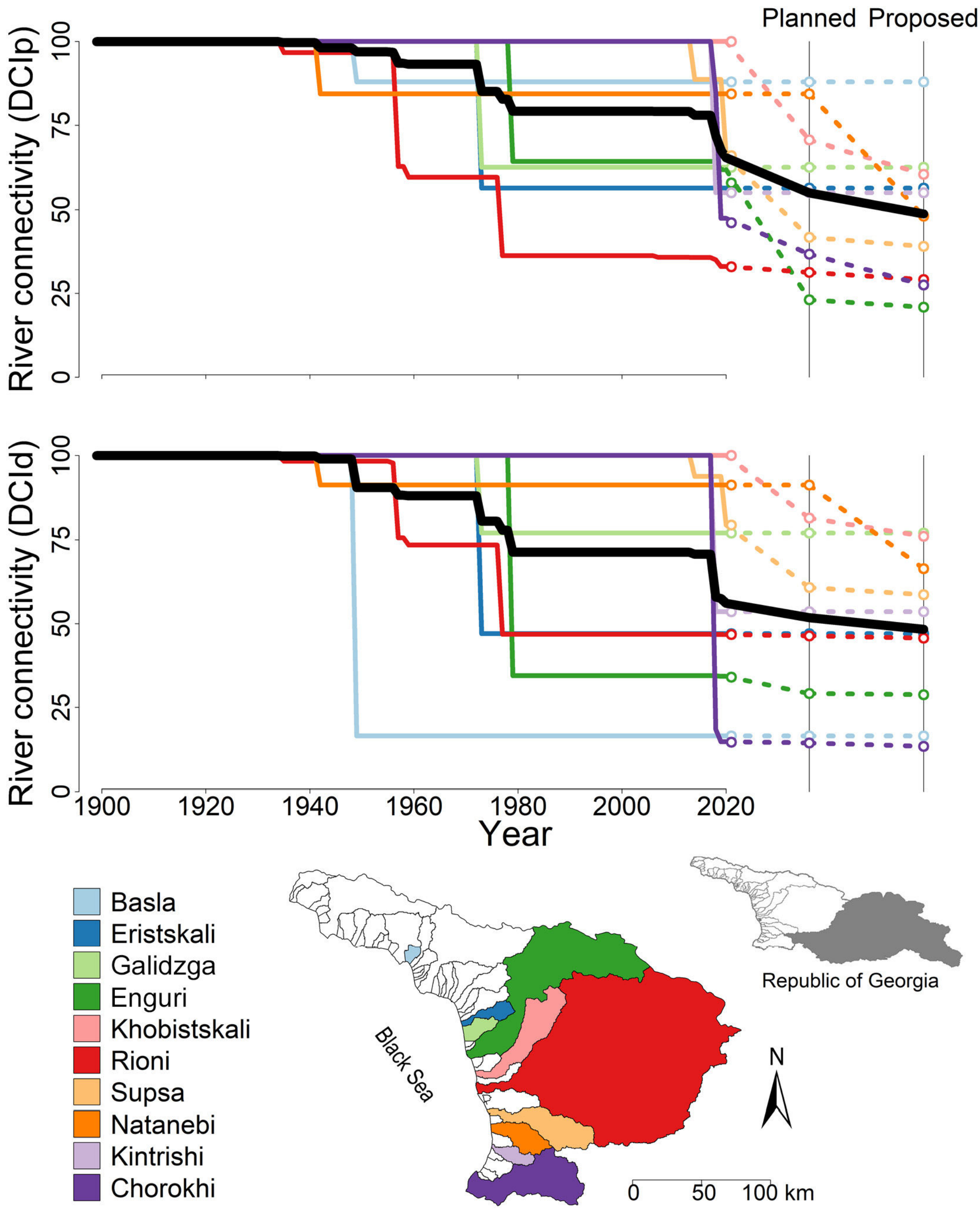

Fig. 3. Trends over time in river connectivity for Black Sea drainages of western Georgia according to calculated DCI_p and DCI_d values, with 100 being a fully connected, free-flowing river network. Colored lines on graph correspond to color coded dammed river basins in the map; undammed basins are represented in white. Trends are shown for hydropower dams already built (continuous lines) and for prospective dams (dashed line). Prospective dams include those under construction and in various licensing stages (planned), and those under feasibility studies (proposed). Averages of dammed basins are summarized by black lines.

accounts for >90% of installed electricity generation capacity in Georgia, making it the dominant source of electricity. In the most recent Hydropower Status Report (IHA, 2020), Georgia's total hydropower capacity was 3271 MW based on 27 large HPPs (installed capacity over 13 MW) and 62 small HPPs (below 13 MW capacity); it added 50 MW that same year. Further development of the hydropower sector in Georgia has been considered a key aspect of energy security and decreased reliance on imported fossil fuels (IHA, 2016). Hydropower in Georgia is increasingly influenced by international support from other European (e.g., Turkey, Norway) and Asian (India, Korea) countries. During summer months, Georgia exports electricity generated by hydropower facilities to neighboring countries, particularly Turkey and Russia. The recently commissioned Paravani Hydropower Project (87 MW) located near the Georgia-Turkey border, for example, is connected to the Georgian and Turkish power grids and was completed with international financing (IHA, 2016). Many other new or planned projects in Georgia are energized by international support, including the Shuakhevi Dam (187 MW), which includes Indian and Norwegian partners. Interconnected hydropower development schemes—such as cascades of multiple dams on one river—have emerged for some Georgian rivers in the past decade, such as the Adjaristskali, Rioni, and Enguri rivers. These interconnected schemes, still largely in planning, are considered important for energy independence in Georgia, especially during winter seasons that correspond with dry periods for river flows.

5.2. Implications of hydropower development to river connectivity and fish conservation

Temporal trends in losses in longitudinal river connectivity in western Georgia, as revealed by our analyses, aligned with distinct periods of hydropower development: pre-Soviet, Soviet, post-independence, and modern Georgia (Fig. 3). Hydropower development during the Soviet period (1921–1991) fragmented the lower portions of the largest river basins, such as the Rioni and Enguri, but rivers in many of the smaller, coastal basins remained longitudinally intact. More recent hydropower development (post-2010) has increased losses in river connectivity in these remaining smaller basins. Considering existing, planned and proposed dams, 10 of 55 basins in western Georgia already have or will have at least one hydropower dam in the future (Fig. 3).

Depending on their position in the river network, hydropower dams have caused major losses in longitudinal riverine connectivity for several western Georgian rivers. For example, the completion of the Enguri Dam in 1978, Georgia's largest hydropower dam (1300 MW), disconnected the lower and montane sections of the Enguri River (Fig. 3). However, small projects have also had big consequences for basin-level connectivity losses. An example is the Kintrishi Dam (6 MW), constructed in 2017, which alone reduced the longitudinal connectivity of the Kintrishi River by 50% for both DCId and DCIp. The recent wave of new hydropower development has exacerbated decreases in river connectivity for diadromous species with new construction near the mouth of some rivers and has fragmented upper portions of some basins. For instance, the Khelvachauri dam (47 MW) was built in 2019 just 13 km from the mouth of the Chorokhi River (also known as Çoruh River, its Turkish name) and is the most downstream dam of the whole basin. In addition, many of the new planned and proposed dams are small and located in parts of river basins relatively distant from the coast, such as the 16 new projects proposed for the uppermost portion of the Enguri Basin – 15 of them below 10 MW. This new wave of small projects is promoting considerable decreases in DCIp in some basins like Enguri,

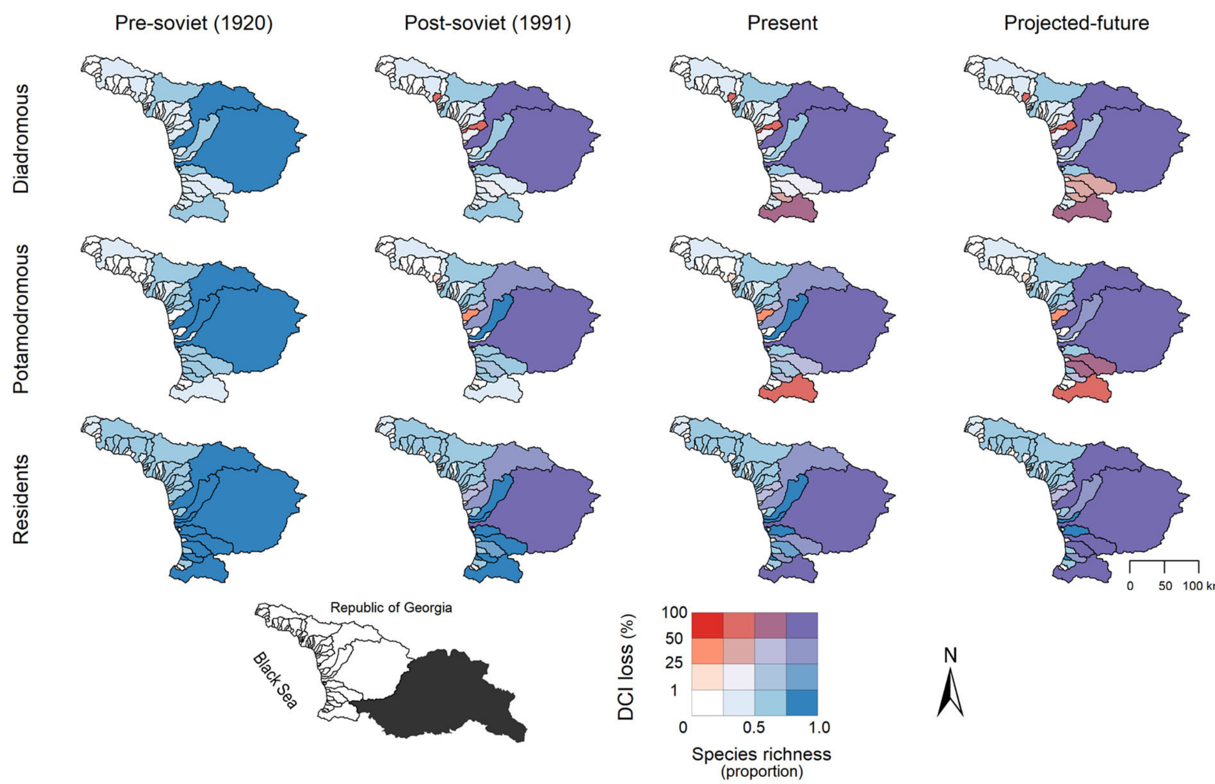

Fig. 4. Set of maps depicting historical and projected-future loss in river connectivity, and fish species richness for all the 55 Black Sea drainages of western Georgia. The four critical periods of hydropower development in Georgia are represented as the pre-soviet (year 1921), post-soviet (year 1991), present-day (2021) and projected-future scenarios (all planned and proposed dams built). Percentage of connectivity loss estimates (i.e., 100 - DCI estimates) are presented for three sets of migratory fish life histories: Diadromous, potamodromous and residents. Fish species richness for these three life histories are represented as proportions calculated based on the pool of species registered in these 55 basins. Full species list, summary statistics and records per basin are presented in Table 1 and Supplementary information.

Chorokhi, and Supsa (Fig. 3).

The most species-rich basins in western Georgia are also those most heavily affected by hydropower development (Fig. 4). We documented 36 fish species from western Georgia, of which 15 are diadromous, 8 potamodromous, and 13 resident species (Table 1). During the Soviet period (192191), the Enguri (31 fish spp.) and Rioni (32 fish spp.) basins were the most heavily fragmented by hydropower development. In the modern period (2010-present), hydropower development has intensified river fragmentation in the Enguri and the Rioni and affected several other large and mid-size river basins, such as the Khobistskali, Supsa, Natanebi, Kintrishi and Chorokhi, which harbor many resident fish species. In contrast, basins located in northwestern Georgia have been less subjected to hydropower development. Whereas they may harbor fewer species than larger basins, some free-flowing rivers, such as the Kodori and Bzipi, may still support populations of migratory species, including sturgeons (*A. gueldenstaedtii*, *A. stellatus* and *H. huso*) and the European eel (*Anguilla anguilla*) – all these species are classified as critically endangered (CR) according to IUCN criteria.

Both resident and migratory species are vulnerable to hydropower development in Georgia. Our analysis indicated that four species (*A. gueldenstaedtii*, *A. sturio*, *Capoeta banarascui*, and *Oxynoemacheilus cemali*) presently have average DCIs below 70 at the level of their geographic range in western Georgia, meaning that connectivity losses exceed 30% of their range. Connectivity losses are expected to increase substantially for these four species and exceed 30% for 10 more species if all the planned and proposed hydropower projects are constructed (Table 1). Species potentially affected include five out of six sturgeon species, four species of diadromous Gobiids, two species of potamodromous Leuciscids (*Abramis brama* and *Alburnus alburnus*), and resident species of the genus *Capoeta* and *Oxynoemacheilus*.

As expected, increasing barrier permeability increases river connectivity estimates for western Georgia basins for both DCId and DCIp metrics (Fig. 5). However, our estimates reveal that significant improvements in basin-level connectivity can only be reached with major improvements in dam permeability. For instance, in all the scenarios very little gain in connectivity occurs with any levels of permeability ranging from zero to 0.2. Although this is a simulation exercise based on key assumptions (e.g., all dams have the same permeability value), these results highlight that fish passages must have much higher efficacy than currently reported to serve as efficient mitigation action that restore basin-level river connectivity.

6. Discussion

In this paper, we have provided an overview of hydropower development in Georgia, an area with interesting political and natural history for which limited studies have been published in the international conservation biology literature. We have offered a window into the challenges for freshwater biodiversity science and conservation in modern Georgia. Using longitudinal river connectivity as an analytical

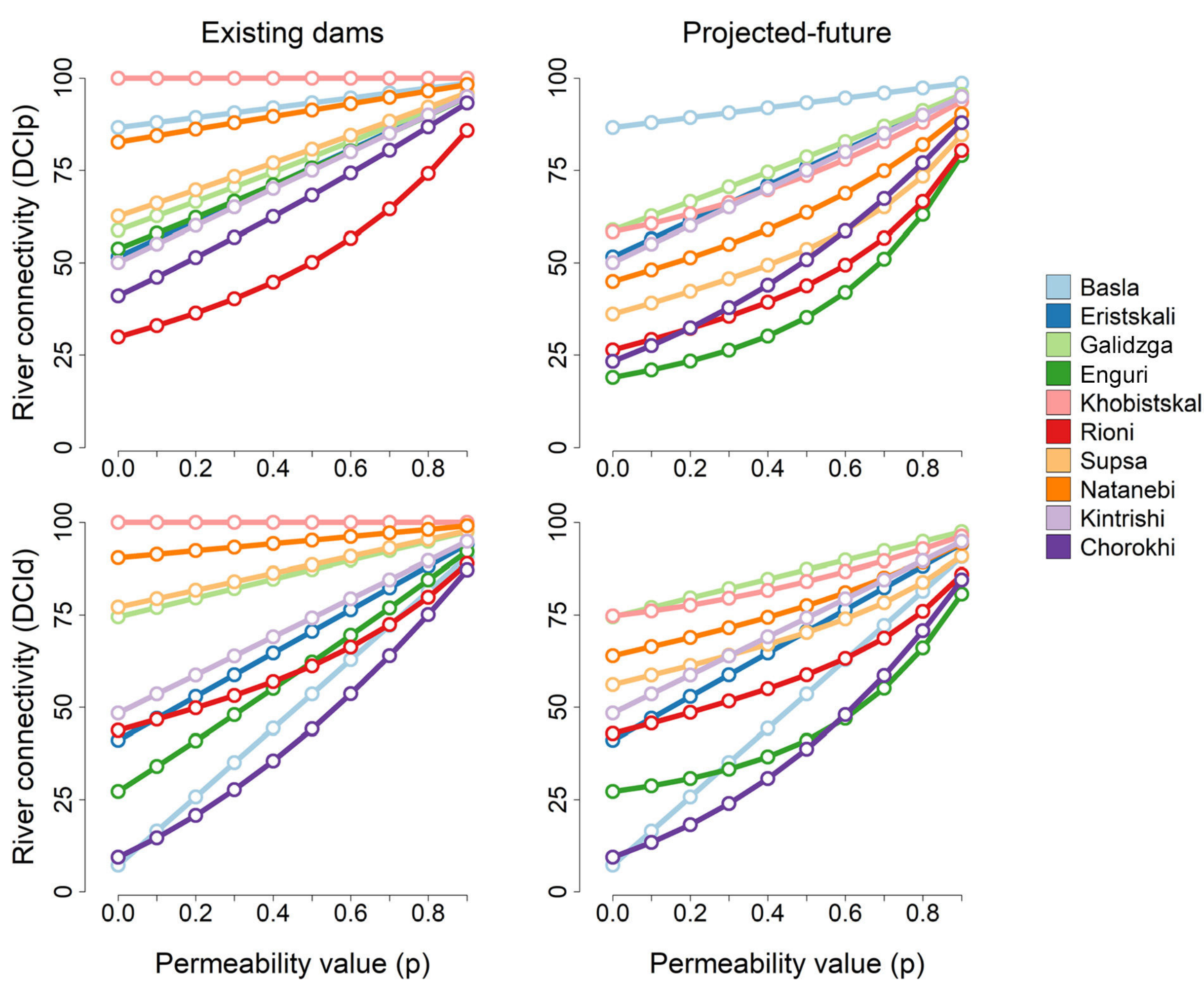

Fig. 5. Sensitivity analysis depicting the relationship between dam permeability parameter (p) and river connectivity estimates for the western Georgia basins. Both equations, DCIp (upper panels) and DCId (lower panels), were fit with permeability values ranging for 0 (i.e., fully impassible) to 0.9 (i.e., 90% passible) for current and projected-future scenarios. The current scenarios include all the existing hydropower dams in 2021 (left panels), and projected-future scenarios include all the planned and proposed dams. Colored lines on graph correspond to color coded dammed river basins.

lens, we illustrated how historical and modern hydropower development has fragmented Georgian rivers and constrained freshwater fish habitat along riverine corridors, especially for migratory species.

Our examination of the trends in hydropower development over time revealed two major periods of intensive dam construction: the Soviet period of the past century and right now (2010-present). Our analysis showed significant losses in longitudinal river connectivity in basins of western Georgia during both of these periods as linked to construction of existing dams, for which reliable information exists. We projected further losses of longitudinal river connectivity under future hydropower development scenarios, considering planned and proposed dams, although the likelihood that all of these dam projects will be completed is uncertain, especially for proposed dams. Many dams listed as proposed today have plans that date back to the Soviet period but were discarded or postponed then because of high risk of environmental damage, threats to human populations, or limited investment capital. It remains to be seen whether those projects will eventually be constructed and, if they do proceed, what kind of mitigation plans for environmental and social impacts will be incorporated. Finally, our exploration of the implications of hydropower development for freshwater fishes was completed using the best available data for freshwater fauna in Georgia. Future improvements of species-level estimates of impacts of dams can be incorporated as more refined data becomes available on fish species distributions, migratory paths, and critical habitats.

Our efforts underscore the fact that the ecological impacts of hydropower development in Georgia remain highly understudied. In our review, we did not encounter targeted studies post-operation of hydropower dams in Georgia on freshwater fauna. However, anecdotal evidence and data from other biodiversity studies can be used to deduce some of their consequences. For example, historically, the Rioni River contained the most important spawning areas for 5 to 6 Black Sea sturgeon species. The construction and operation of the Vartsikhe Dam in the 1970s resulted in the loss of most of their spawning areas. This situation was exacerbated by the construction of additional hydropower dams and by illegal or unsustainable fishing. As a consequence, all sturgeons in the eastern Black Sea are now considered critically endangered (Guchmanidze, 2009). Hydropower facilities outside of Georgia's national borders, such as the Mingachevir Dam on the Kura River in Azerbaijan, also have affected freshwater fauna. The original dam at this site was constructed during the Soviet period and began operation in 1953, with a height of 80 m and a flooded reservoir area of 605 km^2 (15.7 km^3 volume). The dam was recommissioned early this century and its installed generation capacity increased to 420 MW. The Mingachevir Dam's closure effectively split the Kura River Basin into an upper and lower portion and disconnected its headwater region within Georgia from the Caspian Sea. This split was followed by the subsequent extinction of Caspian lamprey and sturgeons in the upper Kura Basin (Demetrashvili, 1963).

In the past decade, the paucity of information on the ecological impacts of dams and concern for their effects on Georgian rivers has helped to catalyze efforts to apply internationally accepted approaches to understanding or mitigating effects of hydropower dams. For example, in response to concerns over the multiple dams that are existing or proposed for the Rioni River, a pilot study of environmental impact assessment for cumulative impacts of hydropower projects was completed in 2016 (Vogel et al., 2016). This effort focused on practical aspects of environmental impact assessment and was intended to support the Ministry of Environment and Natural Resource Protection (MENRP) and the National Environmental Agency (NEA) in their evaluation of hydropower projects. The pilot project tried to align with the European Union's Water Framework Directive's principles for both environmental impact assessment (EIA) and for cumulative impact assessment (CIA), though the CIA was based on existing EIA report's expert judgement and only considered impacts of hydropower projects. The project also included a 5-day training component for Georgian environmental authorities (Vogel et al., 2016). Overall, the CIA of the Rioni River found that the studied segment of the river and its tributaries are at risk for several reasons, for example: a large amount of the river flows will be used by hydropower plants; planned and existing hydropower plants are large in size relative to rivers; and mitigation measures for environmental impacts of hydropower were lacking. Until today, the Rioni CIA pilot project remains the only example, albeit with limited success, of more advanced EIA and impact mitigation planning in Georgia.

Further, rapid hydropower development in Georgia has encouraged several efforts to advance the science and practice of environmental flows—defined as the quantity, timing, and quality of freshwater flows and levels necessary to sustain aquatic ecosystems which, in turn, support human cultures, economies, sustainable livelihoods, and well-being (Arthington et al., 2018a). These efforts have linked Georgia with a larger scientific community of practice on environmental flows and drawn on experiences from similar geographies. Beginning in 2012–13, the Georgian Ministry of Environment and Natural Resources Protection requested support from the United States Agency for International Development (USAID) to develop a methodology for estimation of environmental flows for Georgian rivers. This request occurred around the same time that Georgia signed an association agreement with the European Union and therefore emphasis was given to developing a methodology that would be compatible with the European Water Framework Directive. Experiences from Austria—with its well-established methods and ordinances for environmental flows—and from the U.S. state of Connecticut—with similar migratory fish species assemblages—were considered in the development of a specific framework and step-by-step methodology for environmental flows in Georgia, which took place between 2014 and 17 and involved an international team of environmental flow experts and close collaboration with Georgia's National Environmental Agency, under the Ministry of Environment and Natural Resources Protection of Georgia. Additionally, development of the environmental flows framework and methodology engaged numerous Georgian stakeholders, involved consultation with other government ministries (Energy and Agriculture), and included a detailed piloting of the proposed approach on the Supsa River, where a cascade of hydropower projects are under development. Three of the authors on this paper participated in leadership or supporting roles in these efforts (USAID, 2017).

6.1. *Considerations for future research and conservation*

In light of current and anticipated future trends in hydropower development in Georgia, we articulate four specific research needs for freshwater biodiversity and its conservation.

6.2. *Expansion of biodiversity research and environmental monitoring*

Among the obstacles facing freshwater diversity protection and conservation in Georgia is a lack of relevant expertise and long-term data (i.e., time series); this kind of information is critical for understanding and mitigating the impacts of hydropower development on rivers, including how losses in river connectivity affect freshwater fauna. As a consequence of frequent political and social-economic transitions, Georgia does not yet have a functional, unified infrastructure for collecting and maintaining biodiversity and environmental data. Except for sparse and fragmented historical information, no reference collection or comparable data for evaluating temporal changes in biodiversity exists. An attempt to establish a unified national system for biodiversity evaluation and monitoring has been unsuccessful (NBSAP, 2014), but could be revived with additional support and increased involvement from universities. Indeed, the best source of reliable information on any aspect of biodiversity or biodiversity-related issues is internationally published research papers that are produced by scientists at Georgian universities, but these too are relatively scarce (Mumladze et al., 2020). This particularly concerns freshwater biodiversity, increasingly affected by various anthropogenic activities—such as hydropower, mining, water withdrawals, pollution, and illegal fishing. Additionally, taxonomic expertise on freshwater biota is extremely limited within Georgia, for fishes and especially for invertebrates.

The situation with the Red List of threatened species of Georgia illustrates the kind of challenges facing biodiversity-related research. In 2006, Georgia adopted a Red List of species based on Soviet-era data published in 1982, with a commitment to update the list every decade. However, in 2021, the Red List of species of Georgia remains the same as it was in 2006 and still based on the 1982 publication. Few attempts have been made to advance the understanding of the conservation status of individual species. There is thus an immediate need to encourage biodiversity research and environmental monitoring in Georgia in a nationally coordinated effort.

To address this need, we recommend the development of an infrastructure and program program for biodiversity monitoring, which could start with freshwater biota given the current conservation challenges for rivers in light of hydropower development. An example of the kind of infrastructure and system for biodiversity monitoring that could serve Georgia is the Biodiversity Information System of Colombia (SiB Colombia), which is a national-level effort developed over the past two decades to create an open network of biodiversity data for the country. An effort in Georgia should unite government, non-governmental organizations, and academic institutions within Georgia. However, this effort could also benefit from international partnerships, provided that these partnerships include—in a compulsory way—opportunities for leadership or co-leadership by Georgian scientists and opportunities for training students and early career researchers in Georgia. Also important is to make sure that international standards for biodiversity data are followed in Georgia to improve access and limit duplication of data, and to allow corrections to propagate throughout databases.

6.3. Maintenance or restoration of hydrologic connectivity in Georgian rivers

As many hydropower dams already exist across Georgian rivers, the implementation of efficient fish passage facilities and appropriate flow management are among the only remaining alternatives to restore longitudinal river connectivity in dammed basins in the country. Currently, fish passages are not mandated for hydropower projects in Georgia, even in basins that harbor nationally protected migratory fish species. Therefore, building, maintaining, and monitoring fish passage structures are uncommon practices in Georgia. With the growing development of hydropower dams and other environmental threats in the country, fish passages are an important issue that must be better integrated into policies and regulations related to hydropower. For instance, redesigning and rebuilding fish passages on the Vartsikhe and Gumati dams could be the most efficient measure to conserve sturgeon populations in the lower Rioni River, where most sturgeon species of western Georgia still reproduce.

It is important to highlight that planning and maintaining fish passage structures are not trivial and cheap tasks, and that sturgeons' special needs must be considered in design of fish passages for them to be successful (Jager et al., 2016). Although the efficiency of fish passages has much to improve worldwide (Pelicice and Agostinho, 2008; Olden, 2016; Cooke et al., 2020), some well-designed examples can provide sufficient outcomes to be considered an actual mitigation action. From North American rivers, we know that fish passages for sturgeons must consider things beyond flow and connectivity, such as water quality in reservoirs, and must be designed to facilitate both upstream and downstream movement (Jager et al., 2016). This bidirectionality is often ignored, even though downstream migrations are equally important for the completion of the life cycle of sturgeons and other migratory fish (Pelicice and Agostinho, 2008; Cooke et al., 2020). Georgia has an opportunity to learn from decades of study of fish passages and other conservation solutions for sturgeons and implement recommendations for such structures that increase the likelihood of positive outcomes for sturgeon conservation in rivers with dams (Jager et al., 2016).

In addition to fish passage at hydropower dams, the protection of environmental flows will also be important to the conservation of freshwater fishes in Georgia, as in other parts of the world (Arthington et al., 2018b). Environmental flows are typically assessed for rivers during environmental impact studies and then implemented during hydropower operations to maintain a recommended compensation flow downstream from a dam or in reaches between a dam and turbines over which flow has been diverted. Environmental flows can help reduce losses in connectivity associated with river de-watering and can be combined with fish passage structures to improve possibilities for fish movement through dammed sections of river. At present, Georgia does not have a clear legal framework for integrated water resources management with explicit requirements for assessment and protection of environmental flows. Conventional practice in environmental impact assessments in Georgia has been to set aside 10% of river flow for environmental purposes, calculated as 10% of annual or monthly average flows, depending on the circumstance. This 10% value dates from the Soviet period and was until recently referred to as a “sanitary flow” suggesting its origins may be more related to public health than to environmental concerns.

An earlier project, described in previous sections here, developed a framework and methodology for estimating environmental flows in Georgia with input from Georgian scientists, government authorities, and international cooperation. This methodology drew upon experiences in other places where sturgeons are present, such as North America and Europe. Since the pilot study of the methodology in the Supsa River in 2016, to our knowledge, not much more has advanced on environmental flows in practice in Georgia. We recommend that this framework and methodology for estimating environmental flows be adopted immediately for all new hydropower developments in Georgia. For existing dams, restoration of environmental flows will be needed, but the same framework and methodology could be applied to determine ecological needs as a starting point for discussions on flow restorations. The framework and methodology for environmental flows should be reviewed periodically and adapted as needed as more information becomes available about Georgian rivers and according to global advances in the science and practice of environmental flows.

6.4. Strategic planning of new hydropower development

Multi-objective optimization approaches have been employed in different parts of the world to balance the trade-offs between hydropower benefits and the socioecological costs that different dams may have (Tickner et al., 2017). For instance, it is possible to assess the performance of sets of prospective dams on the trade-offs between energy generation and losses in longitudinal river connectivity, which affect migratory fish (Ziv et al., 2012; Couto et al., 2021). To date, hydropower development in Georgia has not proceeded according to comprehensive basin-level strategic planning. Hydropower dams are rarely, if ever, considered in terms of their cumulative or synergistic effects on a river basin and its connectivity. For example, the first and only pilot project to consider the cumulative impacts of multiple hydropower projects on the Rioni River basin was in 2016 (Vogel et al., 2016). An interesting result of the Rioni pilot project was the finding that river size was inappropriate for the energy production demand of existing and planned hydropower development. In other basins with multiple dams, flow regimes have been strongly affected by hydropower operations, sometimes experiencing near or complete drying of river channels during dry seasons, as occurred in the Chorokhi River during 2010–11 or the Tergi (Terek) River in 2019. Monitoring of the impacts of hydropower development post-operation also rarely takes place.

We recommend the implementation of strategic planning of hydropower development at a basin level, including intergovernmental planning and biodiversity monitoring in the case of transboundary river basins. Maintaining longitudinal river connectivity could be a central theme of strategic planning. Two of the largest river basins in the southern Caucasus region—the Kura and Chorokhi—are shared by multiple countries and have intensive hydropower development, often conducted unilaterally. Strategic planning for hydropower at a basin level should consider both existing and future hydropower developments and could draw on experiences and approaches of other areas also experiencing a proliferation of new hydropower developments for optimization, biodiversity monitoring, and impact assessment and mitigation. We also recommend that strategic planning involve a broad swath of interested parties and stakeholders.

6.5. Strict conservation areas for protection of sturgeons

Sturgeons are widely accepted internationally as a highly threatened species in need of protection (Pikitch et al., 2005). In Georgia, sturgeons are indeed among the species most affected by hydropower development in the 20th century, especially losses in river connectivity. An examination of the historical (based on Barach, 1941; Sharvashidze, 1982; and Elanidze, 1983) and recent records (Guchmanidze, 2012 and reports from Fauna and Flora International's Georgian sturgeon team) of sturgeon in the eastern Black Sea region revealed that their distribution ranges are now restricted to just one-sixth of the reported original extent. Surely, there are other factors that have affected sturgeon populations, such as illegal fishing or poorly controlled bycatch in Georgia's coastal areas of the Black Sea, water pollution from mining, and other small but potentially significant river barriers (Belletti et al., 2020). However, hydropower development likely outweighs all of these other threats. The lower reaches of the Rioni River are considered the only remaining spawning habitats for sturgeons of the eastern Black Sea region, so the Rioni and other rivers of western Georgia are critical pieces of the global puzzle of sturgeon conservation and their habitats must remain intact to avoid species' extinctions.

To this end, we recommend that Georgia begin a process of identifying and assessing the feasibility of the establishment of strict conservation areas along some Georgian rivers, designating them as off limits to new hydropower development. This idea follows similar recommendations as those made by Freyhof et al. (2015) and fit in with global trends towards designation of freshwater protected areas. Potential areas for such protection include the lower reaches of the Rioni and Enguri, as well as other medium to large rivers such as the Bzyb, Kodori, Khobi, and Supsa. All of these rivers (and some others) were historically known to harbor many endangered species such as the European eel (*A. Anguilla*) and sturgeon species (Ninua and Japoshvili, 2008; Table 1). The lack of occurrence data from the last few decades does not mean that sturgeon species are extinct in these basins, as just a few studies have been done to assess their status. Further, segments of other rivers, such as the Kura and Alazani in eastern Georgia, should also be considered for protected status. Although no sturgeon or other diadromous species is known to currently inhabit these rivers since the closure of Mingachevir dam in 1954, the resident ichthyofauna still harbors remarkable diversity.

7. Conclusions

Conservation of Georgia's freshwater biodiversity depends on better understanding of the current status of freshwater species and the environmental conditions in the rivers they inhabit, and careful consideration of the impacts of existing and future hydropower development, particularly its effects on river connectivity. Our aim in this manuscript was to tell the story of Georgian rivers and their freshwater biota to a broader audience, engaging colleagues that may have been previously unaware of this region's biological richness, political history, and conservation challenges. It is our hope that others may find the case of Georgia insightful for understanding global trends in hydropower development, its effects on river connectivity, and its consequences for freshwater biodiversity. It is also our hope that this manuscript helps catalyze more interest from the international scientific and conservation community in the country and in collaborations with Georgian biodiversity scientists, and contribute to an upward trajectory of freshwater biodiversity research across the Caucasus region in the future.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biocon.2021.109359.

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AQUATIC FLORA AND FAUNA

Phylogeography of the Kura Barbel *Barbus cyri* De Filippi as Inferred from mtDNA Data

A. A. Gandlin[a,] *, B. Japoshvili[b], G. Epitashvili[b], N. J. Mustafaev[c], H. R. Roubenyan[d], and B. A. Levin[a,e]

[a] *Papanin Institute for Biology of Inland Waters, Russian Academy of Sciences, Borok, Nekouzskii raion, Yaroslavl oblast, Russia*

[b] *Ilia State University, Institute of Zoology, Tbilisi, Georgia*

[c] *Azerbaijan State Agricultural University, Ganja, Azerbaijan*

[d] *Institute of Hydroecology and Ichthyology, National Academy of Sciences of the Republic of Armenia, Yerevan, Republic of Armenia*

[e] *Cherepovets State University, Cherepovets, Russia*

**e-mail: gandlin.aleksander@yandex.ru*

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Abstract—Caucasian barbels of the genus *Barbus* are a genetically poorly studied group of cyprinids. This article presents the first detailed study of the phylogeography of the Kura barbel *B. cyri* (229 specimens from 52 locations) using two molecular markers of mtDNA, the cytochrome *b* gene and the control region. By analyzing haplotype networks, the complex genetic structure of the population system of the Kura barbel was revealed. The discovered genetic subdivision correlates weakly with the geographical distribution of populations. We suggest that climatic oscillations, Caspian Sea level fluctuations, and the tectonic activity resulted in the repeated secondary contacts, as well as temporary isolation of some populations (e.g., Lenkoran, Sevan, Lower Kura, southern coast of the Caspian Sea) during the Pleistocene—Holocene. At the same time, genetic subisolation of the population of the upper-middle reach of the Kura River from the population in the Lower Kura was revealed. Along with other data on the endemism of the ichthyofauna of the middle reach of the Kura, this finding favors the hypothesis of a local refugium in the Kura basin.

Keywords: phylogeography, *Barbus*, mtDNA, Caucasus, refugium, Pleistocene, Holocene

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INTRODUCTION

Barbels of the genus *Barbus* sensu stricto are a lineage of evolutionary tetraploids in the family Cyprinidae (2n = 100) (Berrebi and Tsigenopoulos, 2003) inhabiting water bodies of Europe, the Caucasus, and the Middle East. The group comprises more than 30 species and is considered complex in taxonomic and evolutionary aspects. Studies on the phylogeny and phylogeography of the European species of barbels revealed complex patterns of their evolutionary history, showing their dispersal from several refugia that existed in the glacial periods, the presence of secondary contacts, and hybridization (Kotlík and Berrebi, 2001; Lajbner et al., 2009; Meraner et al., 2013; Levin et al., 2019). Thus, the group of *Barbus* is of significant interest for evolutionary biology and biogeography. At the same time, the Caucasian barbels represented by five species, *B. ciscaucasicus* Kessler, 1877; *B. cyri* De Filippi, 1865; *B. kubanicus* Berg, 1912; *B. rionicus* Kamensky, 1899; and *B. tauricus* Kessler, 1877, are a poorly studied group (Levin and Rubenyan, 2006; Gandlin et al., 2017; Levin et al., 2019).

The Kura barbel *B. cyri* is the most widespread species of barbels in the Caucasus; it is named from the Kura River near the city of Tbilisi. Its range covers fresh water bodies in the Caspian Sea basin in Transcaucasia, the Kura and Araks rivers (Georgia, Azerbaijan, Armenia, Turkey), Lake Sevan (Armenia), and water bodies of the Southern Caspian Sea and Lake Urmia basin (Khaefi et al., 2018) as well as the Transcaspian region (Atrek River in Iran) (Bănărescu and Bogutskaya, 2003; Khaefi et al., 2017).

The systematic status of the Kura barbel has repeatedly changed. Some authors identified *B. cyri* as the synonym of *B. lacerta* Heckel, 1843 (Berg, 1949; Coad, 1995), named from the Queiq River near the city of Aleppo in Syria, or considered it a subspecies of *B. lacerta* (Dadikyan, 1986; Bănărescu and Bogutskaya, 2003). The other researchers considered it a subspecies of *B. cyclolepis* Heckel, 1837 (Bianco and Bănărescu, 1982). At the same time, several described taxa (*B. toporovanicus* Kamensky, 1899, *B. sursunicus*

Abbreviations: bp, base pairs; PCR, polymerase chain reaction; cyt *b*, cytochrome *b* gene; CR, control region.

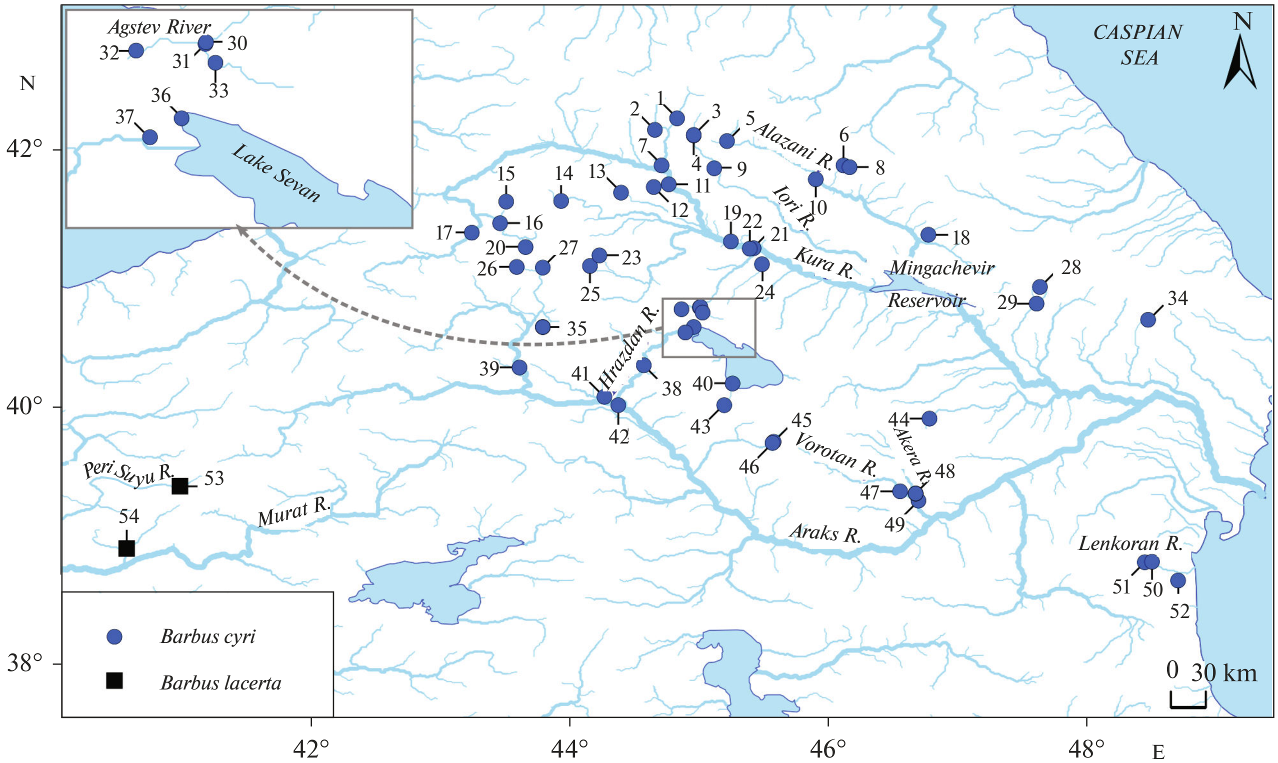

Fig. 1. Map of sampling sites.

Kamensky, 1899, *B. bortschalinicus* Kamensky, 1899 and *B. armenicus* Kamensky, 1899) were synonymized with *B. cyri* (Berg, 1949), and *B. goktschaicus* Kessler, 1877 was proposed to be a junior synonym (Levin et al., 2019). The discussion on the taxonomic status of Kura barbel or its particular populations is apparently explained by the significant morphological variability of the widespread species (Abdurakhmanov, 1962; Levin, 2004). The genetic data may play a key role in understanding the population structure of the species and its taxonomic status, although little information is available in the literature about the genetic polymorphism of the Kura barbel.

Objective—To study the genetic polymorphism of Kura barbel and reconstruct its phylogeography based on the results of molecular genetic analysis.

MATERIALS AND METHODS

A molecular genetic analysis of 52 samples covering most of the range was carried out to reconstruct the evolutionary history of the Kura barbel and clarify the issues of its phylogeography. For comparison, two samples of *Barbus lacerta*, a species closely related to the Kura barbel, were also studied. The study was performed based on the collected materials of the Papanin Institute for Biology of Inland Waters, Russian Academy of Sciences (IBIW RAS). The map of sampling sites is presented in Fig. 1, the geographical coordinates are given in additional materials (Table 1S).

The DNA was extracted from fins fixed in 96% ethanol under the standard salt-extraction method (Aljanabi and Martinez, 1997). Further, we worked with mtDNA, which is successfully used in the study of fish phylogeography (Avise, 2000; Artaev et al., 2021). Sequences of two mtDNA fragments, the cytochrome *b* gene (cyt *b*) and the control region (CR), which successfully used in phylogeographic and phylogenetic studies of barbels, were studied (Tsigenopoulos and Berrebi, 2000; Doadrio et al., 2002, Kotlík et al., 2004; Khaefi et al., 2018; Levin et al., 2019; Zaccara et al., 2019).

The polymerase chain reaction (PCR) was performed in 25-μL of the mixture [1× buffer, 1.5 mM $MgCl_2$, 10 μM of each primer, 0.2 μM of each dNTP, 1 μL of DNA template and 1U of Tag-polymerase (Sileks, Moscow, Russia)].

The cytochrome *b* gene (cyt *b*) fragment was amplified using primers GluDg 5'-TGACTTGAARAACCAYCGTTG-3' (Palumbi, 1996) and H16460 5'-CGAYCTTCGGATTACAAGACCG-3 (Perdices et al., 2002). In some cases, internal primers LA 5'-GCGACTTGAAAAACCACCGTT-3' and CB2H 5'-CCCTCAGAATGATATTTGCCCTCA-3' were used to restore the nucleotide sequence in the beginning of the 5'-end of the cyrochrome *b* gene (Espanhol et al., 2007). The mtDNA control region (CR, according to

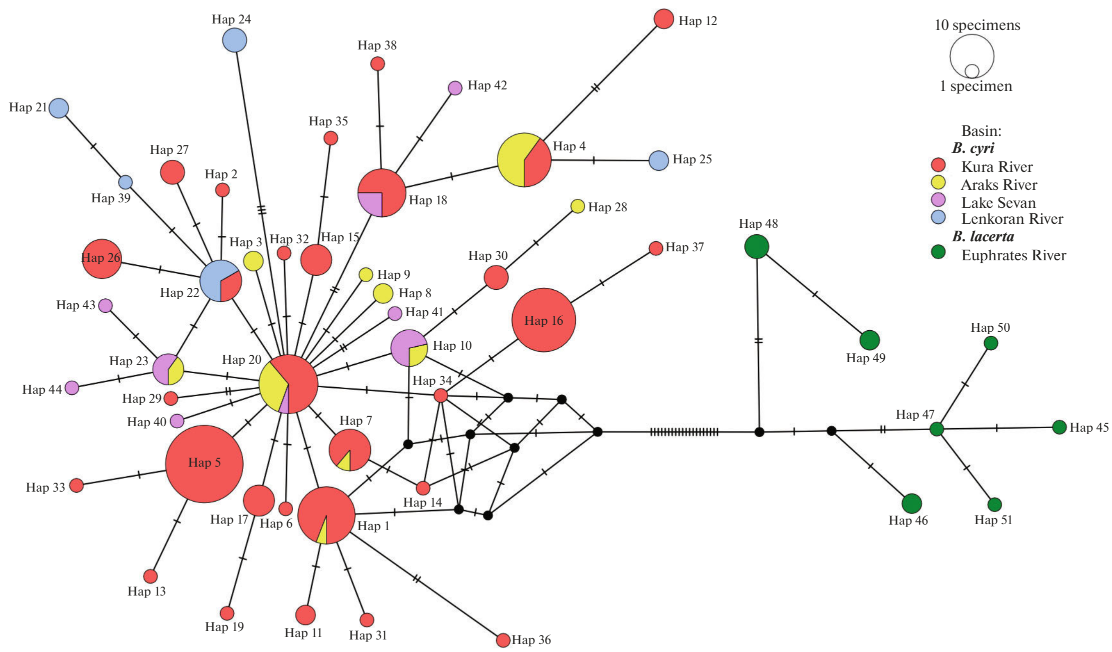

Fig. 2. A haplotype network of *B. cyri* and *B. lacerta* based on 216 sequences of the mtDNA cyt *b* gene (993 bp length). The number of short perpendicular lines on the lines connecting the haplotypes corresponds to the number of nucleotide substitutions; black dots denote hypothetical haplotypes.

BLAST, the amplified fragments also include 18 bp of the tRNA-Pro coding region) was amplified using primers DL623 5'-GGAATAGATATGTTATG-CACTTG-3' (Levin et al., 2013) LProf 5'-AACTCT-CACCCCTAGCTCCCAAAG-3' (Meyer et al., 1994), ESTFOR 5'-CATCGGTCTTGTAATCCGAAGAT-3', and NewCRev_AC 5'-GTTTAGGGGTTTGA-CAAGGATA-3', developed by us. The PCR was performed under conditions described for the cytochrome *b* gene (Levin et al., 2012) and for the control region (Levin et al., 2013).

The PCR products were visualized on 1.5% agarose gel and purified using 96% ethanol and sodium acetate (3M). Sequencing was performed on a ABI3500 automatic sequencer at the IBIW RAS following the manufacturer's instructions. The homologous sites of the sequences were tested for errors with the FinchTV 1.4.0 program (Rothgänger et al., 2005) and aligned using the MEGA7 software (Tamura et al., 2007).

Haplotype networks were built using the Median Joining algorithm (Bandelt et al., 1999) included in the PopART software (Leigh and Bryant, 2015). Genetic differentiation among populations was evaluated by computing the F_{ST} fixation index (Weir and Cockerham, 1984) using the Arlequin v.3.5.2.2 software (Excoffier and Lischer, 2010). Nucleotide and haplotypic diversity, the average number of nucleotide differences were computed with DnaSP v. 6 (Rozas et al., 2017). The average and maximum intra-group, as well as the average intergroup *p*-distance were calculated using the MEGA7 program (Tamura et al., 2007).

A total of 216 sequences of the cyt *b* gene with a length of 993 bp of *B. cyri* and *B. lacerta* were used. They include 62 sequences of the cyt *b* gene of the Kura barbel, published in the NCBI database, nos. MK108197-MK108236, MK108254-MK108266, and MK108279-MK108287 from the study of Levin et al. (Levin et al., 2019), and 154 sequences (MZ547143–MZ547296) obtained in this study. For the first time, 152 CR sequences of *B. cyri* with a length of 652 bp (MZ547297–MZ547448) were sequenced. In addition, 68 CR sequences from NCBI database nos. MF599000–MF599067, published in the study (Khaefi et al., 2018), were used as additional material. Thus, the total number of analyzed CR sequences reached 220.

RESULTS

Phylogeography of the Kura barbel inferred from cyt *b* sequences. Overall, 205 sequences of the cyt *b* gene of Kura barbel *B. cyri* from the basins of the Kura, Araks, and Lenkoran rivers and the Lake Sevan basin belong to 44 haplotypes, while 11 sequences of sister species *B. lacerta* from the Euphrates River basin are represented by 7 haplotypes (Fig. 2, Table 1). The haplotype network of the Kura barbel is star-shaped with haplotype 20 in the center and marginal haplotypes diverging from it. The main haplotype was identified in specimens from three basins (the Kura and Araks rivers and Lake Sevan), the remaining haplotypes were found in representatives of one–two basins. In

Table 1. Intra- and intergroup genetic variation of *B. cyri* and *B. lacerta* as inferred from the cyt *b* gene

Group	*n/H*	*Hd* ± SD	π ± SD	*K*	AID ± SE	MID	NG	Dist. NG ± SE
				Barbus cyri				
Araks River basin	27/10	0.843 ± 0.050	0.0024 ± 0.0002	2.4	0.0024 ± 0.0009	0.006	Lake Sevan basin	0.003 ± 0.001
Kura River basin	147/29	0.908 ± 0.012	0.0026 ± 0.0001	2.6	0.0026 ± 0.0007	0.008	The same	0.003 ± 0.001
Lenkoran River basin	14/5	0.780 ± 0.085	0.0033 ± 0.0007	3.3	0.0033 ± 0.0011	0.007	"	0.003 ± 0.001
Lake Sevan basin	17/9	0.882 ± 0.055	0.0024 ± 0.0003	2.4	0.0024 ± 0.0008	0.005	Araks River basin	0.003 ± 0.001
All basins	205/44	0.938 ± 0.007	0.0028 ± 0.0001	2.7	0.0028 ± 0.0007	0.008	–	–
				Barbus lacerta				
Euphrates River basin	11/7	0.909 ± 0.066	0.0039 ± 0.0004	3.9	0.0039 ± 0.0012	0.007	Kura River basin	0.028 ± 0.005

n/H – sample size/number of haplotypes; *Hd* ± SD, haplotype diversity ± standard deviation; π, nucleotide diversity (per site); *K*, average number of nucleotide differences; AID ± SE, average intragroup *p*-distance ± standard error; MID, maximum intragroup *p*-distance; NG, nearest group; Dist. NG, average *p*-distance to the nearest group (based on 1000 bootstrap replicates).

this regard, the subdivision of the network into separate haplogroups is difficult. The barbel haplotypes from the Kura River (haplotypes 12, 37) and the Lenkoran River (haplotypes 24 and 25) were the most distant (up to five mutations) from the main haplotype (Fig. 2). The barbel haplotypes from the basins of the Araks River and Lake Sevan weakly diverge from the main haplotype.

The minimum haplotype diversity was found in the Lenkoran River basin (Hd = 0.780), the maximum diversity was in the Kura River basin (Hd = 0.908) (Table 1). The minimum nucleotide diversity was found in the basins of Lake Sevan and Araks River ($\pi = 0.0024$), the maximum diversity was in the Lenkoran River basin ($\pi = 0.0033$). The average number of nucleotide differences and the average genetic distance between haplotypes from the Lenkoran River basin are also the largest (K = 3.3, AID = 0.0033) compared to other basins (Table 1, Fig. 2).

Despite a small size of the sample of *B. lacerta*, its haplotype diversity (Hd = 0.780) is comparable to or exceeds that of *B. cyri* from the Kura River basin, which has the highest Hd value among all basins. The nucleotide diversity, the average intraspecies distance, and the average number of nucleotide differences of *B. lacerta* are significantly higher than in *B. cyri* (Table 1). When considering the longer sequences of this marker (1068 bp), almost each of them is unique for *B. lacerta*, although most of them originate from the same locality, the Peri Suyu River. Thus, *B. lacerta* has a high genetic diversity. In general, the barbels of *B. cyril* and *B. lacerta* are well differentiated genetically, as can be seen from the haplotype network. The minimum genetic distance between the haplotypes of these species is 25 nucleotide substitutions.

Phylogeography of the Kura barbel inferred from control region sequences. The haplotype network of the control region of the Kura barbel was constructed using 152 sequences with a length of 652 bp (Fig. 3a). In total, 59 haplotypes were found, 38 of them were represented by unique sequences.

The haplotype network has a complex structure. In view of the network topology, seven haplogroups can be distinguished (Fig. 3a). Haplogroups do not completely correspond to basins or sub-basins, although haplogroup 3 includes haplotypes exclusively from the Kura River basin. Haplogroups 1, 6, and 7 include haplotypes from the Kura and Araks River basins. Haplogroup 2 includes haplotypes from all basins except the Araks River. Haplogroups 4 and 5, which slightly diverge from each other (four mutations), include haplotypes from all basins studied (the Kura, Araks, and Lenkoran rivers and Lake Sevan). It was also found that the barbel haplotypes from Lake Sevan and the Lenkoran River are combined into the same haplogroups: the haplotypes identified in these water bodies belong to haplogroups 2, 4, and 5 (Fig. 3a). Haplogroups of the control region are presented on the map for better visualization of their geographical distribution (Fig. 3b).

All seven haplogroups were found in the Kura River basin, five haplogroups, in the Araks River basin and three haplogroups each in the basins of the Lenkoran and Lake Sevan. Haplogroups have a different distribution: for example, haplogroup 3 occurs exclusively in the upper and middle reaches of the Kura River. There are also haplogroups that are distributed mainly in certain basins: haplogroup 5 is common for the Upper and Middle Kura, Lake Sevan, the Araks and Badara River (Lower Kura); haplogroup 6 is found in the Upper and Middle Kura, the Araks River, as well as in the Badara River (Lower Kura); haplogroup 4 is found mainly in the Lower Kura, Lake Sevan, and the Lenkoran River; haplogroup 1 occurs

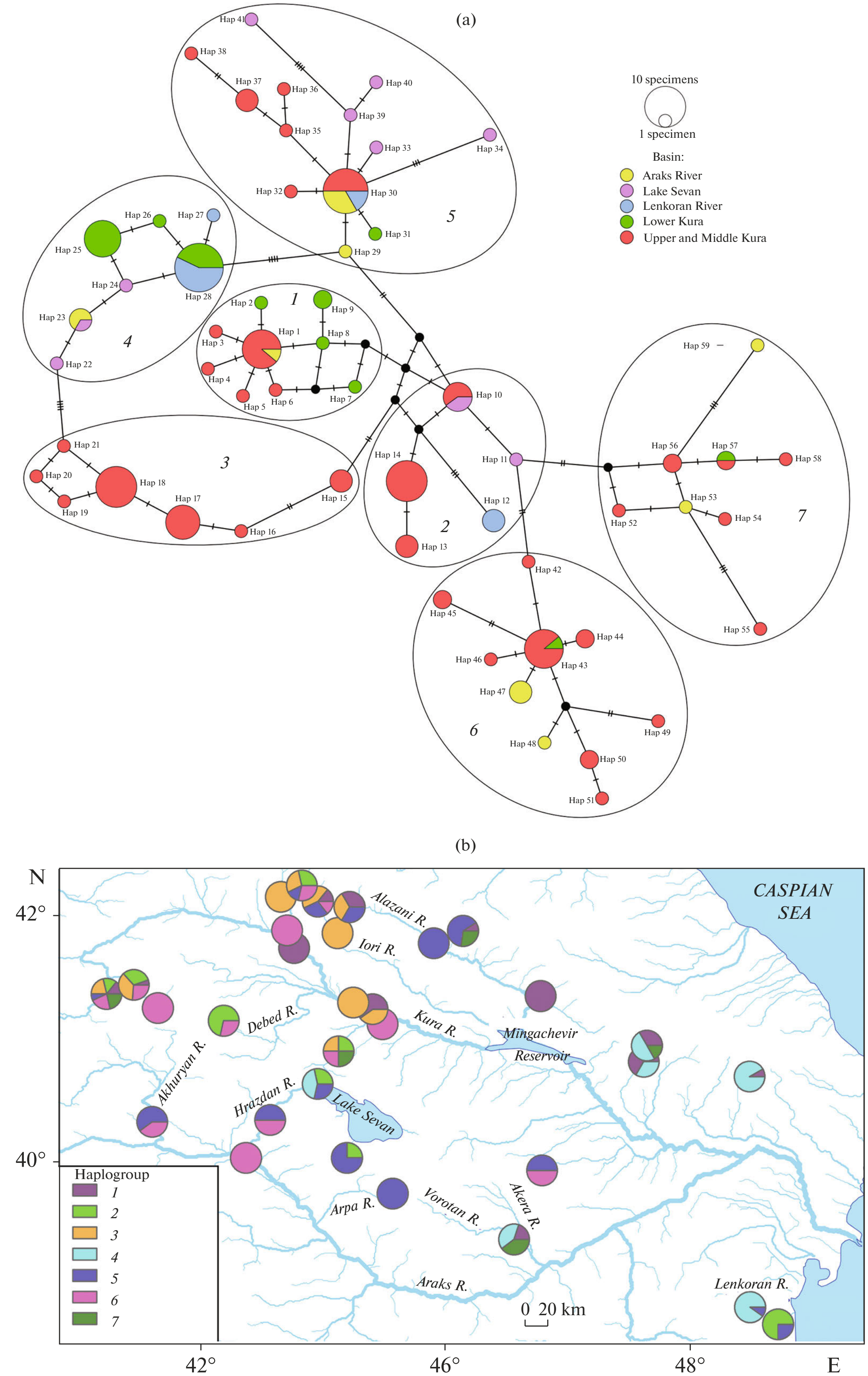

Fig. 3. Phylogeography of barbels *B. cyri* as inferred from 152 sequences of the mtDNA control region (652 bp length): (a) haplotype network (number of short perpendicular lines on the lines connecting haplotypes corresponds to the number of nucleotide substitutions; black dots denote hypothetical haplotypes); (b) geographical distribution of the control region haplotypes of *B. cyri*. (geographically close localities are combined in one). *1–7*, haplogroups.

Table 2. Intra- and intergroup genetic variability of the control region sequences of *B. cyri*

Group	n/H	$Hd \pm$ SD	$\pi \pm$ SD	K	AID ± SE	MID	NG	Dist. NG ± SE
Basin:								
Araks River	14/8	0.890 ± 0.06	0.0086 ± 0.0012	5.6	0.0086±0.0022	0.017	Lenkoran River	0.009 ± 0.002
Kura River	113/43	0.960 ± 0.006	0.0097 ± 0.0002	6.2	0.0096±0.0022	0.02	Araks River	0.010 ± 0.002
Lenkoran River	14/4	0.648 ± 0.116	0.0069 ± 0.0015	4.5	0.0069±0.0021	0.017	Lake Sevan	0.009 ± 0.002
Lake Sevan	11/10	0.982 ± 0.046	0.0093 ± 0.001	6.1	0.0093±0.0023	0.018	Lenkoran River	0.009 ± 0.002
All basins	152/59	0.965 ± 0.005	0.0096 ± 0.0002	6.3	0.0096±0.0022	0.025	–	–

Designations of parameters see in Table 1.

in one locality of the Araks River basin, but is widespread throughout the Kura River basin. In addition, some haplogroups are absent in certain basins (haplogroup 2 is not detected in the Araks River basin). As previously mentioned, the same haplogroups are common in the basins of Lake Sevan and the Lenkoran River.

A rather high haplotype diversity of the Kura barbel was recorded in Lake Sevan, $Hd = 0.982$, compared to that in other basins and in all basins together (Table 2). Almost every sequence is unique for the Lake Sevan basin. The Lenkoran River basin is characterized by low values of almost all genetic diversity indexes compared to other basins ($Hd = 0.648$, $K = 4.5$, AID = 0.0069, $\pi = 0.0069$). The populations from the Kura River basin are characterized by the maximum values of nucleotide diversity, the average number of nucleotide substitutions and the average intra-group distance ($\pi = 0.0097$, $K = 6.2$, AID = 0.0096), which is in line with their presence in each haplogroup of the haplotype network (Fig. 3a).

Pairwise values of the fixation index (F_{ST}) were calculated for sequences of the mtDNA control region from different basins (Fig. 4). The river basin was divided into two sections, the upper-middle and lower reaches of the river, owing to the difference in haplogroup frequencies between the Kura River basin upstream of the Mingachevir Reservoir and downstream of it (Fig. 5).

The maximum F_{ST} value was recorded for the barbel populations within the Kura River basin, between the sections of this river upstream and downstream of the Mingachevir Reservoir ($F_{ST} = 0.27$, $p < 0.05$). The minimum pairwise F_{ST} value was found for samples from the basins of Lake Sevan and the Araks River ($F_{ST} = 0.01$, $p > 0.05$). The differences in the F_{ST} values between the samples of barbels from the upper-middle course of the Kura River and the samples from the other basins were statistically significant. The populations of the Lenkoran River basin were more isolated from the populations of the Araks River basin ($F_{ST} = 0.14$, $p < 0.05$) than from the populations of the lower reaches of the Kura River basin ($F_{ST} = 0.09$, $p > 0.05$) or Lake Sevan ($F_{ST} = 0.09$, $p > 0.05$). At the same time, the populations of the Araks River basin is genetically more isolated from the populations of the Lower Kura ($F_{ST} = 0.19$, $p < 0.05$) than from the populations of the upper and middle reaches of this river ($F_{ST} = 0.10$, $p < 0.05$).

For a better understanding of the phylogeography of the Kura barbel, we constructed a network of haplotypes (Fig. 6) with the addition of shorter sequences of the control region of *B. cyri* from the Iranian part of the Caspian Sea basin (Khaefi et al., 2018), while seven previously assigned haplogroups (Fig. 3a) remained. The haplotypes described by Khanefi et al. (2018) are part of the haplogroups we have identified or are close to them (Fig. 6). Some of the Iranian sequences belong to haplotypes common for barbels from the basins of the Kura, Araks, and Lenkaran rivers and Lake Sevan. It is interesting that haplotypes from the Kalibar river (Araks River basin) are located in different parts of the network at a great distance from each other (5–9 substitutions, AID = 0.011 ± 0.004, MID = 0.019 ± 0.007) as part of different haplogroups. Haplogroups C1–C3 are geographically and genetically close, haplogroups C4 and C5, despite their geographical proximity, have significantly diverged genetically. Barbels from the basin of Lake Urmia are grouped separately from the others.

DISCUSSION

Two mtDNA markers give a rather different population genetic structure of Kura barbel *B. cyri*. Thus, the haplotype network of cyt *b* gene sequences represents the main haplotype and derived haplotypes and haplotype lineages with insufficiently structured haplogroups diverging from it (Fig. 2). Such a structure may be an indication that the Kura barbel spread rather quickly in water bodies and watercourses of the Caucasus. The cyt *b* gene haplotype network also includes *B. lacerta* sequences from two rivers of the Euphrates River basin, represented by seven haplotypes. In general, the genetic diversity of *B. lacerta* is high.

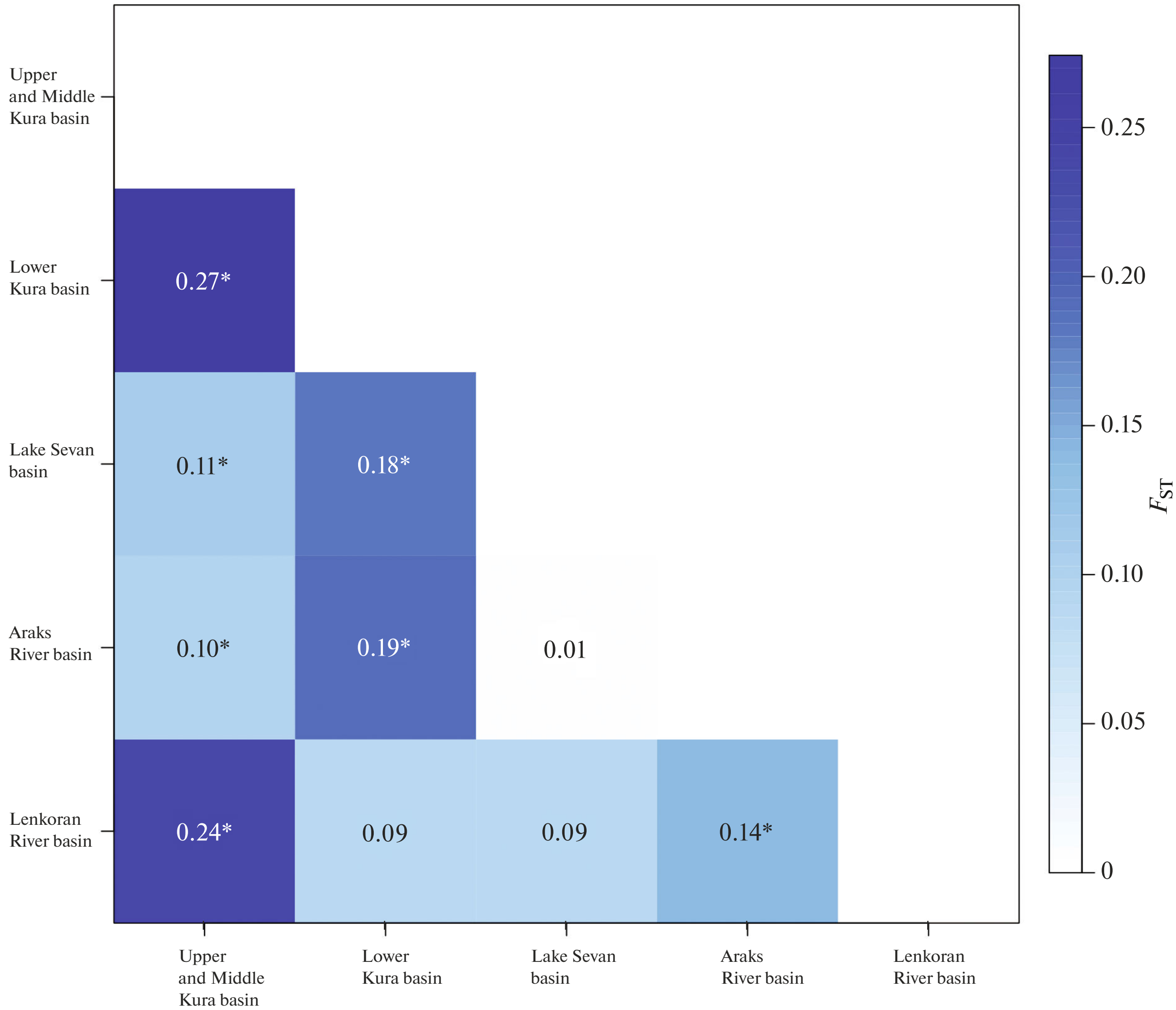

Fig. 4. Matrix plot of pairwise F_{ST} values between barbel populations based on mtDNA control region sequences. The significant F_{ST} values are marked with an asterisk ($p < 0.05$).

At the same time, the analysis of the haplotype network based on non-coding sequences of the control region (Fig. 3a) showed a significant subdivision of the identified haplotypes into a number of haplogroups that are not clearly attributed to a particular geographical region or basin. Despite of a generally weak geographical structuring, a unique group of *B. cyri* haplotypes was found in the Kura River, haplogroup 3 (Fig. 3). It is represented only in the upper-middle reaches of the river that may indicate a possible partial isolation of the populations of the upper-middle and lower reaches from each other. The absence of representatives of haplogroup 4 in the upper-middle reaches that occurs in the lower reach of the Kura River, also indicates the genetic segregation of the Kura populations. The reduced gene flow between populations of the upper-middle and lower reaches of the Kura River is also evidenced by a high value of F_{ST} between them (Fig. 4).

The results are consistent with the information about the endemism of the ichthyofauna in the middle reach of the Kura River. In particular, narrowly distributed endemic fishes *Pseudophoxinus atropatenus* (Derjavin, 1937) (Artaev et al., 2018) and *Cobitis derzhavini* Vasil'eva, Solovyeva, Levin, and Vasil'ev, 2020 (Vasil'eva et al., 2020) inhabit this part of the Kura basin. Apparently, the area of the Middle Kura basin can be considered as a refugium of the aquatic fauna of Transcaucasia.

Although the Lenkoran River basin is isolated from the Kura–Araks river system, the sequences of the Lenkoran barbel still belong to different haplogroups, being at a considerable distance from each other (Table 2, Fig. 2). This may indicate the repeated colonization of the Lenkoran River basin by representatives of different haplogroups, possibly via several pathways. Thus, over the last million years, the level of the Caspian Sea has fluctuated dramatically, which

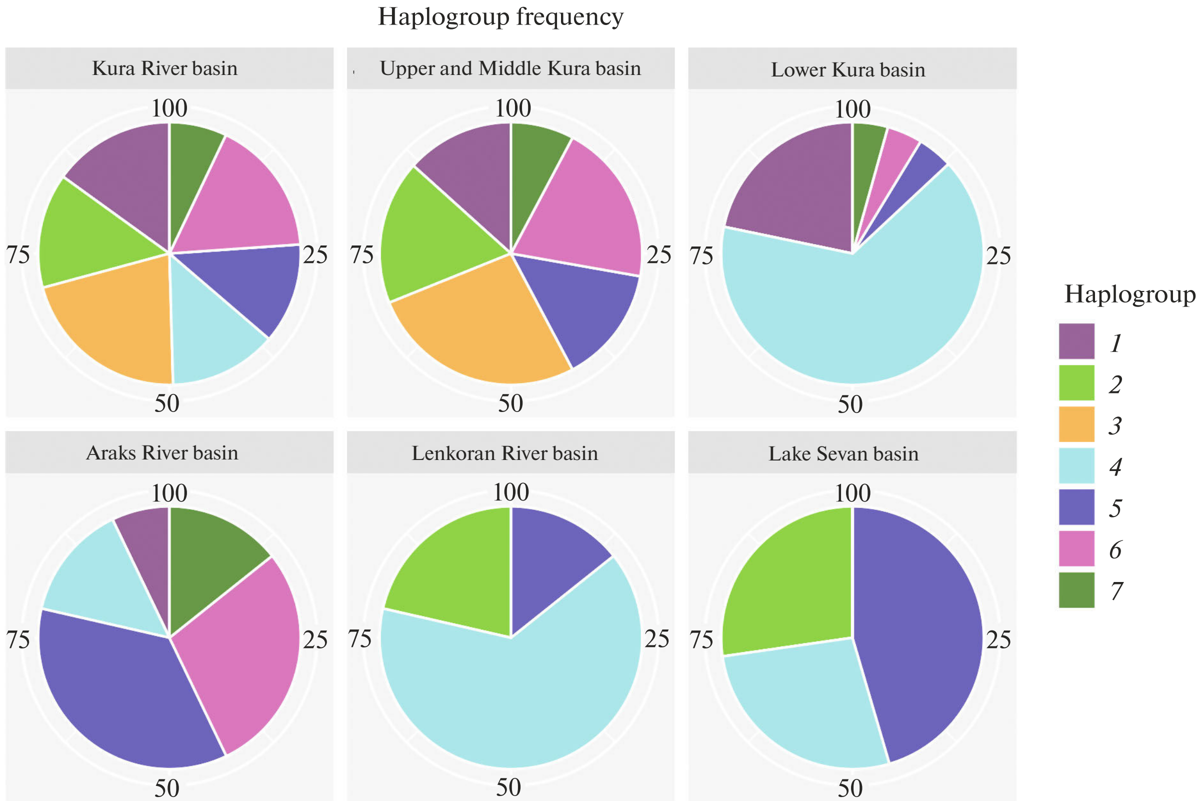

Fig. 5. Diagrams of haplogroup frequences in different basins. For the Kura River basin, the frequencies are calculated for the entire basin and its upper-middle and lower reaches.

was accompanied by changes in salinity in the range of 7–16‰ (Yanina, 2012). The penetration of the Kura barbel into the Lenkoran River basin could be timed to considerable regressions or the initial stages of transgressions of the Caspian Sea, during which its salinity decreased with the possible formation of local freshwater estuaries in the riverine mouths (Svitoch, 2016). As Svitoch (2016) suggests, during the transgressive epochs, the volume of dissolved salts in the Caspian Sea waters was somewhat larger than during the regressive epochs. During the transgressions of the Caspian Sea, the sources of salts were the Northern Caspian salt domes and, probably, the Kara-Bogaz-Gol Bay and salt marches during their flooding. In addition, during the regressions the paleochannels of rivers could be merged downstream, which also represents an opportunity for fish dispersal. The cases of similar connection of paleochannels are known for periods of regressions of the sea and ocean levels (Gulin and Kovalenko, 2010; Swartz et al., 2014).

It is possible that the Lenkoran River basin was connected to the Kura–Araks basin through a system of captures in the upper reaches of the rivers. The similarity between the fauna of the Lenkoran River and the fauna of the Kura–Araks basin is also evidenced by recent genetic studies of riffle minnow *Alburnoides* (Levin et al., 2018). According to pairwise F_{ST} values, the gene flow between the basins of the upper-middle reaches of the Kura and Lenkoran rivers is lower than between the Lenkoran River and other basins (Fig. 4). At the same time, the higher F_{ST} values between the samples of the Lenkoran and Araks rivers compared to those between the Lenkoran and the Lower Kura rivers are apparently explained by the colonization of the Lenkoran River from the Lower Kura region or simultaneously with it.

Sevan is a mountain isolated lake located in the Araks River basin. Isolation of Lake Sevan occurred ~20000 years ago by the formation of a waterfall with a height of 15 m on the Hrazdan River outflowing from the lake (Davydov, 1938; Milanovsky, 1968). A low F_{ST} index between the populations of basins of the Araks River and Lake Sevan indicates their recent genetic isolation. Also, the haplogroup shared with Lenkoran population was detected in Lake Sevan that suggests incomplete lineage sorting (Fig. 3).

According to the data of spore-pollen analysis, the humidity of the climate and the water level in Lake Sevan fluctuated significantly during the Holocene (Sayadyan and Aleshinskaya, 1991). Changes in the water regime of Lake Sevan can be in this case an indicator for the entire South Caucasus region, reflecting fluctuations in the water level of river systems. Climatic oscillations could contribute both to the isola-

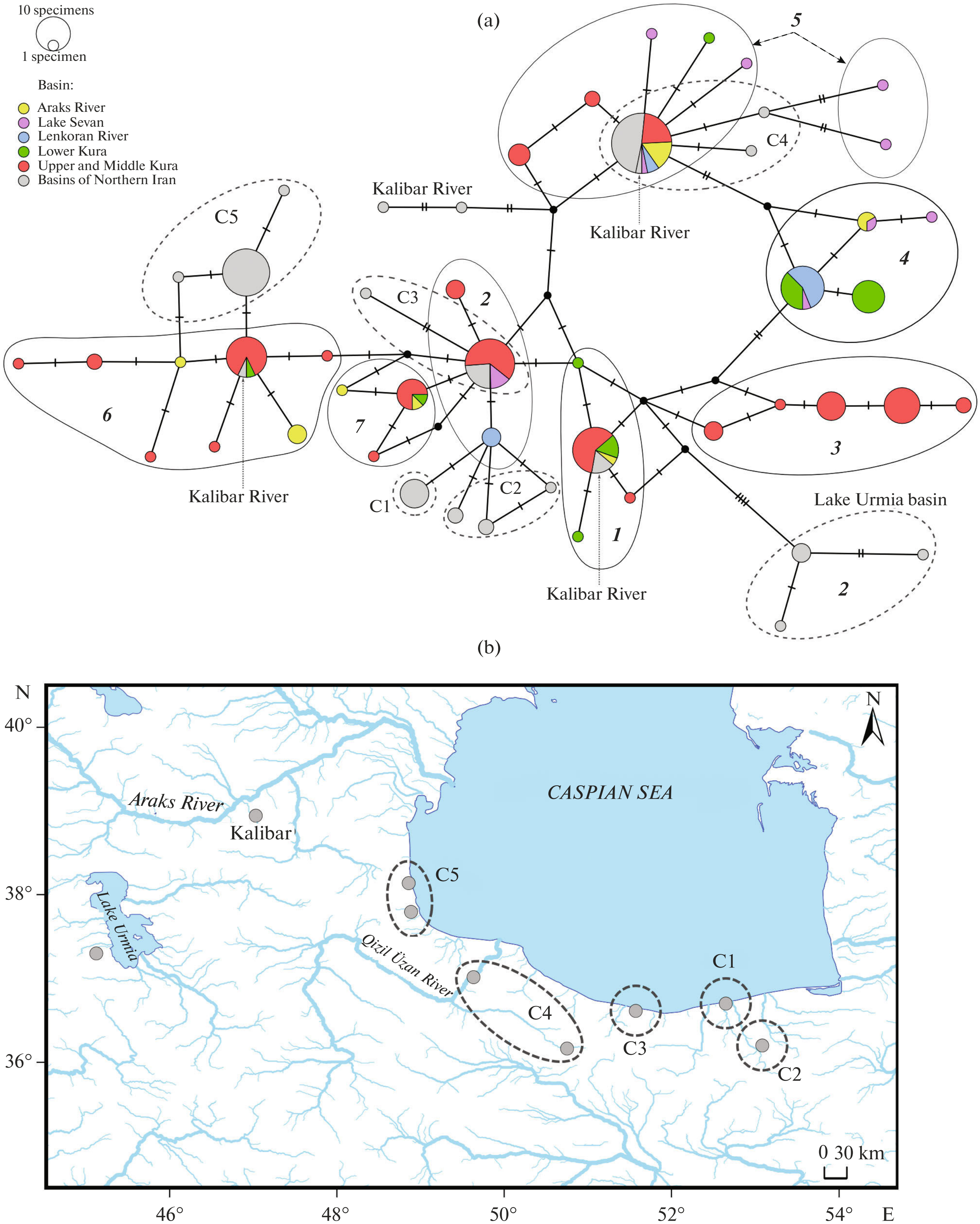

Fig. 6. Phylogeography of barbels *B. cyri* as inferred from 220 sequences of the mtDNA control region (366 bp length combined with sequences from Iran (Khaefi et al., 2018): (a) haplotype network (C1–C5, haplogroups described by Khaefi et al. are outlined with a dotted line, Kalibar River, Lake Urmia Iranian; *1–7*, haplogroups we identified earlier are outlined with a solid line; (b) geographical distribution of haplogroups C1–C5.

tion of certain populations during fragmentation of river networks at the period of dry climate, and result in secondary contacts due to the expansion of the hydrographic network and river captures during the wet climate. In addition, high volcanic activity of the Lesser Caucasus (Milanovskii, 1968; Leonov, 2003) also affected the hydrographic network.

It is worth noting that the isolation of certain populations and secondary contacts are detected also for the Kura barbel of the Southern Caspian (Khaefi et al., 2018). Populations of the Kura barbel of the Iranian coast of the Caspian Sea have many common haplotypes with samples of the Caucasus and the routes of their dispersal are difficult to establish. The repeated dispersal of the Kura barbel along the coast of the Caspian Sea in the Quaternary period can not be excluded.

CONCLUSIONS

This study is the first attempt to consider in detail the phylogeography of barbels of the genus *Barbus* by the example of the Kura barbel *B. cyri* in the Caucasus. The analysis of polymorphism of two mtDNA markers indicates the complex history of dispersal and interaction of populations of the Kura barbel *B. cyri*. The cyt *b* protein coding marker indicates a relatively rapid dispersal across the freshwaters of the Caucasus. According to the analysis of polymorphism of the non-coding region of mtDNA, the control region (CR), the populations of the Kura barbel are genetically subdivided into several groups. The results revealed the genetic segregation of the populations of the upper-middle and lower reaches of the Kura River, as well as the repeated colonization of basins of the Lenkoran River, Lake Sevan, and the Lower Kura River. Probably, there was a gene flow between the basins considered, including the basins of the Iranian coast of the Caspian Sea. The complex genetic structure of the Kura barbel populations apparently was formed during the periods of intense tectonic processes and climatic fluctuations during the Pleistocene–Holocene, which significantly affected the hydrography of river networks and contributed to both secondary contacts and isolation of populations.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest. The authors declare that they have no conflict of interest.

Statement on the welfare of animals. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

SUPPLEMENTARY INFORMATION

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Risk of invasiveness of non-native fishes in the South Caucasus biodiversity and geopolitical hotspot

Levan Mumladze[1], Tatia Kuljanishvili[2], Bella Japoshvili[1], Giorgi Epitashvili[1], Lukáš Kalous[2], Lorenzo Vilizzi[3], Marina Piria[3,4]

1 *Institute of Zoology, Ilia State University, Tbilisi 0162, Georgia* **2** *Department of Zoology and Fisheries, Faculty of Agrobiology, Food, and Natural Resources, Czech University of Life Sciences, 165 00 Prague, Czech Republic* **3** *Department of Ecology and Vertebrate Zoology, Faculty of Biology and Environmental Protection, University of Lodz, 90-237 Lodz, Poland* **4** *University of Zagreb, Faculty of Agriculture, Department of Fisheries, Apiculture, Wildlife Management and Special Zoology, 10000 Zagreb, Croatia*

Corresponding author: Lorenzo Vilizzi (lorenzo.vilizzi@gmail.com)

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Abstract

Aquatic invasions are one of the major threats for freshwater ecosystems. However, in developing countries, knowledge of biological invasions, essential for the implementation of appropriate legislation, is often limited if not entirely lacking. In this regard, the identification of potentially invasive non-native species by risk screening, followed by a full risk assessment of the species ranked as higher risk, enables decision-makers to be informed about the extent of the threats posed to the recipient (risk assessment) area. In this study, 32 non-native extant and horizon fish species were screened for their risk of invasiveness under current and predicted climate conditions for the South Caucasus – a biodiversity and geopolitical hotspot that includes the countries of Armenia, Azerbaijan and Georgia. Overall, the number of very high-risk species increased from four (12.5%) under current climate conditions to 12 (37.5%) under predicted climate conditions. The highest-risk species under both conditions included the already established gibel carp *Carassius gibelio* and topmouth gudgeon *Pseudorasbora parva*, the locally translocated pikeperch *Sander lucioperca* and the horizon North African catfish *Clarias gariepinus*. Under predicted climate conditions, a very high risk of invasiveness was predicted also for the translocated three-spined stickleback *Gasterosteus aculeatus* and Eurasian perch *Perca fluviatilis*, for the already established eastern mosquitofish *Gambusia holbrooki*, ruffe *Gymnocephalus cernua*, sharpbelly *Hemiculter leucisculus* and Nile tilapia *Orechromis niloticus*, and for the horizon pumpkinseed *Lepomis gibbosus* and largemouth bass *Micropterus salmoides*. Future research on the non-native species in the South Caucasus should be conducted both country- and region-wide and should account not only for the high biodiversity, but also for the critical geopolitical situation affecting the study area.

Keywords
Aquatic invasions, AS-ISK, Black Sea, Caspian Sea, climate change, extant, horizon

Introduction

Biological invasions are a major threat to global biodiversity and pose a considerable challenge for human well-being (Mazza et al. 2014; Shackleton et al. 2018). Protecting biodiversity and maintaining ecosystem function involves the allocation of considerable financial resources due to the growing trend of invasion events and the resulting rate of pressure exerted by invasive non-native species (Seebens et al. 2017; Diagne et al. 2020). Additionally, the management and eradication of invasive non-native species once they become established are far more demanding endeavours in terms of costs and related challenges than prevention and early detection (Simberloff et al. 2013). The latter can be achieved through the establishment of country-based regulations to control species translocations and introductions within/amongst countries, supported by actions for eradication (Simberloff et al. 2013). Yet, concerted efforts to mitigate the non-native species invasion process and promote management actions pose overall a challenge at the global scale (Genovesi et al. 2013; Tittensor et al. 2014; CBD 2018).

One of the main reasons hindering effective national and cross-national strategic plans against invasive non-native species is the absence of quality biological data for several countries (Latombe et al. 2017). This is especially true of most developing countries that lack an exhaustive list of invasive non-native species, do not have monitoring capacities and/or have not yet adopted strategies for dealing with such species, which are usually identified long after their entry and establishment in the recipient area (Early et al. 2016). A good example is the South Caucasus biodiversity hotspot, which comprises the countries of Armenia, Azerbaijan and Georgia (Mittermeier et al. 2004). Despite preliminary efforts, there are currently no effective nation-wide initiatives in these countries to provide an inventory of non-native species, nor are there any related monitoring programmes in place. In fact, only incidental academic studies have so far provided some authoritative, albeit in most cases still partial, inventories of non-native plants (Kikodze et al. 2010; Fayvush and Tamanyan 2014; Sharabidze et al. 2018; Abdiyeva 2019), molluscs (Mumladze et al. 2019), insects (Aleksidze et al. 2021) and fishes (Kuljanishvili et al. 2021b). Yet, none of the aforementioned inventories (except for fishes) is complete or up-to-date enough to be useful at the national or cross-national level. Moreover, the impacts of invasive non-native species on the biodiversity and ecosystems of the South Caucasus have not yet been evaluated even for a single species, despite some of the non-native species therein having already caused environmental and economic losses (Diagne et al. 2020).

The South Caucasus is widely recognised as a biodiversity hotspot characterised by a great diversity of landscapes and climate zones that shelter a highly diverse plant and animal biota. Freshwater biodiversity is the most understudied ecological aspect of the South Caucasus (Mumladze et al. 2020), whose watercourses are exploited for hydropower production (Japoshvili et al. 2021) and secondarily for drinking water uptake, fisheries, irrigation and recreational activities. However, the potential threats faced by the freshwater biodiversity and ecosystems of the South Caucasus have not yet been evaluated, hence remain overall poorly understood. Only in a recent study have the effects of existing and planned hydropower plants on the connectivity of fish communities in Georgia been investigated in some detail (Japoshvili et al. 2021). Additionally, in another recent study, an attempt has been made to summarise the diversity, distribution and introduction history of non-native fishes in the South Caucasus based on a literature review and social-media data (Kuljanishvili et al. 2021b).

To understand the potential risk of invasiveness posed by non-native fishes in the South Caucasus, the aims of the present study were to: (i) screen both extant and horizon species and (ii) discuss the resulting species-specific risk ranks of invasiveness also within the current geopolitical situation affecting the study area with a view to implementing future legislation. Notably, this study represents the first risk screening for the South Caucasus and Georgia in particular. It is anticipated that the outcomes of this study will provide for an important step forward in the understanding of the impacts and related risks of environmental/economic losses caused by invasive non-native fishes in this biodiversity and geopolitical hotspot.

Methods

Risk assessment area

The South Caucasus (hereafter, also the 'risk assessment area') is located south of the Great Caucasus mountain range and stretches across the Black and Caspian seas with 80% of its area belonging to the Kura-Aras drainage basin (Caspian Sea Basin) shared with Turkey and Iran and the remaining 20% (western part) to the Black Sea Basin (Fig. 1). The South Caucasus is politically subdivided into the three independent countries of Armenia, Azerbaijan and Georgia. However, the South Caucasus is also an individually distinct geographic unit bordered by the Great Caucasus mountain range in the north (an impassable barrier for most animal species) and the Black and Caspian seas in the west and east, respectively. Whereas the southern border of the South Caucasus, albeit less distinctively identifiable, coincides with the two large rivers Kura-Aras and Chorokh, which originate from the Anatolian Plateau in Turkey. The Kura-Aras River is the longest watercourse and flows into the Caspian Sea, with most of the eastern South Caucasian rivers draining into this river's basin, except for a few short watercourses in the extreme north-east part of Azerbaijan. Unlike Armenia and Azerbaijan, in western Georgia, several small- to medium-sized rivers drain into the

Figure 1. Map of the South Caucasus (Armenia, Azerbaijan, Georgia), representing the risk assessment area, and neighbouring countries.

Black Sea and, at higher altitudes, there are several isolated mountainous lakes with their own independent basins. Amongst these, Lake Sevan in Armenia is the largest and harbours endemic taxa.

The climate of the South Caucasus is continental-mesophilic with strong local variation due to its complex topography. According to the updated Köppen-Geiger climate map (Beck et al. 2018), the warm-temperate climate types without dry season *Cfa* and *Cfb* (with warm and hot summer, respectively) are predominant in the westernmost part of the risk assessment area, which stretches along the entire eastern Black Sea coast and extends through to the Cholchis lowland. In this area, precipitation can be as high as 4000 mm annually (Adjara Region, south-western Georgia). Going further east, at higher altitudes (700–2000 m a.s.l.) the climate changes from cold with no dry season *Dfa* (but with hot summer) to *Dfb* (with warm summer) and *Dfc* (cold summer). Further east and up to the Caspian Sea, the climate becomes drier and corresponds predominantly to the *Bsk* (cold, arid steppe) and *Csa* (temperate dry and hot summer) types, with areas of cold semi-arid climate *Bwk*. In the eastern part, there is also a climate 'island' along the foothills of the southern Great Caucasus corresponding to the *Cfa* type. At the higher altitudes (above 2500 m) of the northern Great Caucasus and southern Lesser Caucasus, the climate changes sharply from cold with no dry season and cold summer (*Dfc*) to polar (*ET*).

Currently, there are 121 freshwater and anadromous fish species known from the South Caucasus (Kuljanishvili et al. 2021b) of which nearly 30% are endemic. The Kura-Aras River is the richest with at least 16 endemic species alone, though some watercourses of the Black Sea Basin also are species-rich, for example, the River Rioni, which is the last spawning ground for at least four sturgeon species (Beridze et al. 2022). Amongst the 121 fish species, ten are currently considered established non-native, whereas an additional five species are intra-regionally translocated (Kuljanishvili et al. 2021b). However, the conservation status of these species is largely unknown and, at the time of writing, there are only 16 species listed in the IUCN as globally threatened under various conservation statuses, with the remaining species still waiting for a comprehensive assessment.

Species selection

In total, 32 freshwater fish taxa (hereafter, for simplicity 'species') were selected for risk screening in the South Caucasus (Table 1). Notably, the marine/brackish water fishes that are frequently entering the lower reaches of rivers in the risk assessment area (i.e. golden grey mullet *Chelon auratus*, leaping mullet *Chelon saliens* and flathhead grey mullet *Mugil cephalus*) or that can survive in isolated freshwater bodies (i.e. black-striped pipefish *Syngnathus abaster*) (Elanidze 1956, 1983; Kuljanishvili et al. 2021c) will also be regarded in this study as 'freshwater species'. The criteria for species selection were as follows (Table 1):

1. Translocated species (n = 8);
2. Non-native species already present in the risk assessment area (n = 14);
3. Non-native 'horizon' species established in neighbouring countries or countries of similar climate to the risk assessment area (n = 5);
4. Non-native species recorded in the risk assessment area, but in the wild (n = 5).

Selection of species based on the first three criteria was according to the most recent non-native species list published by Kuljanishvili et al. (2021b), whereas selection based on the latter criterion relied on literature resources.

Risk screening

Risk screening was undertaken using the Aquatic Species Invasiveness Screening Kit (AS-ISK: Copp et al. 2016b, 2021), which is available for free download at www.cefas.co.uk/nns/tools. This taxon-generic decision-support tool consists of 55 questions: the first 49 questions comprise the Basic Risk Assessment (BRA) and address the biogeography/invasion history and biology/ecology of the species under screening; the last six questions comprise the Climate Change Assessment (CCA) and require the assessor to predict how future predicted climatic conditions are likely to affect the BRA with respect to risks of introduction, establishment, dispersal and impact. For the purposes of the CCA component of the screening protocol, an increase in temperature on average by 2 °C relative to current conditions is predicted for the SCR (Hansen et al. 2010; Beck et al. 2018). Screenings were undertaken on all species by three independent assessors, i.e. the authors BJ, GE and TK (combination 3IA: Vilizzi et al. 2022).

To achieve a valid screening, the assessor must provide for each question a response, a level of confidence for the response (see below) and a justification based on

Table 1. Freshwater fish taxa (for simplicity, 'species') screened for their potential risk of invasiveness in the South Caucasus – the risk assessment area. For each species, the following information is provided: criterion (Crit.) for selection (1 = translocated species; 2 = non-native species already present in the risk assessment area; 3 = non-native 'horizon' species established in neighbouring countries or countries of similar climate to the risk assessment area; 4 = non-native species recorded in the risk assessment area, but in the wild); a priori categorisation outcome into Non-invasive or Invasive. For the a priori categorisation, the results of the related protocol (after Vilizzi et al. 2022) are indicated: (i) FishBase (www.fishbase.org); (ii) Centre for Agriculture and Bioscience International Invasive Species Compendium (CABI: www.cabi.org/ISC) and Global Invasive Species Database (GISD: www.iucngisd.org); (iii) Invasive and Exotic Species of North America list (IESNA: www.invasive.org); (iv) Google Scholar literature search. N = no impact/threat; Y = impact/threat; '–' = absent; n.e. = not evaluated (but present in database); n.a. = not applicable.

Species name	Common name	A priori categorisation						
		Crit.	FishBase	CABI	GISD	IESNA	Google Scholar	Outcome
Ameiurus melas	black bullhead	3	Y	Y	–	–	n.a.	Invasive
Anguilla anguilla	European eel	4	N	Y	–	–	n.a.	Invasive
Carassius gibelio	gibel carp	2	Y	Y	–	–	n.a.	Invasive
Chelon auratus	golden grey mullet	1	N	–	–	–	N	Non-invasive
Chelon saliens	leaping mullet	1	N	–	–	–	N	Non-invasive
Clarias gariepinus	North African catfish	3	Y	Y	Y	–	n.a.	Invasive
Coregonus albula	vendace	2	N	Y	–	–	n.a.	Invasive
Coregonus sp.*	–	2	N	–	–	–	N	Non-invasive
Ctenopharyngodon idella	grass carp	2	Y	Y	Y	Y	n.a.	Invasive
Gambusia holbrooki	eastern mosquitofish	2	Y	Y	Y	–	n.a.	Invasive
Gasterosteus aculeatus	three-spined stickleback	1	N	–	–	–	N	Non-invasive
Gobio artvinicus	Artvin gudgeon	1	N	–	–	–	N	Non-invasive
Gymnocephalus cernua	ruffe	2	–	Y	–	–	n.a.	Invasive
Hemiculter leucisculus	sharpbelly	2	Y	N	–	–	n.a.	Invasive
Hypophthalmichthys molitrix	silver carp	2	Y	Y	Y	Y	n.a.	Invasive
Hypophthalmichthys nobilis	bighead carp	2	Y	Y	Y	Y	n.a.	Invasive
Ictalurus punctatus	channel catfish	4	Y	Y	–	–	n.a.	Invasive
Lepomis gibbosus	pumpkinseed	3	Y	N	–	–	n.a.	Invasive
Micropterus salmoides	largemouth bass	3	Y	Y	Y	–	n.a.	Invasive
Mugil cephalus	flathead grey mullet	4	N	–	–	–	N	Non-invasive
Mylopharyngodon piceus	black carp	4	Y	Y	–	Y	n.a.	Invasive
Oncorhynchus kisutch	coho salmon	4	N	–	–	Y	n.a.	Invasive
Oncorhynchus mykiss	rainbow trout	2	Y	Y	Y	Y	n.a.	Invasive
Oreochromis niloticus	Nile tilapia	2	Y	Y	Y	Y	n.a.	Invasive
Perca fluviatilis	Eurasian perch	1	Y	Y	Y	–	n.a.	Invasive
Pseudorasbora parva	topmouth gudgeon	2	Y	Y	–	–	n.a.	Invasive
Rhinogobius lindbergi	Lin's goby	2	N	–	–	–	N	Non-invasive
Salmo ischchan	Sevan trout	1	–	–	–	–	n.a.	Non-invasive
Salmo trutta	brown trout	2	Y	Y	Y	Y	n.a.	Invasive
Salvelinus fontinalis	brook trout	3	Y	Y	Y	–	n.a.	Invasive
Sander lucioperca	pikeperch	1	Y	Y	–	–	n.a.	Invasive
Syngnathus abaster	black-striped pipefish	1	N	N	–	–	N	Non-invasive

* Reference species for the a priori categorisation: European whitefish *Coregonus lavaretus*.

literature sources. The outcomes are a BRA score and a (composite) BRA+CCA score, which is obtained after adding or subtracting up to 12 points to the BRA score or leaving it unchanged in case of a CCA score equal to 0. Scores < 1 suggest that the species poses a 'low risk' of becoming invasive in the risk assessment area, whereas scores

≥ 1 indicate a 'medium risk' or a 'high risk'. The threshold (Thr) value to distinguish between medium-risk (BRA and BRA+CCA score < Thr) and high-risk (BRA and BRA+CCA score ≥ Thr) species for the risk assessment area is obtained by 'calibration' based on the Receiver Operating Characteristic (ROC) curve analysis (see Vilizzi et al. 2022). A measure of the accuracy of the calibration analysis is the area under the curve (AUC) whose values are interpreted as: 0.7 ≤ AUC < 0.8 = acceptable discriminatory power, 0.8 ≤ AUC < 0.9 = excellent, 0.9 ≤ AUC = outstanding (Hosmer et al. 2013). For the species ranked as high risk, a distinction was made in this study of the 'very high-risk' species, based on an ad hoc threshold weighted according to the range of high-risk scores obtained for the BRA and BRA+CCA. Identification of the (very) high-risk species is useful to prioritise allocation of resources in view of a full risk assessment (Copp et al. 2016a). This examines in detail the risks of: (i) introduction (entry); (ii) establishment (of one or more self-sustaining populations); (iii) dispersal (more widely within the risk assessment area, i.e. so-called secondary spread or introductions); and (iv) impacts (to native biodiversity, ecosystem function and services, and the introduction and transmission of diseases).

For the ROC curve analysis to be implemented, the species selected for screening must be categorised a priori as 'non-invasive' or 'invasive' using literature sources. The a priori categorisation of the species was implemented as per Vilizzi et al. (2022) (Table 1). Confidence levels in the responses to questions in the AS-ISK are ranked using a 1–4 scale and based on the confidence level allocated to each response, a confidence factor (CF) is obtained that ranges from a minimum of 0.25 (i.e. all 55 questions with confidence level equal to 1) to a maximum of 1 (i.e. all 55 questions with confidence level equal to 4). Based on all 55 Qs of the AS-ISK questionnaire, the 49 Qs comprising the BRA and the six Qs comprising the CCA, the CF_{Total}, CF_{BRA} and CF_{CCA} are respectively computed (Vilizzi et al. 2022).

Implementation of the ROC curve analysis followed the protocol described in Vilizzi et al. (2022), with the true/false positive/negative outcome distinction not applied to the medium-risk species, as they can be either included or not into a full (comprehensive) risk assessment depending on priority and/or availability of financial resources. The ROC curve fitting was in two steps. Firstly, separate ROC curves were generated for each of the three independent assessors and differences amongst the resulting three AUCs were statistically tested (Mann-Whitney U-statistic, $\alpha = 0.05$; applet StAR available at http://melolab.org/star/home.php: Vergara et al. 2008). As differences between assessor-specific AUCs were not found, in the second step, a single ROC curve was generated, based on the average species-specific BRA scores of the three assessors. Following ROC analysis, the best threshold value that maximises the true positive rate and minimises the false positive rate was determined using Youden's J statistic, whereas the 'default' threshold of 1 was set to distinguish between low-risk and medium-risk species. Fitting of the ROC curve was with package pROC (Robin et al. 2011) for R x64 v.4.0.5 (R Core Team 2021) using 2000 bootstrap replicates for the confidence intervals of specificities, which were computed along with the entire range of sensitivity points (i.e. 0 to 1, at 0.1 intervals). Differences in CF between components (i.e. BRA and BRA+CCA) were tested with permutational ANOVA. Analysis was implemented in PERMANOVA+ for PRIMER v.7, with normalisation of the data and using a Bray-Curtis dissimilarity measure, 9999 unrestricted permutations of the raw data and with statistical effects evaluated at $\alpha = 0.05$.

Results

There were no differences between the AUCs resulting from the three assessor-specific ROC curves (BJ vs. GE: $P = 0.912$; BJ vs. TK: $P = 0.090$; GE vs. TK: $P = 0.287$): this justified computation of one ROC curve based on the mean BRA scores for the screened species. Accordingly, the ROC curve resulted in an AUC of 0.8213 (0.6310–1.000 95% CI), which indicated that the risk screening was able to distinguish with excellent discriminatory power between invasive and non-invasive fish species for the risk assessment area. Youden's *J* provided the threshold of 18, which was used to calibrate the risk outcomes to distinguish between medium-risk and high-risk species. The AS-ISK report for the 32 screened species is provided as Suppl. material 1.

Based on the BRA outcome scores (Table 2, Fig. 2A):

- 21 (65.6%) species were ranked as high risk and eleven (34.4%) as medium risk;
- Amongst the nine species categorised a priori as non-invasive, one was a false positive (three-spined stickleback *Gasterosteus aculeatus*);
- Amongst the 23 species categorised a priori as invasive, 20 were true positives;
- Of the 11 medium-risk species, eight were a priori non-invasive and three invasive.
- Based on the BRA+CCA outcome scores, hence after accounting for climate change predictions (Table 2, Fig. 2B):
- 23 (71.9%) species were ranked as high risk, eight (25.0%) as medium risk and one (3.1%) as low risk (*Coregonus* sp.);
- Amongst the a priori non-invasive species, four were false positives (*Chelon auratus*, *Gasterosteus aculeatus*, Lin's goby *Rhinogobius lindbergi*, *Syngnathus abaster*) and one a true negative (*Coregonus* sp.);
- Amongst the a priori invasive species, 19 were true positives;
- Of the nine medium-risk species, four were a priori non-invasive and four invasive.

The highest-scoring ('top invasive') species (based on an ad hoc 'very high risk' threshold = 40) were gibel carp *Carassius gibelio*, North African catfish *Clarias gariepinus*, topmouth gudgeon *Pseudorasbora parva* and pikeperch *Sander lucioperca* for both the BRA and BRA+CCA, and eastern mosquitofish *Gambusia holbrooki*, three-spined stickleback *Gasterosteus aculeatus*, ruffe *Gymnocephalus cernua*, sharpbelly *Hemiculter leucisculus*, pumpkinseed *Lepomis gibbosus*, largemouth bass *Micropterus salmoides*, Nile tilapia *Oreochromis niloticus* and Eurasian perch *Perca fluviatilis* for the BRA+CCA only. Overall, the number of very high-risk species increased from four (12.5%) under the BRA to 12 (37.5%) under the BRA+CCA (Figs 2A and B). The CCA resulted in an increase in the BRA score (cf. CCA) for 26 species and in a decrease for the remaining six species (Table 2). Across the three assessors: differences in BRA scores ranged from

Table 2. Risk outcomes for the freshwater fish species screened with the Aquatic Species Invasiveness Screening Kit (AS-ISK) for the South Caucasus. For each species, the following information is provided: a priori categorisation for invasiveness (N = non-invasive; Y = invasive: see Table 1); min, max and mean Basic Risk Assessment (BRA) and BRA + Climate Change Assessment (BRA+CCA) scores with corresponding risk ranks (L = Low; M = Medium; H = High; VH = Very high, based on an ad hoc threshold = 40) and classifications (Class: FP = false positive; TN = true negative; TP = true positive; '–' = not applicable as medium risk: see text for details); difference (Delta) between mean BRA+CCA and BRA scores. Risk ranks are based on a threshold of 18 and computed as: L, with score within the interval [−20, 1[, M [1, 18[, H [18, 40[, VH [40, 68] for the BRA; L [−32, 1[; M [1, 18[, H [18, 40[, VH [40, 80] for the BRA+CCA (note the reverse bracket notation indicating in all cases an open interval).

Species name	A priori	BRA					BRA+CCA						CF		
		Score					Score								
		Min	Max	Mean	Rank	Class	Min	Max	Mean	Rank	Class	Delta	Total	BRA	CCA
Ameiurus melas	Y	26.5	35.0	31.5	H	TP	24.5	47.0	36.8	H	TP	5.3	0.70	0.71	0.61
Anguilla anguilla	Y	1.0	15.0	9.3	M	–	−5.0	17.0	8.0	M	–	−1.3	0.72	0.72	0.71
Carassius gibelio	Y	36.0	52.0	44.0	VH	TP	48.0	64.0	55.3	VH	TP	11.3	0.74	0.75	0.67
Chelon auratus	N	14.0	25.0	17.7	M	–	16.0	20.0	18.3	H	FP	0.7	0.70	0.72	0.46
Chelon saliens	N	13.0	23.0	16.7	M	–	15.0	20.0	17.3	M	–	0.7	0.65	0.68	0.42
Clarias gariepinus	Y	38.0	45.0	40.3	VH	TP	46.0	55.0	49.7	VH	TP	9.3	0.66	0.68	0.51
Coregonus albula	Y	5.0	19.5	11.2	M	–	−3.0	7.5	1.2	M	–	−10.0	0.71	0.72	0.63
Coregonus sp.	N	1.0	18.0	9.0	M	–	−7.0	6.0	−0.3	L	TN	−9.3	0.70	0.71	0.61
Ctenopharyngodon idella	Y	18.0	23.5	20.7	H	TP	14.5	31.5	24.7	H	TP	4.0	0.69	0.72	0.49
Gambusia holbrooki	Y	31.5	38.0	34.5	H	TP	37.5	48.0	43.2	VH	TP	8.7	0.70	0.72	0.51
Gasterosteus aculeatus	N	37.0	38.0	37.7	H	FP	38.0	44.0	41.0	VH	FP	3.3	0.67	0.69	0.50
Gobio artvinicus	N	5.0	14.0	8.7	M	–	7.0	15.0	12.0	M	–	3.3	0.59	0.61	0.44
Gymnocephalus cernua	Y	34.0	46.0	39.3	H	TP	46.0	58.0	50.7	VH	TP	11.3	0.63	0.65	0.50
Hemiculter leucisculus	Y	32.0	35.0	33.8	H	TP	42.0	45.0	43.8	VH	TP	10.0	0.71	0.73	0.58
Hypophthalmichthys molitrix	Y	20.5	24.0	22.8	H	TP	18.5	34.0	28.8	H	TP	6.0	0.65	0.67	0.51
Hypophthalmichthys nobilis	Y	25.5	28.0	26.8	H	TP	19.5	38.0	30.8	H	TP	4.0	0.67	0.68	0.61
Ictalurus punctatus	Y	26.0	33.0	29.0	H	TP	32.0	45.0	39.0	H	TP	10.0	0.64	0.66	0.47
Lepomis gibbosus	Y	25.5	36.0	29.8	H	TP	37.5	46.0	40.5	VH	TP	10.7	0.71	0.72	0.63
Micropterus salmoides	Y	22.0	38.5	31.2	H	TP	34.0	50.5	41.2	VH	TP	10.0	0.70	0.72	0.56
Mugil cephalus	N	6.0	22.0	11.3	M	–	12.0	18.0	14.7	M	–	3.3	0.63	0.66	0.42
Mylopharyngodon piceus	Y	20.0	24.0	22.0	H	TP	28.0	34.0	32.0	H	TP	10.0	0.66	0.69	0.44
Oncorhynchus kisutch	Y	4.0	15.0	11.2	M	–	8.0	17.0	11.8	M	–	0.7	0.64	0.66	0.50
Oncorhynchus mykiss	Y	15.0	26.5	20.2	H	TP	15.0	18.5	16.8	M	–	−3.3	0.63	0.66	0.35
Oreochromis niloticus	Y	24.0	38.0	32.7	H	TP	34.0	48.0	42.0	VH	TP	9.3	0.65	0.68	0.49
Perca fluviatilis	Y	17.0	51.0	32.0	H	TP	23.0	63.0	41.3	VH	TP	9.3	0.66	0.68	0.50
Pseudorasbora parva	Y	32.0	47.0	40.0	VH	TP	44.0	57.0	49.3	VH	TP	9.3	0.77	0.78	0.71
Rhinogobius lindbergi	N	16.0	17.5	16.5	M	–	26.0	28.0	27.2	H	FP	10.7	0.54	0.53	0.63
Salmo ischchan	N	5.0	25.0	16.7	M	–	−7.0	20.0	10.0	M	–	−6.7	0.64	0.65	0.58
Salmo trutta	Y	34.0	39.0	36.0	H	TP	31.0	40.0	36.7	H	TP	0.7	0.63	0.66	0.46
Salvelinus fontinalis	Y	17.0	33.0	23.7	H	TP	17.0	29.0	23.0	H	TP	−0.7	0.70	0.75	0.31
Sander lucioperca	Y	30.0	50.0	43.0	VH	TP	38.0	59.0	46.3	VH	TP	3.3	0.69	0.70	0.58
Syngnathus abaster	N	9.0	27.0	16.3	M	–	5.0	37.0	20.3	H	FP	4.0	0.70	0.72	0.50

0 to 34, with a mean of 11.8, a median of 11.0 and 5% and 95% CIs of 5.1 and 20.2, respectively (Fig. 3A); differences in BRA+CCA scores ranged from 4 to 35, with a mean of 16.1, a median of 13.5 and 5% and 95% CIs of 5.8 and 32.0, respectively (Fig. 3B).

In terms of confidence in responses, the mean CL_{Total} was 2.69 ± 0.03 SE, the mean CL_{BRA} 2.76 ± 0.03 SE and the mean CL_{CCA} 2.11 ± 0.07 SE (hence, indicating

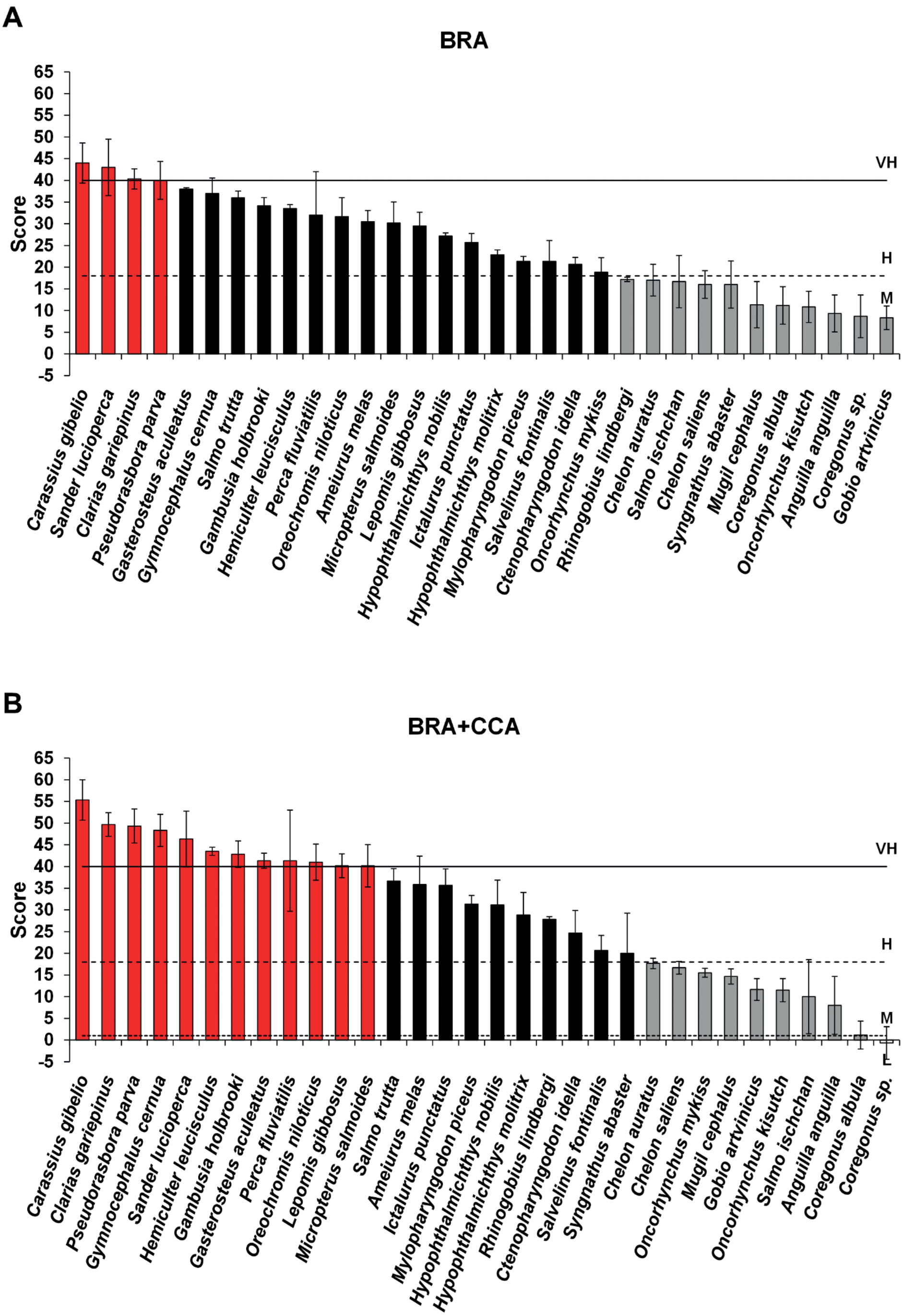
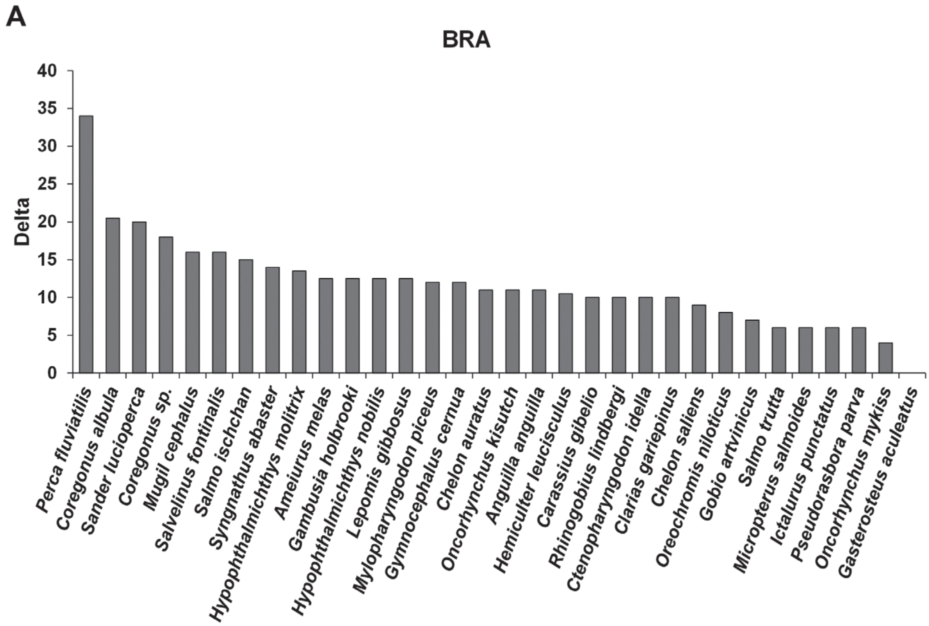


Figure 2. Aquatic Species Invasiveness Screening Kit (AS-ISK) mean outcome scores (± SE) for the species screened for the South Caucasus: **A** Basic Risk Assessment (BRA) scores **B** BRA+CCA (Climate Change Assessment) scores. Red bars = very high-risk species; Black bars = high-risk species; Grey bars = medium-risk species; White bars = low-risk (L) species. Solid line = very high-risk (VH) threshold; Hatched line = high-risk (H) threshold; Dotted line = medium-risk (M) threshold (thresholds as per Table 2).

a medium confidence level). The mean CF_{Total} was 0.673 ± 0.008 SE, the mean CF_{BRA} 0.691 ± 0.008 SE and the mean CF_{CCA} 0.527 ± 0.017 SE. Statistically, the CL_{BRA} was higher than the CL_{CCA} ($F^{\#}_{1,62}$ = 75.44, P < 0.001; # = permutational value).



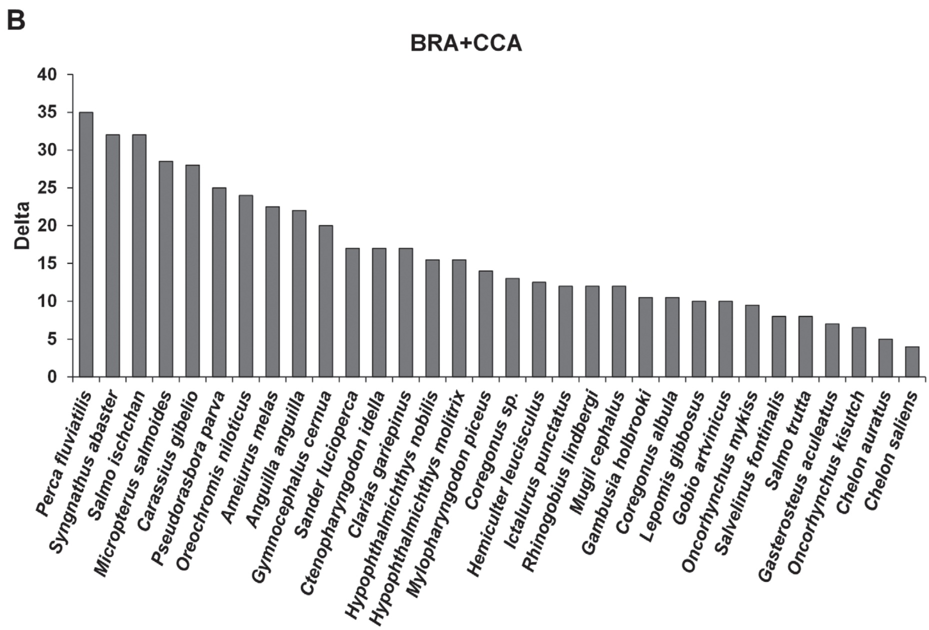


Figure 3. a Between-assessor differences in the BRA scores for the species screened for the South Caucasus **b** same for the BRA+CCA scores. See also Table 2.

Discussion

Risk outcomes

The present study, which is the first to conduct a risk screening for the South Caucasus, was able to identify with excellent discriminatory power the level of risk of invasiveness of the non-native fish species under evaluation. The calibrated threshold value (Thr = 18) in this study can, therefore, be used for future screening of additional non-native fish species in the risk assessment area, as required. Further, this threshold could be refined subject to availability of new biological data on the species screened in this study and/or additional species that may be identified as horizon or recorded in the risk assessment area by future surveys and/or based on more up-to-date climate change scenarios – this is in line with risk analysis as a dynamic, 'work-in-progress' applied field of science (see Vilizzi et al. 2021). Finally, the replication of screenings by three independent assessors in this study to account for the inherent potential bias with expert-based evaluations and the resulting lack of differences in assessor-specific AUCs has further strengthened the reliability of the species-specific risk ranks.

Amongst the screened species, 20 were ranked as carrying a high or very high risk of invasiveness under both current (BRA) and predicted climate conditions (BRA+CCA) (Table 2). These species included the false positive *Gasterosteus aculeatus*, whose high-risk ranking in the present study can, however, be justified with reference to the risk assessment area. In this respect, *G. aculeatus* is a widespread circum-arctic/temperate freshwater fish that naturally occurs in the River Danube mouth and on the Black Sea coast (Piria et al. 2018). This species has expanded its range to the Caspian Sea via the Volga-Don Channel and is now widely established along the Caspian Sea coast (eastern South Caucasus), where it actively enters the lower reaches of rivers (Ibrahimov and Mustafayev 2015). Due to its high tolerance of salinity and temperature and its reproductive and foraging characteristics (e.g. Roch et al. 2018; Candolin 2019), *G. aculeatus* can, therefore, be regarded as a high-risk species for the risk assessment area, despite its a priori non-invasive status elsewhere.

Overall, the a priori invasive species found to carry a high or very high risk of invasiveness (Table 2) may represent a threat to the native species and ecosystems of the South Caucasus. Some of these species are already established in the risk assessment area (Table 1) and, amongst these, *Carassius gibelio* and *Pseudorasbora parva* (both identified as very high risk) are already recognised as posing a serious threat (Kuljanishvili et al. 2021b). *Carassius gibelio* was introduced unintentionally (and probably several times) since the 1980s mainly as a contaminant of stockings of young-of-the-year common carp *Cyprinus carpio* in Lake Paliastomi and in the lakes of the Javakheti Plateau (Japoshvili et al. 2013; Kuljanishvili et al. 2021b). However, other species, including Chinese carps (i.e. bighead carp *Aristichthys nobilis*, *Ctenopharyngodon idella* and silver carp *Hypophthalmichthys molitrix*) and *Cyprinus carpio*, have also been intensively and mostly illegally introduced to other water bodies of the South Caucasus by local anglers for recreational purposes resulting in accidental introductions of *C. gibelio*

(Japoshvili et al. 2013; Kuljanishvili et al. 2021b). Concerning *P. parva*, this species is a typical hitchhiker without any economic value that is spreading on its own through the watercourses of the South Caucasus and is still being translocated as part of cyprinid farming practices (Gozlan et al. 2010; Kuljanishvili et al. 2021b). Overall, both species are currently the most widespread in the risk assessment area, where, in most cases, they form dense populations that dominate in abundance the local fish community (Shoniya et al. 2011; Japoshvili et al. 2013; Pipoyan and Arakelyan 2015; Kuljanishvili et al. 2021b).

A very high risk of invasiveness was also attributed to *Clarias gariepinus* and to locally translocated *Sander lucioperca*. *Clarias gariepinus* is found in neighbouring Turkey and is an invasive predator species that can easily spread once established (Ellender et al. 2015; Weyl et al. 2016). Detrimental effects of this species on the native fauna are, therefore, expected to occur in the South Caucasus as observed elsewhere (Kadye and Booth 2012; Ellender et al. 2015) and can be exacerbated by climate change (i.e. increase in temperatures) as revealed by the augmented BRA+CCA score for this species (Table 2). *Sander lucioperca* is a sought-after species with anglers that is actively translocated within the risk assessment area (Kuljanishvili et al. 2021b). This species has been reported to alter severely the invaded ecosystems in multiple ways including predation, hybridisation and disease transmission (Godard and Copp 2011). However, the species' impact on the native freshwater fauna of the risk assessment area remains unknown.

The threats posed by other established species ranked as carrying a high risk (BRA) or very high risk (BRA+CCA) of invasiveness, such as *Gambusia holbrooki*, *Gymnocephalus cernua* and *Hemiculter leucisculus* and by locally-translocated *Perca fluviatilis*, are still not clearly understood. *Gambusia holbrooki* was one of the first non-native species to be introduced in the South Caucasus for mitigation of the malaria disease (Barach 1941; Elanidze 1983). Since its introduction, this species has formed dense populations in most of the still water bodies of western Georgia and the eastern South Caucasus (Kuljanishvili et al. 2021b). The other two species *G. cernua* and *H. leucisculus* are cryptic invaders for which no invasion/establishment history exists. The former species is far more widespread in the risk assessment area than previously thought (G. Epitashvili, unpublished data), whereas the latter was first detected from the River Rioni in western Georgia in 2020 based on DNA barcoding (Epitashvili et al. 2020). *Perca fluviatilis* has been repeatedly introduced to water bodies of the South Caucasus for recreational angling, although no data are available on its introduction/establishment history, population dynamics or range of expansion (Kuljanishvili et al. 2021b). However, given its predatory lifestyle, this species can severely alter the native fish communities of the risk assessment area (review in Rowe et al. 2008).

Other species ranked as high (or very high) risk included those that are regularly stocked in the risk assessment area, but have not yet established self-sustaining populations, namely grass carp *Ctenopharyngodon idella*, *Hypophthalmichthys molitrix*, bighead carp *Hypophthalmichthys nobilis*, channel catfish *Ictalurus punctatus*, black carp *Mylopharyngodon piceus*, rainbow trout *Oncorhynchus mykiss*, Nile tilapia *Orechromis niloticus* and brown trout *Salmo trutta*. Amongst these species, *O. niloticus* is currently considered a hitchhiker in the South Caucasus, where no information on its deliberate farming is available. This species has been recorded only once in the wild (River Alazani, eastern Georgia), though no established population has been confirmed (Kuljanishvili et al. 2021a). Overall, all of the above species could pose a substantial threat to the local native ecosystems once established (e.g. Dibble and Kovalenko 2009; Martín-Torrijos et al. 2016; DeBoer et al. 2018; Faria et al. 2019), so their stocking should either be avoided altogether or strictly monitored.

The horizon species black bullhead *Ameiurus melas*, *Lepomis gibbosus*, *Micropterus salmoides* and brook trout *Salvelinus fontinalis* were also ranked as high (or very high) risk. However, neither of them has so far been recorded from the risk assessment area, although they are all well known to have expanded their range worldwide as a result of introductions for recreational and aquaculture purposes. Although a proper understanding of their impact on the invaded ecosystems is limited, these species are known to pose substantial threats to the native fish faunas (e.g. Cucherousset et al. 2008; Leunda et al. 2008; Drake 2009; Almeida et al. 2014; Copp et al. 2017).

Of the eleven species found to carry a medium risk of invasiveness (based on the BRA), vendace *Coregonus* sp., *Coregonus albula*, coho salmon *Oncorhynchus kisutch* and *Rhinogobius lindbergi* deserve some special consideration. Both *Coregonus* sp. (a putative hybrid known as *C. lavaretus sevanicus*: Dadikyan 1964, 1986) and *C. albula* have been regularly stocked in the mountainous lakes of Georgia (Javakheti Plateau) and in Lake Sevan, where they are thought to have established self-reproducing populations and are also highly valued commercially (Dadikyan 1964; Japoshvili 2012; Kuljanishvili et al. 2021b). In contrast, *O. kisutch* was released only once into the Caspian Sea in the 1980s and no further information on this species has since been available (Musayev et al. 2004). The small gobiid fish *R. lindbergi*, which is already widespread in the south-western Caspian Sea Basin (Sadeghi et al. 2019; Japoshvili et al. 2020), is not known to pose any risk to the native fauna, although this may be only a provisional expectation. This is because *R. lindbergi* resembles native gobies and occupies a similar ecological niche. Additionally, being a cryptic invader (e.g. Epitashvili et al. 2020), *R. lindbergi* may pose a threat to the native fauna; hence, it should be subject to future monitoring.

The remaining species carrying a medium risk of invasiveness are all native to the South Caucasus and translocated, except for *Mugil cephalus*. Four of these species, namely the mullets *Chelon auratus*, *Chelon saliens* and *Mugil cephalus*, as well as *Syngnathus abaster*, are primarily marine/brackish water species regularly occurring in estuaries or in the lower stretches of rivers. Mullets, which are economically valuable and naturally occur in the Black Sea, were introduced to the Caspian Sea in the early 20th century and, amongst them, *C. saliens* and *C. auratus* have established dense and abundant populations in river mouths (Bogutskaya et al. 2013). Risk screening for the latter two species has also been conducted for the neighbouring Anzali Wetland Complex (Caspian Sea Basin, Iran), with a similar medium-risk rank for *C. saliens*, but a low-risk rank for *C. auratus* (Moghaddas et al. 2021). This was mainly due to the fact that these species are known to reproduce in the Caspian Sea and to use associated estuaries and river mouths only temporarily for feeding (Coad 2017). As per *M. cephalus*, this species is currently not known to be established in the risk assessment area. However, given its high tolerance for water temperature and salinity, *M. cephalus* could reach the Caspian Sea Basin either on its own (i.e. via the Volga-Don Channel) or by translocation for aquaculture purposes (Abo-Taleb et al. 2021). The other eurihaline translocated species *S. abaster*, which is native to the Black and Caspian sea coasts (and with a threatened status in the Caspian Sea: Kolangi-Miandare et al. 2013), was introduced into Tbilisi Reservoir in the 1980s and has since resulted in the establishment of a dense population (Kuljanishvili et al. 2021b). Although not regarded as invasive, *S. abaster* is known for its ability to establish easily in freshwater habitats and affect zooplankton communities by selective feeding (Didenko et al. 2018).

The remaining translocated species ranked as medium risk included migratory European eel *Anguilla anguilla* and the resident species Artvin gudgeon *Gobio artvinicus* and *Salmo ischchan*. *Anguilla anguilla* was recorded from the Caspian Sea Basin in 1964 (Kuljanishvili et al. 2021b) and has been reported to enter various rivers of the risk assessment area, where it occurs, however, at low densities (Abdurakhmanov 1966; Ibrahimov and Mustafayev 2015; Pipoyan 2015). *Gobio arthvinicus* was unintentionally translocated from the Black Sea Basin to the River Kura, where it has most probably spread as a hitchhiker, though it is not used either for recreational or aquaculture purposes. This species is already established in the River Kura Basin (Kuljanishvili et al. 2021b) and its distribution has expanded to other watercourses. In contrast, *S. ischchan* was introduced to water bodies of Azerbaijan and Georgia from Lake Sevan and self-sustaining populations have been reported to cause negative impacts on native Caspian trout *Salmo caspius* (Elanidze 1983; Musayev et al. 2004; Yusifov et al. 2017; Kuljanishvili et al. 2021b).

Overall, under predicted climate change, 12 species in total were ranked as very high risk (Table 2, Fig. 1B). Of these species, eight are already established in the risk assessment area of which three are translocated (i.e. *Gasterosteus aculeatus*, *Perca fluviatilis*, *Sander lucioperca*) and six introduced (i.e. *Carassius gibelio*, *Gambusia holbrooki*, *Gymnocephalus cernua*, *Hemiculter leucisculus*, *Oreochromis niloticus*, *Pseudorasbora parva*) and three are horizon (i.e. *Clarias gariepinus*, *Lepomis gibbosus*, *Micropterus salmoides*). The current lack of legislation for non-native species in the South Caucasus, hence strategies for dealing with the impacts of current invasions, is therefore an issue of even higher concern given the predicted increase in number of very high risk species because of climate change (see below).

Recommendations for future research

In this study, the South Caucasus has been treated as a distinct biogeographic unit rather than a politically defined entity at the country level, hence in line with the preferred approach to the definition of a risk assessment area (Vilizzi et al. 2022). This is because the Kura-Aras Basin is shared amongst all the South Caucasus countries of Armenia, Azerbaijan and Georgia, making establishment of invasive non-native species in any of them likely to result in future potential ecological impacts for all three countries. In addition, the Kura-Aras Basin is also shared between Turkey and the South Caucasus and the same is true for the River Chorokhi between Turkey and western Georgia.

Consequently, any evaluation of the impact of potentially invasive non-native species and the establishment of regulations for their introduction and management should ideally be agreed upon and implemented across all of the South Caucasus countries (if not beyond: cf. Turkey), rather than independently for each of them. Unfortunately, the South Caucasus biodiversity hotspot is also a 'geopolitical hotspot' subject to permanent military tensions, which are likely to be exacerbated in recent times (Muradov 2022). For this reason, it is hardly possible to communicate and discuss the outcomes of environmental issues not only between countries (i.e. Armenia vs Azerbaijan), but also at the country level (e.g. 20% of the territory of Georgia is inaccessible due to Russian occupation), with the result that these long-unresolved political tensions are further aggravating the extent of non-native species management plans across the South Caucasus.

In the European Union, policies, legislation and management approaches have been developed to address the issue of non-native species, based on Regulation (EU) no. 1143/2014 of the European Parliament and of the Council on the prevention and management of the introduction and spread of invasive alien species (Piria et al. 2017, 2021). However, countries outside of the EU do not have an obligation to follow these rules and usually lack national legislation, which represents an additional problem for non-native species management (Piria et al. 2021). Whilst there is no region-wide agreement/management policy related to non-native aquatic species, there is also a major lack of relevant national-level legislation in the three South Caucasus countries (Kuljanishvili et al. 2021b). These all are parties to the Convention on Biological Diversity (CBD: https://www.cbd.int/) and Georgia is, in addition, a party to two other conventions, namely the Convention on the Protection of the Black Sea Against Pollution (http://www.blacksea-commission.org/) and the Convention for the Control and Management of Ships' Ballast Water and Sediments (https://www.imo.org/en). Within these Conventions (and particularly under the CBD), the South Caucasus countries are regularly developing National Biodiversity Strategy and Action Plans (https://www.cbd.int/nbsap/) in which the lack of data/infrastructure dealing with invasive non-native species is being emphasised and relevant targets (e.g. development of invasive non-native species lists, identification of introduction pathways, evaluation of impacts, implementation of legislative measures) are being proposed. However, none of the targets related to (freshwater) non-native species has so far been fulfilled (see, for example, the fifth national reports for Armenia, Azerbaijan and Georgia: https://www.cbd.int/).

Overall, to date, none of the South Caucasus countries has achieved a clear understanding of non-native species management within a national legislation plan (Kuljanishvili et al. 2021b), so that the import/farming of aquatic non-native species for recreational/aquaculture purposes is simply allowed under permission of governmental bodies. In fact, there is no legislative means for banning any particular aquatic non-native species (including highly invasive ones), checking for hitchhikers or restricting translocations. It is, therefore, strongly advocated that both country- and region-wide (i.e. South Caucasus) strategies, legislation acts and related actions for freshwater non-native species should be urgently developed. In this respect, it is recommended that such a strategy should adopt the following overarching conceptual goals:

1. Full risk assessment of any potentially invasive species should focus on those ranked as high risk (or very high risk, depending on availability of resources). Thus, whenever possible, a comprehensive risk screening, as achieved in this study, should be conducted and species-specific risk-rank outcomes presented to decision-makers. In this study, 12 very high-risk species in total (after accounting for climate change predictions) were identified that should be prioritised for follow-up full risk assessment.
2. Knowledge gap analysis and improvement of the legal basis for species introductions related to aquaculture/game fisheries and the pet trade. Ideally, this should be jointly agreed upon by the SCR countries to be fully effective.
3. Early detection and communication of freshwater non-native species is a process already under way, with researchers publishing results about new introductions of potentially invasive non-native species and citizen science platforms regularly receiving data from the general public on the identification of new non-native species. However, data and knowledge developing over time must be standardised in order to be rapidly communicated to stakeholders and decision-makers. In addition, adequate measures should be taken to enhance data collection from all potential sources. For instance, there is currently no information on non-native species available from local markets.
4. Continuous development of an in-depth monitoring scheme (including infrastructure for data collection based on fieldwork, barcoding/metabarcoding approaches, data management and presentation). This is a critical step to understand the history of non-native species colonisation and the accompanying processes related to community perception, including associated costs for damage/mitigation.
5. Prevention of introductions (cf. 'blacklists' of species). Since there is a large amount of data on freshwater non-native species worldwide, it would be straightforward to develop a list of potentially invasive non-native species for the South Caucasus (see Roy et al. 2019). In this regard, risk screening could help further refinement of the taxa list and via a similar exercise given in the present study. Control of the introduction of the most (if not all) threatening species could then be implemented by the local governments. As such practice already exists elsewhere (Essl et al. 2011; Gederaas et al. 2012; Poeta et al. 2017; Battisti et al. 2019), it should be discussed and adopted also within the countries of the South Caucasus.
6. Impact assessment research, including that related to already established invasive non-native species. Currently, there is no evaluation of the economic/environmental costs related to freshwater non-native species, nor for terrestrial ones. This makes it difficult to assess the effects of non-native species on local communities and to manage in an optimal way available resources to prevent/mitigate non-native species introductions. The results of this study can, therefore, be used to prioritise the list of fish species in the South Caucasus to be evaluated for impact assessment.

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Supplementary material 1

Combined AS-ISK report including the 96 screenings for the 32 fish species screened for the South Caucasus

Authors: Levan Mumladze, Tatia Kuljanishvili, Bella Japoshvili, Giorgi Epitashvili, Lukáš Kalous, Lorenzo Vilizzi, Marina Piria

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Taxonomy & Inventories

Ponticola alasanicus sp. n. (Gobiiformes, Gobiidae) from the Alazani River Basin, Georgia

Giorgi Epitashvili[‡], Bella Japoshvili[‡], Levan Mumladze[‡]

‡ Ilia State University, Tbilisi, Georgia

Corresponding author: Giorgi Epitashvili (giorgi_epitashvili@iliauni.edu.ge)

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Abstract

Background

The South Caucasus Region and Georgia, in particular, is a biodiversity hotspot and characterised by high diversity of landscapes and ecosystems, as well as high levels of endemism. At the same time, diversity of freshwater organisms in the region remains poorly studied, including fishes. The freshwater fish fauna of the South Caucasus Region consists of 119 fish species, of which 13 species belong to the order Gobiiformes. It should be noted that gobies are amongst the poorly studied taxa in Georgia and probably unknown/undescribed species still living in the Georgian freshwater ecosystems which requires further research.

New information

Ponticola alasanicus, a new species is described from the Alazani River, western Caspian Sea Basin, Georgia. It is distinguished from its congeners in the Caspian and Black Sea Basins by having the following features: dorsal fin with VI-VII spines and 15½-16½ branched rays, anal fin with 10½-12½ branched rays; lateral line with 48-55 scales;

laterally compressed body with dark brown and black blotches - scales ctenoid; first and second dorsal fins almost touching with dorsal fins bases; head large, depressed, wider than deep, its length approaches almost 3.4th of standard length; nape scaled completely; cycloid scales cover upper part of opercle, cheeks noticeably swollen; snout longer than eye, eye diameter 4.5 times its head length; lower jaw slightly protruding; upper lip is uniform; pelvic disc short, elongated and flat, not reaching the anus; the pectoral fins extends vertically through first branched dorsal fin; caudal fin rounded. *Ponticola alasanicus* sp. n. belongs to *P. syrman* group and it is separated by a minimum Kimura 2-parameter distance of 3.5, 3.6 and 4.8% from *P. syrman*, *P. iranicus* and *P. patimari*, respectively.

Keywords

Ponticola alasanicus sp. n., freshwater gobies, DNA barcoding, taxonomy, Western Caspian Sea Basin

Introduction

The family Gobiidae Cuvier, 1816 is one of the most diverse group of vertebrates and includes 257 valid genera with more than 2000 species (Fricke et al. 2022). Gobies are small-sized fish that mostly inhabit saline and brackish waters; few of them are found in freshwater habitats around the world (Kottelat and Freyhof 2007, Tutman et al. 2020). In Europe, freshwater gobies are most diverse in the Mediterranean, Black and Caspian Sea Basins (Kottelat and Freyhof 2007). The Black and Caspian Seas belong to the Ponto-Caspian biogeographical region (Vasil'eva et al. 2015), part of which is recognised as a Caucasus biodiversity hotspot (Zazanashvili et al. 2020). The freshwater fish fauna of the South Caucasus Region consists of 119 fish species, of which 13 species belong to the order Gobiiformes, which are united into two families and seven genera (Kuljanishvili et al. 2020). The genus *Ponticola* Iljin, 1927 is restricted to the Black and Caspian Sea Basins and does not enter the Marmara or Mediterranean Seas (Freyhof 2012), though the species *P. kessleri* has reached the northern seas and river systems via artificial channelling (Thiel et al. 2017). Within the South Caucasus and particularly in Georgian freshwaters, the genus *Ponticola* is most numerous and represented by four valid species: *P. constructor* (Nordmann, 1840), *P. cyrius* (Kessler, 1874), *P. gorlap* (Iljin, 1949) and *P. syrman* (Nordmann, 1840) (Kuljanishvili et al. 2020). Within this genus, six more species have been described in the last decades from the Caucasus and Irano-Anatolian biodiversity hotspots, including *P. rhodioni* (Vasil'eva & Vasil'ev 1994a) from the rivers flowing into the Black Sea, north of the Bzyb' Mountain Range and in the Kuban Basin, *P. rizensis* (Kovačić and Engín 2008) and *P. turani* (Kovačić and Engín 2008) from the south-eastern Black Sea Basin; *P. iranicus* Vasil'eva, Mousavi-Sabet & Vasil'ev, 2015; *P. patimari* Eagderi, Nikmehr & Poorbagher, 2020 and *P. hircaniaensis* Zarei, Esmaeili, Kovačić, Schliewen & Abbasi, 2022 from the southern Caspian Sea Basin (*Vasil'eva and Vasil'ev 1994b*, Kovačić and Engín 2008, Vasil'eva et al. 2015, Eagderi et al. 2020, Zarei et al. 2022).

During a recent field collection event in 2020 in eastern Georgia, we collected goby specimens from the Alazani River, which were preliminarily identified as *P. cyrius*. However, later CO1 DNA barcoding showed substantial differences with other known *Ponticola* species from Georgia and surrounding regions. Based on further detailed investigation of the collected material, in the following, we decide to provide a description of a new *Ponticola* species from eastern Georgia.

Study area

The Alazani River is one of the largest in the Kakheti Region (eastern Georgia), which currently drains into the Mingachevir Reservoir. The first detailed ichthyological research on this river was conducted in the 1950s and 20 fish species were recorded (Elanidze 1951). The same author noted that there were no native goby species found in the Alazani River and, in his view, the distribution area of Kura goby - *P. cyrius* did not reach as far as Alazani. Since then, sporadic ichthyological investigations have been conducted (Epitashvili et al. 2020) and there are about 30 fish species currently known for the river, including the invasive Amur goby – *Rhinogobius lindbergi* Berg, 1933.

All along the Alazani River, floodplain riparian forests have developed where small streams originate drained from the surrounding mountains. Such tributaries are often called “Psha” amongst Georgian locals (which means cold clear stream). The water temperatures in these Pshas are constant throughout the year and thus create perfect living conditions for many aquatic species. The biodiversity of these streams is generally poorly understood.

Materials and methods

Data collection

During the regular ichthyological fieldwork in August 2020, one of those Psha, Tsitsmatiant Psha, was sampled and specimens of gobies of the genus *Ponticola* were collected. Fish were caught using the electrofishing device EFGI 650 (http://www.electric-fishing.de/efgi6_e.html) under a permission from the Ministry of Agriculture and Nature Protection of Georgia (permission number: 5768/01). In total, five specimens of gobies were collected in the location and after anaesthesia with MS-222 of a subsample of the collected fishes, a fin-clip was taken and stored in 99.9% molecular grade ethanol for molecular genetic study and specimens were fixed in 70% ethanol for morphological investigation. In 2020-2022, we also conducted three additional field surveys in different seasons at the Tsitsmatiant Psha and collected an additional 15 goby specimens and preserved these in the same way. The collected specimens are kept in the collection of Hydrobiology and Ichthyology Department of the Institute of Zoology of Ilia State University under the instituional code HID-ISU.

DNA barcoding

The genetic study was conducted under the ongoing project - Caucasus Barcode of Life (CaBOL - https://ggbc.eu/) with standard CaBOL protocol. In particular, genomic DNA was extracted from pectoral fin clips using a BioSprint96 magnetic bead extractor (Qiagen, Hilden, Germany). PCRs, targeting the standard DNA barcode region COI, were carried out in 20 µl reaction volumes including 2 µl undiluted DNA template, 0.8 µl of each primer (10 pmol/µl; LCO1490-JJ: 5'-CHACWAAYCATAAAGATATYGG-3' and HCO2198-JJ: 5'-AWACTTCVGGRTGVCCAAARAATCA-3', (Astrin and Stüben 2008, Astrin et al. 2016)), 2 µl 'Q-Solution' and 10 µl 'Multiplex PCR Master Mix' (Qiagen, Hilden, Germany). Cycler temperatures for PCR reaction: thermal conditions included denaturation at 95°C for 1 min, followed by first cycle set (15 cycles): 94°C for 30 sec, annealing at 55°C for 1 min (−1°C per cycle) and extension at 72°C for 1:30 min. Second cycles set (25 cycles): 94°C for 35 sec, 45°C for 1 min, 72°C for 1:30 min, followed by one cycle at 72°C for 3 min and final extension step at 72°C for 5 min. Purification of PCR products and bidirectional sequencing were conducted at either BGI (Hong Kong, China) or Macrogen Europe Laboratories using the amplification primers. Voucher specimens are kept in the ichthyological collections at the Institute of Zoology of Ilia State University (ISU). Extracted genomic DNA is deposited in the ISU Biobank.

Molecular data analysis

Data processing and sequence assembly were performed using the Geneious Pro v.7 (Drummond et al. 2011) and the Muscle algorithm (Edgar 2004) was used to align the DNA barcodes after screening for indels or stop codons (Epitashvili et al. 2020). All newly-generated DNA sequences with acceptable quality (with less than 1% ambiguous bases and free of stop codons) were submitted to the Barcode of Life Data System (BOLD, http://v4.boldsystems.org/), including relevant metadata, where they were automatically assigned Barcode Index Numbers (BINs). The sequences will be accessed via the dataset "Freshwater Fishes of Georgia" (SCCBO) in the BOLD Systems.

In addition to the newly-generated DNA barcodes, we included all published DNA barcodes of *Ponticola* spp. deposited at BOLD and NCBI GenBank (Table 1) that originated from Georgia and the Ponto-Caspian biogeographic region. As outgroups, *Neogobius fluviatilis* (accession number: MW564554) and *Proterorhinus* sp. (accession number: MW564522) were retrieved from GenBank (Epitashvili et al. 2020).

Table 1.

List of public CO1 sequences used for molecular analyses taken from BOLD and NCBI GenBank with information on Accession Number, drainage and country of origin.

Species	**Drainage**	**Published by**	**GenBank/BOLD Acc. N.**
Ponticola kessleri	Elbe River (Germany)	Thiel et al. (2017)	KX857485
P. kessleri	Elbe River (Germany)	Thiel et al. (2017)	KX857486
P. ratan	Sea of Azov (Ukraine)	Neilson and Stepien (2009)	FJ526848
P. cephalargoides	Dniester River (Ukraine)	Neilson and Stepien (2009)	FJ526813

Species	Drainage	Published by	GenBank/BOLD Acc. N.
P. rhodioni	Vostochnyy Dagomys River (Russia)	Neilson and Stepien (2009)	FJ526842
P. eurycephalus	Odessa Bay (Ukraine)	Neilson and Stepien (2009)	FJ526814
P. syrman	Caspian Sea, Kiashahr beach (Iran)	Zarei et al. (2021)	MW393618
P. syrman	Caspian Sea, Babolsar beach (Iran)	Zarei et al. (2021)	MW393617
P. gorlap	Volga River (Russia)	Karabanov et al. (2022)	MT833801
P. gorlap	Caspian Sea (Azerbaijan)	Neilson and Stepien (2009)	FJ526817
P. constructor	Galitsga River, Black Sea basin (Georgia)	Epitashvili et al. (2020)	MW564519
P. constructor	Kintrishi River, Black Sea basin (Georgia)	Epitashvili et al. (2020)	MW564390
P. cyrius	Kura River (Georgia)	Epitashvili et al. (2020)	MW564540
P. cyrius	Aragvi River (Georgia)	Epitashvili et al. (2020)	MW564261
P. iranicus	Caspian Sea, Siah Darvishan River (Iran)	Zarei et al. (2021)	MW393605
P. iranicus	Caspian Sea, Siah Darvishan River (Iran)	Zarei et al. (2021)	MW393606
P. patimari	Caspian Sea, Chalous (Iran)	Eagderi et al. (2020)	MW256419
Neogobius fluviatilis	Rioni River, Black Sea basin (Georgia)	Epitashvili et al. (2020)	MW564554
Proterorhinus sp.	Kintrishi River, Black Sea basin (Georgia)	Epitashvili et al. (2020)	MW564522

Subsequently, we evaluated sequence divergence and relationships between *Ponticola* spp, based on uncorrected *p*-distance. A Neighbour-Joining tree based on K2P (Kimura 2-parameter model) distances with 1000 bootstrap replicates was constructed to visualise the phylogenetic relationships amongst specimens (Fig. 1). Analyses were performed using MEGA 11 software (Tamura et al. 2021) and statistical tools provided by BOLD Systems (Ratnasingham and Hebert 2007).

Morphological examinations

All measurements were made using a digital caliper with a precision of 0.1 mm. We used a character set commonly used for the study of the Caucasian gobies (Vasil′eva and Vasil′ev 1994a). For counts and meristic and plastic measurements, we also followed methodologies given in Kottelat and Freyhof (2007), Armbruster (2012), Vasil'eva et al. (2015) and Eagderi et al. (2020). The last two branched rays articulating on a single pterygiophore in the second dorsal (D2) and anal (A) fins are noted as “1½”. For the lateral line system, we used terminology from Miller (1986). We calculated mean values and standard deviations for the sample, including the holotype and four paratypes, on all morphometric characters for comparative morphological analysis (see Table 2).

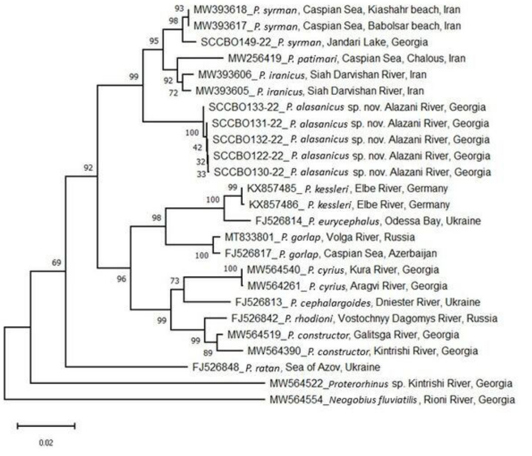

Figure 1. doi

Phylogenetic relationships are presented by the Neighbour-Joining tree, based on the mitochondrial COI barcode region using the Kimura 2 Parameter (K2P) distance model with other default parameters provided by Mega 11 software. Numbers near nodes indicate bootstrap support values from 1000 replicates. The analyses involved 25 COI nucleotide sequences of 14 goby species from the Black, Azov and Caspian Sea Basins.

Table 2.

Morphometric characters (ranges, means and standard deviations) of type specimens of *Ponticola alasanicus* sp. n.

	Holotype + Paratypes (n = 5)		
	Holotype	Paratypes	
Character in mm		Range	Mean±SD
Total body length	73.5	52.0-73.5	65.7±7.86
Standard body length	61.0	42.0-61.0	54.6±7.23
Number of anal fin branched rays	12.0	10.5-12.5	11.7±0.81
Number of the second dorsal fin branched rays	16.0	15.0-16.0	15.5±0.32
In percent of SL			
Maximum body depth	23.8	21.6-24.6	23.1± 1.22
Least depth of caudal peduncle	12.9	12.0-14.3	13.04±0.76
Predorsal distance	36.2	35.6-39.3	36.82±1.36
Length of the second dorsal fin base	32.9	29.4-33.3	32.38±1.50
Height of the second dorsal fin	14.4	11.8-14.4	13.04±1.02
Pre-anal distance	57.4	56.7-60.8	58.44±1.71
Length of anal fin base	24.6	21.6-24.6	22.82±1.19

	Holotype + Paratypes (n = 5)		
	Holotype	Paratypes	
Length of pectoral fin	27.5	22.5-27.5	24.78±1.86
Length of ventral disc	19.7	16.7-19.7	17.96±1.09
Caudal peduncle length	18.0	17.1-19.0	18±0.64
Width of caudal peduncle at the anal fin	6.6	6.6-8.5	7.6±0.67
Minimum width of caudal peduncle	4.1	3.9-4.9	4.38±0.40
Head length	29.5	27.1-32.1	29.28±1.66
In percent of HL			
Horizontal diameter of eye	22.2	22.2-25.6	23.24±1.25
Pre-orbital distance	28.3	28.3-31.2	29.7±0.94
Postorbital distance	60.6	55.6-60.6	58.3±2.12
Interorbital distance	16.7	14.8-17.5	16.56±0.93
Width of upper lip	45.0	44.1-48.1	45.66±1.52
Head depth at nape	50.0	43.3-53.1	47.92±3.62
Head width	75.0	66.7-81.2	74.54±4.72

Comparative materials: ***Ponticola syrman***: ISU - SCCBO149-22 – 1007278, 168 mm SL, 205 mm TL, 1, Georgia: Caspian Sea Basin, Jandari Lake, Kvemo Kartli Region, N 41.438641 E 45.208572.

Ponticola cyrius: ISU - HIDPcy1, 54-99 mm SL, 65-122 mm TL, 5, Georgia: Caspian Sea Basin, Kvabliani River, Samtskhe-Javakheti Region, N 41.656865 E 42.776685.

ISU - HIDPcy10, 41-105 mm SL, 51-126 mm TL, 5, Georgia: Caspian Sea Basin, Kura River, Samtskhe-Javakheti Region, N 41.322334 E 43.229994.

ISU - HIDPcy13, 74 mm SL, 91 mm TL, 1, Georgia: Caspian Sea Basin, Aragvi River, Mtskheta-Mtianeti Region, N 42.035527 E 44.749659.

Ponticola constructor: ISU – HIDPco2, 47-77 mm SL, 57.5-92 mm TL, 6, Georgia: Black Sea Basin, Tskaltsitela River, Imereti Region, N 42.269336 E 42.741677.

ISU - HIDPco8, 46-69 mm SL, 56.5-85 mm TL, 3, Georgia: Black Sea Basin, Sulori River, Imereti Region, N 42.081774 E 42.522275.

ISU - HIDPco11, 40-88.5 mm SL, 46-104.5 mm TL, 3, Georgia: Black Sea Basin, Charnali River, Adjara Region, N 41.582625, E 41.625455.

Abbreviations used: TL, total length; SL, standard length; HL, lateral head length.

Collection codes: ISU – HIDPcy2, Ilia State University – Hydrobiology and Ichthyology Department.

Data resources

In total, we obtained mitochondrial cytochrome oxidase subunit I (CO1) sequences from five specimens of *Ponticola alasanicus* sp. n. collected in the Tsitsmatiant Psha stream (right tributary of the Alazani River). For molecular analysis, we downloaded COI sequences of *P. cyrius*, *P. constructor*, *P. syrman*, *P. gorlap*, *P. iranicus*, *P. patimari*, *P. kessleri*, *P. ratan*, *P. rhodioni*, *P. cephalargoides* and *P. eurycephalus* (Table 1). COI sequences of *Neogobius fluviatilis* and *Proterorhinus* sp. from the Georgian Black Sea Basin (Epitashvili et al. 2020) were used as outgroups (Fig. 1). A Neighbour-Joining phylogenetic tree, based on COI sequences, placed *Ponticola alasanicus* sp. n. in a group that includes *P. syrman*, *P. iranicus* and *P. patimari*, making it the fourth species, in the so-called *P. syrman* group (for more details see Eagderi et al. (2020)). The new species is separated by a minimum K2P distance of 3.5, 3.6 and 4.8%, from *P. syrman*, *P. iranicus* and *P. patimari*, respectively (Table 3).

Table 3.

Estimates of evolutionary divergence (%) over sequence pairs between *Ponticola* species distributed in the south-western Caspian and Georgian Black Sea Basins. Analyses were conducted using the Kimura 2-parameter model.

Species	**N**	1	2	3	4	5	6	7
P. syrman	**1**							
P. alasanicus sp. n.	**2**	3.52						
P. constructor	**3**	7.22	8.25					
P. cyrius	**4**	7.86	8.47	4.96				
P. gorlap	**5**	7.01	8.03	6.78	6.58			
P. iranicus	**6**	1.89	3.59	7.74	8.17	7.63		
P. patimari	**7**	2.94	4.77	7.84	8.27	7.41	1.95	

In the following, we provide a morphological description of the new *Ponticola* species.

Taxon treatment

Ponticola alasanicus, sp. n.

- ZooBank 5BC69115-FABA-4455-B10F-E3D3E28207AA
- https://pubmed.ncbi.nlm.nih.gov/36177146/

Materials

Holotype:

a. scientificName: *Ponticola alasanicus* Epitashvili, Japoshvili & Mumladze, 2023; originalNameUsage: *Ponticola alasanicus*; taxonID: SCCBO130-22 – 1007251; kingdom: Animalia; phylum: Chordata; class: Actinopterygii; order: Gobiiformes; family: Gobiidae; taxonRank: Species; vernacularName: Alazani goby; genus: *Ponticola*;

scientificNameAuthorship: Epitashvili, Japoshvili and Mumladze 2023; locationID: Alazani River; higherGeographyID: Kakheti Region; higherGeography: Caucasus; continent: Eurasia; waterBody: Western Caspian Sea Basin; country: Georgia; countryCode: GE; stateProvince: Kakheti; county: Telavi; municipality: Telavi; locality: Shakriani; verbatimLocality: Telavi-Eniseli connecting bridge; maximumElevationInMeters: 347; verbatimLatitude: 41.988808; verbatimLongitude: 45.577726; samplingEffort: 1; eventDate: 11-Aug-2020; year: 2020; month: August; day: 11; habitat: River; individualID: HIDPal1; individualCount: 1; sex: male; lifeStage: adult; preparations: fin clip; establishmentMeans: native; catalogNumber: HIDPal1; occurrenceRemarks: Captured during the electro fishing; recordedBy: Giorgi Epitashvili; occurrenceStatus: present; previousIdentifications: *Ponticola cyrius*; associatedSequences: BOLD:AEO718; identifiedBy: Giorgi Epitashvili; dateIdentified: Bella Japoshvili; Levan Mumladze; identificationRemarks: DNA barcoding; type: PhysicalObject; language: EN; rightsHolder: Ilia State University; institutionID: ISU Ilia State University; collectionCode: Actinopterygii; ownerInstitutionCode: ISU; occurrenceID: 90162319-7839-579C-9EB1-E1F5C8E0D687

Paratypes:

a. scientificName: *Ponticola alasanicus* Epitashvili, Japoshvili & Mumladze, 2023; originalNameUsage: *Ponticola alasanicus*; taxonID: SCCBO122-22 – 1007242; kingdom: Animalia; phylum: Chordata; class: Actinopterygii; order: Gobiiformes; family: Gobiidae; taxonRank: Species; vernacularName: Alazani goby; genus: *Ponticola*; locationID: Alazani River; higherGeographyID: Kakheti Region; higherGeography: Caucasus; continent: Eurasia; waterBody: Western Caspian Sea Basin; country: Georgia; countryCode: GE; stateProvince: Kakheti; municipality: Telavi; locality: Shakriani; verbatimLocality: Telavi-Eniseli connecting bridge; maximumElevationInMeters: 347; verbatimLatitude: 41.988808; verbatimLongitude: 45.577726; samplingEffort: 1; eventDate: 11-Aug-2020; year: 2020; month: August; day: 11; habitat: River; individualID: HIDPal1; individualCount: 1; preparations: fin clip; establishmentMeans: native; catalogNumber: HIDPal1; occurrenceRemarks: Captured during the electro fishing; recordedBy: Giorgi Epitashvili; occurrenceStatus: present; previousIdentifications: *Ponticola cyrius*; associatedSequences: BOLD:AEO718; identifiedBy: Giorgi Epitashvili; identificationRemarks: DNA barcoding; type: PhysicalObject; language: EN; rightsHolder: Ilia State University; institutionID: ISU Ilia State University; collectionCode: Actinopterygii; ownerInstitutionCode: ISU; occurrenceID: 503A9C0F-959F-5928-BBB4-2D352EADA131

b. scientificName: *Ponticola alasanicus* Epitashvili, Japoshvili & Mumladze, 2023; originalNameUsage: *Ponticola alasanicus*; taxonID: SCCBO131-22 – 1007252; kingdom: Animalia; phylum: Chordata; class: Actinopterygii; order: Gobiiformes; family: Gobiidae; taxonRank: Species; vernacularName: Alazani goby; genus: *Ponticola*; locationID: Alazani River; higherGeographyID: Kakheti Region; higherGeography: Caucasus; continent: Eurasia; waterBody: Western Caspian Sea Basin; country: Georgia; countryCode: GE; stateProvince: Kakheti; municipality: Telavi; locality: Shakriani; verbatimLocality: Telavi-Eniseli connecting bridge; maximumElevationInMeters: 347; verbatimLatitude: 41.988808; verbatimLongitude: 45.577726; samplingEffort: 1; eventDate: 11-Aug-2020; year: 2020; month: August; day: 11; habitat: River; individualID: HIDPal1; individualCount: 1; preparations: fin clip; establishmentMeans: native; catalogNumber: HIDPal1; occurrenceRemarks: Captured during the electro fishing; recordedBy: Giorgi Epitashvili; occurrenceStatus: present; previousIdentifications: *Ponticola cyrius*; associatedSequences: BOLD:AEO718; identifiedBy: Giorgi Epitashvili; identificationRemarks: DNA barcoding; type: PhysicalObject; language: EN; rightsHolder: Ilia State University; institutionID: ISU Ilia State University; collectionCode: Actinopterygii; ownerInstitutionCode: ISU; occurrenceID: EB90F6D3-169C-56EB-9E5B-68C90403BEDE

c. scientificName: *Ponticola alasanicus* Epitashvili, Japoshvili & Mumladze, 2023; originalNameUsage: *Ponticola alasanicus*; taxonID: SCCBO132- 22 – 1007253; kingdom: Animalia; phylum: Chordata; class: Actinopterygii; order: Gobiiformes; family: Gobiidae; taxonRank: Species; vernacularName: Alazani goby; genus: *Ponticola*; locationID: Alazani River; higherGeographyID: Kakheti Region; higherGeography: Caucasus; continent: Eurasia; waterBody: Western Caspian Sea Basin; country: Georgia; countryCode: GE; stateProvince: Kakheti; municipality: Telavi; locality: Shakriani; verbatimLocality: Telavi-Eniseli connecting bridge; maximumElevationInMeters: 347; verbatimLatitude: 41.988808; verbatimLongitude: 45.577726; samplingEffort: 1; eventDate: 11-Aug-2020; year: 2020; month: August; day: 11; habitat: River; individualID: HIDPal1; individualCount: 1; preparations: fin clip; establishmentMeans: native; catalogNumber: HIDPal1; occurrenceRemarks: Captured during the electro fishing; recordedBy: Giorgi Epitashvili; occurrenceStatus: present; previousIdentifications: *Ponticola cyrius*; associatedSequences: BOLD:AEO718; identifiedBy: Giorgi Epitashvili; identificationRemarks: DNA barcoding; type: PhysicalObject; language: EN; rightsHolder: Ilia State University; institutionID: ISU Ilia State University; collectionCode: Actinopterygii; ownerInstitutionCode: ISU; occurrenceID: D59DD48D-957E-5FA7-BCB6-CAD685B8C637
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Other materials:

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sex: female; lifeStage: adult; preparations: fin clip; reproductiveCondition: pregnant; establishmentMeans: native; catalogNumber: HIDPal2; occurrenceRemarks: Captured during the electro fishing; recordedBy: Giorgi Epitashvili; occurrenceStatus: present; previousIdentifications: *Ponticola cyrius*; associatedSequences: BOLD:AEO718; identifiedBy: Giorgi Epitashvili; identificationRemarks: Morphology & DNA barcoding; type: PhysicalObject; language: EN; rightsHolder: Ilia State University; institutionID: ISU Ilia State University; collectionCode: Actinopterygii; ownerInstitutionCode: ISU; occurrenceID: 60686D75-310B-5299-86C3-4F1010A91CFA

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Description

Holotype: ISU - SCCBO130-22 – 1007251; Male, 73.5 mm TL, 61.0 mm SL; Georgia, Kakheti Region, Western Caspian Sea Basin, Tsitsmatiant Psha, right tributary of the Alazani River, near the Telavi-Eniseli connecting bridge, N41.988808, E45.577726; (collecting date: 11-Aug-2020).

Paratypes: ISU - SCCBO122-22 – 1007242, ISU - SCCBO131-22 – 1007252, ISU - SCCBO132- 22 – 1007253, ISU - SCCBO133-22 – 1007254, 52.0 – 71.0 mm TL, 42.0 – 59.0 mm SL, same data as holotype.

Additional materials used in morphological analyses: ISU - HIDPcy2, male, 71.0 mm TL, 58.5 mm SL; ISU – HIDPal2, female, 45.0 mm TL, 38.0 mm SL; ISU – HIDPal2, female, 69.0 mm TL, 57.0 mm SL; ISU – HIDPal2, female, 63.0 mm TL, 52 mm SL; HID – Pal2, male, 74.0 mm TL, 59.0 mm SL, same data as holotype/ paratypes. **Remark**: these specimens were captured during resampling in 26-Dec-2020 and 30-May-2022 in the same location.

Description: D_1 VI, D_2 I / 16½, A I / 12½, l.l. 53. Body laterally compressed, gradually tapering from head to tail; minimum body depth almost 1.4 times less than caudal peduncle length; lateral parts of the body covered by ctenoid scales; first and second dorsal fins almost touching with their bases; second dorsal fin of uniform height; head large, depressed, wider than deep, its length approaches almost 3.4th its SL; nape scaled completely; cycloid scales cover upper part of opercle, cheeks noticeably swollen; snout longer than eye; eye diameter 4.5 times its HL; lower jaw slightly protruding; upper lip is uniform; pelvic disc elongated, not concave but flat, its length about 1.6 times less than ventro-anal distance, not reaching anus; pectoral fins reach base of first dorsal branched ray; caudal fin rounded.

The colouration of the preserved holotype yellowish-grey with dark brown and black blotches on the body (Fig. 2). The upper part of the head is dark, the throat white with yellow-marble colouration; the same colouration is present in the upper and lower parts of the whole body; on the last part of the caudal peduncle, there is a dark spot in the shape of a cross; the predorsal area is linearly concave in the middle with dark brown and black scales; vertically arranged uneven spots follow the lateral sides of the whole body; ventral disc with white/marble colouration, pectoral, dorsal, anal and caudal fins yellowish-grey; first and second dorsal-fin with some brown stripes and blotches; first three unbranched dorsal fin rays with dark stripes and, in upper part, transparent/white; dark brown spots on the upper part of the pectoral fin base. Body proportions and morphometric characters are given in Table 2.

Figure 2. doi

Ponticola alasanicus sp. n. holotype; ISU - SCCBO130-22 – 1007251, male, 73.5 mm TL, Georgia, Tsitsmatiant Psha (Alazani River Basin). Scale = 1 cm.

Extended description based on all type specimens: Overall meristic characteristics: D_1 VI-VII, D_2 I / 15½-16½, A I / 10 ½-12½; l.l. 48-55. All studied specimens have a relatively short body, laterally compressed at caudal peduncle, the maximum length (TL) was fixed as 73.5 mm in holotype; 15½-16½ branched dorsal rays; 10½-12½ branched anal rays; scales in lateral line series from 48 to 55; minimum body depth is 1.3–1.5 times its caudal peduncle length; head relatively large, depressed, wider than deep, its length 3.1-3.7 times its SL; head width slightly larger than depth at nape, interorbital distance short from 2.0 to 3.0 mm; lower jaw slightly protruded, upper and low jaws same length; lips almost uniform, upper lip slightly swollen; pelvic disc short, elongated and flat, not reaching anus. In our samples, the largest specimen was a male with 59.0 mm SL and 74.0 mm TL. Sexual dimorphism is not pronounced, except that males are slightly longer than females.

Colouration: Live specimens have yellowish-grey colour with dark brown and black blotches on the body (Fig. 3). Some goby species, for example, *P. hircaniaensis* (Zarei et al. 2022) distributed in the southern Caspian Basin have a similar colouration. Studied specimens after fixation in 96% molecular grade ethanol are usually yellowish-grey with dark brown and black blotches on the body. Some specimens also have brownish colouration on the head. The abdomen is white/marbled. Pelvic disc with white/grey colouration; pectoral, dorsal, anal and caudal fins yellowish-grey; first and second dorsal-fin with some brown/dark or grey stripes and blotches; first dorsal fin ray with dark stripes and, in upper part, transparent/white in some specimens; dark brown spots on the upper part of the pectoral fin base.

Figure 3. doi

Live specimen of *Ponticola alasanicus* sp. n. from the type location.

Head lateral-line system in *Ponticola alasanicus* sp. n. is similar to the other known species from the genus *Ponticola* (Fig. 4): neuromast organs form seven transverse rows, four (*1-4*) before, one (*5*) in parallel and two (*6* and *7*) above hyomandibular row *b*, row 7 consists of several papillae before anterior oculoscapular pore α; three (C, C_1 and C_2) under PN and lacking row *a*. One hyomandibular row (S_1) above PN and another one (S_2) above AN, respectively. Lacking papillae row *5i* and *6i*. Row *5* and *6* long, papillae row (*O*) above oculoscapular pore γ. Sub-orbital longitudinal row *d* consists of three parts: the anterior d_1 oblique, following the border of the upper lip and not reaching below the anterior origin of d_2, the posterior longitudinal row d_2 and d_3 are related to each other. Anterior and posterior oculoscapular canals and pre-opercular canals present with pores σ, λ, κ ω, α, β, ρ, θ, τ and γ, δ, ε, respectively. Anterior oculoscapular pore ρ and posterior oculoscapular pore θ well-separated, row X_1 between them, row X starts from pore β and reaches pore θ.

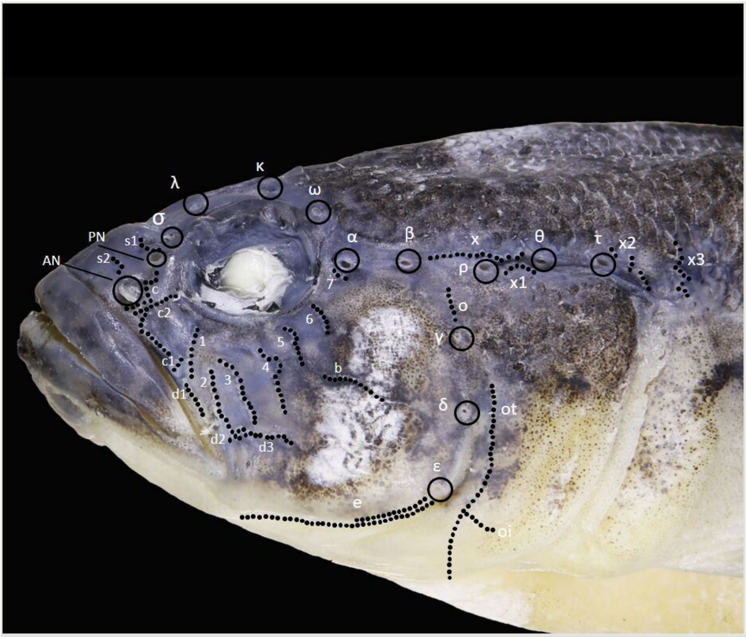

Figure 4. doi

Diagram of head lateral-line sensory system in the holotype of *Ponticola alasanicus* sp. n.; pores are designated by Greek letters, rows of neuromasts (genipores/sensory papillae) by Latin letters and Arabic numerals; AN - anterior nostril, PN - posterior nostril.

Diagnosis

D_1 – VI, D_2 I / 15½-16½, A I / 10½ – 12½; nape scaled completely, scales cycloid and ctenoid, cycloid scales covering upper part of opercle; lateral line system with sub-orbital row *d* continuous; predorsal area is linearly concave in middle; first dorsal fin with oblique black stripe between first two or three rays, the tip of the first 3-4 rays is transparent white; species has one large dark spot at the base of the pectoral fin; ventral disc has oval/elongated shape, short, not reaching anus.

Differential diagnosis: *Ponticola alasanicus* sp. n. is distinguished from other species of the genus *Ponticola* entering freshwater habitats in the Caspian Sea Basin by the following characters:

It can be distinguished from *P. syrman* by fewer scales in mid-lateral series (48-55 vs. 57-67+2-3); relatively short body (maximum size 73.5 mm TL vs. 220 mm TL); cycloid scales on the nape (vs. ctenoid scales); the ventral disc is slightly shorter (16.7 -19.7 vs. 19.0-20.0% SL) and shorter pre-anal distance (56.7-60.8 vs. 61.3-64.4% SL); *P. alasanicus* sp. n. does not have transverse infraorbital papillae rows below longitudinal hyomandibular row *b* (vs. *P. syrman* has three rows).

Ponticola alasanicus sp. n. is distinguished from *P. iranicus* by relatively small body-size (42.0-61.0 mm SL vs. 65.0-83.0 mm SL); fewer scales in lateral series (48-55 vs. 55-60); pectoral fin reaching to second dorsal fin (vs. not reaching); minimum body depth almost 1.4 times less than caudal peduncle length (vs. 1.5-1.9 times in *P. iranicus*); interorbital distance particularly large from 1.3 to 1.5 mm in eye diameter compared to 0.3-0.8 mm in *P. iranicus*; absence of transverse infraorbital papillae rows bellow longitudinal hyomandibular row *b* (vs. *P. iranicus* has two rows: *5i* and *6i*).

Ponticola alasanicus sp. n. differs from *P. patimari* by a ventral disc that is noticeably shorter (16.7-19.7 vs. 20.2-26.0% SL), not reaching anus (vs. reaching in some specimens of *P. patimari*); additionally, there are seven transverse rows in *P. alasanicus* sp. n. comapred to six transverse rows in *P. patimari*).

Ponticola alasanicus sp. n. is also distinguished from the recently described *P. hircaniaensis* Zarei, Esmaeili, Kovačić, Schliewen & Abbasi, 2022 from the southern Caspian Sea Basin by a lower number of scales in the mid-lateral series (48-55 vs. 52-59) and a relatively short head depth at nape (43.3-53.1 vs. 70.9-81.0%).

Ponticola alasanicus sp. n. is distinguished from *P. gorlap* by having a longer maximum body depth (21.6-24.6 vs. 15.0-18.1% SL); a longer least depth of caudal peduncle (12.0-14.3 vs. 7.8-9.4% SL); cycloid scales on the nape and predorsal area (vs. ctenoid scales in *P. gorlap*); less scales in mid-lateral series (48-55 vs. 68-72 + 3-4); interorbital distance large (1.3-1.5 times vs. 0.8-0.9 times in eye diameter) and noticeably small body length (vs. TL in *P. gorlap* is almost three times larger).

Ponticola alasanicus sp. n. differs from *P. cyrius* by having an elongated and flattened ventral disc (vs. round and concave ventral disc); anterior membrane of ventral disc with shallow, slightly excised lobes (vs. deeply cut and angular in *P. cyrius*); *P. alasanicus* sp. n. has longer least depth of caudal peduncle (12.0-14.3 vs. 9.4-10.0% SL); shorter head width (66.7-81.2 vs. 81.4-100.0%); predorsal area with dark brown and black colouration (vs. marbled).

Ponticola alasanicus sp. n. is distinguished from all other *Ponticola* species (e.g. *P. constructor*, *P. rhodioni*, *P. rizensis* and *P. turani*) occurring in the Caucasian Black Sea Basin by following characters: *P. alasanicus* has fewer branched rays in dorsal fin 15½-16½ vs. 16½-19½ in *P. constructor* and *P. rhodioni* and 17½-19½ in *P. turani* and *P. rizensis*, respectively. *P. alasanicus* is also easily distinguished from the aforementioned gobies by noticeably short body (maximum reported SL = 61 mm) and elongated, short and flat-shaped ventral disc (vs. round and concave, with relatively long size ventral disc).

Etymology

The species is named after the Alazani River.

Distribution

Ponticola alasanicus sp. n. is endemic to the Alazani River Basin and currently known from the type locality (Tsitsmatiant Psha) and also from the small swamps near Shakriani (N42.001151 E45.600603) and Mshvidobiani (N41.76147 E46.130432) villages. Most likely inhabiting similar tributaries and habitats of the aforementioned river basin.

Ecology

The new species inhabits small to medium-sized rivers called “Psha” (which means clear, cold water in Georgian and which can be used for drinking) with sand and plant substrate (Fig. 5). These rivers rise from the ground and are typical for the Mtkvari, Alazani and Iori River lowlands.

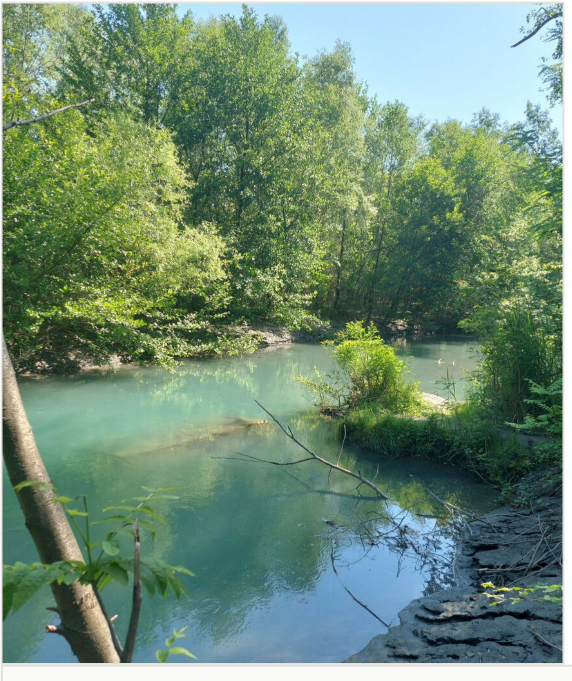

Figure 5. doi

Type location - Tsitsmatiant Psha stream, right tributary of the Alazani River, typical habitat of *Ponticola alasanicus* sp. n.

Conservation

The new species has a small range (i.e. less than 20 km^2) and is known only from the restricted area of the Alazani River Basin. The surrounding area of the known occurrences is within agricultural land where extensive use of pesticides and fertilisers is common. In addition, water intake from the river and illegal fishing are frequent (personal observation). Thus, we assume strong anthropogenic impacts on the habitat. Currently, the population trend is unknown. However, due to its extremly small range and high anthropogenic pressure, the conservation status of *Ponticola alasanicus* sp. n. can be regarded as vulnerable (IUCN 2012).

Biology

Inhabits lowland rivers and swampy habitats with sand and plant bottom. Often found near submerged trees and roots. Feeds on aquatic invertebrates. Spawns in May-June.

Discussion

Research on the diversity of freshwater fishes of the South Caucasus Region and particularly in Georgia has been done with more or less intensity in past years. Recent molecular-genetic studies (Epitashvili et al. 2020) and annotated checklist of the freshwater fishes of the South Caucasus Region (Kuljanishvili et al. 2020) have revealed several new species as well as taxonomic rearrangement in freshwater fish fauna of the region. For instance, less than half of the fish species distributed in Georgian inland waters were studied with the aid of molecular genetics methods. Accordingly, it is expected that the fish diversity in Georgia is much higher and this study is one example of that. Therefore, the description of new species is to be expected in the future. Freshwater ecosystems and their organisms are amongst the most vulnerable in the world as well as in Georgia and further studies, including the use of genetic methods, will be important in order to fully understand the diversity of Georgian fishes which will also help us in their protection.

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Author contributions

GE, BJ and LM developed the conception and design of the study, collected material and analysed data. LM supported the research logistics. GE and LM drafted manuscript. All authors participated in revising the article and contributed with intellectual content. All authors gave final approval of the submitted version.

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Research Article

New records and range expansions of invasive fish species in Georgia

Giorgi Epitashvili, Bella Japoshvili and Levan Mumladze
Institute of Zoology, Ilia State University, Tbilisi, Georgia
Corresponding author: Levan Mumladze (levan.mumladze@iliauni.edu.ge)

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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

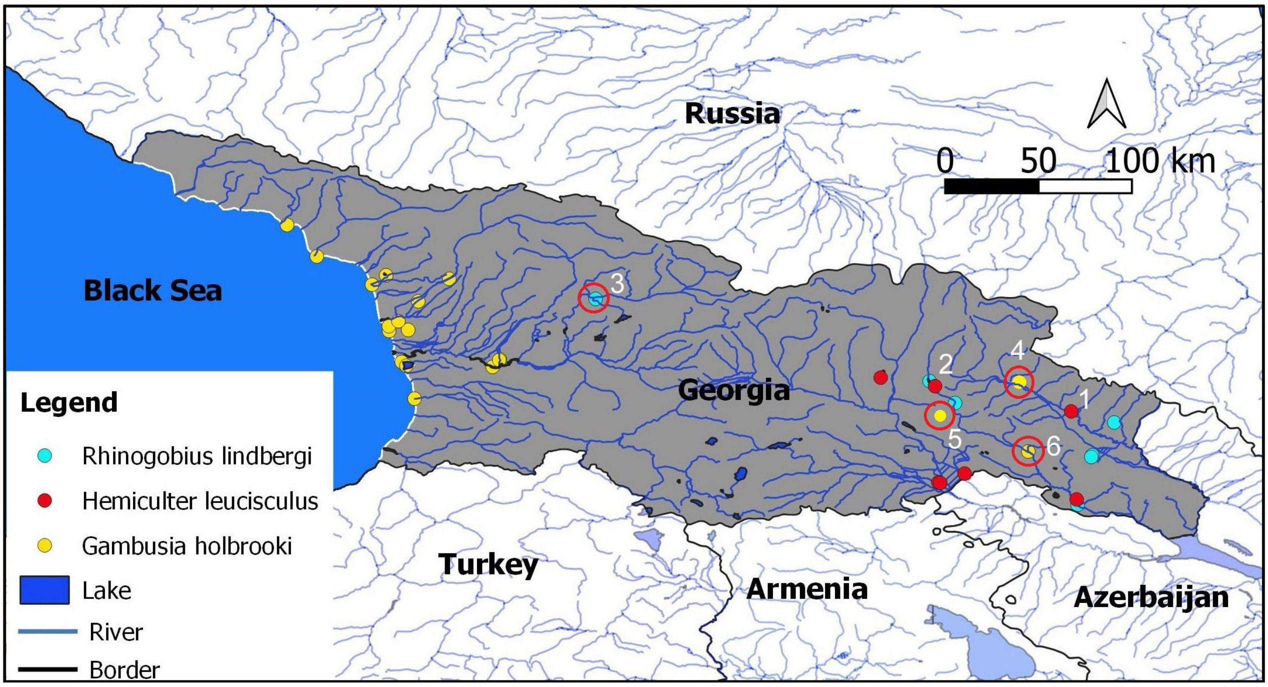

Figure 1. Map of Georgia with sample collection localities marked with colored dots. The red circles border new places where the *G. holbrooki* and *R. lindbergi* were observed for the first time. Particular localities mentioned in the main text are denoted with numbers: 1 – Alazani River (41.831858N; 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:
http://www.reabic.net/journals/bir/2023/Supplements/BIR_2023_Epitashvili_etal_SupplementaryMaterial.xlsx

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Research Article

Distribution, community composition and vulnerability of fish species in the Algeti River Basin (Georgia)

Giorgi Epitashvili[1], Giorgi Ramazashvili[2], Bella Japoshvili[1], Levan Mumladze[1]

1 *Ilia State University, Institute of Zoology, Tbilisi, Georgia*
2 *The National Environment Agency, Ministry of Environmental Protection and Agriculture of Georgia, Tbilisi, Georgia*
Corresponding author: Giorgi Epitashvili (giorgi_epitashvili@iliauni.edu.ge)

Abstract

This study presents the first comprehensive survey of fish species' diversity and distribution in the Algeti River and its reservoir in Georgia, a region within the Caucasus biodiversity hotspot. The research, conducted between 2016 and 2022, identified 19 fish species, including nine native to the Kura River basin and ten introduced or invasive species. The construction of a hydro power plant (HPP) dam in the 1980s and other subsequent anthropogenic activities have significantly altered the river ecosystem, creating distinct upstream, reservoir, and downstream sections with varying fish communities.

The study reveals a concerning ongoing impact of anthropogenic factors on the Algeti River's fish fauna and whole ecosystem. The dam impedes fish migration; in addition, pollution, particularly in the mid-downstream section, has led to habitat degradation and a decline in native species. The introduction of non-native species, including predatory and bioturbator species, poses a substantial threat to the native fish populations.

Our findings highlight the urgent need for conservation action to mitigate these threats. While the river's headwaters are within a protected area and some species are on the Red List of Georgia, alone these designations are insufficient safeguard. Effective conservation strategies must include stricter enforcement against illegal activities like poaching, as well as better pollution control and habitat management to preserve the Algeti River's biodiversity. This baseline study provides critical data for future research and conservation planning in this ecologically important region.

Key words: Artificial barriers, Freshwater, HPP dam, Invasive, Reservoirs, South Caucasus

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Introduction

According to the World Wide Fund for Nature's 2021 report, freshwater habitats occupy just 1% of the Earth but support almost 19000 fish species, or over 50% of the world's fish diversity (WWF, 2021; Fricke et al. 2025). Freshwater fisheries are the main source of protein for 200 million people in Asia, Africa, and South America and provide jobs and livelihoods for millions of families. In addition, freshwater fish stocks provide two of the world's largest global industries: recreational fishing, which generates 100 billion US dollars annually, and the aquarium fish trade, which has an annual turnover of 30 billion US dollars as aquarium fish are the most popular pets in the world (WWF, 2021). In addition to the fisheries, freshwater ecosystems and their biodiversity represent a valuable natural resource from economic, cultural, scientific, and educational perspectives (Strayer, 2010). It is probably not surprising that the freshwater ecosystems are being degraded at a faster rate than any other terrestrial ecosystems (Dudgeon et al. 2006) due to multiple factors, most of which are of human origin. The non-exhaustive list of these factors includes water pollution from domestic waste, sewage, industrial waste, gravel mining and the construction of hydropower plants. In addition, flow modification and fragmentation, water abstraction and overconsumption, the introduction of non-native and invasive species, as well as poaching, all impact on freshwater ecosystems (Simon et al. 1995; Kuylenstierna et al. 1998; Leprieur et al. 2008; Gherardi 2010; Arthington et al. 2016). As a result, freshwater biodiversity is declining at an alarming rate, populations of vertebrates in freshwaters have declined by 80% over the past 50 years; twice the amount of decline in marine and terrestrial ecosystems (Darwall et al. 2018).

Freshwater fish species are seriously threatened in many parts of the world, the main causes include habitat modification, invasive species, pollution, and overfishing (Miller et al. 1989; Leidy and Moyle 1998). Of the 117 native fish species found in the South Caucasus region's freshwaters (including migratory species), 12% are considered threatened according to the IUCN Red List (https://www.iucnredlist.org); the status of four species are defined as vulnerable (VU), one species as endangered (EN), and nine species as critically endangered (CR) (Kuljanishvili et al. 2020; Epitashvili et al. 2020; Freyhof et al. 2021; Epitashvili et al. 2023a). Determining risk assessments of freshwater habitats, conservation planning, development of fisheries management tools, and conservation improvement are important challenges for scientists, managers and citizens, especially in light of existing threats and given future climate change scenarios (Arthington et al. 2016). The threats and challenges mentioned above are particularly prominent in the developing regions of the world, which include the so-called biodiversity hotspots in the Caucasus region, and in particular, in Georgia. Although Georgia is a small country (up to 70,000 km^2), it is rich in freshwater ecosystems and resources. It has a dense network of rivers draining into the Black and Caspian Seas and over 640 lentic freshwater bodies (Abkhazava 1975), most of which are found in Eastern Georgia. In addition, there are 34 irrigation and nine hydropower reservoirs in Georgia (Ninua et al. 2013; Berdzenishvili 2017).

One of these Georgian rivers, the Algeti, is located in the Kvemo Kartli region (Caspian Sea basin), within the territorial boundaries of Tetritskaro and Marneuli municipalities (South-Eastern Georgia) (Fig. 1). The river originates on the southern slopes of the Lesser Caucasus mountains, in the Algeti National Park, 1900 metres above sea level (Gamma Consulting LLC 2016). The total length of the river is 118 km, and the catchment area is about 762 km^2 (Lomsadze et al. 2016). The Algeti River is notable for the abundance of tributaries; it has 188 tributaries with a total length of 508 km (Gamma Consulting LLC 2016). Despite the extent of the river and its tributaries, the fish fauna of the Algeti River has never been studied and documented. In the early 1980s, an Algeti HPP was built on the river. In the absence of any prior studies of the fish communities, it is not possible to quantify the impact of the dam and reservoir on the fish community or on the ecosystems of the river.

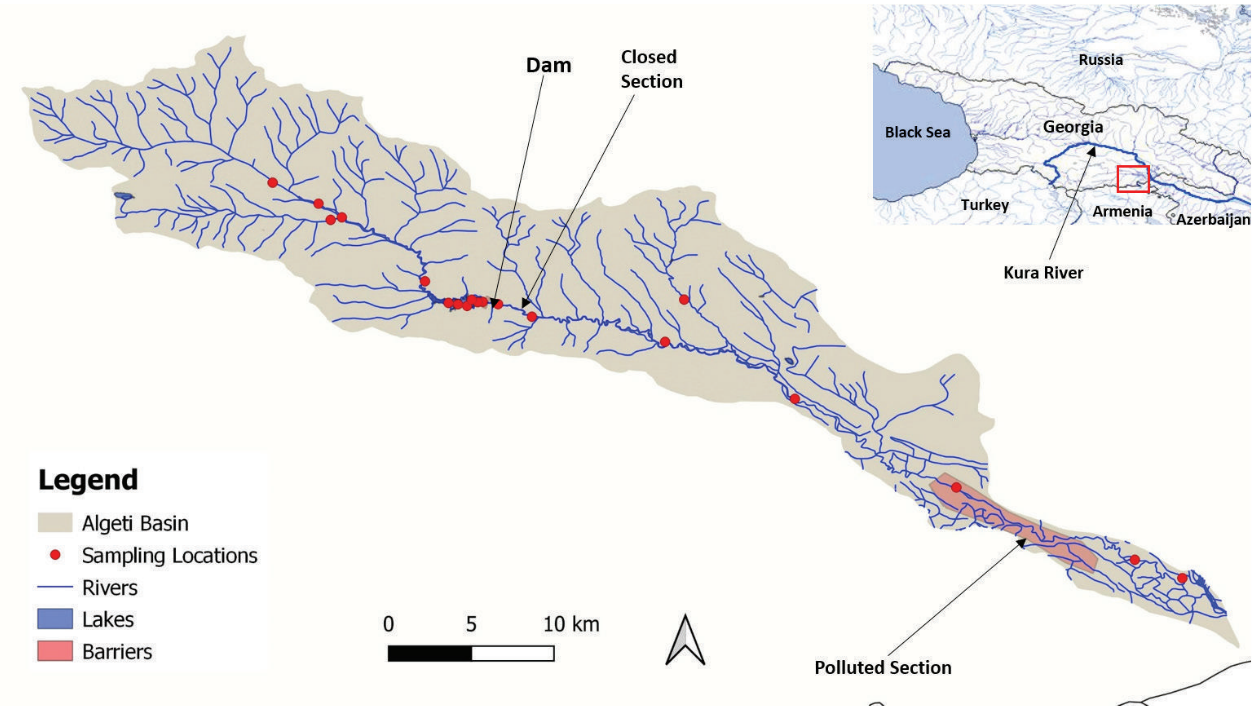


Figure 1. Algeti River basin with sampling locations.

Given these circumstances, our research aimed to establish a baseline of fish species diversity and distribution in the Algeti River and reservoir. An attempt has also been made to evaluate the impact of the dam on fish species' distribution and identify other anthropogenic factors affecting the river ecosystems.

Materials and methods

Fish were collected from the Algeti River between 2016 and 2022 from 17 localities. The aim of the fish sampling was to make an inventory of fish species occurring in the river, as well as to observe the distribution pattern of species above and below the dam and in the reservoir. Accordingly, the sampling was optimised to cover as much of the study area as possible (Fig. 1; Suppl Mater 1). Fish samples were collected in accordance with the requirements of Georgian legislation (Order No. 21 of the Minister of Environmental Protection of Georgia; October 17, 2011; Tbilisi; "On approval of the rules for fishing for scientific-research purposes") (https://matsne.gov.ge). Depending on the characteristics of the habitat, the following fishing equipment was used: an electric fishing device - EFGI 650 (http://www.electric-fishing.de/index_e.html) in streams and rivers (shallow waters), cast and frame nets, and fishing rods. All specimens were identified in the field to species level using standard morphological characters (e.g., Kottelat and Freyhof 2007) and then released back to the water. After anaesthesia with MS-222, only a few specimens (1 to 10) for each species were preserved in 96% molecular grade ethanol for further morphological investigation in the laboratory, museum collection, and DNA barcoding purposes. As no barcoding results have yet been generated, this topic will not be discussed further in this manuscript.

Table 1. Fishes of Algeti River and Algeti Reservoir.

N	Common name	Scientific name	IUCN	RLG	UpS	Res	DoS
1	Kura barbell	*Barbus cyri* (De Filippi, 1865)	NE	NE	+	–	+
2	South Caspian sprilin	*Alburnoides eichwaldii* (De Filippi, 1863)	LC	NE	+	+	+
3	Kura loach	*Oxynoemacheilus brandtii* (Kessler, 1877)	DD	NE	+	–	+
4	Khramulya	*Capoeta capoeta* (Güldenstädt, 1773)	LC	NE	+	+	+
5	Caspian trout	*Salmo caspius* (Kessler, 1877)	NE	VU	+	–	–
6	Kura chub	*Squalius agdamicus* (Kamensky 1901)	NE	NE	+	+	+
7	Common carp	*Cyprinus carpio* (Linnaeus, 1758)	VU	NE	–	+	–
8	Gibel carp	*Carassius gibelio* (Bloch, 1782)	NE	NE	–	+	+
9	European catfish	*Silurus glanis* (Linnaeus, 1758)	LC	NE	–	+	–
10	River perch	*Perca fluviatilis* (Linnaeus, 1758)	LC	NE	–	+	–
11	Rainbow trout	*Oncorhynchus mykiss* (Walbaum, 1792)	NE	NE	–	–	+
12	Golden loach	*Sabanejewia aurata* (De Filippi, 1863)	LC	VU	–	–	+
13	Colchic gudgeon	*Gobio caucasicus* (Kamensky, 1901)	LC	NE	–	–	+
14	Stone moroko	*Pseudorasbora parva* (Temminck & Schlegel 1846)	LC	NE	–	+	+
15	European bitterling	*Rhodeus amarus* (Bloch, 1782)	LC	NE	–	–	+
16	Kura bleak	*Alburnus filippii* (Kessler, 1877)	LC	NE	–	+	+
17	Sharpbelly	*Hemiculter leucisculus* (Basilewsky, 1855)	LC	NE	–	–	+
18	Pike-perch	*Sander lucioperca* (Linnaeus, 1758)	LC	NE	–	+	–
19	Monkey goby	*Neogobius fluviatilis* (Pallas, 1814)	LC	NE	–	–	+

+ / - indicates the presence and absence of a species in a specific section of the study area. IUCN – International Union for Conservation of Nature; RLG – Red List of Georgia; UpS – Upstream Section; Res – Reservoir; DoS – Downstream section; EX – Extinct; EW – Extinct In The Wild; CR – Critically Endangered; EN – Endangered; VU – Vulnerable; NT – Near Threatened; LC – Least Concern; DD – Data Deficient; NE – Not Evaluated.

Results

Fish diversity in the Algeti Basin

A total of up to 350 individuals, representing 19 species across 19 genera and 10 families, were recorded in the Algeti River and reservoir (Table 1 and Suppl Mater 1). Of these, nine species are native to the Kura River basin (Fig. 2), while six (*Cyprinus carpio*, *Silurus glanis*, *Sander lucioperca*, *Perca fluviatilis*, *Gobio caucasicus*, and *Neogobius fluviatilis*) were introduced from Western Georgia (Black Sea drainage), and four (*Pseudorasbora parva*, *Carassius gibelio*, *Hemiculter leucisculus*, and *Oncorhynchus mykiss*) are invasive (Fig. 3) in the Caucasus region (Kuljanishvili et al. 2020, 2021). Notably, *N. fluviatilis*, not native to the Caspian Sea drainage, was widely introduced into Armenia in the late 20th century (Pipoyan and Tigranian 1998). Its first record in Eastern Georgia was in 2021 in the Debeda River, near the Georgia-Azerbaijan border (own unpublished data); thus, this Algeti record is the second evidence of its expanded range in the region. This species likely spread into the Algeti independently from Armenian populations or was introduced as a hitchhiker with *P. fluviatilis* from Western Georgia, a common, though often illegal, practice by anglers. Among the invasive species in the Algeti River and reservoir, *O. mykiss* appears to have escaped from a fish farm downstream of the Algeti HPP dam. The other invasives were likely introduced by anglers. The HPP dam on the Algeti River lacks a fish pass, isolating upstream populations from downstream ones. Furthermore, the reservoir's mass of water creates distinct habitats absent in the river. Consequently, the Algeti River basin can be divided into three relatively

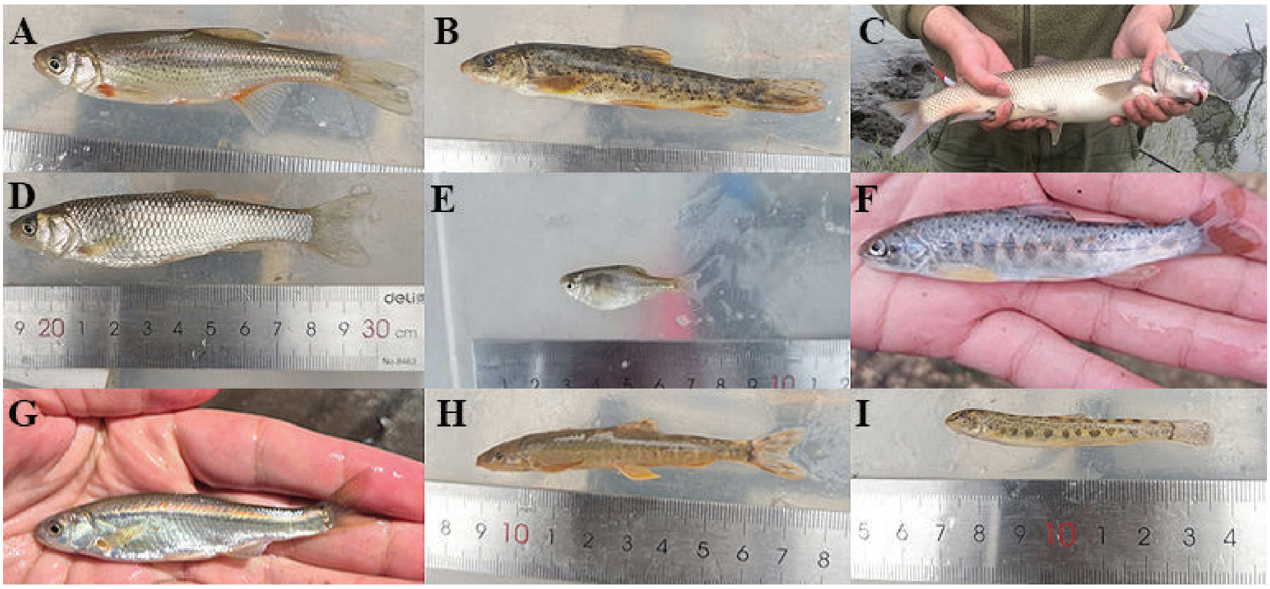


Figure 2. Native fish species to the Algeti River basin. **A** – Kura spirlin (*Alburnoides eichwaldii*); **B** – Kura barbell (*Barbus cyri*); **C** – Khramulya (*Capoeta capoeta*); **D** – Kura chub (*Squalius agdamicus*); **E** – European bitterling (*Rhodeus amarus*); **F** – Caspian trout (*Salmo caspius*); **G** – Kura bleak (*Alburnus filippii*); **H** – Kura loach (*Oxynoemacheilus brandtii*); **I** – Golden loach (*Sabanejewia aurata*). © Epitashvili G and Ramazashvili G.

Figure 3. Introduced/invasive species to the Algeti River basin. **A** – Gibel carp (*Carassius gibelio*); **B** – Common carp (*Cyprinus carpio*); **C** – Monkey goby (*Neogobius fluviatilis*); **D** – Sharpbelly (*Hemiculter leucisculus*); **E** – Stone moroko (*Pseudorasbora parva*); **F** – Colchic gudgeon (*Gobio caucasicus*); **G** – Rainbow trout (*Oncorhynchus mykiss*); **H** – Zander (*Sander lucioperca*); **I** – European perch (*Perca fluviatilis*); **J** – Wels catfish (*Silurus glanis*). © Epitashvili G and Ramazashvili G.

isolated parts: (1) the upstream section above the reservoir, (2) the reservoir itself, and (3) the downstream section below the reservoir (Fig. 4).

Upstream section of the Algeti River

The upstream Algeti section spans from the river's headwaters in Algeti National Park, situated on the Trialeti ridge's southern slopes near Kldekari Castle, to the Algeti Reservoir. The reservoir's variable water level marks the lower boundary, or confluence point. The Algeti river's headwaters, within the National Park, are well-protected from human impact. This upper section features a steep, narrow riverbed, largely covered by boulders and large sandstones, and with a fast flow rate. Near Manglisi village, the riverbed's slope decreases, and the speed of flow becomes more reduced. Here, the riverbed widens, and the water temperature significantly rises due to the approximately 900-metre elevational drop from the headwaters. Seasonal floods contribute to substantial downstream accumulation of debris, sludge, and household waste. This section is home to six fish species (Table 1); notably, *Salmo caspius* is the only species found within the National Park, with its downstream limit located a few kilometers upstream from the reservoir (N41.627245, E44.479057). *Alburnoides eichwaldii* and *Barbus cyri* are prevalent throughout the upstream section, excluding the headwaters; *Capoeta capoeta* ranges from the reservoir to Algeti village (N41.668247, E 44.391128), while *Oxynoemacheilus brandtii* is found only near the Manglisi bridge (N41.675471, E44.379027), and *Squalius agdamicus* is restricted to the section from the reservoir to near Abrameti village (N 41.604459, E 44.488717), at 800 metres above sea level. Notably, this marks its upper distribution limit within the river.

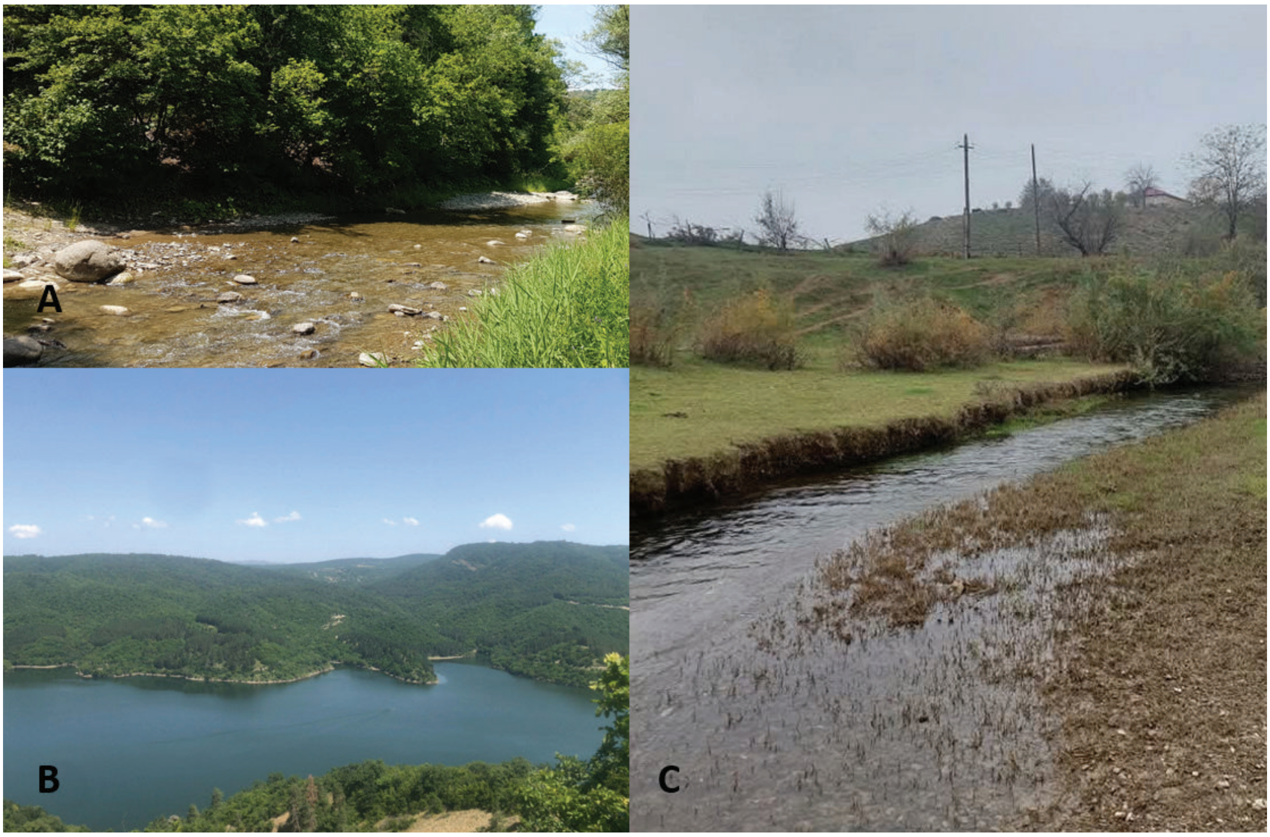
The Reservoir

The Algeti Reservoir was commissioned in 1983, primarily to store water for irrigation. At maximum water level, the reservoir's surface area reaches 2.3 km², and its maximum depth is about 42 m. Locals report that prior to the dam's construction, this section of the river was known for its abundant fish, with poaching being common. The lack of a fish passage on the dam has severely disrupted fish migration between upstream and downstream areas.

A survey of the reservoir revealed ten fish species (Table 1). The most abundant species caught in the Algeti Reservoir is *Perca fluviatilis* (33.5% of the total catch), introduced from Western Georgia. Four other species, *Carassius gibelio*, *Squalius agdamicus*, *Capoeta capoeta*, and *Silurus glanis*, are also common, each representing 10-17% of the total catch (Fig. 5). The remaining species, *Sander lucioperca*, *Cyprinus carpio*, *Alburnoides eichwaldii*, *Alburnus filippii*, and *Pseudorasbora parva*, are relatively rare, comprising less than 2% of the total catch.

Downstream section of the river

The downstream section of the river begins at the dam and extends to Kesalo II village in the Marneuli municipality, where it joins the Mtkvari (Kura) River. Water is discharged from the reservoir through three main pipes and one emer-



Figures 4. A – Upstream part of the Algeti River (Near vill. Algeti); **B** – Algeti Reservoir; **C** – Downstream part of the Algeti River (Near the Kura River confluence). © Epitashvili G and Ramazashvili G.

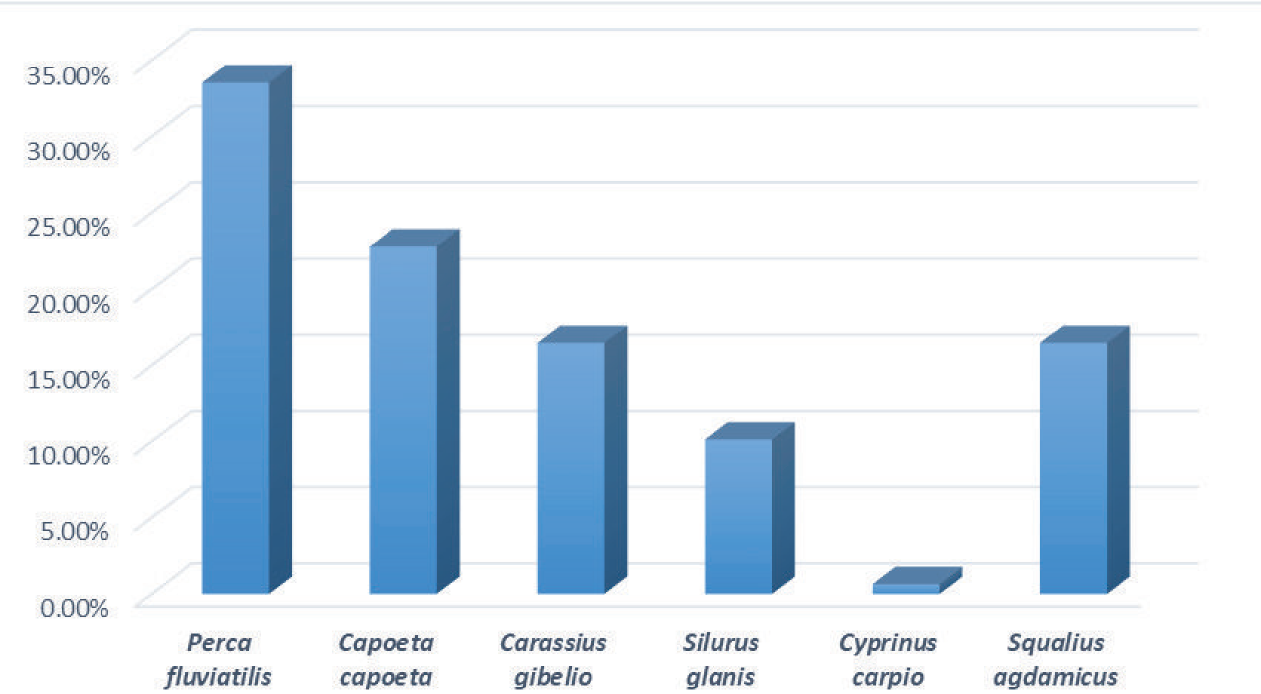


Figure 5. Percentage distribution of commercially important fish in the Algeti Reservoir during the 2020-2021 survey.

gency pipe. The first main pipe connects to the lower riverbed, the second to the former HPP (no longer operational), and the third to the reclamation station. Although not studied, we assume the pipes' flow is rapid enough to impede upstream fish migration. The downstream section is characterised by numerous human-modified habitats. Anthropogenic impacts, such as wastewater, household waste pollution, and riparian forest degradation, are prevalent and intensify towards the Kura confluence.

This section, with 14 fish species (Table 1), is the most diverse. Fish species distribution is heterogeneous. Only three species — *Alburnoides eichwaldii*, *Barbus cyri*, and *Oncorhynchus mykiss*, are found immediately below the dam. The latter entered the river from a trout farm near Tbisi village (N41.588762, E44.538311), just hundred of metres from the dam. In the mid-downstream section, near Marabda village (N41.518376, E44.756174), eight species were caught: *A. eichwaldii*, *B. cyri*, *Gobio caucasicus*, *Alburnus filippii*, *Squalius agdamicus*, *Capoeta capoeta*, *Oxynoemacheilus brandtii*, and *Sabanejewia aurata*. The water level drops significantly from the Marneuli municipality border to the Kura confluence, due to irrigation use ("Reclamation of Georgia" LLC, 2021). Simultaneously, household waste and sewage water pollution dramatically increase, with recent detections of heavy pollution (ammonium compounds, sulphates, phenols, heavy metals, and petroleum products) (Lomsadze et al. 2016). A field survey in this area (N41.403818, E45.001482) yielded no fish species. In the lower downstream section near Kesalo II village (N41.390930, E 45.033301), seven species were observed: *C. capoeta*, *B. cyri*, *Rhodeus amarus*, *Pseudorasbora parva*, *Carassius gibelio*, *Hemiculter leucisculus*, and *Neogobius fluviatilis*. These species, except *C. capoeta* and *B. cyri*, are unique to this section, indicating their entry into the Algeti from the Kura River.

Discussion

Notes on fish distribution, ecology and vulnerability in Algeti River

Human intervention has significantly impacted the species composition and distribution of fish in the Algeti River. Overall, the native fauna has been severely altered throughout the river's length. The dam and reservoir construction created extensive habitats for native species like *Capoeta capoeta*, *Alburnoides eichwaldii*, *Squalius agdamicus*, and *Alburnus filippii*, now more abundant than expected in the corresponding river sections. *Capoeta capoeta* populations from the reservoir migrate upstream to spawn in the river's fast currents, a typical behaviour supported by the age distribution in our catch. Conversely, *A. eichwaldii* is abundant in the upstream Algeti River, suggesting it spends much of its life cycle there and reproduces in the reservoir's shallow areas. This view is supported by *A. eichwaldii* individuals only being observed in the reservoir during their spawning period (May-June), at maturity stages 3 and 4. *Squalius agdamicus*, considered a riverine species, is found in all three river sections. However, it is primarily restricted to the river mouth and reservoir, with few individuals caught in the lower upstream flow. It appears to use the reservoir as a feeding environment, due to the higher abundance of prey in this area of the catchment, an assessment confirmed by size distribution (upstream individuals were much smaller than those in the reservoir). *Alburnus filippii*, has a similar distribution to *S. agdamicus*, and does not seem to utilise the upstream river; its population is limited to the reservoir. Two other small-bodied native species, *Barbus cyri* and *Oxynoemacheilus brandtii*, which likely originally occurred throughout the river, now have isolated populations in the upstream and downstream sections. The dam and the heavily polluted river section near Marneuli act as insurmountable barriers for these species. *Salmo caspius* also only occurs in the isolated upstream population. In the downstream section, *A. filippii*, *Rhodeus amarus*, *Gobio caucasicus*, and *Sabanejewia aurata* are restricted to the river mouth, and do not approach the dammed area. The range of these species within the Algeti River appears heavily contracted due to anthropogenic factors (dam, pollution, and introduced species).

The introduction of non-native species poses a threat to native fauna and the environment, although quantifying this impact remains challenging due to the lack of baseline data. Nevertheless, the abundance of non-native species, including aggressive predators like *Perca fluviatilis* and strong bioturbators like *Carassius gibelio*, has a significant impact. Non-native species distribution shows at least six species in the downstream section and another six in the reservoir, while the upstream section remains free of non-native species. This pattern appears attributable to limited local species releases and the less favourable environmental conditions for non-native species upstream. Only *Cyprinus carpio* was introduced legally into the Algeti Reservoir; this was done for aquaculture purposes soon after the reservoir was put into operation, about 40 years ago (pers. obs.). However, its population is now endangered. Fishing efforts in the last two years yielded only one individual in 2021, and local anglers confirm (pers. comm.) the severe depletion of the carp population. The introduction of other species into the Algeti River system occurred either accidentally or intentionally and illegally by anglers. Deliberate illegal introductions include *C. gibelio* (an older introduction, possibly accidental with *C. carpio* fry), *Silurus glanis* (approximately 16 years ago), *P. fluviatilis* (2016-2017), and *Sander lucioperca* (2022), all represent species of interest to anglers (data from local fishermen and social networks; see also Kuljanishvili et al. (2020), Mumladze et al. (2022), Epitashvili et al. (2023b)). According to local fishermen, the populations of all species in the reservoir, important for fishing, have recently declined and are now at extremely low densities due to the introduction of *P. fluviatilis*. Accidental introductions include *Pseudorasbora parva*, *Hemiculter leucisculus*, *Neogobius fluviatilis*, *Gobio caucasicus* and *Oncorhynchus mykiss*. These species might have spread as hitchhikers with other species or entered the river independently via the Kura River. All of them, except *O. mykiss*, are recorded in the downstream part of the river, while *O. mykiss* appears to have escaped from nearby trout hatcheries.

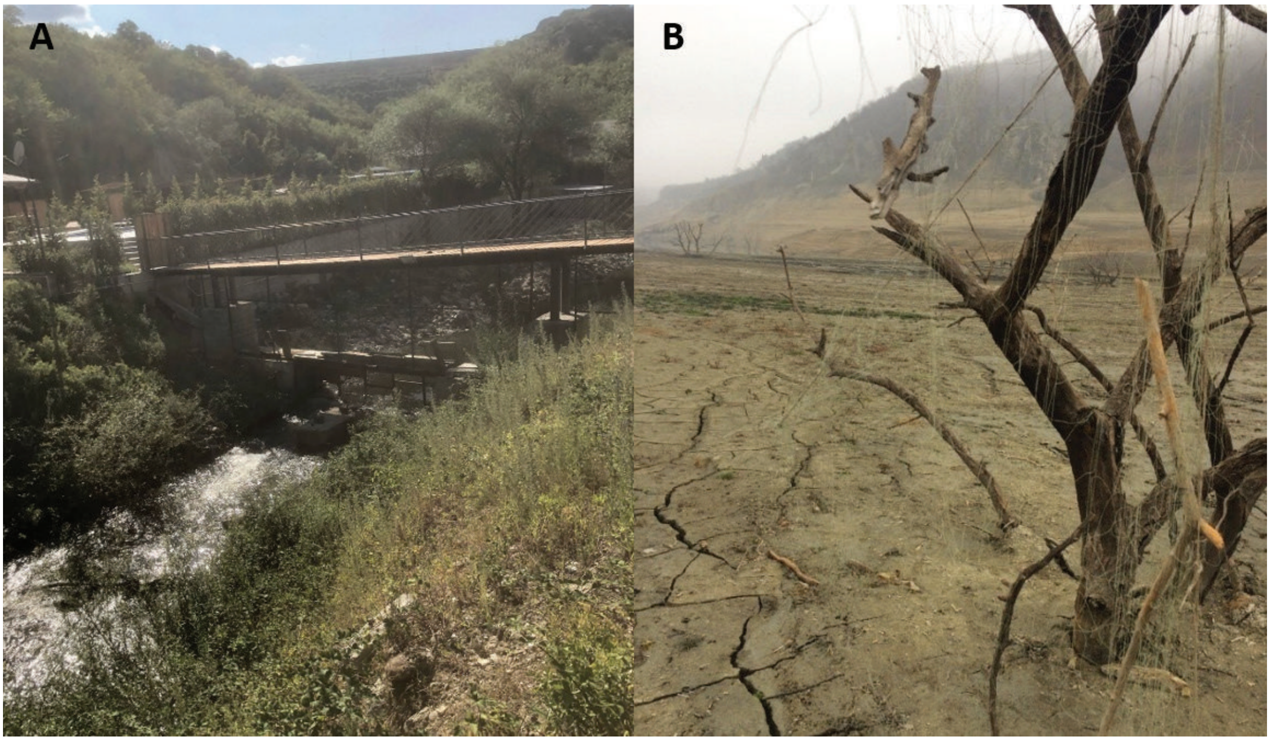
Threats and conservation of the Algeti River fishes

The fish fauna and ecosystems in the Algeti River basin are under significant anthropogenic pressure. The most pronounced threats include habitat fragmentation, pollution, invasive species, and illegal activities (poaching, non-native species releases, and habitat modification), which collectively exacerbate the effect. The dam's construction, lacking a proper fish passage, severely limits native fish migration (at least towards upstream) and results in population isolation. Additionally, another barrier near Tbisi village, a steel construction built by a nearby restaurant owner (Fig. 6A) for rainbow trout releases, is illegal and violates Article 48 of the "Code of Administrative Offenses of Georgia" (https://matsne.gov.ge), further disrupting connectivity. In the mid-downstream section, there is heavy pollution from household waste and sewage peaks at the Marneuli municipality. The riverbed in this area is covered with municipal sediment (sludge, fine fraction), reaching 50-70 cm deep in some places. This section is nearly sterile, devoid of fish and even macroinvertebrates. These barriers likely cause complete isolation of the upstream segment and the reservoir. Several native species to Kura River basin, such as *Luciobarbus mursa*, *Acanthobrama microlepis*, and *Alburnus chalcoides*, are still present in the Kura River and would be expected to utilise the Algeti River, but do not enter this section due to the pollution. The remaining native species upstream of the reservoir are threatened with local extinction. The isolated headwater trout population is particularly vulnerable, and within Algeti National Park, a decline in the trout population is clear despite the lack of comprehensive monitoring data, even though the National Wildlife Agency of Georgia has released trout fry multiple times between 2020-2025.

Non-native and invasive species pose another substantial threat, significantly impacting the river and replacing native fauna. In the reservoir, non-native species, including the invasive crayfish *Pontastacus leptodactylus* (Blaha et al. 2021), which was found in *Silurus glanis* gut contents, have been introduced; leading to the establishment of a homogenised biota with widespread non-native taxa, while the local fauna experiences significant declines. Currently, half of the fish species in the river are introduced. However, beyond speculations and opinions from local anglers, research-based understanding of non-native species' effects on local fauna and ecosystems is lacking.

Poaching remains a significant negative factor throughout the river and reservoir. For instance, during periods of lowered water levels in the Algeti Reservoir, numerous abandoned gill nets were observed (Fig. 6B). Although this poaching primarily targets introduced species, abandoned nets (and other poaching activities), coupled with limited connectivity and pollution, pose a severe threat to Algeti native fish species. In addition, habitat modification, degradation, and water abstraction have also impacted the Algeti River. In most sections, the surrounding riparian forests have been cleared, leading to excessive sediment input during rainy periods. Artificial deepening or modification of the riverbed is also common, especially near settlements. Near the Marneuli municipality, the irrigation system extracts water from the river to irrigate 2034 hectares of agricultural land ("Reclamation of Georgia" LLC, 2021), significantly affecting fish migration and water quality. Consequently, downstream of Marneuli municipality, the river flow diminishes significantly to ecological flow during dry seasons.

In conclusion, the ecological condition of the Algeti River basin is critical. Therefore, it is essential to implement necessary measures to mitigate negative pressures and preserve the habitats and biodiversity of this water body. The river's headwaters are part of a protected area system, and two species found in the river – *Sabanejewia aurata* and *Salmo caspius* – are listed as vulnerable (VU) in the Red List of Georgia. However, these conservation designations alone are insufficient to safeguard local fish fauna. Greater efforts, such



Figures 6. A – closed section of the Algeti River near Tbisi village; **B** – a snapped net at the bottom of the Algeti Reservoir. © G. Ramazashvili

as law enforcement against poaching and other illegal activities, pollution control, and habitat modification management, are needed to mitigate biodiversity loss in the Algeti River.

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The authors have declared that no competing interests exist.

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Author contributions

GE, GR, BJ, LM – research conceptualisation; GE, GR – investigation and data collection; GE, GR, LM – data analysis and interpretation; GE, GR – writing – original draft in Georgian and translation; LM – writing final draft; BJ, LM – writing, review and editing.

Author ORCIDs

Giorgi Epitashvili https://orcid.org/0000-0001-5921-5362
Bella Japoshvili https://orcid.org/0000-0003-0966-0622
Levan Mumladze https://orcid.org/0000-0002-2172-6973

Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

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Supplementary material 1

Presence-absence data of Fish in Algeti River

Authors: Epitashvili et al.

Data type: occurrence

Explanation note: Detailed information on fish species composition from Algeti river and reservoir.

Link: https://doi.org/10.3897/caucasiana.4.e155494.suppl1

Conservation profiles for native freshwater fish species of Georgia (Southern Caucasus region), compliant with the IUCN Red List of Threatened Species

GIORGI EPITASHVILI‡ *, BELLA JAPOSHVILI‡, LEVAN MUMLADZE‡

‡Institute of Zoology, Ilia State University, Tbilisi, Georgia

correspondence: giorgi_epitashvili@iliauni.edu.ge

Abstract

In this study, we provide a first estimation of freshwater fish vulnerability in Georgia. Within the framework of the study, the conservation statuses of 84 local fish species were assessed based on the IUCN Red List criteria. Out of 84 native species evaluated, there is no reliable information on range and population trends for 10 species (11.9%). Their conservation status was determined as Data Deficient (DD). Of the remaining species: 39 are numerous and widespread. Their conservation status was defined as Least Concern (LC). One species, *Acipenser sturio* considered as extinct at national level, as it has not been observed in Georgia for the last decades. Remaining 34 species showed a restricted or fragmented range and small populations, which can be attributed to habitat destruction or degradation and invasive species. Also, illegal fishing is one of the main threat for all native fish species in the country. Taking into account all the factors mentioned above, the conservation statuses of these species were determined as Near Threatened (NT), Vulnerable (VU), Endangered (EN) and Critically Endangered (CR). We identified the most important habitats according to the number of vulnerable species, where the Rioni River occupies the first place with up to 22 threatened species. Expansion of the network of protected areas, dismantling of old dams, restoring original habitats of threatened species, strengthening control over illegal fishing, also further monitoring and research of invasive, data deficient and vulnerable species will be prioritized for further conservation measures.

Keywords:

Freshwater fishes; Southern Caucasus; Eastern Black Sea basin; Vulnerability assessment; Extinction risk; IUCN Red List

Introduction

Fish is most diverse group in the vertebrate and includes up to 36 953 valid species, from which nearly 18 793 are inhabiting in freshwater ecosystems (Fricke *et al.* 2024). Despite this impressive diversity, many species are under threat of extinction due to overexploitation of wild stocks, habitat fragmentation/lost, environmental pollution, spread of alien species, etc. (Dudgeon *et al.* 2006; Collen *et al.* 2014). For instance, in Europe almost 40% of freshwater fish species are under threat of extinction and therefore, many of them are included in the European Red List (Freyhof & Brooks 2011). Generally, regional and global red lists are one of the important mechanisms for protecting vulnerable species and they are used to assess the extinction risk of species based on quantitative criteria developed by the IUCN (Verreycken *et al.* 2014).

Diversity of freshwater fishes is particularly high in the tropics, however, several regions in the Earth's temperate zone should be noted as a refugee and "hot spots" for rear and endemic species such as sturgeons. Among such biodiversity hotspots is the Caucasus region, and Georgia whose territory is characterized by abundance of water resources and therefor freshwater fish species (Kuljanishvili *et al.* 2020; Epitashvili *et al.* 2020). The Georgian ichthyofauna belongs to the eastern Black Sea and western Caspian Sea basins from where Rioni River should be mentioned as one of the last refugee and spawning river in the world for critically endangered sturgeons (Bacalbasa-Dobrovici & Holčík 2000; Kuljanishvili *et al.* 2020; Epitashvili *et al.* 2020). From 20th century to the present, strong environmental changes occurred in Georgia as a result of increasing land consumption, deforestation, water pollution, road and hydropower dam constructions (Epitashvili *et al.* 2020; Japoshvili *et al.* 2021). As a result, negative impacts on the Georgian freshwater ecosystems have likely occurred. Another problem was taxonomic uncertainties in many species, which was partially resolved by the annotated checklist of freshwater fishes of Southern Caucasian countries and first molecular assessment of Georgian freshwater fish species (Kuljanishvili *et al.* 2020; Epitashvili *et al.* 2020). However, major gaps are still existing in the accurate knowledge of species distribution and conservation status within the Georgian inland waters. Nowadays, up to 100 freshwater fish species are known from Georgia and most of them are not evaluated by the IUCN red list criteria. Only 15 fish species are still assessed in the red list of Georgia from which some of them are included with incorrect taxonomy (https://matsne.gov.ge/) (for details see Tab. 1). It is worth noting that the Georgian Red List (RLG) was approved in 2006 and since then, conservation statuses for the fish species have not been renewed.

Table 1. National Red List of Georgian fishes. The species included in the old scientific names are marked in red.

* Please note that all trout species distributed in Georgia were included in the national red list as single species –*Salmo trutta fario*

N	Species names in Georgian Red List	Valid scientific name	IUCN	Red List of Georgia (RLG)
1	*Huso huso* (Linnaeus, 1758)	*Huso huso* (Linnaeus, 1758)	CR	EN
2	*Acipenser sturio* (Linnaeus, 1758)	*Acipenser sturio* (Linnaeus, 1758)	CR	CR
3	*Acipenser nudiventris* Lovetsky, 1828	*Acipenser nudiventris* Lovetsky, 1828	CR	EN
4	*Acipenser stellatus* Pallas, 1771	*Acipenser stellatus* Pallas, 1771	CR	EN
5	*Acipenser gueldenstaedti* Brandt & Ratzeberg, 1833	*Acipenser gueldenstaedti* Brandt & Ratzeberg, 1833	CR	EN
6	*Acipenser persicus* Borodin, 1897	*Acipenser persicus* Borodin, 1897	CR	EN
7	**Salmo trutta fario* Linnaeus, 1758	**(Salmo caspius; S. ciscaucasicus; S. rizeensis)*	LC	VU
8	*Salmo trutta labrax* Pallas, 1814	*Salmo labrax* Pallas, 1814	LC	EN
9	*Rutilus frisii* (Nordmann, 1840)	*Rutilus frisii* (Nordmann, 1840)	LC	VU
10	*Varicorhinus sieboldi* (Steindachner, 1864)	*Capoeta sieboldii* (Steindachner, 1864)	LC	VU
11	*Sabanejewia aurata* (De Filippi, 1863)	*Sabanejewia aurata* (De Filippi, 1863)	LC	VU
12	*Neogobius fluviatilis* (Pallas, 1814)	*Neogobius fluviatilis* (Pallas, 1814)	LC	VU
13	*Alosa caspia paleostomi* Eichwald, 1838	*Alosa tanaica* (Grimm, 1901)	LC	VU

14	*Aspius aspius taeniatus* Berg, 1949	*Leuciscus aspius* (Linnaeus, 1758)	LC	VU
15	*Nemachilus angorae alasanicus* Elanidze, 1983	*Oxynoemacheilus lenkoranensis* (Abdurakhmanov, 1962)	DD	VU
IUCN - The categories are formulated as follows:				
EX – Extinct; **EW** – Extinct in Wild; **CR** – Critically Endangered; **EN** – Endangered; **VU** – Vulnerable; **NT** – Near Threatened; **LC** – Least Concern; **DD** – Data Deficient ; **NE** – Not Evaluated				

Another problem in the RLG is the inconsistency of conservation statuses with the IUCN red list of globally vulnerable species (for instance - sturgeons). As given in the Table 1, all sturgeons are included in the IUCN red list as critically endangered (CR) and in the Georgian red list, only *Acipenser sturio* Linnaeus, 1758 has appropriate status (CR) and relatively lower statuses of the other species (EN). In addition to the above mentioned species, conservation statuses at national level does not have such vulnerable species, such as *Luciobarbus capito* (Güldenstädt, 1773) (IUCN status – VU); *Anguilla anguilla* (Linnaeus, 1758) (IUCN status – CR) and etc.

The aim of this paper was to fill these gaps and assess the conservation statuses for all native freshwater fish species distributed in Georgia according to the IUCN red list criteria. Also, to identify the most vulnerable reservoirs and river basins according to the threatened species common there and suggest future conservation actions critical for the survival of threatened species. We hope that this study serves as a baseline for future investigation and conservation of freshwater ecosystems and species diversity in the country.

Materials and Methods

Species data were collected from all bibliography on Georgian fishes published until March 2024. We also used social networks (Facebook, YouTube, fishermen's forums and webpages), own unpublished data and additional information collected from various projects. For all analyses we used IUCN redlisting tools (the R package red). The R package red performs a number of spatial analyses based on either observed occurrences or estimated ranges for each species we have such data. Functions also include calculating Extent of Occurrence (EOO), Area of Occupancy (AOO), mapping species ranges, species distribution modelling using different parameters and etc. (Cardoso 2017; Cardoso *et al*. 2017). The R package red includes algorithms to facilitate the assessment of extinction risk of species according to the IUCN red list criteria (http://www.iucn.org).

From the data we have (literary data and collection sites – e.g. coordinates for each species), we used two main criteria to assess species vulnerability: area of occupancy (AOO) and extent of occurrence (EOO). Accordingly, to determine conservation statuses for all native freshwater fishes distributed in Georgia, we relied on one of the five main criteria suggested by IUCN, namely criterion B: Geographic range in the form of either B1 (extent of occurrence) and/or B2 (area of occupancy) (http://www.iucn.org). Within this study, the EOO and AOO were calculated in one of two ways: for endemic and range restricted species for which we assumed to know the full range, these values were classified as observed, the minimum convex polygon encompassing all observations used to calculate the EOO and the 2x2 km cells known to be occupied used to calculate the AOO. When the EOO was smaller than the AOO, it was made equal as per the IUCN guidelines (Cardoso *et al*. 2017; IUCN Standards and Petitions Subcommittee 2022). Species distribution maps were prepared in Google Earth (Google Earth Pro, 2024) (suppl. materials see below).

Discussion

Out of 84 native fish species evaluated, there is no reliable information on range and trends for 10 species (11.9%). Some of them (e.g. *Alosa maeotica*, *Ponticola gorlap*, *Chelon labrosus*, *Chelon ramada*, etc.) were not observed in Georgia at all, although they are mentioned in the new checklist (Kuljanishvili *et al*. 2020). It is necessary to carry out additional works, including genetic studies, to clarify their existence and conservation status in Georgia. Accordingly, their current status was determined as Data Deficient (DD). From the remaining 74 species: 39 are numerous and widespread, with an estimated EOO > 10 000 km^2 and AOO > 100 km^2. Their conservation status was defined as Least Concern (LC). One species, *Acipenser sturio* has not been observed in Georgia for the last decades. Accordingly, this species was assessed as Regionally Extinct (RE). Of the remaining 34 species, most of them showed a restricted or fragmented range and small populations, which can be attributed to habitat destruction/degradation or invasive species (e.g. *Pseudorasbora parva*, *Carassius gibelio*, *Rhinogobius lindbergi*, *Gambusia holbrooki*, *Hemiculter leucisculus*, etc.), which are important competitors for these species. Also, illegal fishing is one of the most important threat for all native fish species in the country. Taking into account all the factors mentioned above, the statuses of these species were determined as Near Threatened (NT), Vulnerable (VU), Endangered (EN) and Critically Endangered (CR) (Table 2; Diagram 1).

Table 2. Conservation statuses of native freshwater fishes of Georgia.

Taxa	Conservation Status at National Level
Acheilognathidae	
Rhodeus amarus (Bloch, 1782)	**NT**
Rhodeus colchicus Bogutskaya & Komlev 2001	**LC**
Acipenseridae	
Acipenser gueldenstaedtii Brandt & Ratzeburg 1833	**CR**
Acipenser nudiventris Lovetsky, 1828	**CR**
Acipenser persicus/colchicus Marty, 1940	**CR**
Acipenser stellatus Pallas, 1771	**CR**
Acipenser sturio Linnaeus, 1758	**RE**
Huso huso (Linnaeus, 1758)	**CR**
Anguillidae	
Anguilla anguilla (Linnaeus, 1758)	**CR**
Atherinidae	
Atherina caspia Eichwald 1831	**LC**
Clupeidae	
Alosa immaculata Bennett, 1835	**VU**
Alosa maeotica (Grimm, 1901)	**DD**
Alosa tanaica (Grimm, 1901)	**LC**
Clupeonella cultriventris (Nordmann, 1840)	**LC**
Cobitidae	
Cobitis saniae Eagderi, Jouladeh-Roudbar, Jalili, Sayyadzadeh & Esmaeili, 2017	**EN**
Cobitis satunini Gladkov 1935	**NT**
Sabanejewia aurata (De Filippi, 1863)	**NT**
Cyprinidae	
Barbus cyri De Filippi, 1865	**LC**

Barbus rionicus Kamensky, 1899	LC
Capoeta banarescui Turan, Kottelat, Ekmekçi & Imamoglu, 2006	VU
Capoeta capoeta (Güldenstädt, 1773)	LC
Capoeta sieboldii (Steindachner, 1864)	VU
Cyprinus carpio Linnaeus, 1758	LC
Luciobarbus capito (Güldenstädt, 1773)	VU
Luciobarbus mursa (Güldenstädt, 1773)	VU
Esocidae	
Esox lucius Linnaeus, 1758	LC
Gasterosteidae	
Gasterosteus aculeatus Linnaeus, 1758	LC
Gobiidae	
Babka gymnotrachelus (Kessler, 1857)	VU
Mesogobius batrachocephalus (Pallas, 1814)	DD
Neogobius fluviatilis (Pallas, 1814)	LC
Neogobius melanostomus (Pallas, 1814)	LC
Ponticola alasanicus Epitashvili, Japoshvili & Mumladze, 2023	EN
Ponticola constructor (Nordmann, 1840)	LC
Ponticola cyrius (Kessler, 1874)	LC
Ponticola gorlap (Iljin, 1949)	DD
Ponticola syrman (Nordmann, 1840)	VU
Proterorhinus nasalis (De Filippi, 1863)	LC
Gobionellidae	
Knipowitschia caucasica (Berg, 1916)	LC
Knipowitschia longecaudata (Kessler, 1877)	LC
Gobionidae	
Gobio artvinicus Turan, Japoshvili, Aksu & Bektaş 2016	DD
Gobio caucasicus Kamensky, 1901	LC
Romanogobio macropterus (Kamensky, 1901)	VU
Leuciscidae	
Abramis brama (Linnaeus, 1758)	NT
Acanthobrama microlepis (De Filippi, 1863)	NT
Alburnoides eichwaldii (De Filippi, 1863)	LC
Alburnoides fasciatus (Nordmann, 1840)	LC
Alburnus alburnus (Linnaeus, 1758)	LC

Alburnus chalcoides (Güldenstädt, 1772)	LC
Alburnus derjugini Berg, 1923	LC
Alburnus filippii Kessler, 1877	LC
Alburnus hohenackeri Kessler, 1877	DD
Ballerus sapa (Pallas, 1814)	DD
Blicca bjoerkna (Linnaeus, 1758)	NT
Chondrostoma colchicum Derjugin, 1899	LC
Chondrostoma cyri Kessler, 1877	VU
Leuciscus aspius (Linnaeus, 1758)	VU
Leucaspius delineatus Heckel, 1843	DD
Petroleuciscus borysthenicus (Kessler, 1859)	LC
Phoxinus colchicus Berg, 1910	LC
Rutilus frisii (Nordmann, 1840)	EN
Rutilus lacustris (Pallas 1814)	LC
Scardinius erythrophthalmus (Linnaeus, 1758)	DD
Squalius agdamicus (Kamensky 1901)	LC
Squalius orientalis Heckel, 1847	LC
Vimba vimba (Linnaeus, 1758)	LC
Moronidae	
Dicentrarchus labrax (Linnaeus, 1758)	LC
Mugilidae	
Chelon auratus (Risso, 1810)	LC
Chelon labrosus (Risso, 1810)	DD
Chelon ramada (Risso, 1810)	DD
Chelon saliens (Risso, 1810)	LC
Mugil cephalus Linnaeus, 1758	LC
Nemacheilidae	
Oxynoemacheilus brandtii (Kessler, 1877)	LC
Oxynoemacheilus cemali Turan, Kaya, Kalayci, Bayçelebi & Aksu 2019	EN
Oxynoemacheilus phasicus Freyhof, Kaya, Epitashvili & Geiger 2021	VU
Percidae	
Perca fluviatilis Linnaeus, 1758	LC
Sander lucioperca (Linnaeus, 1758)	VU
Petromyzontidae	
Lampetra ninae (Naseka, Tuniyev & Renaud 2009)	NT

Salmonidae	
Salmo caspius Kessler, 1877	VU
Salmo ciscaucasicus Kessler, 1877	EN
Salmo labrax Pallas 1814	VU
Salmo rizeensis Turan, Kottelat & Engin 2010	EN
Siluridae	
Silurus glanis Linnaeus, 1758	LC
Syngnathidae	
Syngnathus abaster Risso, 1827	LC
Tincidae	
Tinca tinca (Linnaeus, 1758)	EN

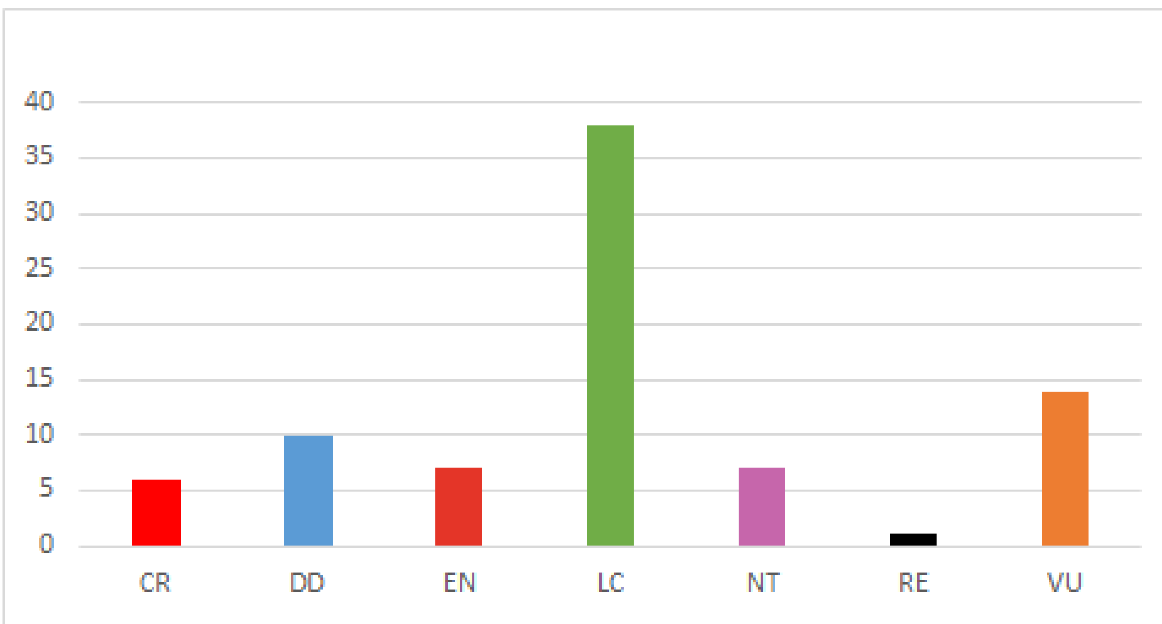

Diagram 1. Visualization of conservation statuses of Georgian freshwater fish species.

According to the number of vulnerable species, we have identified the following water bodies: most of the vulnerable and threatened species are currently distributed in the Rioni River, which is largest in Georgia and one of the most important habitat for critically endangered sturgeons globally. We identified 22 vulnerable species in the Rioni River basin, from which six species are considered as Critically Endangered (CR) by IUCN (e.g. European eel - *Anguilla anguilla* and sturgeons: *Huso huso, Acipenser gueldenstaedtii*, *A. nudiventris*, *A. persicus/colchicus* and *A. stellatus*). The Rioni River is also home to many endemic species, including the newly described Rioni loach - *Oxynoemacheilus phasicus*, which is endemic to the Rioni and Enguri River basins (Freyhof *et al*. 2021). All this once again emphasizes the importance of Rioni as a river with high ecological value.

The Mtkvari (Kura) River basin (including Iori and Alazani river basins) is also distinguished by the number of threatened and endemic fishes, where we identified 15 vulnerable species. Among them, the globally vulnerable species, *Luciobarbus capito*, is noteworthy. Due to illegal fishing, habitat degradation/fragmentation and invasive species, some fish populations in the Mtkvari River basin, e.g. *Luciobarbus mursa*, *Chondrostoma cyri*, *Leuciscus aspius* and *Sander lucioperca* decreased significantly. Moreover, the *Chondrostoma cyri* has completely disappeared from the Iori River, which is one of the main rivers in the Mtkvari River basin. In recent years, an expansion of the area of the Caucasian gudgeon – *Gobio caucasicus* has been observed in Eastern Georgia. This species was believed to live in the Black Sea basin (Elanidze, 1983; Ninua *et al*. 2013). It probably got into the Mtkvari basin through accidental translocation, where it successfully established sustainable populations. Probably one of the reasons why the area and number of South Caucasian gudgeon - *Romanogobio macropterus* decreased in Mtkvari River basin is *G. caucasicus* which managed to occupy its niche. It is worth noting the newly discovered species from the Alazani River, Alazani goby – *Ponticola alasanicus*, which is currently known from the three location and represents endemic species to this river (Epitashvili *et al.* 2023). The EOO of the mentioned goby is less than 55 km^2 and AOO is about 12 km^2. Population is small and has restricted range. Based on all this, its status was defined as Endangered (EN).

The Enguri is third diverse river by threatened fish species in Georgia. At least 14 vulnerable species are living here. We did not include sturgeons in this number because we do not have confirmed reports whether they still use this river for spawning or not.

The situation is more or less similar in the Khobistskali River, where 13 threatened species are found. In terms of the number of threatened fishes, Chorokhi River basin is the fifth among the Georgian rivers with 11 vulnerable species. As for lakes and reservoirs, the most diverse in this regard is Paliastomi Lake, where at least 13 threatened fish species are living. The rest of the data is given in the Diagram 2.

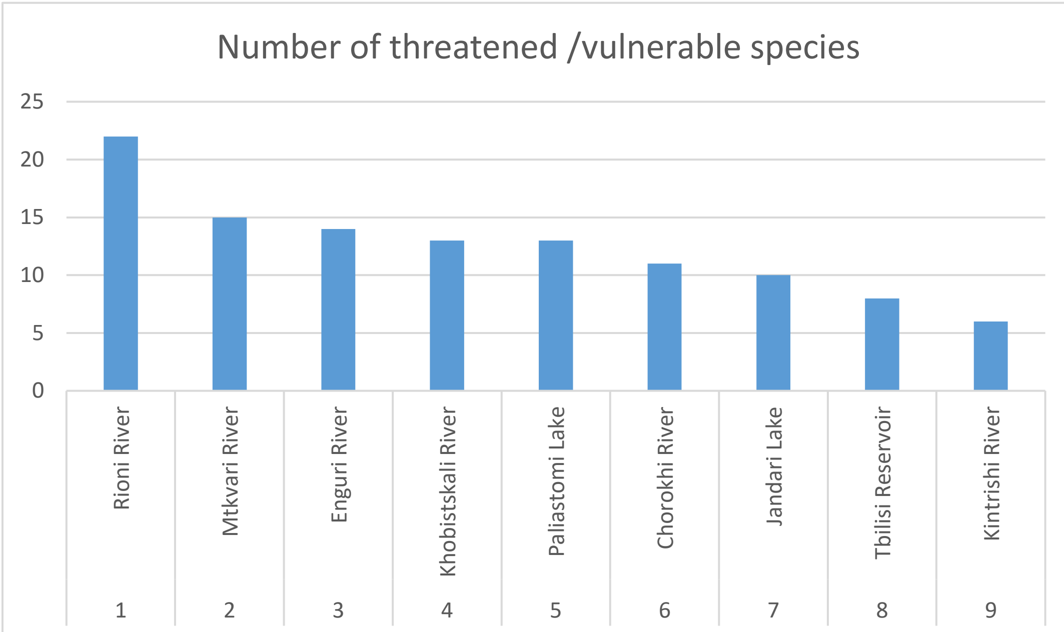

Diagram 2. The main river basins and lakes of Georgia with approximate number of threatened fish species.

Freshwater fish and their habitats are one of the most vulnerable globally (Dudgeon *et al.* 2006; Ahmed *et al.* 2022). The effects of illegal fishing, habitat degradation/fragmentation by HPP dams, gravel quarries, waste waters, etc. also spread of alien species are one of the prevailing threats accross the world affecting numerous species (Kuylenstierna *et al.* 1998; Simon *et al.* 1995; Gherardi, 2010). This effects were never studied for Georgian fishes, despite the fact that all these threats are relevant in the country as well. As mentioned above, the Red List of Georgia was created in 2006, where up to 15 fish species were included. Since then, for almost 18 years, the mentioned red list has not changed. The update process of RLG is currently underway, where we have assessed new fish species according to the IUCN Red List criteria. It was within the scope of this study that the vulnerability of freshwater fishes distributed in Georgia was assessed for the first time and accordingly, the results of this study were used in the process of updating the national red list.

Among all other conservation measures suggested, the restoration of wild habitats and control of invasive species are often a priority to guarantee the survival of threatened species (Cardoso *et al*. 2017). In Georgia, some conservation actions (for instance - trout restoration project) have been undertaken by the local authorities (e.g. Agency of Wildlife - Ministry of Environmental Protection and Agriculture of Georgia) and NGO's (FFI; WWF - Georgia, etc.) (which mainly concerns the conservation of sturgeons and their main habitat – Rioni River). Similar conservation measures have almost never been implemented for other fish species in Georgia. However, it is important not only to ensure the continuity of conservation actions, but also to monitor their effectiveness by assessing population changes in fish communities as they already proved to be effective bioindicators in many freshwater ecosystems (Chovanec *et al*. 2003; Dietrich *et al*. 2022; Marin *et al*. 2023). For some species (e.g. sturgeons, Asp - *Leuciscus aspius* and Kura nase - *Chondrostoma cyri*) ex-situ conservation, restocking and reintroduction could be a last resort or insurance against extinction. As for the extinct *Acipenser sturio*, it should be reintroduced in the historical habitats.

The network of protected areas in Georgia has increased in recent years and covers some important areas (such as Kolkheti lowland) where vulnerable and endemic fish species are found. However, many rivers and reservoirs still needs to be protected, including sturgeon spawning habitats in the Rioni and Khobistskali rivers. Also, from the point of view of the development of protected areas, Jandari Lake, the Enguri, Chorokhi and Alazani river basins and the upper part of Mtkvari River are important places.

In conclusion, although up to 40 native fish species in Georgia are more or less in a good condition and have a wide range as well as numerous populations, monitoring and protection of their habitats will be vitally important, as other vulnerable species live with them.

As mentioned above, there are some species requiring urgent attention and protection measures. These include the range restricted species (such as sturgeons, also *Cobitis saniae*, *Ponticola alasanicus*, etc.) or species with high economic and ecological value (e.g. *Anguilla anguilla*, *Alosa immaculata*, *Leuciscus aspius*, *Luciobarbus capito*, *L. mursa*, etc.), which should be considered in future conservation and species recovery programs.

Expansion of the network of protected areas, dismantling of old dams, restoring original habitats of threatened species, strengthening control over illegal fishing and monitoring of invasive species will be priority.

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Author contributions

GE, BJ and LM developed the conception and design of the study, collected material and analyzed data. LM supported the research logistics. GE drafted manuscript. All authors participated in revising the article and contributed with intellectual content. All authors gave final approval of the submitted version.

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Fishes of the Caucasus ecoregion

Levan Mumladze, Giorgi Epitashvili, Bella Japoshvili

Institute of Zoology, Ilia State University, Tbilisi, Georgia
K. Cholokashvili ave. 3/5. 0169, Building S, Room S611

levan.mumladze@iliauni.edu.ge

Review of Fish Species Inventory

The Caucasus ecoregion, a biodiversity hotspot, enjoys an abundance of water resources and a corresponding diversity of freshwater species (Myers et al. 2000). The study of the Caucasus region's fish fauna has a lengthy history. From the initial publications by Gmelin, Pallas, and Eichwald in the 18th century (Elanidze, 1983) to the present day, numerous papers and books detailing fish diversity, distribution, ecology, and evolution have been consistently published. Consequently, fish are among the most thoroughly studied groups within the Caucasus ecoregion regarding species composition and distribution and even life history. However, driven by economic priorities, 20th-century fish research primarily aimed to maximize economic benefits from inland waters. To achieve this, numerous non-native species (*Coregonus albula* (Linnaeus, 1758), *C. lavaretus* (Linnaeus, 1758), *C. peled* (Gmelin, 1789), *Hypophthalmichthys molitrix* (Valenciennes, 1844), *H. nobilis* (Richardson, 1845), *Ctenopharyngodon idella* (Valenciennes, 1844), *Cyprinus carpio* Linnaeus, 1758, *Oncorhynchus mykiss* (Walbaum, 1792), and other species, including hitchhikers) were introduced into various water bodies, alongside extensive translocations of native species within the Caucasus region. These activities have led to the widespread establishment of invasive species, such as *Pseudorasbora parva* (Temminck & Schlegel, 1846) and *Carassius gibelio* (Bloch, 1782) throughout the region brought heavy, though not well documented, impact on freshwater ecosystems (Kuljanishvili et al. 2021).

From the 20th century to the present, particularly during the 1990s, the region has experienced significant environmental changes due to increased land consumption, deforestation, water pollution, illegal fishing, and the construction of roads and hydropower plants (Freyhof et al. 2020; Epitashvili et al. 2020; Japoshvili et al. 2021; Gabrielyan et al. 2022). These changes have likely had negative impacts on the Caucasus freshwater environment, resulting in substantial shifts in fish diversity and composition. Notably, following the collapse of the Soviet Union, there was a nearly 20-year hiatus in fish systematics and diversity research. Consequently, the taxonomy and distribution of many species require clarification and updating.

The Caucasus ecoregion currently supports 182 freshwater fish species, including 24 introduced species. This ichthyofaunal composition reflects a blend of influences from the adjacent Caspian and Black Sea basins, with numerous species sharing close affinities with those beyond the Caucasus boundaries. The region is a significant center for freshwater fish endemism, notably within the Kura-Ara(k)s river systems, harboring 26 endemic species, and the Kuban river, with 11.

Perhaps one of the most notable elements of Caucasus fish diversity is the sturgeons (Acipenseridae), once represented by up to seven species (Freyhof et al. 2020). Sturgeons historically were and continue to be under significant threats caused by overfishing and habitat modifications, ultimately leading to the extinction of several lineages or driving species to the verge of extinction (Ustaoglu & Okumuş 2004). The lamprey fauna comprises 4 species, of which *Lampetra ninae* is a Black Sea coastal endemic, *Caspiomyzon wagneri* is restricted to the Caspian basin, and *Lampetra lanceolata* is found in northern Anatolian sites. The Kuban's lamprey, often identified as *Eudontomyzon mariae*, requires taxonomic revision, potentially revealing another endemic lineage (Freyhof et al. 2020; Artaev et al. 2021). Anadromous species, such as shads (Clupeidae) and trout (Salmonidae), are notable for their commercial and recreational value, respectively. Molecular analyses challenge traditional morphological classifications, suggesting recent speciation in trout and potential species flocks in shads, prompting further research (Ninua et al. 2018; Epitashvili et al. 2020; Levin et al. 2021).

In general, Cypriniformes dominate freshwater fish diversity, with Acheilognathidae (2 species), Cobitidae (10 species), Cyprinidae (15 species), Gobionidae (11 species), Leuciscidae (38 species), Nemacheilidae (10 species), Tincidae (1 species), and Xenocyprididae (5 species) forming the core of the region's ichthyofauna.

Taxonomic ambiguities persist, particularly within Nemacheilidae and Leuciscidae, but the broader fauna is well-documented. Minor families, including Anguillidae (1 species), Atherinidae (1 species), Esocidae (1 species), Gasterosteidae (2 species), Lotidae (1 species), Moronidae (1 species), Mugilidae (6 species), Percidae (6 species), Siluridae (1 species), and Syngnathidae (2 species), lack regional endemics. Introduced families, such as Adrianichthyidae (1 species), Cichlidae (4 species), Coregonidae (2 species), Ictaluridae (1 species), Poeciliidae (1 species), Polyodontidae (1 species), Serrasalmidae (1 species), and Xenocyprididae (5 species), contribute to the region's aquatic biodiversity.

Gobiidae and Gobionellidae, with 27 native (six endemic) and one introduced species, are integral to both freshwater and coastal ecosystems. Recent taxonomic updates have refined species classifications, reflecting ongoing research and molecular insights. For example, within Nemacheilidae, the Oxynoemacheilus genus has undergone revisions based on molecular data, clarifying species boundaries and distributions, and resulting in the description of a new species. Similarly, Leuciscidae species, such as those within the Alburnus and Squalius genera, have been re-evaluated, leading to taxonomic adjustments. These refinements underscore the dynamic nature of fish taxonomy and the importance of continued research in the Caucasus.

Among the 182 species counted for the Caucasus ecoregion, 3 species (*Rhodeus caspius, Oxynoemacheilus phasicus, Ponticola alasanicus* (Esmaeili et al. 2020; Freyhof et al. 2021; Epitashvili et al., 2023)) were described after 2020, indicating potential gaps in our understanding of true diversity (a last review of Caucasus fish fauna by Freyhof et al. (2020) also pinpointed this).

There are 57 species (31.3%) endemic to the region. A few taxa considered here as endemic (e.g., Gobiidae) still remain taxonomically unresolved, requiring further investigation. In addition, some freshwater systems and taxa also need to be investigated to complete our knowledge. Given these circumstances, the current understanding of species diversity, including endemism rates, might change in the future, but likely insignificantly. The advance in molecular methodologies since 2000 has revolutionized our comprehension of the region's fish diversity, alongside the increased research efforts across the Caucasus, Europe, Iran, and Turkey. Yet, taxonomic intricacies remain, promising future discoveries and potential revisions.

Countrywise species distribution within the hotspot

Armenia. In the Armenian part of the Caucasus region, 42 species of freshwater fish are recorded. Freyhof et al. (2020) mentioned that one species of lamprey is also inhabiting water bodies of Armenia, however, at this stage, we do not have any material or source to confirm this. One species (*Salmo ischchan*) is endemic to Armenia but has been introduced to Azerbaijan and Georgia and to Central Asia. In General, the most important single freshwater body in Armenia is Lake Sevan - a largest and closed freshwater lake in the Caucasus. It hosts the endemic trout species *Salmo ischchan* which is close to extinction in the wild; this taxon was once represented by four “forms” which most likely were different species. Artificial drainage of the lake in the mid-1950s for irrigation and hydropower caused irreversible changes in ecosystem functioning and biodiversity, destroying the lake’s globally unique diversity of trout. Currently, only two “forms” of Sevan trout survive, largely by artificial breeding and subsequent release into the lake. Action is being taken to restore the lake, the original diversity of trout cannot be restored because several “forms” are extinct or have hybridised with each-other (Kuljanishvili et al. 2020; Levin et al. 2021).

Azerbaijan. From Azerbaijan, 96 species are so far reported, four of them endemic to Azerbaijan (*Cobitis derzhavini, Oxynoemacheilus lenkoranensis, Pseudophoxinus atropatenus, Pseudophoxinus sojuchbulagi*). One of these species – *O. lenkoranensis* - needs a critical taxonomic review and one endemic species – *P. sojuchbulagi* - seems to be extinct.

Georgia. In Georgia, 106 species are recorded, two of which (*Oxynoemacheilus phasicus* and *Ponticola alasanicus*) are endemic to Georgia. The existence of some species mentioned in Kuljanishvili et al. (2020) is doubtful due to their distribution area and lack of materials (e.g., *Barbus ciscaucasicus*, *Capoeta kaput*, *Oxynoemacheilus bergianus*, *O. veyseli*, *Chelon labrosus*, *C. ramada*), requiring additional research in the future. The fish fauna of Georgia represents a mix of Black Sea and Caspian Sea fauna. In the Black Sea part, the Rioni River is the most significant, with over 40 fish species (Epitashvili et al. 2020; Beridze et al. 2023). Additionally, it appears that the Rioni is the last spawning river for sturgeons in the eastern Black Sea region. The entire eastern Georgia (including the Greater and Lesser Caucasus) drains into the Caspian Sea through the Kura River and its tributaries (also, a small northern part belongs to the Terek River basin). The Kura River (with its tributaries) is a very important transboundary river, crossing three countries (Turkey, Georgia, Azerbaijan), and harbors one of the most important and diverse fish fauna in the region.

Iran. In the Gilan region of Iran, 82 species are found, none of which are endemic. Notable freshwater ecosystems in the region include the Sefid River and the Anzali wetland, where endemic and relict species of the Caucasus ecoregion inhabit, including sturgeons, *Sabanejewia caspia*, etc.

Russia. In the Russian part of the Caucasus ecoregion, at least 136 species occur, and 14 of them are endemic to the Russian Caucasus (*Clupeonella abrau*, *Sabanejewia caucasica*, *Sabanejewia kubanica*, *Barbus kubanicus*, *Gobio holurus*, *Gobio kubanicus*, *Romanogobio ciscaucasicus*, *Romanogobio parvus*, *Romanogobio pentatrichus*, *Alburnoides kubanicus*, *Chondrostoma kubanicum*, *Chondrostoma oxyrhynchum*, *Petroleuciscus aphipsi*, *Salmo ezenami*). Most of the endemism occurs in the Kuban River, which is a separate freshwater ecoregion according to Abell et al. (2008).

Turkey. In the Turkish Caucasus, 92 species are recorded, and four of them are endemic to the Turkish Caucasus (*Ponticola rizensis*, *Ponticola turani*, *Leucalburnus satunini, Oxynoemacheilus cyri*).

The conservation status of these aquatic vertebrates, though incompletely documented, underscores the Caucasus's critical stewardship role. Sturgeons, historically paramount in conservation focus due to their spawning in Black and Caspian Sea tributaries, continue to face poaching despite legal protections, with their current status obscured by conflicting reports. Spawning persists in Georgia's Rioni River, with potential reproduction in Azerbaijan's Kura and Iran's Sefid Rivers. However, the unchecked proliferation of hydropower projects poses a grave threat to both endemic and vulnerable species, even within protected zones, severely compromising the region's biodiversity.

Threats and conservation of fish in the Caucasus

Globally threatened species of freshwater fish in the Caucasus

There are 21 freshwater fish species, and two marine species, listed as globally threatened – vulnerable (VU), endangered (EN), and critically endangered (CR) – in the Caucasus Ecoregion (Table 1). *Pseudophoxinus sojuchbulagi,* which has not been observed for decades, is likely extinct. Furthermore, *Acipenser sturio* and *A. nudiventris* appear no longer to spawn in the Caucasus. Most important from a global perspective are six threatened species that are endemic to the Caucasus region (*Clupeonella abrau*, *Ponticola rizensis, P. turani*, *Pseudophoxinus atropatenus*, *Salmo ischchan*, *S. ezenami*), as well as the threatened sturgeons potentially still spawning in the area. Not all species have been reassessed for their conservation status at a regional scale, and several assessments are outdated and require renewal.

Important places for the conservation of freshwater fish in the Caucasus

The freshwater environment and biota of the Caucasus ecoregion as a whole are under significant human pressure, and a region-scale conservation strategy is crucial. However, given resource limitations, research and evidence-based prioritization must occur. Freyhof et al. (2015a) suggested several areas of particular importance from a conservation viewpoint, and here we also emphasize a few freshwater areas from slightly different perspectives.

The Kura River (Turkey, Georgia, Azerbaijan, Armenia) and its drainage, including the Arax River, are the only transboundary rivers stretching across four countries. They are also among the most stressed, due to numerous human impacts, including pollution, overexploitation of fish and water, poaching, and damming. At the same time, they are still inhabited by a number of endemic species. One of them, *Pseudophoxinus sojuchbulagi*, may already be extinct (or close to extinction) (Artaev et al. 2018), while *Leucalburnus satunini* is the only endemic genus (and species) for the Caucasus. The habitats of the latter are still in relatively good condition due to limited attractiveness for industrial projects; however, they must be protected to avoid the loss of this unique species (Freyhof et al. 2020).

The Rioni River (Georgia) is perhaps the most significant single unit for conservation prioritization, as it is the only critical habitat for the surviving sturgeon (*Acipenser persicus/colchicus*, *Acipenser nudiventris*, *Acipenser stellatus*, *Acipenser gueldenstaedtii*, and *Huso huso*) populations in the eastern Black Sea basin. With a large diversity of fishes occurring in the Rioni River, including endemic species (Beridze et al. 2023; Epitashvili et al. 2023), the Rioni River is under heavy and progressive anthropogenic pressure.

Another particularly important area is the Anzali wetland (Iran), which is the estuary of the Sefid River and is among the few habitats of its kind still preserved in wild conditions. The most common freshwater fish species of the southern Caspian Sea basin use this wetland for spawning, and it is of exceptional importance for regional fish reproduction. It is also remarkable for hosting a population of *Sabanejewia caspia*, a small loach found at no more than three places worldwide (the other two are in Azerbaijan).

And finally, the Kuban River and its tributaries (Russia) are particularly interesting water bodies for their unique fish diversity, including a number of endemic species. These include *Petroleuciscus aphipsi, Alburnoides kubanicus*, *Barbus kubanicus*, *Chondrostoma kubanica*, *Gobio kubanicus*, *Romanogobio parvus*, *Romanogobio pentatrichus*, and *Sabanejewia kubanica*. These species are of exceptional value for fish conservation. All except two are rheophilic inhabitants of fast-flowing, natural rivers with gravel bottoms. Sadly, these important areas for fish conservation have been degraded by the construction of dams, which inhibit the spawning migration of sturgeons and other migratory fishes, which were abundant in former times. The few wild river sections that remain are vulnerable to many threats, and large sections should be protected.

Threats and perspective in fish conservation

In the Caucasus, as throughout the West Palearctic, populations of large and long-distance migratory fish species, such as sturgeons, have vanished or nearly vanished due to dam construction and overfishing. These two threats remain acute across the region, with an increasing trend in hydropower plant development. Combined with other factors, loss of habitat connectivity due to damming may cause significant fish biodiversity loss and biotic homogenization (biased towards tolerant and mostly introduced taxa) across the ecoregion (Japoshvili et al. 2021). Consequently, the longest free-flowing sections of rivers must be urgently considered for appropriate conservation.

Other significant threats include pollution (a massive regional challenge) from municipal and industrial wastewater, as well as heavily polluted discharge from agricultural lands; water abstraction, especially in the eastern Caucasus; habitat destruction by gravel mining, which is widespread in the region; and invasive species used in commercial and recreational fisheries.

Nevertheless, the conservation status of many freshwater fish in the Caucasus is expected to be relatively good compared to other regions in Europe and the Middle East, due to the presence of still natural or only slightly disturbed habitats (mainly at higher elevations). Such rivers provide habitat for groups of rheophilic fishes, to which most endemic species belong.

In contrast to rivers, lentic water systems are generally in very poor condition. For example, the largest closed lake, Sevan in Armenia, underwent long-term habitat modification, pollution, overfishing, and invasive species stress. As a result, the lake ecosystem and native biota experienced irreversible damage and are still under significant anthropogenic pressure (Gabrielyan et al. 2022). Another case is Paliastomi Lake in the Colchis Lowland, which, due to its unique and great diversity, has been given protected lake status. However, overfishing, pollution, and poaching have never been reduced there.

Alongside these anthropogenic changes, climate change is an important factor threatening freshwater fish habitats and fish diversity within the region. Accordingly, it is important to consider all these factors in combination to safeguard fish diversity.

Within the region, freshwater habitats are considered the most important habitat type in all countries within the Caucasus ecoregion, with numerous important local regulatory acts. At the same time, Caucasian countries are members of international conventions such as RAMSAR (https://www.ramsar.org/), and a number of water bodies are designated as important freshwater areas. However, the main problem hindering the effectiveness of any single conservation activity in the long term remains law enforcement at a regional scale.

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