Limnology and Freshwater Biology 2022 (5): 1639-1642 DOI:10.31951/2658-3518-2022-A-5-1639

Short communication

ISSN 2658-3518
LIMNOLOGY FRESHWATER BIOLOGY
www.limnolfwbiol.com

Comparative analysis and reconstruction of phylogenetic position of sunbleak *Leucaspius delineatus* (Heckel, 1843) from the Irkutsk Reservoir

Kulakova N.V.[1], Bukin Yu.S.[2], Denikina N.N.[2*], Bogdanov B.E.[2], Dzyuba E.V.[2]

[1] *Siberian Institute of Plant Physiology and Biochemistry of the Siberian Branch of the Russian Academy of Sciences, 132 Lermontov Str., Irkutsk, 664033, Russia*
[2] *Limnological Institute of the Siberian Branch of the Russian Academy of Sciences, 3 Ulan-Batorskaya Str., Irkutsk, 664033, Russia*

ABSTRACT. This work was aimed to a comparative analysis and reconstruction of the phylogenetic position of sunbleak *Leucaspius delineatus* (Heckel, 1843) from the Irkutsk Reservoir. Determination of a species specific fragment of the *Cox1* gene allowed to reveal a unique common haplotype present in all specimens analyzed. Comparative analysis and phylogenetic reconstruction based on nucleotide sequences available in genetic databases showed that the studied species is represented by three phylogenetic lineages. Intraspecific differences of nucleotide sequences of fragments of the *Cox1* gene amounted to 5.5%, and within phylogenetic lineages - $<$ 3%. The studied fishes were related to the genetic lineage widely distributed in Europe and northwestern Russia. It is shown that all specimens with 100% genetic similarity belong to the same genotype, which is due to recent introduction of sunbleak into Pre-Baikal and to the “founder effect”.

Keywords: *Leucaspius delineatus*, invasive species, *Cox1* gene, Irkutsk Reservoir, Baikal Region

1. Introduction

Nowadays, the popularity of studies on invasive species of hydrobionts has increased considerably worldwide (Novoa et al., 2020; Kovalenko et al., 2021). For this reason, molecular genetic studies are particularly important in developing standards for monitoring and in assessing of long-term trends in ecosystem status, including the proxies describing expansion and impacts of invasive species, and in predicting genetic trends and potential consequences of their invasion (Darling et al., 2017; Pérez-Portela et al., 2018; Belle et al., 2021; Simberloff, 2021).

Sunbleak *Leucaspius delineatus* (Heckel, 1843) is a small-sized freshwater species of family Leuciscidae (Fig. 1). The natural habitat of sunbleak is the rivers of Eastern Europe. In Russia, it occurs in all Baltic rivers east of the Neva River; in some lakes of the Onega and Northern Dvina basins; in the Caspian Sea basin (the Volga River, from the upper reaches to the mouth, rivers of the Republic of Dagestan to the Kuma River); in the basins of the Black and Azov Seas – the Don and Kuban’ Rivers as well as in rivers on the shore of the Black Sea (Atlas …, 2003). Currently, sunbleak is spreading far beyond its natural areal (Reshetnikov et al., 2017) due to unintended introduction and subsequent self-propagation. Sunbleak penetrated into Pre-Baikal water bodies in 1973 together with fish breeding material of carp *Cyprinus carpio* Linnaeus, 1758, brought into a Kudareya pond (the basins of the Kuda and Angara Rivers) from the fish farm “Zerkal’ny” in Novosibirsk Region (Demin, 1997; Demin and Abramenok, 1998). It is now known that sunbleak inhabits the mainstream of the Angara and Irkut Rivers, as well as in the downstream of the Kaya and Olkha Rivers (Matveev and Samusenok, 2009; Ponkratov, 2014; Yuriev et al., 2021).

Fig.1. Sunbleak *Leucaspius delineatus* (Heckel, 1843).

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*Corresponding author.
E-mail address: denikina@lin.irk.ru (N.N. Denikina)

Received: July 28, 2022; ***Accepted:*** October 17, 2022;
Available online: November 11, 2022

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The appearing of sunbleak in the Irkutsk Reservoir was noted earlier (Yuriev et al., 2021), it results probably as well from an unintended introduction. Due to this fact, it is important to study genetic peculiarities of sunbleak in local ichthyocenoses. The aim of the study is to perform a comparative analysis and phylogenetic reconstruction of sunbleak from the Irkutsk Reservoir.

2. Materials and methods

The catching site (52°12’37’’N, 104°25’28’’E) is located in the Irkutsk Reservoir at the Angara River (Fig. 2). The fishes were caught with hooks at the depth of 2-3 m in July-August, 2019.

Euthanasia of fish was done with an overdose of anesthetic (GOST 33219-2014, 2016) using a 2% lidocaine solution (Lidocaine Bufus, Renewal, Russia). The samples wGOST 33219-2014ere handled in ice in the laboratory and stored at the temperature of -20°C until analysis. The mass and standard lengths of the studied fish (average ± SE) were 2.6 ± 0.2 g and 5.8 ± 1.4 cm, respectively.

Molecular genetic analysis was performed on 20 mature specimens. For DNA extraction, 25 mg of muscle tissue was collected from each specimen. Each sample was used for DNA extraction with the DNA-sorb-AM extraction kit (AmpliSens, Russia) according to the manufacturer’s instructions. The primers COIintF_MiSeq: 5´tcgtcggcagcgtcagatgtgtataagagacagggwacwggwtgaacwgtwtayccycc, dgHCO2198_MiSeq: 5´gtctcgtgggctcggagatgtgtataagagacagtaaacttcagggtgaccaaaraayca3´ (Leray et al., 2013) were used to amplify the *Cox1* gene fragment. The sequence is registered in the GenBank database under the number MZ818000.

The sequence obtained was aligned with all sequences of the *Cox1* gene of sunbleak presented in the GenBank database using the Clustal W program (Larkin et al., 2007).

Evolutionary model selection (Nei and Kumar, 2000) and visualization of phylogenetic relationships were performed using MEGA7 software (Kumar et al., 2016). Evolutionary history was inferred by a maximum likelihood method based on the Kimura’s two-parameter model (Kimura, 1980), using the discrete gamma distribution to model differences in evolutionary rate among sites (K2 + G).

3. Results and discussion

Determination of the species specific fragment of the *Cox1* gene allowed identification of a single haplotype found in all specimens. Comparative analysis and phylogenetic reconstruction based on the nucleotide sequences accessible in the genetic databases showed that the studied species is represented by three phylogenetic lineages (Fig. 3). The nucleotide differences of the fragments of the *Cox1* gene in sunbleak in the comparative data analysis from the GenBank database were 5.5%, and within the phylogenetic lineages the nucleotide sequences differed by < 3%.

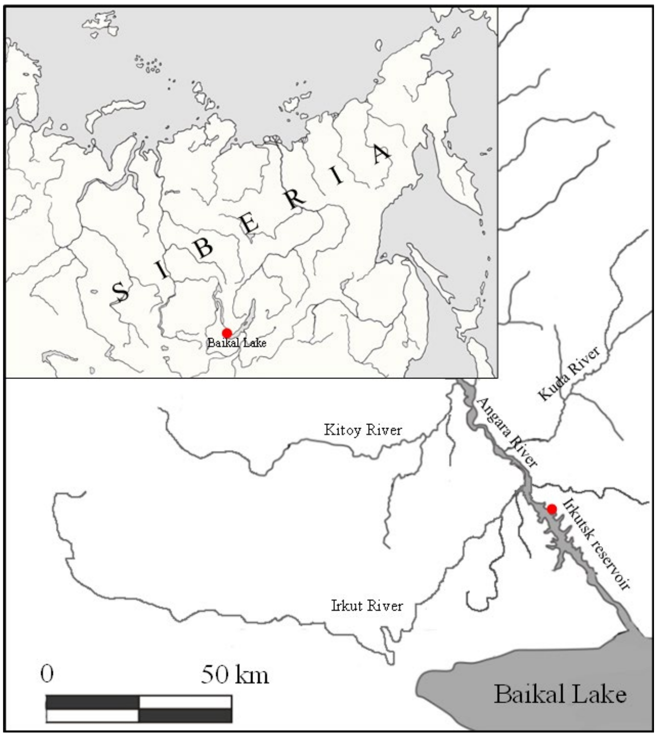

Fig.2. Sampling site, the Irkutsk Reservoir (the Angara River).

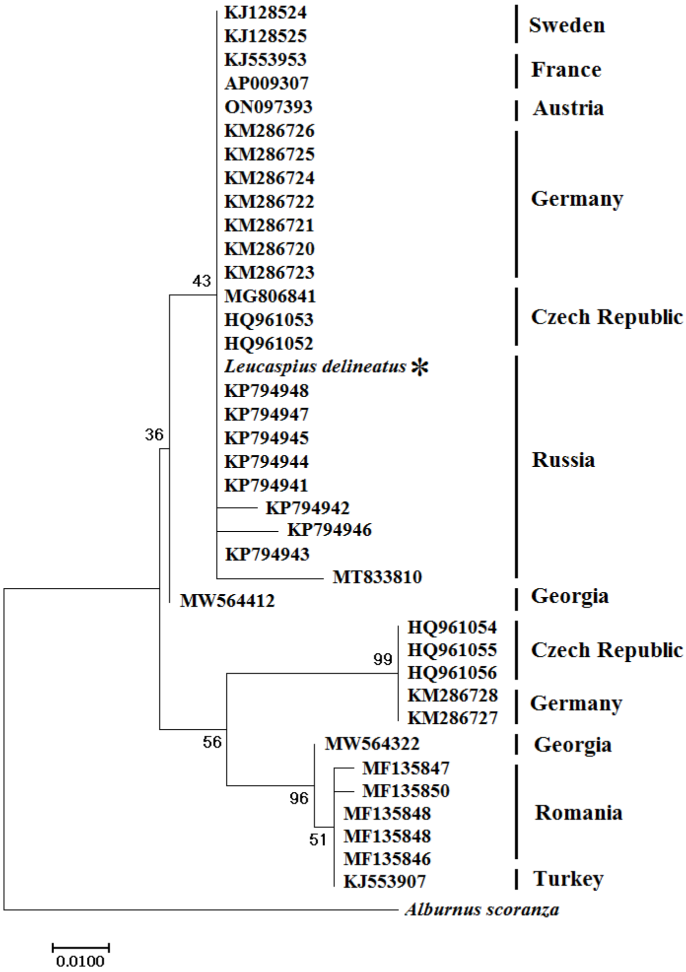

Fig.3. Phylogenetic reconstruction based on *Cox1* gene fragment. *L. delineatus* from the Irkutsk Reservoir (the Angara River) is marked with *.

The previously obtained data of morphological and molecular genetic analyses of sunbleak from the basin of the Pechora River indicate a common mechanism of the origin of the populations of this species on the territory of the Komi Republic and their long-term inhabiting the Pechora basin (Rafikov et al., 2015; Rafikov, 2018). Geographical variability of populations from water bodies of the basins of the Vychegda and Pechora Rivers results from natural species expansion and from its long-term living under the conditions of local landscape-geographical zones (Rafikov, 2018). No genetic differences were found between sunbleak from the Irkutsk Reservoir (the Angara River) and the Pechora River. The specimens we studied were found to be genetically close to fish inhabiting the Bröl River and other German creeks, the Rhone River in France, Lake Skvatsjon in Sweden, the Eger and Jordanka Rivers in the Czech Republic and the Vychegda River in the European part of Russia.

Two other phylogenetic lineages of sunbleak are reliably recognisable as separate clades in the phylogenetic tree. Their distribution is related to Romania – Turkey and the Czech Republic – Germany, respectively (Fig. 3). Two genetically different lineages of sunbleak inhabit the same water bodies: in the Czech Republic (the Eger River) and Germany (creeks).

4. Conclusions

We did not find any genetic differences between sunbleak from the Irkutsk Reservoir (the Angara River) and the Pechora River. This fact confirms the information on occasional introduction and expansion of the species from the European part of Russia into Siberian water bodies. Nowadays, the only one genetic variant in sunbleak, which adapted successfully to hydrological conditions of Siberian water bodies, is found. It is likely that they first inhabited bodies of water in Eastern Europe and from there expanded their range eastward. It is also probable that it was this genetic lineage that proved more successful in adaptation to the new habitat conditions. The lack of genetic diversity in the fragment studied is probably due to the “founder effect” – a decrease in genetic diversity when new areas are inhabited by a small number of representatives of this species, i.e. when a small number of fish are introduced. Sunbleak is an alien species in Siberian water bodies, and its adaptability and food concurrence as well as expansion just near Lake Baikal now require the greatest attention and careful study.

Acknowledgements

The work was supported by the State projects No. 121032300224-8, 121032300196-8.

Conflict of interest

The authors declare that they have no competing interests.

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