Unravelling origin and diversity of a non-native fish in Iran; Hemiculter leucisculus (Basilewsky, 1855) and its response to climate change

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Research Article

Abstract

Non-native and invasive species are the second leading cause of species extinction and one of the major threats to freshwater ecosystems. *Hemiculter leucisculus* is one of the introduced species of Iran's aquatic ecosystems for many years (1967) that has been reported from Iran in the Anzali wetland for the first time. The aim of the current study is to assess the diversity and origin of *H. leucisculus* a non-native species and also its response to current and future climate change in Iran's freshwater. To this, we collected the specimen from the species distribution range in the country and performed the mitochondrial gene, cytochrome b (cytb) combining them with the 84 GenBank(NCBI) sequences. In addition, an ensemble model approach was used for future Species Distribution Modelings (SDMs) and it was estimated according to two Shared Socio-economic Pathways (SSPs): 126 and 585 of the MRI-ESM2 based on CMIP6. According to our results, all the samples examined in the current study originated from the same loctlity, the river basins of china. The results also show a high genetic and haplotype diversity that can have a positive effect on the success of its non-native in different catchments of inland waters of Iran. Afterward, the result of SDM maps, clearly illustrated that climate change would significantly affect the distribution of *H. leucisculus* and it showed a sharp tendency to expand over time in its distribution areas.

Introduction

From the twentith century onward, with the growth of the human population especially human trade and travel, natural barriers such as mountains, oceans, deserts and rivers, that allowed the intricate coevolution of species and the development of unique ecosystems have been breached (Vitule et al. 2019; Abdoli et al. 2022).

One of the important current worldwide biodiversity crises is non-native organisms and biological invasions that have been characterized by an unprecedented rate of species loss (McGeoch and Jetz 2019; Padial et al. 2020). In fact, these species potentially also impose a huge cost on forestry, fisheries, agriculture and other human enterprises, as well as on human health (Taylor 2002; Cambray 2003; Ricciardi and Kipp 2008).

It documented that among all types of ecosystems, freshwater ecosystems are damaged more than others by economic and environmental impacts from non-native and invasive species (Mills et al. 2003), as the intensity of human activity has been positively related to the number of non-native fish species (Leprieur et al. 2008).

To date, in Iran 25 invasive freshwater fish species that belong to 10 orders and 14 families have been reported (Abdoli 2022). *Hemiculter leucisculus* (Basilewsky, 1855) is the only sawbelly that belongs to the hemicultrine group(middle-sized cyprinids) and is one of the non-native freshwater fish that the first time was reported from the Anzali wetland in Iran (1967) (see also Holčík and Razavi 1992; Kiabi et al. 1999; Abdoli 2000; Jolodar and Abdoli 2004; Coad 2010). The main origin range of this species is from far

eastern region of Russia through China to Korea and Vietnam and it has been assumed been introduced into Iran by accident along with commercial shipments of Chinese (Abdoli 2000; Mustafayev et al. 2015; Nitta et al. 2017).

The history of the introduction of *H. leucisculus* to inland waters of Iran about 20 years after the report showed that the species like most other exotic species have sharply been distributed in other catchments area (Abdoli 2016; Rezamand and Patimar 2017). Due to its characteristics such as high reproductive capacity, rapid sexual maturity, short life cycle and resistance to various environmental conditions, it can become invasive in the environments where it has been introduced (coad 2020). Indeed, *H. leucisculus* will easily be able to change its type of food during food competition with other fish (Coad and Hussain 2007; Abdoli et al. 2022).

Therefore, having the ability to predict the results of the introduction of a non-native fish species and having the correct tools for its management is so essential (Bagheri et al. 2016; Khosravi et al. 2020).

The lack of comprehensive knowledge regarding the distribution and origin of *H. leucisculus* in Iran requires detailed investigations with wide sampling using cutting-edge approaches. Hence, due to being non-native to *H. leucisculus*, it could important to study the genetic background. Recent advances in molecular biology quickened the development of effective DNA-based methods that could be used in the detection and monitoring of alien fish species (Chen et al. 2017). Using the phylogeny method is the best tool to identify the introduced species in Iran by molecular analyses, and discover the origin of the introduced *H. leucisculus* in Iran. In addition, using the simulated present and future Spatio-temporal predictions such as Species Distribution Models (SDMs) of non-native species can help diagnose the impact of climate change on the non-native species in freshwater ecosystems and how to predict their distribution for better management.

Although some authors have documented the *H. leucisculus* (Holčík and Razavi 1992; Kiabi et al. 1999; Abdoli 2000; Patimar et al. 2008; coad 2010; Esmaeili(a) et al. 2011; Zareian et al. 2015; Radkhah and Eagderi 2015; Radkhah et al. 2016; Rezamand and Patimar 2017; Mousavi-sabet et al. 2019; Abdoli et al. 2022), no study to date integrating molecular phylogeny and SDMs to discover the origin and predict of climate change of *H. Leuciscus* in Iran.

To do so, the aim and specific objectives of this study are to (a) find out the origin and distribution of the *H. leucisculus*, (b) identify haplotypes and genetic diversity of *H. leucisculus* in Iran and (c) predict potential *H. leucisculus* distribution based on climate change scenarios.

Material And Methods

Taxon Sampling and Laboratory Procedures

To provide a comprehensive phylogeny of *H. leucisculus*, fishes were obtained from four different recorded localities in Iran containing Karun River, Mahabad Dam, Chitgar artificial lake and gorganrud

(Fig. 1). The fishes were euthanized and clips of left pectoral fins were removed and then preserved in 96% ethanol. Collection and locality data for sampled fishes are described in Table. S1.

DNA Amplification and Sequencing

To DNA extraction, high-salt method was performed (Sambrook et al. 1989). A partial sequence of cytochrome b (cytb) gene was selected for molecular analysis. The desired gene was amplified with forward F08_F (5' GACTTGAAAAACCACCGTTG-3') and reverse E08_R (5' CTCCGATCTCCGGATTACAAGAC – 3') primers (Wang et al. 2021). Polymerase Chain Reactions (PCRs) were performed with 1 µl template DNA (50–100 ng), 0.5 µl of each primer, 12.5 µl Master Mix Red (Ampliqon) and 10.5 µl of ddH2O up to 25 µl of reaction mix. The amplification of the PCR, carried out on an MJ Mini™ thermocycler (Bio-Rad), started with an initial denaturation at 95°C for 2 min, followed by 35 cycles consisting of second denaturation at 95°C for 1 min, annealing at 56°C for 30 s and elongation at 72°C for 30 s; the last step was final elongation at 72°C for 10 min. The quality of PCR products were examined with agarose gel 1% stained with Safe-Red ™. The suitable amplicons were sent to Pishgam Inc, for purification and sequencing.

In this study, apart from the newly determined sequences, additional sequences for 84 species were downloaded from GenBank (Table. S1).

Data Analyses

Aligned nucleotide sequences were edited using with Geneious Prime® v. 2021.0.0 (Biomatters, www.geneious.com). To find the phylogenetic position of our study, we obtained 82 additional sequences from Genbank which were added to our dataset (see Table S1). To alignments of the gene dataset, MAFFT v. 7 (https://mafft.cbrc.jp/alignment/server/) was used in a final 617 bp alignment and *Hemiculter beleekeri* was chosen as outgroup.

We used the PartitionFinder v.2 (Lanfear et al. 2016) to best-fit partitioning scheme.

The phylogenetic tree from maximum likelihood (ML) and Bayesian Inference (BI) was inferred by MrBayes v.3.2 (Huelsenbeck and Ronquist 2001). Analysis was performed with Markov Chain Monte Carlo (MCMC) sampling for six million generations and parameters were saved every 100 iterations, which produced 6001 trees during the analysis. Finally, 10% of trees were discarded as burn-in, and the remaining trees were used to reconstruct the 50% majority-rule consensus tree.

The performance of each run and evaluate convergence explored using Tracer v.1.7 (Rambaut et al. 2009).

DnaSP v.5 was used to determine molecular diversity indices that contains the number of haplotypes (H), haplotype diversity (Hd), number of polymorphic sites (s) and nucleotide diversity (π). These diversity indices were estimated for the regional clades based on cytb.

A median-joining (MJ) network was constructed using NETWORK v.10.2 (Bandelt et al. 1999) to find the possible origins of introductions for each detected specimens in Iran.

Environmental Variables and Model Construction

For Species Distribution Modeling (SDM), we collected 93 records of occurrence from three sources: (a) our field surveys (16 occurrence points) carried out in different parts of the study area (2021e2022), (b) the Global Biodiversity Information Facility (GBIF) and direct observations up until the 18th of September 2022 (48 occurrence points) and (c) distribution records compiled by published books and papers (29 occurrence points), then removed duplicated data. This selection process reduced our occurrence records to 77 data points that were used for the distribution modeling approach.

We extracted 19 substantial bioclimatic variables (bio1-bio19) from a 30 arc-seconds (~ 1 km) resolution dataset in WorldClim-Global Climate data for the *H. leucisculus* habitat (http://www.worldclim.org). In addition, slope data from the digital elevation model (DEM) of Iran as an additional geographical input and a Human Footprint Model (HFM) (Sanderson et al., 2002) to evaluate the anthropogenic effects on the *H. leucisculus* habitat were used.

All layers were projected onto the UTM grid, with WGS1984 datum. This bioclimatic and environmental variables are used in the assessment of freshwater fish species distribution (Warren et al. 2013; Hong et al. 2022). To bioclimatic variables the Principal Component Analysis (PCA) was tested for multicollinearity among predictors by calculating coefficients and used an r < 0.80 criteria to select which variables in the distribution models for the present study. The final set of variables were as follows: BIO1 = annual mean temperature; BIO7 = Temperature annual range; BIO8 = Mean Temperature of Wettest Quarter; BIO9; Mean temperature of driest quarter, BIO13 = Precipitation of wettest period; BIO14 = Precipitation of driest period and BIO16 = Precipitation of wettest quarter. The future distribution pattern of *H. leucisculus* for the year 2080 (average of 2061–2080 period) was estimated according to two Shared Socio-economic Pathways (SSPs): 126 and 585 of the MRI-ESM2 and was estimated based on CMIP6.

For model fitting and evaluation, many pseudo-absence were used in the Create Random Point tool in ArcGIS 10.8 to provide more accurate predictions. An ensemble model approach was used to *H. leucisculus* distribution model (Thuiller et al., 2009) using R v. 4.1.3 (R Development Core Team 2014) with the BIOMOD2 package (Thuiller et al. 2016). The nine modelling techniques including: the Generalized Linear Model (GLM), Generalized Boosting Method (GBM), Maximum Entropy (MaxEnt), Classification Tree Analysis (CTA), Artificial Neural Network (ANN), Surface Range Envelops (SRE), Random Forest (RF), Multivariate Adaptive Regression Splines (MARS), and Flexible Discriminant Analysis (FDA), were applied.

Area Under the receiver operating Curve (AUC = ROC), Cohen's Kappa (KAPPA) metrics and the True Skill Statistic (TSS) were evaluated to model performance (Allouche et al. 2006; Zipkin et al. 2012).

Results

Reconstruction of Phylogeny

In the current study, to investigate the situation of individuals belonging to the *H. leucisculus* species, a phylogeny tree was constructed based on 14 sequences studied and 84 sequences from the native distribution area of the species. The datasets, with 617 bp length included partial sequences of cytb.

The Bayesian topology inferred by partitioned analysis was adopted for explaining the *H. leucisculus* phylogeny. The constructed intra- species phylogenetic trees from cytb showed the same topologies for both ML and BI trees. Relative to the outgroup, the *Hemiculter bleekeri* clustered as sister monophyletic lineages. The phylogenetic trees demonstrate that the *H. leucisculus* group are well separated from each other with high support values. According to the Phylogenetic tree, the *H. leucisculus* group was considered a well-supported monophyletic group as expected, and three distinct clades were revealed, the Iranian samples belonged to the 2rd clade (Fig. 2). The samples of this study belong to the china catchment clade and mainly the Yangtze River, which indicates the origin of the individuals studied in Iran.

Haplotype Network Analysis and Genetic Diversity

The parsimony haplotype NETWORK (Fig. 3) for 98 *H. leucisculus* specimens (14 specimens from Iran and 84 specimens from the native distribution area) showed separated haplogroups and different haplotypes for cytb. The NETWORK illustrated that two of the individuals belonging to Iran's specimen (op11 and op12) formed a separate haplogroup. Other specimens in Iran's samples were settled in the Chinese haplogroup.

Molecular diversity indices contain the number of haplotypes (H), haplotype diversity (h), number of polymorphic sites (s) and nucleotide diversity (π) based on cytb were shown in Table 1.

Table 1

Molecular diversity indices based on Cyt b for *H. leucisculus* and its regional populations, including Number of sequences (N), the number of haplotypes (H), haplotype diversity (h), nucleotide diversity (π), and the number of polymorphic sites (S)

Hemiculter leucisculus	Number of sequences	Number of haplotypes (h)	Haplotype diversity (Hd)	Nucleotide diversity (π)	Number of polymorphic sites (S)
Iranian population	14	5	0.731	0.00337	6
Chinese population	82	34	0.937	0.01792	70

Table 2

Evaluation of nine applied models predicting *H. leucisculus* distribution in Iran freshwater using AUC, TSS and KAPPA.

	SRE	MARS	FDA	GLM	MaxEnt	ANN	CTA	GBM	RF
AUC	0.99	0.90	0.96	1	0.98	1	0.96	0.97	0.99
TSS	0.74	0.70	0.72	0.89	0.89	0.86	0.72	0.77	0.89
KAPPA	0.87	0.78	.078	0. 89	0.92	0.81	0.88	.089	.090

Modelling the Present and Future Distribution

In all models techniques for *H. leucisculus* AUC (range of 0.90e1.00), KAPPA (range of 0.78e0.92) and TSS (range of 0.70e0.89) were high predictive capacity. However, for *H. leucisculus* GLM, ANN, MaxEnt and RF were the best performing models, with AUC, KAPPA and TSS > 0.80 (Table. 2).

The greatest contribution to model performance were BIO8 = mean temperature of wettest quarter (38.68%), BIO 1 = annual mean temperature (23.14%), BIO 7 = temperature annual range (14.26%) and BIO 13 = Precipitation of Wettest Month (10.89) (Table. 3).

Based on the future climate change scenarios (SSP126 and 585), suitable habitats will sharply increase over time across most of the *H. leucisculus* range (Fig. 4), and it tend to shift a wide range where human population density is more than other areas.

Table 3. Uncorrelated predictors and mean of their contributions (%) in nine *H. leucisculus* distribution models in Iran freshwater.

Variables	Relative importance (%)	SD
BIO 8 (Mean Temperature of Wettest Quarter)	38.68	9.03
BIO 1 (Annual Mean Temperature)	23.14	6.12
BIO 7 (Temperature Annual Range)	14.26	5.08
BIO 13 (Precipitation of Wettest Month)	10.89	4.23
BIO 9 (Mean Temperature of Driest Quarter)	4.85	2.23
BIO 16 (Precipitation of Wettest Quarter)	2.89	1.68
Human Footprint	2.56	1.26
BIO 14 (Precipitation of Driest Month)	1.39	0.79
DEM	1.34	0.63

Discussion

This study highlighted the role of genetic data in the identification of the origin of *H. leucisculus* a non-native species in Iran. In fact, we investigated the origin of the introduction of the *H. leucisculus* in Iran and tried to explain how the suitable habitat will change using the SDM approach.

According to our results and the investigation of the genetic diversity of the *H. leucisculus* species in Iran, by using the cytb sequences and combining them with the sequences in the GenBank (NCBI), it was determined that there is no difference between Iran's samples and all belong to a clade. As well as, it was also illustrated that there is no significant relationship between the sampling areas of the species in Iran and their origin in the native distribution area. The current study clearly showed the origin and the ancestral of all the samples are in the catchments of China and mostly the Yangtze River. In the other words, it can be theorized that China is the origin and native distribution range of *H. leucisculus*. These results were in line with Wang et al. (2021) findings.

Haplotype Network Analysis and Genetic Diversity
Although there is no strong documentation about the mechanisms of *H. leucisculus* introduction to Iran, it has been guessed that the *H. leucisculus* could be accidentally introduced with other cyprinid species with higher commercial values (Abdoli et al. 2022).
The haplotype network of *H. leucisculus* indicated that all Iranian specimens except for two were positioned with samples in china (Fig. 3). Due to the identification of haplogroups for all 98 specimens studied in different regions, out of the 14 individuals studied in this research, two individuals (op 11, op 12) from the Karun River formed two separate haplogroups. It can indicate the potential of increasing population diversity of *H. leucisculus* compared to its native distribution area and it may constitute separate haplotypes specific to Iran in the future. Based on haplotype network, almost there is one

haplotype for every two individuals sampled and if the number of sampling areas and the number of samples increases, the number of haplotypes will probably increase. Moy et al (2022) for non-native species resulted that high natural diffusion and human translocation could facilitate gene flow and high genetic diversity between river basins, which may help maintain the invasive potential. based on the observed results of the haplotype network, especially for Karun and the existence of separate haplogroups for this population, guessed according to being the long karun river, this river longest and the highest discharge river in Iran, *H. leucisculus* easily expands all over Karun River. As with expention of *H. leucisculus* in Karun River, it can constitute separate haplotypes specific in the future.

It highlighted, the molecular diversity indices, the haplotype and genetic diversity within the species were high (Table. 1). Also according to Table 1, the π of *H. leucisculus* in the Iranian population was low (0.00337) whereas, in the Chinese population was high (0.01792). The reason may have been affected by the founder effect during the invasion of the Iranian population, resulting in lower genetic diversity than in the Chinese population (Yangtze River basin). This result was like Xu et al (2001) that believed having genetic diversity for non-native species will help to adapt to new habitats and maintain new population sizes.

Kolbe et al (2004) mentioned one of the most important features affecting the success of invasive species is haplotype diversity. The haplotype result showed that by removing one haplotype from the population of *H. leucisculus* in Iran, other haplotypes have replaced it, and this proves that it has had invasive success in Iran's inland freshwaters.

Johnston et al (2009) founfed one of the important factors in the genetic diversity of invasive species probably is that Propagule Pressure (PP). In fact, PP is the result of the combination of the number of individuals participating in reproduction at any time the invasive species is introduced and the number of introductions. In the other words, an increase of individuals participating in reproduction probably has a direct effect on genetic diversity and releasing large numbers of individuals into the new environment significantly increases the probability will be maintained the genetic diversity of the introduced source population. In some cases, multiple introductions can lead to more high genetic diversity compared to the native distribution area of the invasive population and basically, the reason for this phenomenon is equivalent to the increase in the number of people participating in reproduction (fertilization). New genotypes can adapt to the features of the new environment in a different way from the initially introduced populations and respond to environmental selection pressures. The combination of newly created genotypes with the original invader population can increase the success capacity of these species.

Species Distribution Modeling (Present–Future)

Our study clearly showed the impact of climate change on the distribution of non-native *H. leucisculus* in Iran's freshwater. The current maps show the suitable distribution for the species, which could occur in a wider distribution range especially in some regions out of the recorded areas (Fig. 4). According to Carosi et al (2019), species can experience four scenarios under climate-change effects (i.e. reduction, expansion, both, or stable) in their habitats. Based on the output maps of Climate change modelling under SSP scenarios, it will be predicted that climate change would significantly affect the distribution of *H. leucisculus* as it showed a sharp tendency to expand over time in its distribution areas (Fig. 4). In

connection with our results, Mamun et al (2018) predicted future climate-change effects on an invasive alien species *Micropterus salmoides* in the Korean peninsula for 2050 and 2100. According to their results, the potentially suitable habitats for *M. salmoides* are most likely to increase by 2050 and 2100. Moreover, the output of the modelling absolutely showed that in areas with high human population density, the probability of *H. leucisculus* distribution in the future is also more. This is due to the high human activities, trade and travel in these areas.

The expansions of *H. leucisculus* into upstream and downstream areas are likely to be the effect of the temperature rise and due to the warm water of this species. It was expected that climate change would benefit this species; temperature illustrated the most important role for model predictions in line with these results, Hong et al (2022) with SDM tools applied to explore predicted range shifts in two invasive alien fish species under future climate-change scenarios. They plainly presented that range shifts increased under climate-change scenarios and temperature is the most reason for the expansion of alien fishes.

It is mentioned that with expansions of alien species the vulnerability of native species will probably be more significant (Hansen et al. 2017; Abdoli et al. 2022; Kim et al. 2022) and it results in lowering the species diversity and degrading the sustainability of native fish species(Jang et al. 2006).

Conclusions

H. leucisculus is an interesting example of non-native species in Iran's inland freshwater. Integrating the phylogeny method and SDMs allowed us to unveil the successful biological invasion of *H. leucisculus* species in the Iran's inland freshwater. Our results supplied the origin of specimens of this study and explain how the suitable habitat under climate change scenarios. Although the information related to the expansion of invasive *H. leucisculus* under climate change in this study can help organize and establish strategic countermeasures and priority area data for ecosystem conservation, introduction sources and pathways should be studied with more genes and more samples.

Declarations

FA: Supervision (lead); Funding acquisition (lead); Conceptualization (lead); data curation (lead); Methodology (equal); Writing − Review & Editing (equal); Resources (equal).

AA: Supervision (lead); Funding acquisition (lead); Conceptualization (lead); data curation (lead); Methodology (equal); Writing − Review & Editing (equal); Resources (equal).

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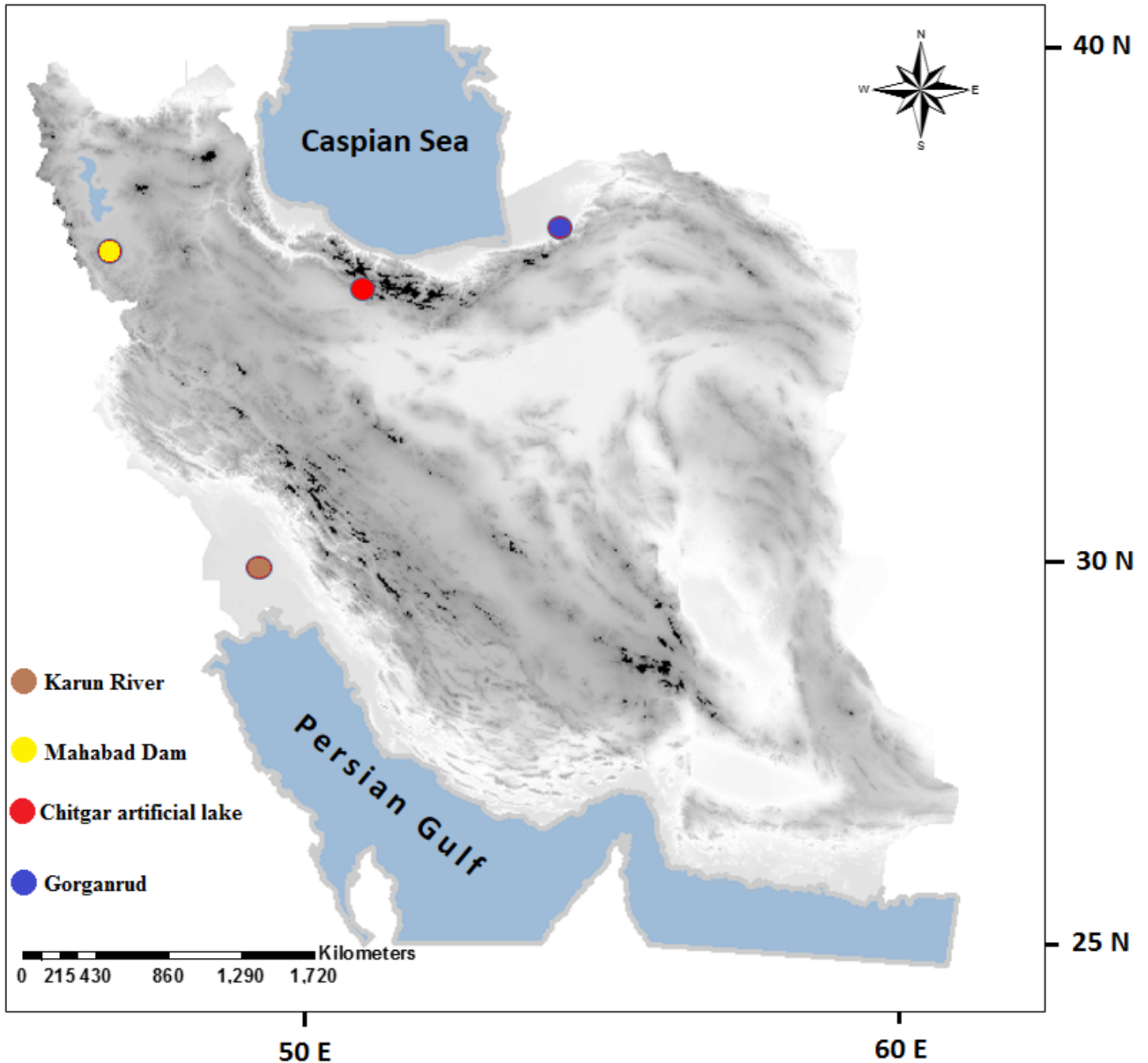
Figures

Figure 1

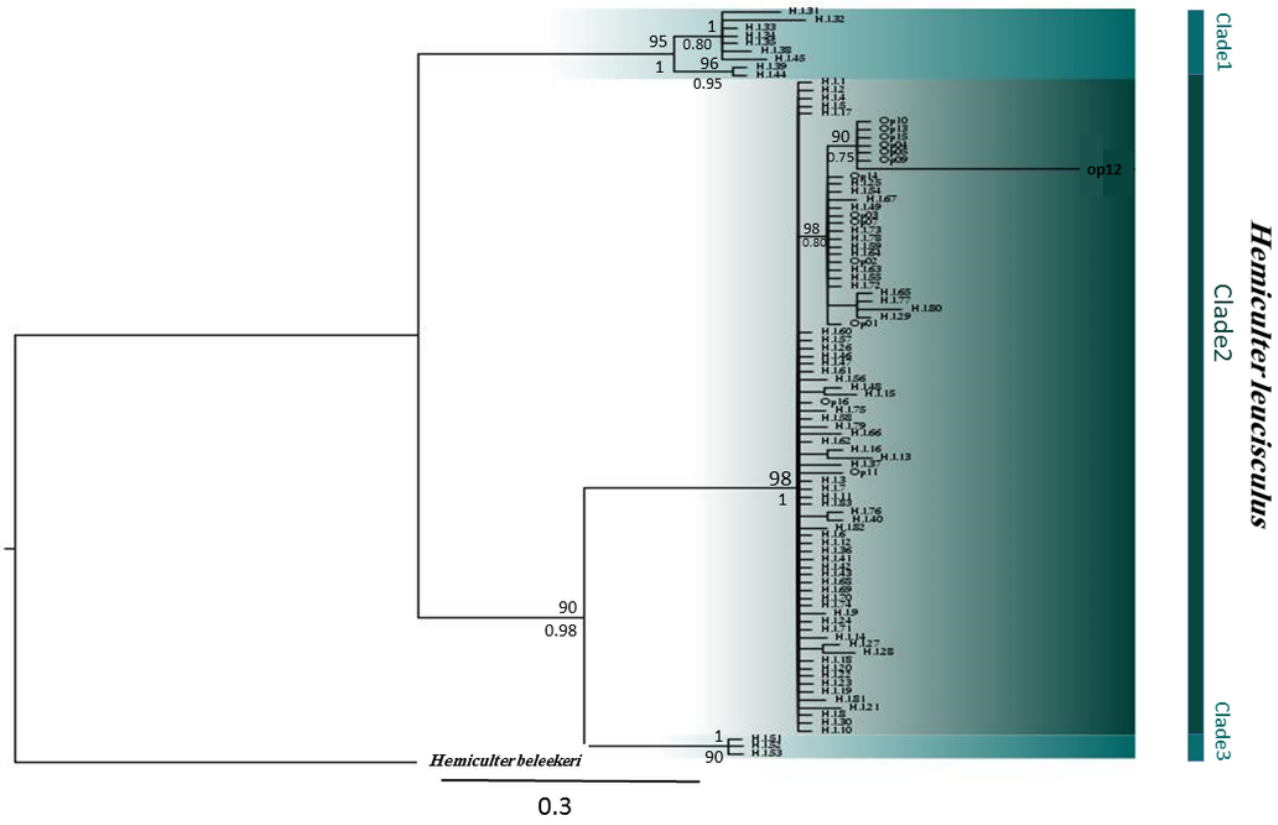Locations of sampling stations *H. leucisculus* in Iran.

Figure 2

Phylogenetic tree reconstructed for *H. leucisculus* based on the cytb. For each node, nodal supports indicate BI posterior probabilities (top) and ML bootstrap support (in percent, base).
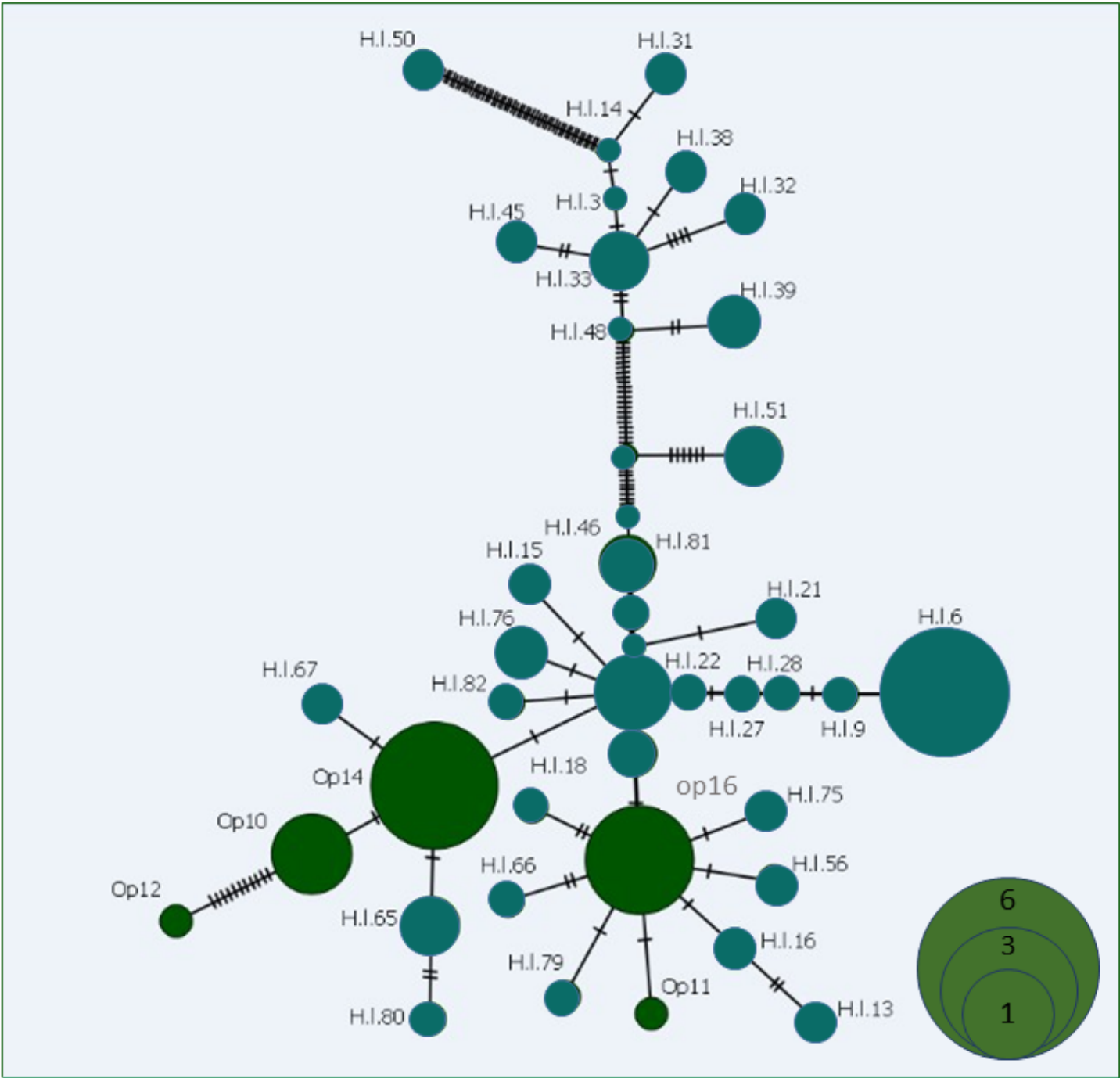
Figure 3

Median-joining (MJ) haplotype network. Each circle represents a unique haplogroup, and its size reflects the number of individuals expressing that haplotype. Crosshatches indicate the number of nucleotide differences between haplotypes.
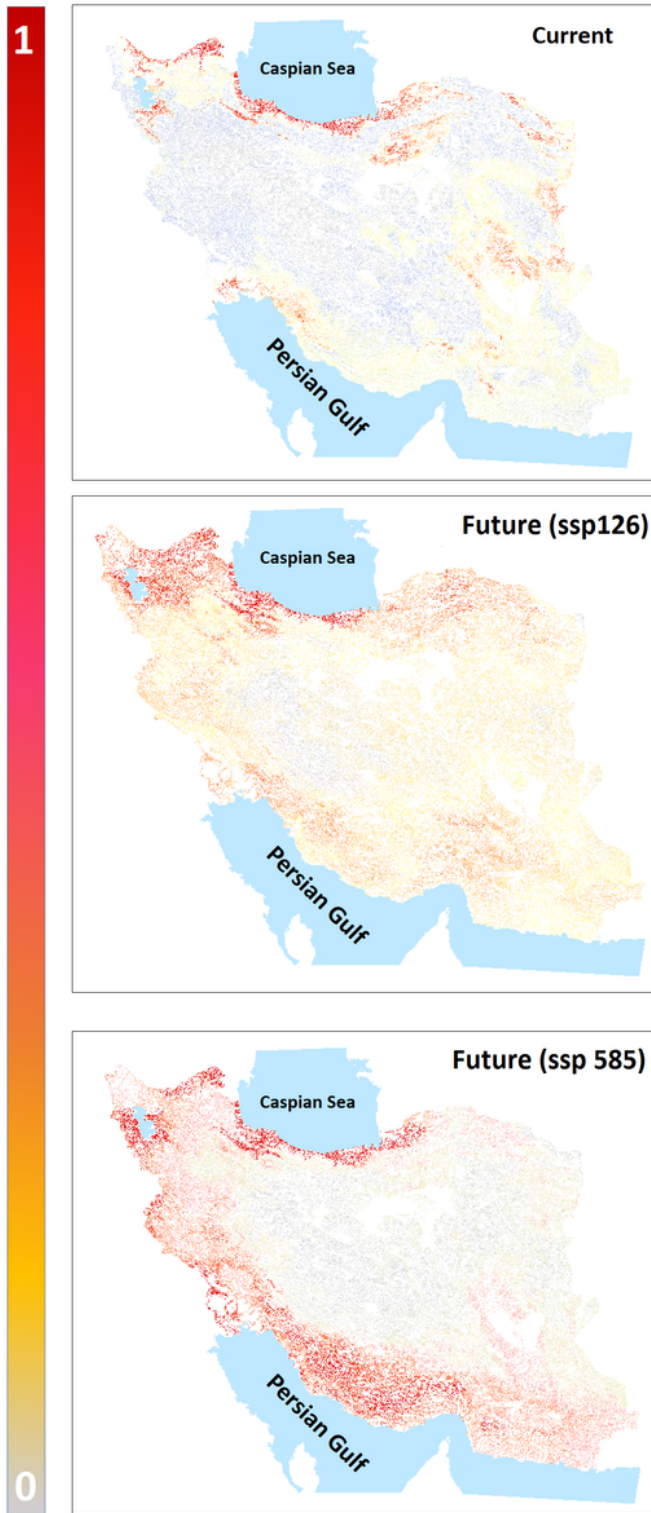
Figure 4

The potential distribution range of the *H. leucisculus* in Iran freshwater under current and future climate projection (2061-2080), SSP126 and 585.

Supplementary Files

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