The Silent Threat of a Non-native Oligochaete Species in Iran's freshwater: Assessment of the Diversity and Origin of Eiseniella tetraedra (Savigny, 1826) and its Response to Climate Change

Maryam Azimi

Maryam Bagheri

Hadi Khoshnamvand (✉ h_khoshnamvand@sbu.ac.ir)

Asghar Abdoli

Faraham Ahmadzadeh

Research Article

Abstract

Oligochaetes are the most abundant benthic taxa in aquatic ecosystems that play an important role in food webs and energy transmission. The aim of the current study is to assess the origin and diversity of *Eiseniella tetraedra* a non-native species of Lar National Park in Alborz Mountain ranges of Iran and also its response to current and future climate change. To this, we collected the specimen (23 samples) from Lar National Park Rivers and performed the mitochondrial gene, mitochondrial Cytochrome c oxidase subunit I (COI) combining them with 117 sequences from Jajroud, Karaj River in Iran and native regions from GenBank (NCBI). We also used an ensemble model approach for Species Distribution Modelings (SDMs) that 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 Spanish rivers and there was no unique haplotype found in Lar National Park. Moreover, The results also show high haplotype diversity that can have a positive effect on the success of its non-native in the different freshwater of Iran. As well as, the result of SDMs maps illustrated that climate change would significantly affect the distribution of *E. tetraedra* and it showed a sharp tendency to expand and verified the invasion power of the *E. tetraedra* in Iran's freshwater ecosystems over time.

Introduction

Oligochaete species belonging to annelid worms occur in marine and terrestrial ecosystems. About one-third of the almost 5,000 known Oligochaete species is aquatic and semiaquatic (Suriani et al. 2007; Krieger et al. 2010). With some exceptions, these groups of oligochaetes are small in size, ranging from 1 mm to a few centimetres in length (Martin et al. 2008). Regarding the idea that earthworms have a limited capacity to autonomously disperse (Sakai et al. 2001), it has been thought that the cosmopolitan dissemination of some oligochaetes (about 110 species) will often be due to human activities or animal-mediated transportation (Costa 2013).

The occurrence of introduced species may cause ecological changes in the ecosystem if they can adapt to new habitats, leading to potential negative interaction with the native species (Gozlan and Newton 2009). Several researches demonstrated that introduced Oligochaete worms have remarkable impacts on the ecosystem. Once introduced earthworms become invasive, and they can cause changes in microorganisms fauna of soil, competition with native species and loss of biodiversity resulting in economic losses and detrimental effects on habitats (Eisenhouer 2010; Blouinet al. 2013; Craven et al. 2017). Furthermore, management options for controlling invasive species are generally troublesome and presumably threaten native species. Hence, clarifying the population structure of exotic species is essential to decrement their harmful effects on the ecosystem.

Parallel to the worldwide status, Iranian inland waters are exposed to habitat degradation and species introductions (either intentionally (e.g., for soil remediation or commercial applications) or inadvertently (e.g., in soil associated with horticultural and agricultural products) likely decrease endemic populations (Abdoli et al. 2022).

So far, 20 species of Oligochaetes have been identified from Iran (Jablonska and Pesic 2014; Nazarhaghighi et al. 2015). No molecular surveys have been conducted to clarify whether these species are native or exotic. Just recently, Javidkar et al (2020) reported a non-native Oligochaete *Eiseniella tetraedra* (Savigny, 1826) from two protected rivers in the southern Alborz Mountains. They deduced European origin for Iranian populations that were transported to this country by anthropogenic activities.

Population genetic structure is the distribution of genotypes in space and time and is determined by both historical and current evolutionary processes (Hewitt and Butlin 1997). The previous study (Javidkar et al. 2020) reported genetic variation in *E. tetraedra* populations in Iran that may be related to the successful establishment and colonization of the species in new habitats. However, the origin and distribution paths have not fully been investigated. For example, whether current populations are derived from a single introduction or are the result of several successive waves.

Besides molecular analysis, Species Distribution Models (SDMs) can act as a useful tool to predict the distribution of species according to climate change. In fact, SDMs significantly relate species presence points with climate and topographical data, unveiling species-to-environment connections that are responsible for shaping species distribution patterns (Ashrafzadeh et al. 2022). However, for non-native species, it has been firmly documented that SDMs approach could be a valuable proactive tool to distinguish invasion potential (Guisan and Thuiller 2005; Reshetnikov and Ficetola 2011; Gallien et al. 2012; Banha et al. 2017; Godefroid et al. 2019; Nunez et al. 2019).

To this end, the aim of the current study is to integrate the mitochondrial marker and SDMs method to investigate the origin and differentiation of *E. tetraedra* populations from two protected rivers (Jajroud and Karaj) and one national park (Lar) in Southern Alborz Mountains and predict potential *E. tetradra* distribution based on climate change scenarios to find how invasion potential.

Material and Methods

Taxon Sampling and Laboratory Procedures

A total of 23 specimens of *E. tetradra* from 12 stations were collected from the rivers of Lar National Park in Iran (Fig. 1). A small piece of the end of the tail was dissected for each specimen then all tissues for DNA extraction were preserved in 96% ethanol and at -20°C. Locality and collection data for *E. tetraedra* are explained in additional file: Table S1.

DNA Amplification and Sequencing

The DNA was extracted from tissues using a standard high-salt method (Sambrook et al. 1989). The partial mitochondrial Cytochrome c Oxidase subunit I (COI) was amplified for all specimens using the universal primers forward LCO1490 (5´-TACTC-AACAA-ATCAC-AAAGA-TATTG-G-3´) and reverse HCO2198 (5´-TAAAC-TTCAGGGTGA-CCAAAAAATC-A-3´) (Shekhovtsov et al. 2016). Polymerase Chain Reactions (PCRs) were conducted 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 DNA was performed with an initial denaturation period of 3 min at 94°C followed by 34 cycles of 94°C for 30 s, primer-specific annealing temperature of 48°C (Additional file 2: Table S2) for 30 s, 72°C for 1 min and a single final extension at 72°C for 5min. The quality of PCR products were assessed with agarose gel 1% stained with Safe-Red™. The suitable amplicons were sent to Pishgam Inc, for purification and sequencing.

Sequence Analyses

Initially, the nucleotide sequences were edited by BioEdit V.2.34 (Hall 1999), aligned by Geneious Prime® V. 2021.0.0 (Biomatters, www.geneious.com) and optimized by eye using MEGA X (Kumar et al. 2018). We extracted 117 sequences from Genbank which were added to our dataset (Table S1). The final gene dataset was 528 bp and MAFFT v. 7 (https://mafft.cbrc.jp/alignment/server/) was used for alignment.

Phylogenetic Analyses and Haplotype Network

Phylogenetic analyses of *E. tetraedra* of the **COI** data were reconstructed using maximum-likelihood (ML) and Bayesian inference (BI) approaches. *Hermodice carunculata* downloaded from GenBank was used as outgroup. The Akaike information criterion was used to select Nucleotide substitution models (GTR + I + G) in MrModeltest v 2 (Nylander 2004).

For BI and ML analyses, two independent runs and four Markov chains (three heated chains and a single cold chain) using the best-fit models were performed in MrBayes v 3.1.2 (Huelsenbeck and Ronquist 2001). Each run was conducted 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 the trees were discarded as burn-in and the remaining trees were used to reconstruct the consensus tree. Tracer v.1.7 (Rambaut et al. 2009) was implemented to the performance of each run and evaluate convergence. To edit and visualis phylogenetic tree, FigTree v.1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/) was used.

Also, we used NETWORK v.10.2 (Bandelt et al. 1999) to construct a median-joining network for **COI**.

Genetic Diversity and Demographic Analysis

The number of haplotypes (H), number of polymorphic sites(s), haplotype diversity (h) and nucleotide diversity (π) of each population were extracted by DnaSP v5 (Librado and Rozas 2009).

To demographic history analyses, we used selective neutrality test of Fu's Fs statistics (Fu 1997) and Tajima's D (Tajima 1989) based on **COI** to find evidence of recent expansion for each lineage using Arlequin v.3.5 (Excoffier and Lischer 2010). A Mismatch Distribution (MMD) analysis was separately performed for each population to estimate the frequency distribution of the pairwise nucleotide differences, assuming a sudden expansion with spatial parameters. The test was performed using Arlequin v.3.5.

Environmental Variables and Model Construction

Occurrence Data

For Species Distribution Modeling (SDMs), a total of 127 *E. tetraedra* locations were compiled between 2019 e2023 through multiple sources (a) direct field surveys (b) the Global Biodiversity Information Facility (GBIF) website, and (c) distribution recorded by published papers.

We visually checked occurrence points with those collected from the literature review in ArcGIS and to reduce spatial autocorrelation in the occurrence points all them filtered with inaccurate spatial information using the package "CoordinateCleaner" (Zizka et al. 2019) in the R v 4.1.3. This process reduced our presence records to 98 points that were available for the habitat modeling approach.

Environmental Variables

We used 19 environmental variables that were used to affect the spatial range of a species (Hermes et al. 2018). All bioclimatic variables (Bio1-Bio19) were downloaded from the WorldClim-Global Climate Database (https://www.worldclim.org/) with a resolution ~ 1 km^2 (30-arc second) for both current (1970–2000) and future (2061–2080) climatic scenarios. Slope data was extracted from the Digital Elevation Model (DEM, http://www.worldclim.org) as an additional geographical input to provide a measure of topographic heterogeneity. In addition, Human Footprint Model offered by Sanderson et al (2002) to evaluate quantifies anthropogenic effects on the *E. tetraedra* habitat. All layers with WGS1984 datum were projected onto a UTM grid and resampled resolution at 1 km^2. A principal Component Analysis (PCA) was estimated for multicollinearity among predictors by calculating coefficients (r < 0.75) and criteria to select which essential variables in the distribution models for the present study.

Eventually, the remaining input variables for the modelling were as follows: Annual Mean Temperature (Bio1), Temperature Annual Range (Bio7), Mean Temperature of Wettest Quarter (Bio 8), Mean Temperature of Driest Quarter (Bio 9), Precipitation of Wettest Month (Bio 13), Precipitation of Driest Month (Bio 14), Precipitation of Wettest Quarter (Bio 16), Human Footprint and Slope.

For future mapping of the suitable climate of *E. tetradra* under future climate change, we extracted the bioclimatic data from MRI-CGCM3 (Meteorological Research Institute, Japan) and used two scenarios Shared Socio-economic Pathways (SSPs): 126 and 585 based on CMIP6. An ensemble model approach was applied to *E. tetraedra* distribution model (Thuiller et al. 2009) using BIOMOD2 package (Thuiller et al. 2016) in R v. 4.1.3 (R Development Core Team. 2014). The ensemble model was formed using nine modelling techniques: Generalized Boosting Method (GBM), the Generalized Linear Model (GLM), Maximum Entropy (MaxEnt), Flexible Discriminant Analysis (FDA), Random Forest (RF), Classification Tree Analysis (CTA), Multivariate Adaptive Regression Splines (MARS), Surface Range Envelops (SRE) and Artificial Neural Network (ANN). To provide more accurate predictions we created many pseudo-absences (n = 220 points) with five replicates per model (Hamid et al. 2018; Dar et al. 2021).

We also evaluated model performance using Area Under the receiver operating Curve (AUC = ROC), the True Skill Statistic (TSS) and Cohen's Kappa (KAPPA) metrics.

Results

Phylogenetic Analyses and Haplotype Network

The datasets, with 528 bp length included partial sequences of **COI** to investigate the position of individuals belonging to the *E. tetraedra* in a phylogeny tree was constructed. The tree based on 140 sequences examinaed in this research contain Lar National Park (23 sequences), Jajroud (40 sequences) and Karaj river (40 sequences) and also 37 sequences from the native distribution area of the species was drawn.

The constructed intraspecies phylogenetic trees based on **COI** showed similar topologies for both ML and BI trees and revealed well-supported monophyletic lineages (only BI tree shown, Fig. 2). In association with the outgroup, the *Hermodice carunculata* clustered as sister monophyletic lineages.

Based on the phylogenetic tree, the *E. tetradra* group was considered a well-supported monophyletic group as expected and the Iranian samples were well placed together with other samples of the population of origin. (Fig. 2).

The phylogenetic trees depict that the *E. tetraedra* group are well separated with high support values. The samples of this study belong to the Spanish catchment clade, which indicates the origin of the individuals studied in Iran.

The parsimony haplotype NETWORK for 140 *E. tetraedra* specimens (103 specimens from Iran and 37 specimens from the native distribution area) showed separate haplogroups and different haplotypes for **COI** by recognizable mutations (Fig. 3). It showed five haplogroups and 40 haplotype for 140 *E. tetraedra* from the distribution area. Also, according to the results, four haplotypes were verified for Lar National Park as they were shared with Jajroud and Karaj, and Lar National Park region did not have a specific haplotype.

Genetic Diversity and Demographic Analysis

Demographic analyses for four localities from all regions were considered. The result of molecular diversity indices depicts the number of haplotypes (H), haplotype diversity (h), number of polymorphic sites (s) and nucleotide diversity (π) based on **COI** in Table 1.

Table 1

Molecular diversity indices based on Cyt b for *E. tetraedra* 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)

Locality	Number of sequences(N)	Number of haplotypes (H)	Haplotype diversity (h)	Nucleotide diversity (π)	Number of polymorphic sites (S)
Lar River	23	4	0.597	0.05502	68
Jajroud	34	7	0.770	0.05614	70
Karaj river	30	10	0.867	0.05953	86
Spain	40	19	0.868	0.05148	85

Tajima's D and Fu's fs analysis were not significant for each population (Lar National Park population p < 0.88, Jajroud population p < 0.98, Karaj river population p < 0.89 and Spanish population p < 0.64) (Table 2). In addition, The MMD diagrams for all population indicated a multimodal pattern (Additional file: Fig. 1).

Table 2

Tests for population expansion for proposed subspecies of *E. tetraedra*

	Tajima's D	Tajima's D p-value	FS	FS p-value
Lar River	1.04	0.88	2.91	0.88
Jajroud	2.73	0.98	11.96	0.99
Karaj river	1.06	0.89	-0.31	0.49
Spain	0.11	0.64	-1.3	0.37

Table 3

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

	SRE	MARS	FDA	GLM	MaxEnt	ANN	CTA	GBM	RF
AUC	0.98	0.91	0.97	1	0.99	1	0.95	0.96	0.98
TSS	0.74	0.71	0.74	0.88	0.86	0.89	0.71	0.76	0.88
KAPPA	0.82	0.77	.079	0.89	0.94	0.82	0.88	.089	.091

Modelling the Present and Future Distribution

In all pattern predictions of models for *E. tetraedra* AUC (0.90-1.00), TSS (0.70–0.89) and KAPPA (0.78–0.92) were good to excellent predictive capacity. Also, the best performing models for *E. tetraedra* were MaxEnt, GLM, ANN, and RF with AUC, TSS and KAPPA > 0.80 (Table. 3).

In among variables BIO 1 (34.3), BIO 9 (24.8), BIO 14 (16.6) and Footprint (10.7) were the greatest contribution to model performance (Table. 4).

Table 4. Uncorrelated predictors and mean of their contributions (%) in nine *E. tetraedra* distribution models in Iran freshwater.

Variables	Relative importance (%)	SD
BIO 1 (Annual Mean Temperature)	34.3	9.03
BIO 9 (Mean Temperature of Driest Quarter)	24.8	6.85
BIO14 (Precipitation of Driest Month)	16.6	5.61
Human Footprint	10.7	4.22
BIO 7 (Temperature Annual Range)	3.2	2.21
BIO13 (Precipitation of Wettest Month)	3.1	1.57
Slope	2.8	1.23
BIO16 (Precipitation of Wettest Quarter)	2.4	1.1
BIO8 (Mean Temperature of Wettest Quarter)	2.1	0.83

According to the future climate change scenarios (SSP126 and 585) in our models projected the increase of suitable areas under all climate scenarios over time and suitable habitats will sharply increase over time across most of the *E. tetraedra* range (Fig. 4), as it tends to shift a wide range in the Western and almost the Southern of Iran.

Discussion

This study clearly reports the role of genetic data in the identification of the origin of *E. tetraedra* a non-native species in Iran freshwater ecosystem. In fact, we investigated the origin of the introduction of the *E. tetraedra* in Iran and tried to explain how the suitable habitat will change using the SDMs approach. The beginning of studies on aquatic Oligochaeta back to 1920 (Stephenson 1920) and after about 100 years the Iranian fauna of aquatic Oligochaeta is inadequately known and limited to just a few studies (Egglishaw 1980; Ahmadi et al. 2012; Javidkar et al. 2019). Based on previous studies, 20 Oligochaeta species have been verified in Iran until 2015 (Jabłon´ska and Pesˇic´ 2014). Considering the area, mountainous landscapes, geographical features and specific hydrological characteristics of Iran, it seems that there will be an increase in the number of these species in the future.

Latif et al (2009) identified *E. tetraedra* based on morphology as a non-native species with European and Palearctic origin from Haraz and Chalus rivers in Iran. Then Javidkar et al (2019) reported the first molecular attempts to discover the aquatic oligochaetes in Iran and they confirmed the non-native of the species by combining samples from Jajroud and Karaj with sequences from NCBI from studies elsewhere in the world for the species.

Until this study and Javidkar et al (2020), the name of this species has not been listed in the native aquatic oligochaetes of Iran and our results reported *E. tetraedra* as a non-native species in Lar national park freshwater ecosystem. In line with our results, a number of researchers have reported *E. tetraedra* as a non-native species in other regions (Brinkhurst et al. 1960; Wood and James 1993; Martinsson et al. 2015; Sosa et al. 2017; Kim et al. 2017; Javidkar et al. 2020).

Haplotype and Genetic Diversity

The results of the haplotype network clearly showed that the samples of Lar National Park, Jajroud and Karaj rivers did not have a specific haplotype and haplotypes of the current study are shared with Jajroud and Karaj rivers. To explain this phenomenon three hypotheses can be suggested; (a): *E. tetraedra* was independently introduced into all three habitats in Iran, (b) initially, *E. tetraedra* was introduced in Lar National Park, and then transferred to Jajrud and Karaj river, and its diversity and abundance decreased over time in Lar National Park; considering the Karaj river has the highest haplotype diversity and specific haplotype and also from Karaj river to Jajroud and Lar National Park, the haplotype diversity decreases, the third hypothesis is proposed, (c) *E. tetraedra* was initially introduced the Karaj river and was transferred to Jajroud and then to Lar National Park. However, based on the evidence and results, the third hypothesis is stronger. As well as, according to studies, altitude is one of the important limiting factors of distribution for the species as the abundance and diversity of Oligochaeta decrease with increasing altitude (Salome et al. 2011). Considering that the Lar National Park is located at an altitude of about 3000 meters, it seems that the species was not native to the region and accidentally transferred to the area.

The molecular diversity indices depicted the haplotype and genetic diversity within the species were almost high (Table. 1). Also according to Table 1, the π of *E. tetraedra* in the three Iranian populations were 0.05502e0.05953 and in the Spanish population was 0.05148 that it showed almost no different genetic diversity in all populations and the Iranian population has not low genetic diversity than the Spanish population. Therefore, not having low genetic diversity compared to the origin population, can express the invasivation of the species in the introduced areas. Xu et al (2001) mentioned that for non-native species genetic diversity is so necessary to adapt to new habitats and maintain new population sizes. In addition, having high haplotype diversity one of the most important features affecting the success of invasivation of the species (Kolbe et al. 2004). The result (Table. 1) showed that *E. tetraedra* haplotype diversity in Iran's freshwater is increasing and it will have invasive success in Iran's freshwaters.

Species Distribution Modeling (Present–Future)

Our study showed the impact of climate change on the distribution range of non-native *E. tetrahedra* in Iran's freshwater ecosystems. Carosi et al (2019) believed that species can experience four reactions under climate-change effects (i.e. expansion, reduction, both, or stable) in their habitats.

The current map for *E. tetrahedra* clearly shows the suitable distribution for the species, that could occur in a wider distribution range especially in some regions out of the recorded areas (Fig. 4). Based on the outcome future maps of climate change modelling under SSPs scenarios, it will be predicted that climate change would significantly affect the distribution of *E. tetrahedra* as maps 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, regarding the output of the modelling, it seems where human population density is high, these areas are probably more affected by the species in the future. This is maybe due to the high human activities, travel and trade in these areas.

The temperature increase is an effective factor in the expansion of *E. tetrahedra* in Iran's freshwater ecosystems as expected climate change would benefit the species. In fact, temperature and precipitation were the most important role in model predictions. Based on the studies, *E. tetraedra* is expanding in most regions of the world and usually prefers humid habitats (Latif et al. 2009; Ezzatpanah et al. 2010; Mirmonsef et al. 2011; Yousefi et al. 2009). Therefore, it may be possible to justify their distribution in the humid regions of the country, including the northern and southern regions. Hong et al (2022) with SDMs tools mentioned temperature as the most reason for the expansion of range shifts in two invasive alien species under future climate-change scenarios.

However, the result of SDMs explicitly illustrated the invasion power of the *E. tetrahedra* in Iran's freshwater ecosystems over time. 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 freshwater species (Shi et al. 2010).

One of the main drivers of worldwide biodiversity loss is biotic exchange in ecosystems by invasive species (Butchart et al. 2010). Although we did not appraise the effects of *E. tetraedra* as a non-native on other non-oligochaete species, studies e.g Migge-Kleian et al (2006) and Ziemba et al (2016) have shown the negative effects of non-native earthworms across trophic levels. According to the evidence in the present study and the identification of the success of Oligochaetes species in terms of being invasive in the river systems of Iran, it is assumed that freshwater ecosystems may be quite vulnerable to Oligochaetes of Western Palearctic origin and taking into account the negative consequences on native species, careful management strategies and regulations can help to mitigate these risks. It is essential that governments and individuals alike take a proactive approach to preventing the spread of invasive species and work to protect native ecosystems. Additionally, quarantine policies must be strictly enforced to help ensure that no potentially damaging organisms are imported into new environments.

CONCLUSIONS

E. tetraedra as an Oligochaete species is an interesting example of non-native species in Iran's freshwater. Integrating the phylogeny method and Species Distribution Models (SDMs) allowed us to unveil the successful biological invasion of *E. tetraedra* in the Iran's freshwater. Our results provided the origin of specimens of this study and supplied a novel approach to assessing the biological invasion of *E. tetraedra* under climate change. Although, the current study presented evidence for the invasion *E. tetraedra*, the information can help establish strategic and priority area data for ecosystem conservation.

Declarations

Funding

not applicable.

Availability of data and materials

The accession numbers for the datasets that were analyzed in this study can be found in supplementary data.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

Additional accessibility data is provided as Additional file.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

MB: Conceptualization (supporting); formal analysis (lead); methodology (lead); project administration (equal); software (lead); visualization (supporting); writing–original draft (lead); MA: Conceptualization (supporting); formal analysis (lead); software (lead); visualization (supporting); writing–original draft (lead); HK: Conceptualization (supporting); formal analysis (lead); software (lead); visualization (supporting); writing–original draft (lead); FA: Conceptualization (lead); data curation (lead); formal analysis (equal); methodology (equal); supervision (lead); writing–original draft (equal); writing–review and editing (equal) and A A:Conceptualization (lead); data curation (lead); formal analysis (equal); methodology (equal); supervision (lead); review and editing (equal).

Acknowledgments

References

1. Butchart SHM, Walpole M, Collen B, van Strien A, Scharlemann JPW, Almond REA, Baillie J E M, Bomhard B, Brown C, Bruno J, Carpenter KE, Carr GM, Chanson J, Chenery AM, Csirke J, Davidson NC, Dentener F, Foster M, Galli A, Galloway JN, Genovesi P, Gregory RD, Hockings M, Kapos V, Lamarque JF, Leverington F, Loh J, McGeoch MA, McRae L, Minasyan A, Herna´ndez Morcillo M, Oldfield TEE, Pauly D, Quader S, Revenga C, Sauer J R, Skolnik B, Spear D, Stanwell-Smith D, Stuart S N, Symes A, Tierney M, Tyrrell T D, Vie´ J C, Watson R (2010) Global biodiversity: indicators of recent declines. *Sci* 328:1164–1168. https://doi:10.1126/SCIENCE.1187512

2. Migge-Kleian S, Mclean MA, Maerz J C, Heneghan L (2006) The influence of invasive earthworms on indigenous fauna in ecosystems previously uninhabited by earthworms. *Bio Inva* 8: 1275–1285. https:// doi:10.1007/S10530-006-9021-9

3. Ziemba J L, Hickerson C A, Anthony C D (2016) Invasive Asian earthworms negatively impact keystone terrestrial salamanders. *PloS One* 11:e0151591. https://doi:10.1371/JOURNAL.PONE.0151591

4. Hermes C, Keller K, Nicholas RE, Segelbacher G, Schaefer HM (2018) Projected impacts of climate change on habitat availability for an endangered parakeet. *Plos One* 13: e0191773.

5. Kolbe JJ, Glor RE, Schettino LR, Lara AC, Larson A, Losos BJ (2004) Genetic variation increases during biological invasion by a Cuban lizard. *Nature* 431: 177–181.

6. Xu C, Zhang W, Lu B, Chen J (2001) Progress in studies on mechanisms of biological invasion. *Bio Sci* 4: 430–438. https://doi:10.17520/biods.2001063.

7. ShiW, Geng Y, Ou X (2010) Genetic diversity and invasion success of alien species: Where are we and where should we go? *Bio Sci* 6: 590–597. https://doi: 10.3724/SP.J.2010.590

8. Krieger KA, Stearns AM (2010) Atlas of the Aquatic Oligochaete Worms (Phylum Annelida: Class Clitellata: Superorder Microdrili) Recorded at the Old Woman Creek National Estuarine Research Reserve and State Nature Preserve. Ohio National Center for Water Quality Research Heidelberg University Tiffin, Ohio, USA p. 32

9. Suriani AL, Franca RS, Pamplin PAZ, Marchese M, Lucca JV, Rocha O(2007) Species richness and distribution of oligochaetes in six reservoirs on Middle and Low Tiete river (SP, Brazil). *Act Limno Bra* 19: 415 - 426.

10. Hall T A (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98NT. Nucleic Acids Symposium Series 41: 95–98.

11. Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. *Mol Bio and Evol* 35(6): 1547–1549. https://doi: 10.1093/molbev/msy096.

12. Nylander JAA (2004) MrModeltest Version 2. Program Distributed by the Author. Evolutionary Biology Centre, Uppsala University, Uppsala.

13. Godefroid M, Meurisse N, Groenen F, Kerdelhué C, Rossi J P(2019) Current and future distribution of the invasive oak processionary moth. *Bio Inva* 22: 523–534. https ://doi.org/10.1007/s1053 0-019-02108 -4.

14. Gallien L, Douzet R, Pratte S, Zimmermann N E Thuiller W (2012) Invasive species distribution models—how violating the equilibrium assumption can create new insights. *Glo Eco and Bio* 21: 1126–1136. https ://doi.org/10.1111/j.1466-8238.2012.00768 .x

15. Nunez-Mir G C, Guo Q, Rejmanek M, Iannone B V, Fei S (2019) Predicting invasiveness of exotic woody species using a traits-absed framework. *Eco Let* 100: e02797. https ://doi.org/10.1002/ecy.2797.

16. Ashrafzadeh MR, Khosravi R, Mohammadi A, Naghipour AA, Khoshnamvand H, Haidarian, M, Penteriani V (2022) Modeling climate change impacts on the distribution of an endangered brown bear population in its critical habitat in Iran, *Sci of the Tot Envi* 837: 155753. https://doi.org/10.1016/j.scitotenv.2022.155753.

17. Nazarhaghighi F, Timm T, Mousavi Nadoushan R, Shabanipour N, Fatemi MR, Moradi MA (2014) Oligochaetes (Annelida, Clitellata) in the Anzali International Wetland, north-western Iran. *Est J of Eco* 63(3). https://doi:10.3176/ECO.2014.3.02.

18. Shekhovtsov S V, Berman D I, Bazarova N E, Bulakhova N A, Porco D, Peltek S E (2016) Cryptic genetic lineages in Eisenia nordenskioldi pallida (Oligochaeta, Lumbricidae). *Euro j of soil bio* 75: 151-156. https://doi:10.1016/J.EJSOBI.2016.06.004

19. Blouin M, Hodson M E, Delgado E A, Baker G, Brussaard L, Butt K R, Dai J, Dendooven L, Peres G, Tondoh J E, Cluzeau D, Brun JJ (2013) A review of earthworm impact on soil function and ecosystem services. *Euro j of soil bio* 64: 161–182. https://doi:10.1111/EJSS.12025.

20. Costa D, Timmermans MJ, Sousa JP, Ribeiro R, Roelofs D, Van Straalen NM (2013) Genetic structure of soil invertebrate populations: collembolans, earthworms and isopods, *Appli Soil Eco* 68: 61-66. https://doi:10.1016/J.APSOIL.2013.03.003

21. Craven D, Thakur MP, Cameron EK, Frelich LE, Beauséjour R, Blair RB, Blossey B, Burtis J, Choi A, Dávalos A, Fahey TJ, Fisichelli NA, Gibson K, Handa IT, Hopfensperger K, Loss SR, Nuzzo V, Maerz JC, Sackett T, Scharenbroch BC, Smith SM, Vellend M, Umek LG, Eisenhauer N (2017) The unseen invaders: introduced earthworms as drivers of change in plant communities in North American forests (a meta-analysis). *Glo Chan Bio* 23: 1065-1074. https://doi.org/10.1111/gcb.13446

22. Eisenhauer N, Ferlian O, Craven D, Hines J, Jochum M (2019) Ecosystem responses to exotic earthworm invasion in northern North American forests. *Res Ide and Out* 5: e34564. https://doi.org/10.3897/rio.5.e34564

23. Gozlan R E, Newton A C (2009) Biological invasions: Benefits versus risks. *Science* 324: 1015. https://doi:10.1126/science.324_1015a.

24. Hewitt G M, Butlin R K (1997) Causes and consequences of population structure. In: KrebsJ, Davies, Neds. Behav Ecology: An Evolutionary Approach. Oxford: Blackwell 350–372.

25. Jabłońska A, Pešić V (2014) Five species of aquatic oligochaetes new to Iran with an updated checklist. *Ocean and Hydro Stu* 43: 100-105. https://doi:10.2478/S13545-014-0121-3.

26. Martin P, Martinez-Ansemil E, Pinder A, Timm T, Wetzel M J (2008) Global diversity of Oligochaetous clitellates('Oligochaeta'; Clitellata) in freshwater. *Hydro* 595: 117–127. https://doi:10.1007/S10750-007-9009-1.

27. Sakai AK, Allendorf FW, Holt JS, Lodge DM, Molofsky J, With KA, BaughmanS, Cabin RJ, Cohen JE, Ellstrand NC, McCauley DE, O'Neil P, Parker IM, Thompson JN, Weller SG (2001) The population biology of invasive species. *Ann Rev of Eco Evol and Syst* 32: 305–332.

28. Sambrook J, Fritsch E F, Maniatis T (1989) Molecular cloning: A laboratory manual (2nd ed.). New York, NY: Cold Spring Harbor Laboratory Press.

29. Huelsenbeck JP, Ronquist F(2001) MRBAYES: Bayesian inference of phylogenetic trees, *Bioin* 17: 754–755.

30. Rambaut A, Drummond A J (2009) Tracer: MCMC trace analysis tool, version 1.5. Retrieved from http://tree.bio.ed.ac.uk/softw are/tracer

31. Bandelt HJ, Forster P, Röhl A (1999) Median-joining networks for inferring intraspecific phylogenies, *Mol Bio and Evol* 16(1):37-48. doi: 10.1093/oxfordjournals.molbev.a026036. PMID: 10331250.

32. Librado P, Rozas J (2009) DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. *Bioin* 25: 1451–1452.

33. Fu Y X (1997) Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics* 147(2): 915–925. https://doi.org/10.1093/genet ics/147.2.915.

34. Tajima F (1989) Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics* 123(3): 585–595. https:// doi.org/10.1093/genet ics/123.3.585.

35. Excoffier L, Lischer H E L (2010) Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Mol Eco Res* 10: 564–567. doi:10.1111/j.1755-0998.2010.02847.x

36. Zizka A, Herdean A, Silvestro D, Andermann T, Azevedo J, Ritter C D, Edler D, Farooq H, Ariza M, Scharn R, Svantesson S (2019) Coordinate cleaner: Standardized cleaning of occurrence records from biological collection databases *Meth in Eco and Evol* 10: 744–751. https://doi.org/10.1111/2041-210X. 13152.

37. Sanderson EW, Jaiteh M, Levy MA, Redford KH, Wannebo AV, Woolmer G (2002) The human footprint and the last of the wild: the human footprint is a global map of human influence on the land surface, which suggests that human beings are stewards of nature, whether we like it or not. *Am Inst Biol Sci* 52: 891e904.

38. Thuiller W, Lafourcade B, Engler R, Araújo MB (2009) BIOMODea platform for ensemble forecasting of species distributions. *Ecography* 32: 369e373.

39. R Development Core Team (2014) R: a Language and Environment for Statistical Computing, R Foundation for Statistical Computing, Vienna.

40. Thuiller W, Georges D, Engler R, Breiner F, Georges MD, Thuiller CW (2016) Package 'biomod2'. https://cran.r-project.org/package¼biomod2.

41. Hamid M, Khuroo AA, Charles B, Ahmad R, Singh CP, Aravind NA (2018) Impact of climate change on the distribution range and niche dynamics of Himalayan birch, a typical treeline species in Himalayas. *Biodi and Cons*. https://doi.org/10.1007/s10531-018-1641-8.

42. Dar SA, Singh SK, Wan HY, Kumar V, Cushman SA, Sathyakumar S (2021) Projected climate change threatens Himalayan brown bear habitat more than human land use. *Anim Cons* 24 (4): 659–676. doi:10.1111/acv.12671

43. Stephenson J (1920) On a collection of Oligochaeta from the lesser known parts of India and from eastern Persia. Mem of the Indi Mus 7: 191–261.

44. Egglishaw HJ (1980) Benthic invertebrates of streams on the Alburz Mountain Range near Tehran, Iran. *Hydro* 69: 49–55. doi:10. 1007/BF00016535

45. Ahmadi R, Aliyev A, Seidgar M, Bayramov A, Ganji S (2012) Macroinvertebrate communities differences on riverine parts and reservoirs of Zarrineh River. *Amer J of Agri and Biol Sci* 7: 71–75. doi:10.3844/AJABSSP.2012.71.75

46. Javidkar M, Abdoli A, Ahmadzadeh F, Nahavandi Z, Yari MB (2020) Molecular evidence reveals introduced populations of Eiseniella tetraedra (Savigny, 1826) (Annelida, Lumbricidae) with European origins from protected freshwater ecosystems of the southern Alborz Mountains. *Mar and Fresh Res* 72: 44. https://doi.org/10.1071/MF20004

47. Jabłon´ska A, Pesˇic´ V (2014) Five species of aquatic oligochaetes new to Iran with an updated checklist. *Oceano and Hydro Stu* 43: 100–105. doi:10.2478/S13545-014-0121-3

48. Latif R, Ezzatpanah S, Malek M, Parsa H (2009) Earthworms of the Central Elburz Mountains, Iran. *Ira J of Anim Bios* 5 (2): 1-15. doi:10.22067/IJAB.V5I2.3332.

49. Banha F, Gama M, Anastácio PM, (2017), The effect of reproductive occurrences and human descriptors on invasive pet distribution modelling: Trachemys scripta elegans in the Iberian Peninsula. *Eco Mode* 360: 45–52. https://doi.org/10.1016

50. Peterson AT (2003) Predicting the geography of species' invasions via ecological niche modeling. *The Quar Revi Of Biol* 78: 419–433. https://doi.org/10.1086/378926.

51. Guisan A, Thuiller W (2005) Predicting species distribution: offering more than simple habitat models. *Eco Lett* 8: 993–1009. https://doi.org/10.1111/j.1461-024 8.2005.00792.x.

52. Reshetnikov AN, Ficetola GF (2011) Potential range of the invasive fish rotan (Perccottus glenii) in the Holarctic. *Biol Inva* 13: 2967–2980. https://doi.org /10.1007/s10530-011-9982-1.

53. Poulos HM, Chernoff B, Fuller PL, Butman D, (2012), Ensemble forecasting of potential habitat for three invasive fishes. *Aqua Inva* 7: 59–72. https://doi.o rg/10.3391/ai.2012.7.1.007.

54. Kulhanek S A, Ricciardi A, Leung B (2011) Is invasion history a useful tool for predicting the impacts of the world's worst aquatic invasive species? *Ecol Appli* 21:189–202. doi:10.1890/09-1452.1.

55. Larson B M H, Kueffer C, ZiF Working Group on Ecological Novelty (2013) Managing invasive species amidst high uncertainty and novelty. *Tren in Ecol and Evol* 28:255–256. doi: 10.1016/j.tree.2013.01.013.

56. Simberloff D, Martin J, Genovesi P, Maris V, Wardle DA, Aronson J, Courchamp F, Galil BS, García-Berthou E, Pascal M, Pyšek P, Sousa R, Tabacchi E, Vilà M (2013) Impacts of biological invasions: what's what and the way forward. *Tren in Ecol and Evol* 28: 58-66 . doi:10.1016/j.tree.2012.07.013.

57. Davis MA, Chew MK, Hobbs RJ, Lugo AE, Ewel JJ, Vermeij GJ, Brown JH, Rosenzweig ML, Gardener MR, Carroll SP, Thompson KA, Pickett ST, Stromberg JC, Tredici PD, Suding KN, Ehrenfeld JG, Grime JP, Mascaro J, Briggs JC (2011) Don't judge species on their origins. *Nature* 474: 153-154. doi:10.1038/474153a

58. Mills E, Casselman J, Dermott R (2003) Lake Ontario: food web dynamics in a changing ecosystem (1970- 2000) .*Cana J of Fish and Aqua Sci* 60: 471–490.

59. Leprieur F, Beauchard O, Blanchet S, Oberdorff T, Brosse S (2008) Fish invasions in the world's river systems: when natural processes are blurred by human activities. *PLoS Biol* 6:e28. doi: 10.1371/journal.pbio.0060028.

60. Brinkhurst R O (1960) Studies on the functional morphology of Gerris najas Degeer (Hem. Het. Gerridae). In Proceedings of the Zoological Society of London . Oxford, UK: Blackwell Publishing Ltd 133 (4): 531-559

61. Wood H B, James S W (1993) Native and introduced earthworms from selected chaparral, woodland, and riparian zones in southern California. Gen. Tech. Rep. PSW-142. Albany, CA: US Department of Agriculture, Forest Service, Pacific Southwest Research Station. 20 p: 142.

62. Martinsson S, Cui Y, Martin P J, Pinder A, Quinlan K, Wetzel M J, Erséus C (2015) DNAbarcoding of invasive European earthworms (Clitellata: Lumbricidae) in south-western Australia. *Biol Inva* 17(9):

2527-2532. doi:10.1007/s10530-015-0910-7

63. Sosa I, Marchán D F, Novo M, Almodóvar A, Cosín D D (2017) Bless this phylogeographic mess– Comparative study of Eiseniella tetraedra (Annelida, Oligochaeta) between an Atlantic area and a continental Mediterranean area in Spain. *Euro J of Soil Biol* 78: 50-56. doi:10.1016/J.EJSOBI.2016.11.006

64. Kim Y N, Dickinson N, Bowie M, Robinson B, Boyer S (2017) Molecular identification and distribution of native and exotic earthworms in New Zealand human-modified soils. New *Ze J of Ecol* 41(2): 218-225. doi:10.20417/NZJECOL.41.23.

65. Salomé C, Guenat C, Bullinger G, Gobat JM, Bayon Rcl (2011) Earthworm communities in alluvial forests: Influence of altitude, vegetation stages and soil parameters, *Pedobiol* 54: 89-98. https://doi.org/10.1016/j.pedobi.2011.09.012.

66. Carosi A, Padula R, Ghetti L, Lorenzoni M (2019) Endemic freshwater fish range shifts related to global climate changes: a long-term study provides some observational evidence for the Mediterranean Area. *Water* 11(11): 23-49. DOI:10.3390/w11112349.

67. Mamun d, Kim S, Guk AK (2018) Distribution pattern prediction of an invasive alien species largemouth bass using a maximum entropy model (MaxEnt) in the Korean peninsula. *J of Asia-Pac Biod* 11(4): 516-524. https://doi.org/10.1016/j.japb.2018.09.007.

68. Ezzatpanah S, Latif R, Malek M, Salehi H (2010) Earthworm fauna of the western Mazandaran province, Iran (Oligochaeta: Lumbricidae, Megascolecidae). *Zoo in the Mid East* 2: 67-74. doi:10.1080/09397140.2010.10638459

69. Mirmonsef H, Malek M, Latif R (2011) The earthworm fauna of Tehran Province, Iran; an ecological characterization. *Irani J of Anim Biosys* 7(2).

70. Yousefi Z, Ramezani M, Akbari Mohamadi S. Kh, Mohammadpour R A, Nemati A (2009) Identification of earthworms species in Sari Township in Northern Iran, 2007-2008. *J of Appli Sci* 9 (20): 3746-3751. doi:10.3923/JAS.2009.3746.3751.

71. Hong S, Jang I, Kim D, Kim S, Park HS, Lee K (2022) Predicting Potential Habitat Changes of Two Invasive Alien Fish Species with Climate Change at a Regional Scale, *Sustain* 14: 6093. doi:10.3390/su14106093

72. Hansen GJA, Read JS, Hansen JF, Winslow LA (2017) Projected shifts in fish species dominance in Wisconsin lakes under climate change, *Glo Chan Biol* 23: 1463–1476. doi:10.1111/gcb.13462.

73. Abdoli A, Valikhani H, Nejat N, Khosravi M (2022) Non-native freshwater fishes of Iran (Identification, Impacts, Management), ISBN: 978-964-479-203-8.

74. Kim Z, Shim T, Ki SJ, An KG, Jung J (2022) Prediction of three-dimensional shift in the distribution of largemouth bass (Micropterus salmoides) under climate change in South Korea, *Ecol Indic* 137: 108731. doi:10.1016/j.ecolind.2022.108731
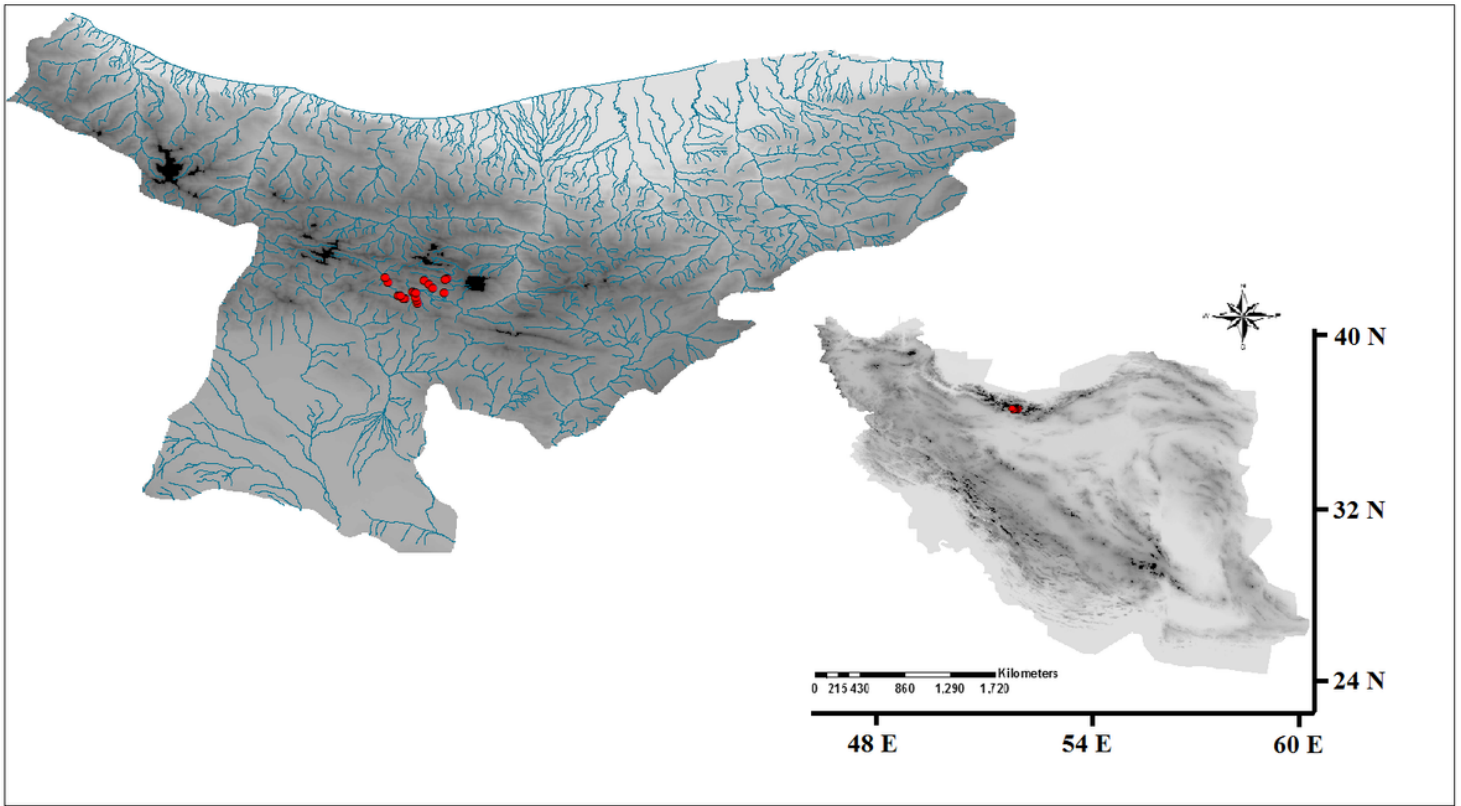
Figures

Figure 1

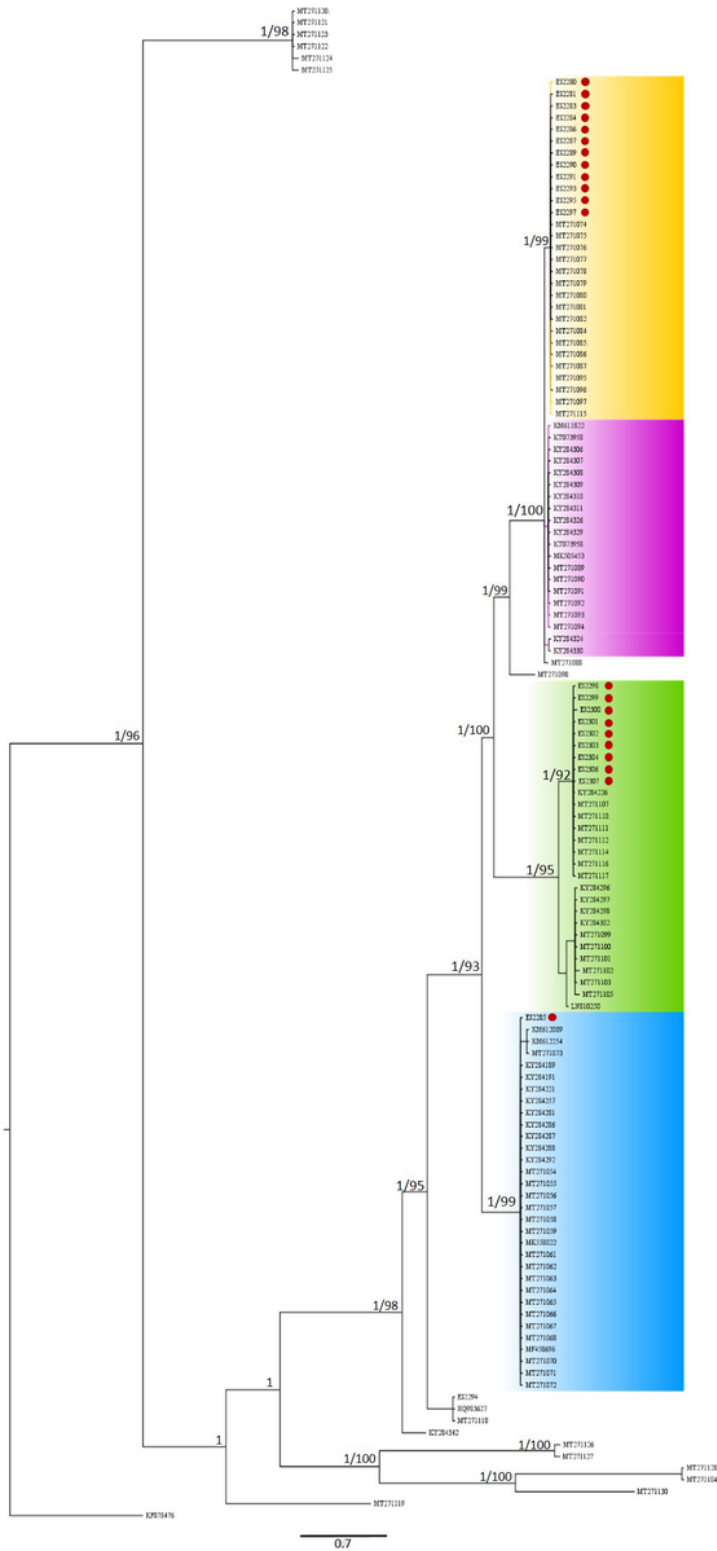Locations of sampling stations *E. tetraedra* in current study.

Figure 2

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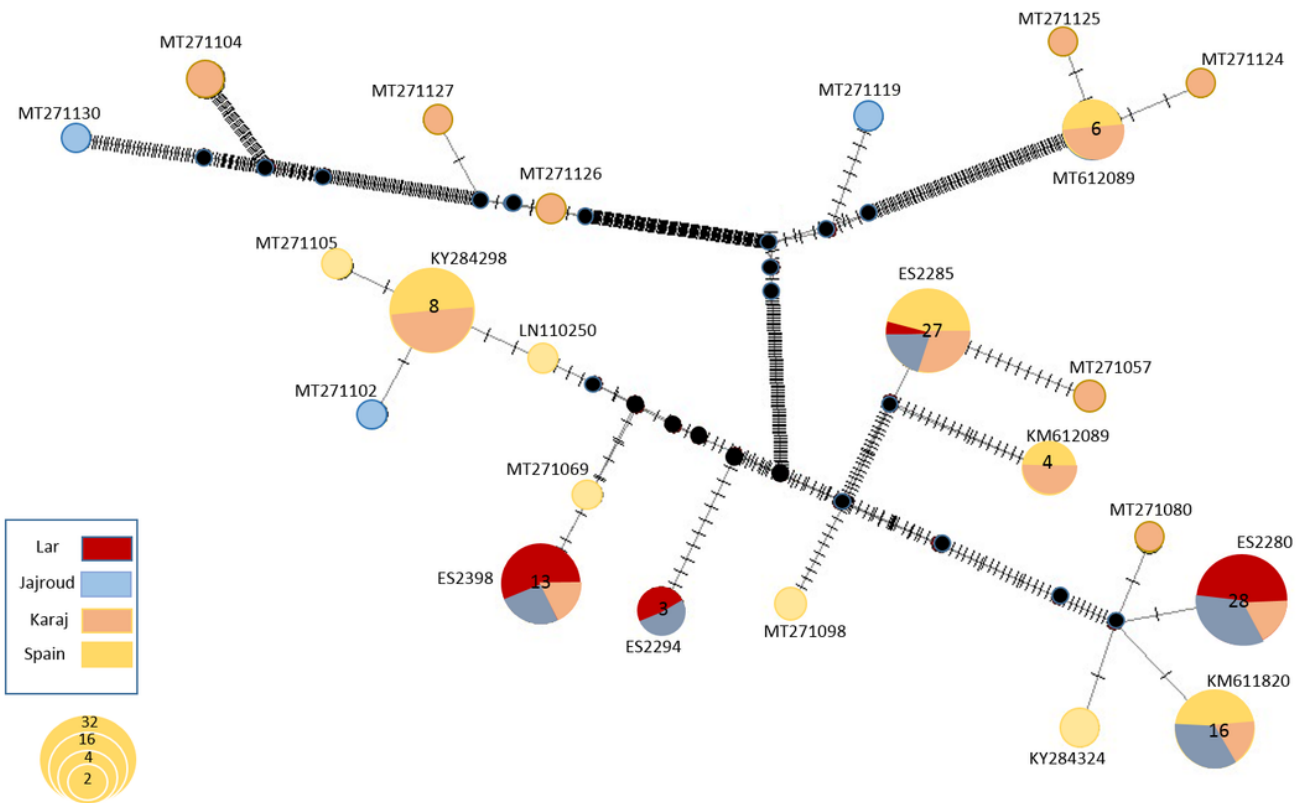
Figure 3

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.
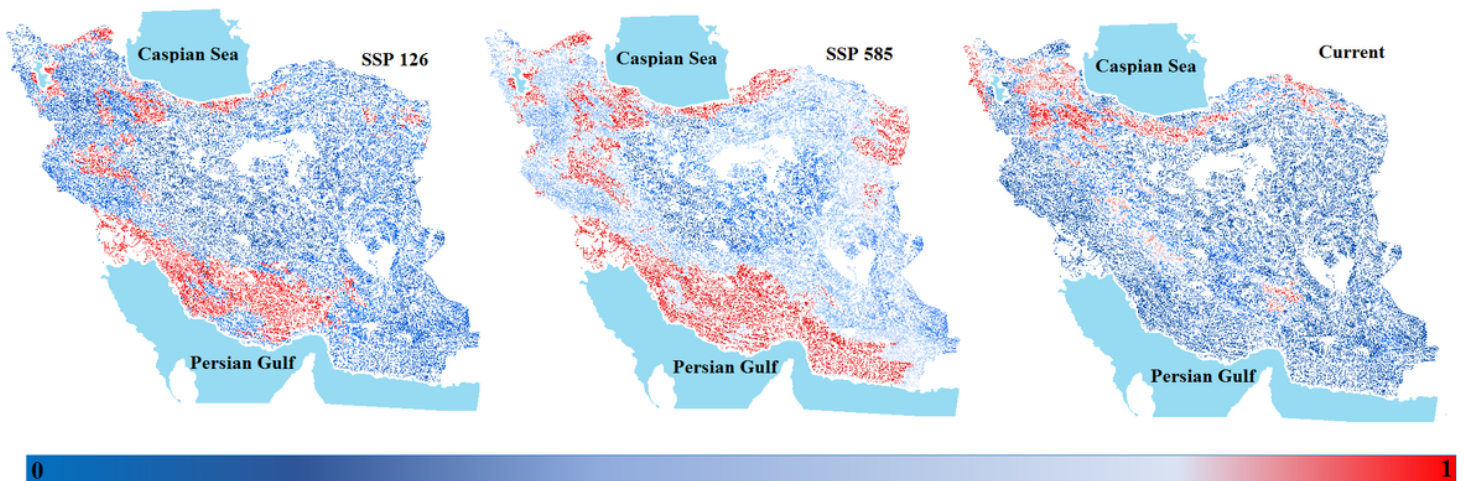

Figure 4

Dynamic changes in the suitable habitat of *E. tetraedra* in the freshwaters of Iran under current and two future climate scenarios -2061-2080 (ssp 126 and ssp 585) based on MRI-CGCM3 model.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- suplement.docx