ELSEVIER

What happens in ice age, does not stay in ice age: Phylogeography of *Bombus terrestris* revealed a low genetic diversity amongst the Eurasian populations

Yasaman Ranjbaran [a,1], Dennis Rödder [b,2], Reihane Saberi-Pirooz [a,3], Faraham Ahmadzadeh [a,*,4]

[a] *Department of Biodiversity and Ecosystem Management, Environmental Sciences Research Institute, Shahid Beheshti University, G.C., Evin, Tehran, Iran*
[b] *LIB, Museum Koenig Bonn, Bonn, Germany*

A B S T R A C T

The objective of this research was to assess the genetic diversity and phylogeography of *Bombus terrestris* and examine the historical events that shaped its contemporary genetic structures using the COI mitochondrial marker. Specimens of the species were collected from its distribution range alongside the Alborz Mountain range, and GenBank sequences from the Eurasian distribution range were incorporated into the dataset. The COI sequences were employed in Bayesian and Maximum Likelihood analyses to generate phylogenetic trees for the species populations and to investigate the evolutionary history of the species. Additionally, species occurrence points and climate data were utilized in Species Distribution Modeling (SDM) analyses to reconstruct the species range under past, present, and future climate conditions. The ML and BI trees yielded similar topologies, indicating extremely low genetic diversity and a homogeneous structure in the species population distribution range in Eurasia. Demographic analyses suggested that the species may have experienced a bottleneck during the last glacial maximum in Eurasia, followed by a recent expansion. The SDM analyses revealed significant fluctuations in the species range in the past and expansion under present conditions. Given the high dispersal ability of the species, the population expansion rate has surpassed the rate of developing new genetic diversity, and the estimated polymorphic sites for the species are likely relatively recent. This low level of genetic variation can also be attributed to the absence of geographical barriers and the excellent flying ability of the queen bee, leading to sustained gene flow throughout the entire continent. Despite the general correlation between larger populations and higher genetic diversity, bumblebees can expand their population size without increasing genetic diversity when residing in resourceful habitats.

* Corresponding author.
  E-mail address: F_Ahmadzade@sbu.ac.ir (F. Ahmadzadeh).
[1] ORCID: https://orcid.org/0000-0002-4682-4270
[2] ORCID: https://orcid.org/0000-0002-6108-1639
[3] ORCID: https://orcid.org/0000-0002-9210-3570
[4] ORCID: http://orcid.org/0000-0001-7152-8484

1. Introduction

Genetic diversity is a crucial factor in the survival and adaptability of species and low levels of this diversity may limit the potential of adaptation to any change or imbalance in the environment (Frankham, 2005) and populations with reduced genetic diversity face a greater risk of extinction (Markert et al., 2010; Keller and Waller, 2002). Given the significant influence of genetic factors on wild populations and the correlation between genetic deterioration and threatened species (Frankham, 2003), it is crucial to assess and evaluate genetic diversity for the purpose of maintaining and conserving biodiversity (Rivers et al., 2014).

One of the determining factors that shaped biogeographic patterns and genetic structure of many species is climatic oscillations (Hewitt, 2004; Amiri et al., 2021). Species responded differently to the associated temperature and precipitation fluctuations in climate oscillations and experienced restriction or expansion in their distributional ranges. Glacial and interglacial cycles restricted their range and shifted them towards refugia, and interglacial cycles allowed them to recolonize and expand their ranges (Provan and Bennett, 2008; Saberi-Pirooz et al., 2021). Phylogeographic information and population genetic analysis can unveil significant factors that have formed contemporary biodiversity and its geographical distribution (Ahmadzadeh et al., 2020).

Bumblebees (*Bombus*) are important plant pollinators of temperate and high-elevation regions (Michener, 2000) and due to their crucial contribution to ecosystem services, are regarded as keystone species (Goulson et al., 2008). In addition to sustaining agricultural production, bumblebees are also influential in maintaining the diversity and quality of natural and semi-natural vegetation (Kwak et al., 1998) that will support species like herbivorous insects and seed-eating birds and mammals (Sepp et al., 2004). These important species have been tremendously affected worldwide by human-driven environmental changes (e.g., habitat loss, agricultural intensification, use of pesticides, the introduction of new parasites, and climate change (e.g., Silva et al., 2020) and the interaction between all these factors of decline. Some species have experienced a decline in genetic diversity at a local scale (Woodard et al., 2015), which might increase the risk of inbreeding and hinder the ability of populations to cope with environmental change (Goulson et al., 2008; Maebe et al., 2015). A range shift towards higher latitudes or altitudes has been witnessed in some pollinators in response to rapid climate change (Ghisbain et al., 2021; Parmesan and Yohe, 2003; Parmesan et al., 1999). However, most bumblebee species have failed to shift outside of their northern borders and climate change has taken a heavy toll on their southern range (Kerr et al., 2015). Bumblebees have been experiencing declines in their historical range and climate change is transforming their habitats so rapidly that puts them at an increasing extinction risk (Rasmont et al., 2021; Leroux et al., 2013; Loarie et al., 2009).

Bombus terrestris or Buff-tailed Bumblebees which is vastly distributed in the western Palearctic (Rasmont et al., 2008), acts as an efficient pollinator for a wide range of Eurasian native plants (Dafni et al., 2010). The species has a wide range of food plants and forages on any available plant (Rasmont and Adamski, 1995). The species is supposedly not declining and has been vastly spread through domestication (Rasmont et al., 2008).

This abundant species is native to Iran and has had a consistent presence in the alpine and elevated regions as well as the lower foothills in the Alborz Mountain range for at least the past 70 years (Monfared et al., 2007). Although the bumblebee fauna of the Alborz Mountain range, including the buff-tailed bumblebee are well known from previous studies through morphological identification (Pittoni, 1937; Skorikov, 1938; Reinig, 1939; Popove, 1967; Baker, 1996; Monfared et al., 2007; Monfared et al., 2008), no research has been performed to identify the genetic structure of the species in Iran. Despite the long history of research on this taxon, the species has still a lot of unknown aspects, and the phylogenetic status of the Iranian populations are not clearly resolved. Previous studies, which illustrated a homogeneous genetic structure in the Eastern Eurasian mainland populations (Estoup et al., 1996; Widmer et al., 1998; Woodard et al., 2015), lacked any data from Eastern Eurasia populations such as Iran and the Far East. Therefore, this study aims to determine the phylogenetic position of Iran's population of *B. terrestris* within the Eurasian populations and to assess the phylogeographic patterns of the species in its distribution range using the mitochondrial COI marker, combined with Species Distribution Models (SDMs).

2. Material and methods

2.1. Sample collection

We collected 22 specimens from the species distribution range in the Alborz Mountains in Iran in 12 localities (Table S1, Fig. 1). The specimens were trapped using insect nets and preserved in 96% ethanol and stored at 4 °C for long-term preservation.

2.2. Laboratory procedures

The specimens were identified according to the identification keys of Tahmasbi et al. (2008) and Cappellari et al. (2018). The identification and sorting of specimens were carried out using the ZSM1001 stereomicroscope.

Total genomic DNA was extracted with a standard high-salt protocol (Sambrook et al., 1989). In this study, we used cytochrome c oxidase subunit I (COI), which is a gene known for its high mutation rate. It is widely utilized for genetic identification purposes in various taxa, including bumblebees (Williams et al., 2012a, 2012b; Geue and Thomassen, 2020; Silva et al., 2020). The amplification for the COI mitochondrial marker was carried out using the LCO 1490 and HCO 2198 primers (Folmer et al., 1994). The Polymerase Chain Reactions (PCRs) were performed on an MJ Mini™ thermocycler (Bio-Rad) and the reaction mix was prepared in a total volume of 25 μl containing 12.5 μl of Master Mix Red (Ampliqon, Copenhagen, Denmark), 0.5 μl of each primer, 10.5 μl ddH$_2$O, and 1 μl of template DNA (50–100 ng). PCRs began with an initial denaturation step at 94 °C for 5 min, followed by 40 cycles. Each cycle consisted of denaturation at 94 °C for 30 s, annealing at 48 °C for 30 s, and extension at 72 °C for 1 min. Finally, there was a single
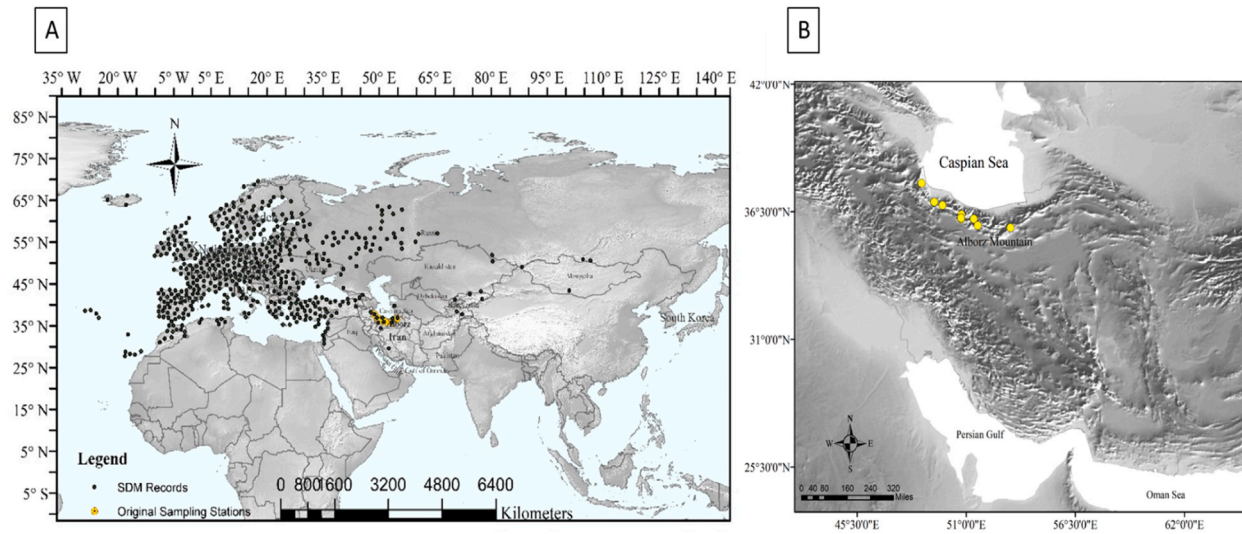
Fig. 1. The distribution points of *B. terrestris* used in the present study. A) original sampling stations, the additional sequences from NCBI and the species' presence records that are used in the SDM are shown in the map, B) the sample sites in Iran.

additional extension step at 72 °C for 5 min. PCR products were visualized on 1% agarose gel. The suitable amplicons were sent to ZistFanavari Pishgam Inc. (Pishgam, Iran) for purification and sequencing.

The quality of chromatograph sequences was checked using Geneious Prime® (V. 2021.1.1). The nucleotides were translated into amino acid to confirm an open reading frame (i.e., no stop codons). The edited sequences were queried in NCBI (https://blast.ncbi.nlm.nih.gov/) by the Standard Nucleotide Basic Local Alignment Search Tool (BLASTn) (Altschul et al., 1990) to determine taxonomic identities.

Sequences were then deposited in the GenBank database (Table S2).

2.3. Alignment and phylogenetic analyses

For phylogenetic analyses, 223 additional sequences from across the *B. terrestris* distribution range were obtained from the NCBI (www.ncbi.nlm.nih.gov) and added to the existing dataset generated in the present study (Table S2). We chose *Apis mellifera* as an outgroup as the closest relative of the *Bombus* genus inside Hymenoptera and *Bombus ignitus* was selected as a second outgroup as it is closely related to *B. terrestris* (Cameron et al., 2007). Due to the extensive size of our dataset, we opted to utilize a phylogenetic tree using haplotypes (haplotype tree). To construct the haplotype tree, we employed dnaSP v.5.10.01 (Librado & Rozas, 2009) to determine the distinct haplotypes. Overall, 19 haplotypes were recognized through the dataset (see Table S2). The haplotypes were aligned using the MAFFT algorithm (Katoh et al., 2002) using the online version of the website (https://mafft.cbrc.jp/alignment/server/). The best fit-nucleotide substitution model was selected under Akaike's information criterion (Akaike, 1974) using MrModeltest v.2 3 (Nylander, 2004). As a result, the best fit model for the COI mitochondrial gene was HKY+G (G = 0.38). A Maximum Likelihood (ML) tree was reconstructed using IQ-Tree v. 1. 6. 12 (Nguyen et al., 2015) under the HKY+G model with 1000 pseudo-replicates to assess the confidence of branches using the ultrafast Bootstrap (UFB) approach (Hoang et al., 2018). Bayesian (BI) analysis was carried out by MrBayes v.3.2 (Huelsenbeck and Ronquist, 2001) with 3 million generations and four chains and a sampling frequency of 0.01. Finally, 10% of the posterior samples were discarded as burn-in. Subsequently, convergence to the stationary distribution was evaluated using Tracer v.1.6 (Rambaut et al., 2007).

2.4. Population structure and demographic analyses

An analysis of molecular variance (AMOVA) was conducted among hypothetical groups of populations based on standardized estimates of genetic differentiation (F_{st}) with 10000 permutations using Arlequin v.3.5 (Excoffier and Lischer, 2010) to assess genetic differentiation in *B. terrestris*. The theoretical populations were determined by considering two factors: the species' geographical distribution encompassing Iran, Eastern Asia, and Western Europe, and the ecological similarity of specific regions such as North-Alborz, South-Alborz, Eastern Asia, and Western Europe, which were treated as separate groups.

Molecular diversity indices, including the number of haplotypes (H), haplotype diversity (*h*), and nucleotide diversity (π), were assessed for COI separately using Arlequin v.3.5.

The demography of the *B. terrestris* population was investigated with neutrality analyses, calculating Tajima's *D* (Tajima, 1989), and Fu's *Fs* (Fu, 1997) indices with the same program. To estimate the frequency distribution of the pairwise nucleotide differences, a Mismatch Distribution (MMD) analysis was performed, assuming a sudden expansion with spatial parameters.

The parsimony haplotype network was constructed using TCS v.1.2.1 (Crandall et al., 2000). Uncorrected genetic distance matrices among haplotypes were determined using MEGA X (Kumar et al., 2018).

2.5. Species distribution modeling (past-present-future)

2.5.1. Species records

A total of 78401 georeferenced localities' information were compiled during our fieldwork, from available literature sources, and the Global Biodiversity Information Facility (https://doi.org/10.15468/dl.kt7pbz). The reliability of all records was assessed by mapping them in DIVA-GIS 7.4 (Hijmans et al., 2005, available at http://www.divagis.org). To avoid potential sample biases the occurrence records were thinned using a spatial filter of 50 km using the package spThin for R (Aiello-Lammens et al., 2015). In total, 516 unique records were available for model building covering the known geographic range of the species.

2.5.2. Climate data and variable selection

The 19 so-called bioclim variables with a grid cell resolution of 2.5 arc.min were obtained from the CHELSA database (Karger et al., 2017; https://chelsa-climate.org/), which represents historical monthly weather data as the averages of the period of 1979–2013 (available through Brown et al., 2018; http://www.paleoclim.org/). The multicollinearity of predictors was assessed using the Variance Inflation Factor (VIF; vistep function of the package usdm; Naimi et al., 2014) in R, allowing only a threshold of 10. The final subset of variables included: Temperature Annual Range (bio7), Mean Temperature of Wettest Quarter (bio8), Mean Temperature of Warmest Quarter (bio10), Precipitation of Wettest Month (bio13), Precipitation of Driest Month (bio14), and Precipitation of Coldest Quarter (bio19).

As paleoclimatic scenarios, we downloaded the respective bioclimatic variables for the following time steps; Pleistocene: late-Holocene, Meghalayan (4.2–0.3 ka); mid-Holocene, Northgrippian (8.326–4.2 ka); early-Holocene, Greenlandian (11.7–8.326 ka); Younger Dryas Stadial (12.9–11.7 ka); Bølling-Allerød (14.7–12.9 ka); Heinrich Stadial 1 (17.0–14.7 ka); Last Interglacial (ca. 130 ka) (all paleoclimatic scenarios are available from Brown et al., 2018; http://www.paleoclim.org/, v1.0).

The following future scenarios of the storylines ssp126, ssp370, and ssp585 as can be expected for 2070–2100 were obtained from the CHELSA database (Karger et al., 2017; https://chelsa-climate.org/): GFDL-ESM4, IPSL-CM6A-LR, MPI-ESM1–2-HR, MRI-ESM2–0, and UKESM1–0-LL. Averages of each bioclimatic predictor per ssp storyline were used for future projections.

2.5.3. SDM analyses

For model fitting, we used Maxent v. 3.4.4 (Phillips et al., 2004; Phillips et al., 2006). Models were trained within an environmental background defined by a radius of 500 km around each occurrence record. In order to find the optimal settings for model fitting, we tested several regularization multipliers (from 0.5 to 2.5 in 0.1 steps, 5 and 10) and feature classes (L, LP, LQ, LH, LT, LQP, LQH, LQT, LPH, LPT, LHT, LQPT, LQHT, LPHT, LQPHT; L = Linear, P = Product, Q = Quadratic, H = Hinge, T = Threshold). Therefore, using Maxent's raw output, we calculated the corrected Akaike Information Criterion [AICc; (Warren & Seifert, 2011)] for each of the 25 replicates of each combination of model settings. The best combination of settings was determined by choosing the lowest average AICc over the respective replicates and an average $AUC_{Test} > 0.7$ [AUC = Area under the ROC curve (Lobo et al., 2008; Phillips et al., 2009; Elith & Graham, 2009). AUC was calculated internally in Maxent with an 80% data split for model training and 20% used for model testing (bootstrap).

Finally, the best fitting model (Regularization = 0.9, Features = -p -q -h threshold=true; Parameters = 66, AICc = 7037.240247) was replicated 100 times using the same bootstrap settings as above, wherein the cloglog output was chosen. AUC was computed for model evaluation.

The final models were projected in geographic space and averages across the 100 replicates were used for further processing. Multivariate Environmental Similarity Surfaces (MESS) maps were computed in R to determine potential extrapolation areas. The potential distributions were rescaled to 0–1 after applying a presence-absence threshold of 10% training omission to distinguish between suitable and unsuitable areas. For the current and past scenarios, we also computed stability through time maps by summarizing all potentially suitable areas across the current and past scenarios.

3. Results

3.1. Phylogenetic analyses

The final length of the mtDNA COI gene that was used for all the analyses after the edition and alignment was 450 bp. The ML and BI phylogenetic (haplotype) trees (Fig. 2) generated similar topologies for all the individuals of the *B. terrestris* species in its distribution
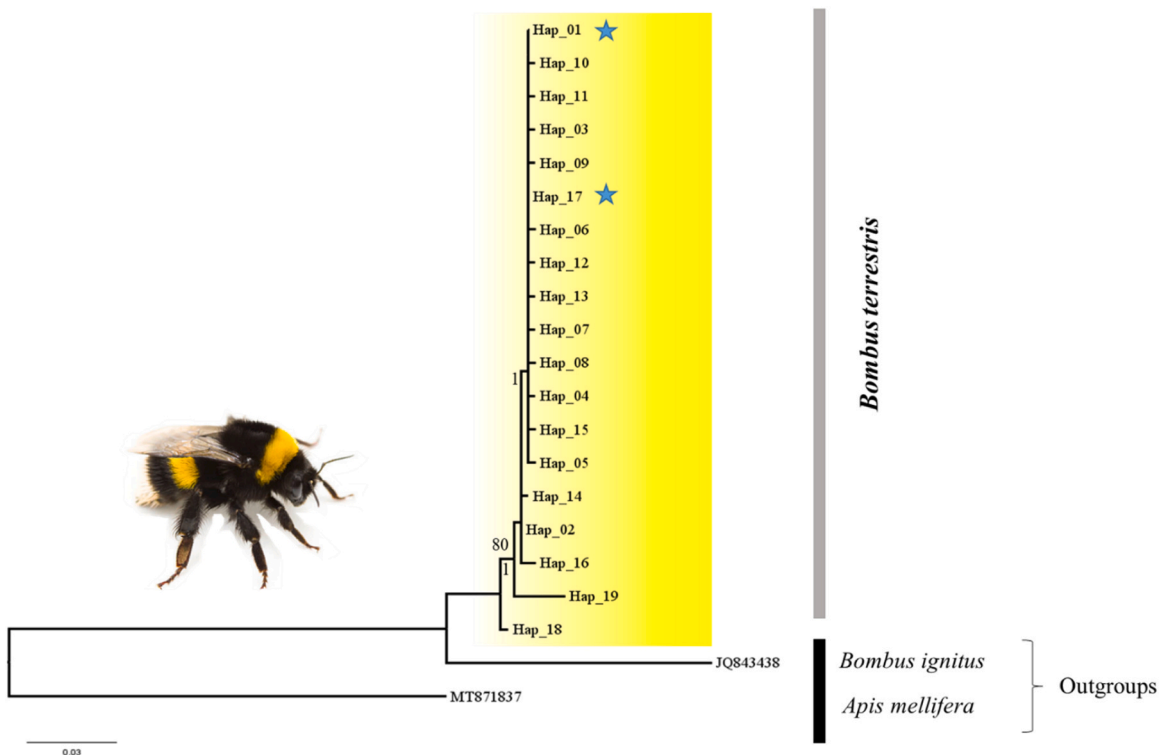

Fig. 2. The final Phylogenetic (haplotype) Tree of *B. terrestris* based on COI mitochondrial marker and according to the ML and the BI methods. The bootstrap values that are above 80 are shown below the branches and posterior probabilities are shown above the branches. Blue stars indicate haplotypes found in the study.

range in Eurasia. According to the phylogenetic trees that were based on the COI mitochondrial marker, all individuals assigned to one population and no apparent genetic differentiation has occurred except two haplotypes belonging to Spain (Hap_18) and Italy (Hap_19) showed more variations compared to others. Iran's specimen appeared to form part of the same genetic lineage as others and only one individual (ES1765-Hap_17) from Gilan province differed by a single nucleotide mutation.

3.2. Population structure

The AMOVA analysis was conducted on two theoretical groups. Upon initial examination, the diversity between the hypothetical populations of Iran, Eastern Asia, and Western Europe was estimated to be 3.76%, which was significantly lower than the diversity within each population, measured at 96.24%. When comparing these populations with another set of hypothetical populations representing North-Alborz, South-Alborz, Eastern Asia, and Western Europe, it was observed that there was slight diversity between these populations, showing 1.2% inter-population diversity. Most of the variation was yet again observed within populations, with 98.8% intra-population diversity.

3.3. Demographic analysis of Bombus terrestris

As all the individuals demonstrated one genetic structure across the range of study area, all specimens form part of a single population. The MMD diagram for *B. terrestris* illustrated a skewed unimodal pattern (Fig. 3).

Molecular diversity indices within *B. terrestris* were evaluated for 240 individuals and showed 19 haplotypes (H), a haplotype diversity (h) equivalent to 0.317, a nucleotide diversity (π) of 0.0012, and 24 polymorphic sites (S).

The analysis of Fu's fs was insignificant ($p > 0.05$) and Tajima's D was significant ($p < 0.05$, -2.327) for the population of *B. terrestris*.

3.4. Haplotype network and genetic distances

The parsimony haplotype network demonstrated a single haplogroup for 240 specimens (Fig. 4). The haplogroup included 19 parsimony haplotypes in which, the ancestral haplotype contained 198 individuals and had the highest frequency. This haplotype included 16 specimens from Iran (alongside the Alborz Mountain range) and other individuals from several countries (see Table S2). A single specimen from Iran (Gilan/Damash) was placed into a unique haplotype (Hap_17). Two haplotypes from Spain (Hap_18) and Italy (Hap_19) were positioned distantly with several step mutations from the ancestral haplotype.

The genetic distance was calculated between haplotypes of the *B. terrestris* (Table 1). The maximum genetic distance among the haplotypes was about 2.2%. The genetic distance between all haplotypes except one haplotype from Italy (Hap 19, which showed a genetic distance from 1.6% to 2.2% to others) was calculated to be less than 1.1%.
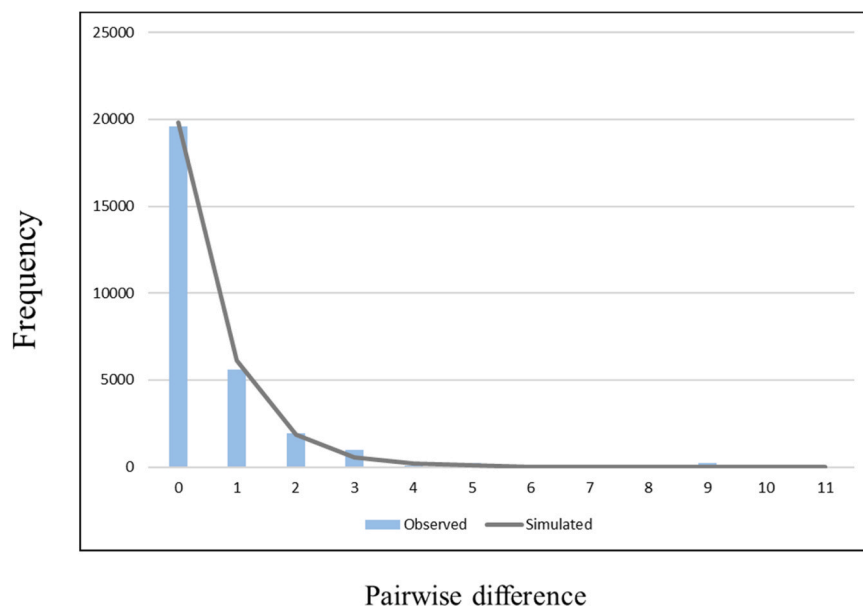

Fig. 3. Mismatch distributions of simulated frequencies (line) within the *B. terrestris* compared with the observed frequencies under the sudden expansion model using COI. The MMD diagram shows a recent expansion.
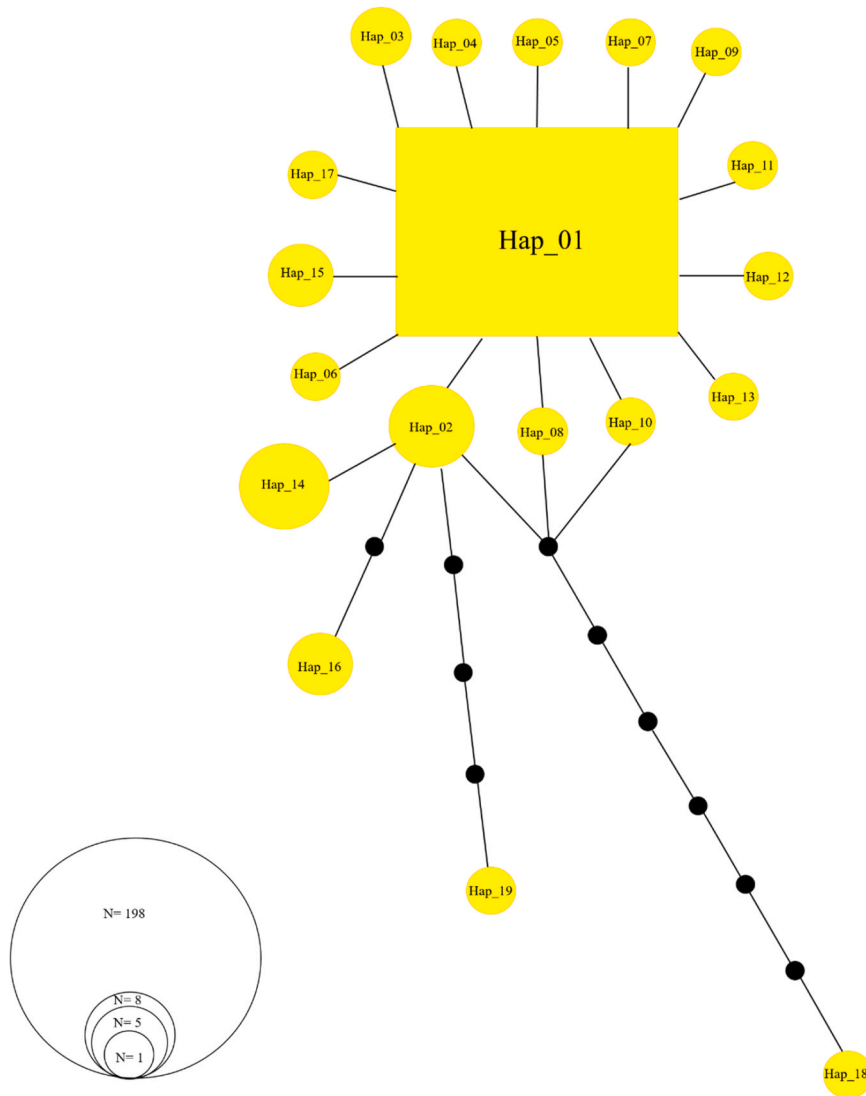
Fig. 4. The parsimony haplotype network of the *B. terrestris* based on COI. The black points represent the missing haplotypes.

3.5. Species distribution modeling

Using the optimal model settings, the SDMs had an overall good performance ($AUC_{Training} = 0.831$, $AUC_{Test} = 0.801$). On average Temperature Annual Range had the highest contribution (bio7, 55.8%), followed by Precipitation of Coldest Quarter (bio19, 24.9%), Mean Temperature of Warmest Quarter (bio10, 9.5%), Precipitation of Wettest Month (bio13, 5.8%), Mean Temperature of Wettest Quarter (bio8, 2.2%), and Precipitation of Driest Month (bio14, 1.6%).

The potential distribution covers the current range of the species quite well, although some parts of the species' eastern range, especially in continental Russia, are underpredicted (Fig. 5 A). The historical range predictions suggest several range restrictions and expansions, mainly towards the coastlines of the Mediterranean Sea and the Iberian Peninsula with subsequent large range expansions (Fig. 5 B, C, D, E, F, G, and H). The stability through time analysis identified these areas as potential refugia, wherein the potential distribution was rather unstable across the Alborz Mountains (Fig. 6). Only a few extrapolation areas occur in the past and future scenarios. In the near future, the model projections suggest a northward range shift and across all three ssp story lines the suitability at the southern range margins may be reduced (Fig. 7).

4. Discussion

In this study, we investigated the historical phylogeography of buff-tailed bumblebee populations from Eurasia and evaluated the species genetic structure to clarify the species variation in different geographical regions and to comprehend its evolutionary history.

Table 1
The uncorrected genetic distance between haplotypes of *B. terrestris* based on COI.

|	Hap_01	Hap_02	Hap_03	Hap_04	Hap_05	Hap_06	Hap_07	Hap_08	Hap_09	Hap_10	Hap_11	Hap_12	Hap_13	Hap_14	Hap_15	Hap_16	Hap_17	Hap_18	Hap_19
Hap_01																			
Hap_02	0.002																		
Hap_03	0.002	0.004																	
Hap_04	0.002	0.004	0.004																
Hap_05	0.002	0.004	0.004	0.004															
Hap_06	0.002	0.004	0.004	0.004	0.004														
Hap_07	0.002	0.004	0.004	0.004	0.004	0.004													
Hap_08	0.002	0.004	0.004	0.004	0.004	0.004	0.004												
Hap_09	0.002	0.004	0.004	0.004	0.004	0.004	0.004	0.004											
Hap_10	0.002	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004										
Hap_11	0.002	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004									
Hap_12	0.002	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004								
Hap_13	0.002	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004							
Hap_14	0.004	0.002	0.007	0.007	0.007	0.007	0.007	0.007	0.007	0.007	0.007	0.007	0.007						
Hap_15	0.002	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.007					
Hap_16	0.007	0.004	0.009	0.009	0.009	0.009	0.009	0.009	0.009	0.009	0.009	0.009	0.009	0.007	0.009				
Hap_17	0.000	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.004	0.002	0.007			
Hap_18	0.009	0.007	0.011	0.011	0.011	0.011	0.011	0.011	0.011	0.011	0.011	0.011	0.011	0.009	0.011	0.009	0.009		
Hap_19	0.018	0.016	0.020	0.020	0.020	0.020	0.020	0.016	0.020	0.016	0.020	0.020	0.020	0.018	0.020	0.020	0.018	0.022	
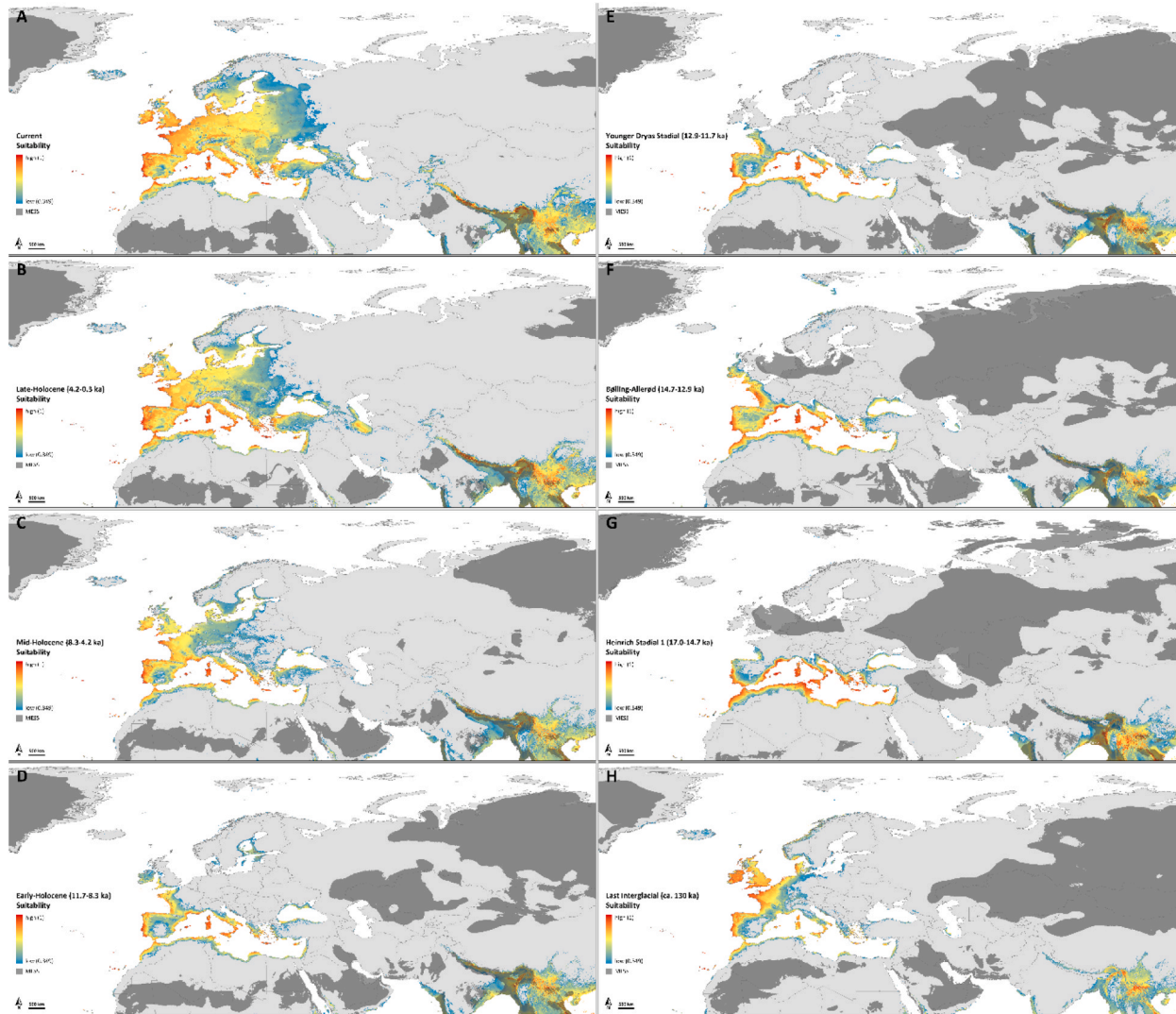
Fig. 5. Current and past potential distribution of *B. terrestris*. Warmer colors indicate higher environmental suitability. A Current climatic conditions, B Late-Holocene, C Mid-Holocene, D Early-Holocene, E Younger Dryas Stadial, F Bolling-Allerod, G Heinrich Stadial 1 and H Last Interglacial.
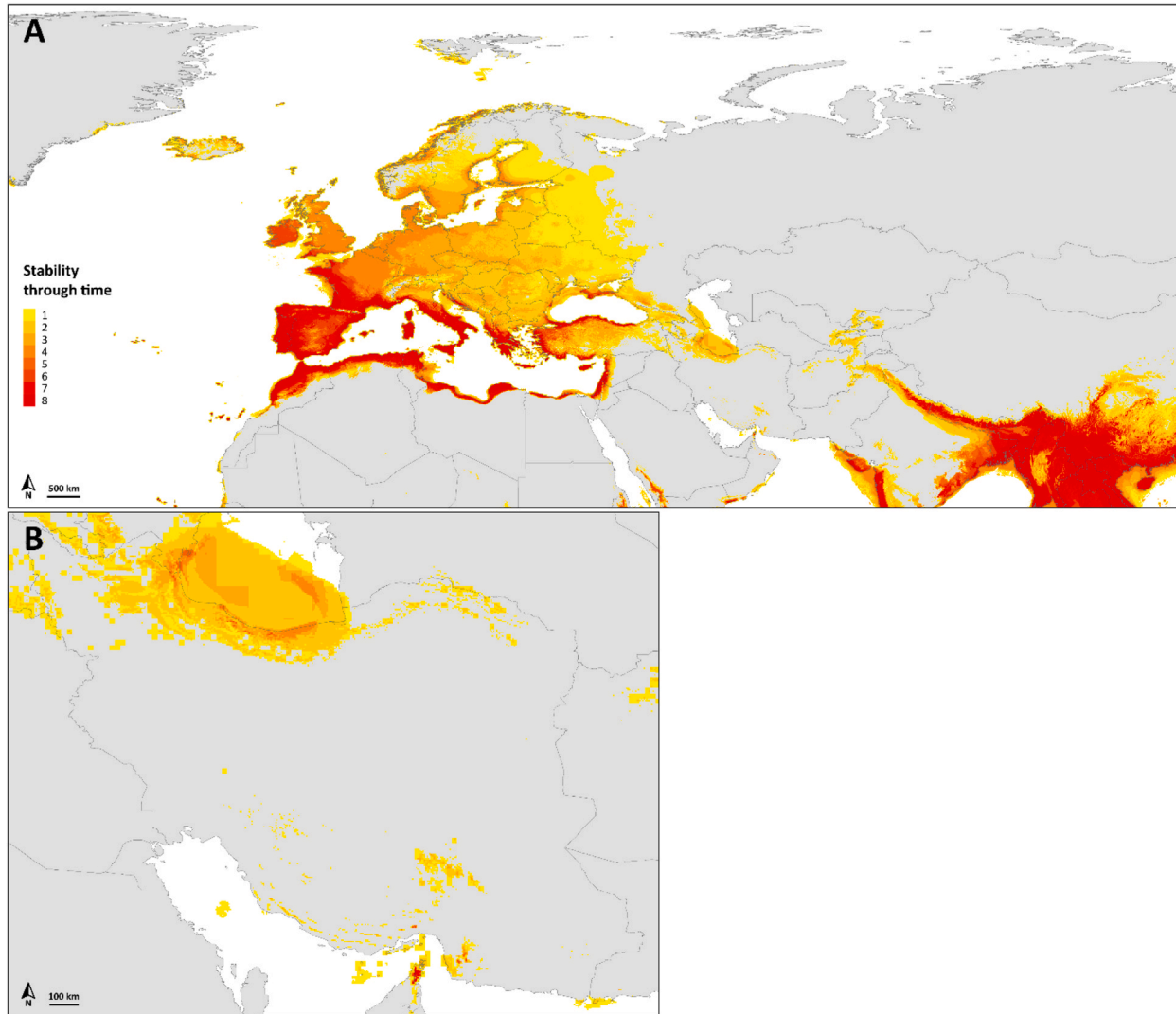
Fig. 6. Stability of the potential distribution through time (present to last Interglacial). A Across the current realized distribution and B across the Albroz Mountains in Iran. A score of eight suggest that the species may have been present through all eight time slices.
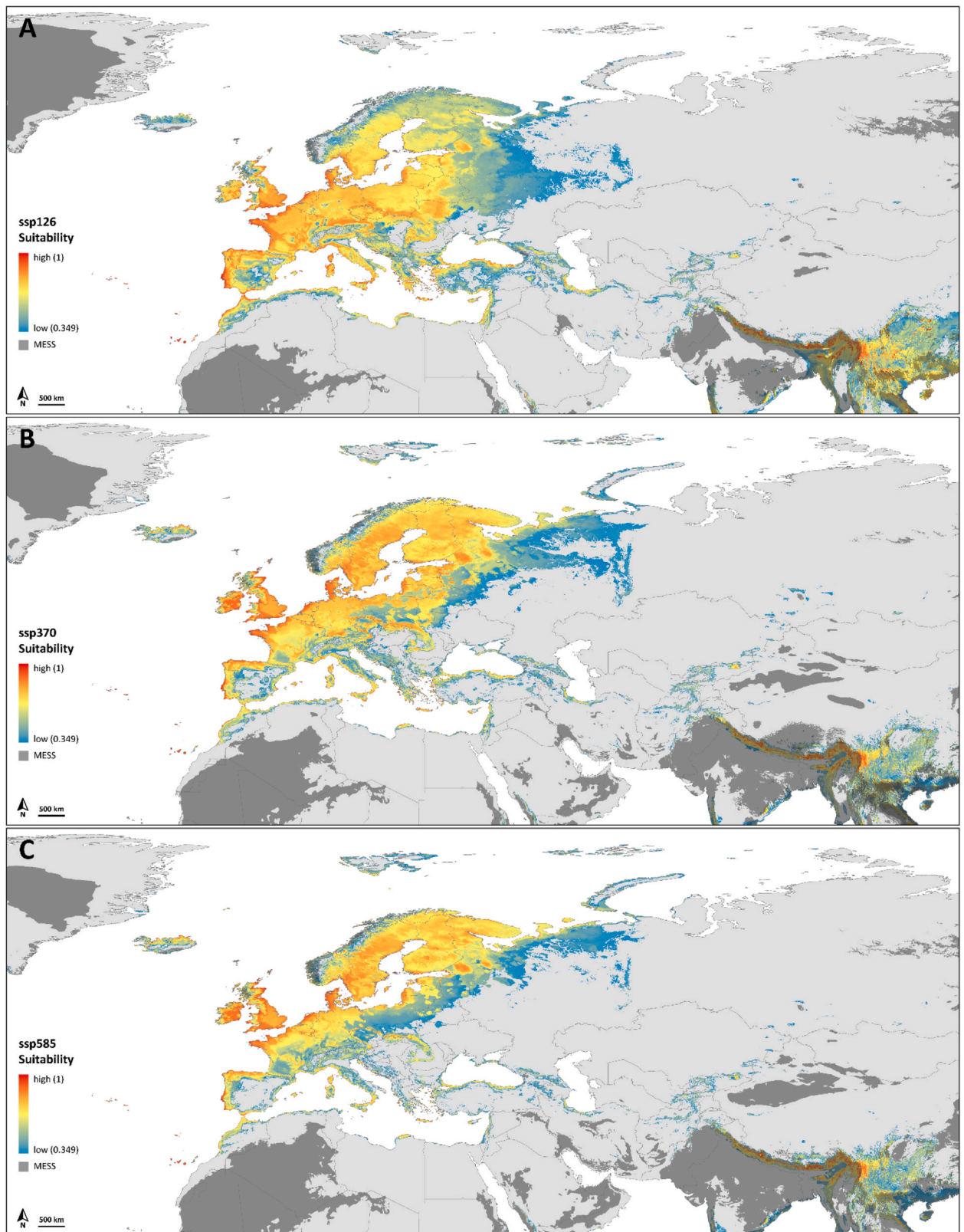
Fig. 7. Future potential distribution of *B. terrestris* across the IPCC story lines ssp126 (A), ssp 360 (B) and ssp585 (C). Warmer colors indicate higher environmental suitability.

We also used species distribution modeling to assess the potential distribution under past, present, and future climate scenarios to illustrate a picture of the species potential range under climate change.

The studies regarding bumblebees in Iran and *B. terrestris* in particular, were limited to morphological studies. Several studies (e.g., Pittoni, 1937; Skorikov, 1938; Monfared et al., 2005; Tahmasbi et al., 2008; Monfared et al., 2009) have confirmed the occurrence of the species through morphological identification in the Alborz Mountain range in Iran. However, up to the present date, no genetic evaluation has been conducted on the population or phylogenetic position in the country. The lack of genetic confirmation can result in the underestimation of the existence of hidden species and a limited understanding of genetic variation within species in these regions.

4.1. Phylogeography of Bombus terrestris

This study was the first attempt to clarify the phylogeography of *B. terrestris* in Iran. We aimed to fill in the void by sampling the species in its range in Iran and to obtain the DNA sequences in order to depict a more comprehensive picture of its genetic structure. To assess the species genetic structure in Iran and its position with regards to other populations across the species distribution range, we used the COI sequences generated in the present study and combined them with sequences obtained from NCBI. The phylogenetic analyses showed no significant genetic structure, regardless of geographic position, although slight differentiation can be observed through the presence of three haplotypes (Fig. 2). The relatively homogenous genetic structure of continental European populations might be a sign of a lack of effective geographical barriers across the region (Estoup et al., 1996).

Our results are aligned with the results of Estoup et al. (1996) in which, the genetic variation of the island and continental populations of the species has been evaluated in Europe using 10 microsatellite loci and partial sequences of COII. Although this study found no significant genetic differentiation in continental populations, their results showed that the island populations are slightly more differentiated than continental ones.

The study by Widmer et al. (1998) investigated the genetic structure of *B. terrestris* in Madeira and the Canary Islands using microsatellites and mitochondrial DNA (cytochrome b). Their results also confirmed the higher genetic differentiation amongst island populations compared to continental ones. Their results suggested that ancestral haplotypes occurred on the Canary Islands, whereas derivative haplotypes were found on the European continent.

Our findings not only align with prior research but also contribute genetic data from populations located in the southern Caspian Sea region and along the Alborz Mountain Range. This information supports the previous hypothesis of shallow genetic structure within continental populations.

4.2. Haplotype network

The results of the haplotype network demonstrate an ancestral haplotype (Fig. 4) that can be found in several countries. However, some samples showed unique haplotypes. Our findings are in accordance with the results of Silva et al. (2020) in which, it was proved that one haplotype (Hap _ 01) is the most common among 10 European haplotypes throughout the distribution range for the species with five haplotype unique to the Iberian Peninsula.

Being known as a Mediterranean hotspot, Iberia is the origin of several endemic species and lineages (Hewitt, 2011). Hence, the diversity of the species in this region and having unique haplotypes can be expected. Overall, examining the haplotype network suggests the presence of genetic sub-structuring, albeit shallow. Notably, the ancestral haplotype includes many samples from several locations. To delve deeper into this, additional research using faster-evolving genetic markers would be required.

4.3. Genetic distance

The genetic distance between haplotypes was estimated to be not higher than 1.1% and the only exception was the unique haplotype of Italy (see Table 1). The homogenous structure and shallow genetic diversity can indicate that insufficient time has passed for evolution to occur at the COI gene. Additionally, the species could have suffered from a bottleneck event during the last ice age. Another reason can be the result of continuous and adequate gene flow that is great enough to connect the local populations across a vast geographical area (Estoup et al., 1996). The *B. terrestris* queens are physically capable of long-distance flights and may be found kilometers away from their birthplace. This phenomenon has been witnessed in the spring migration of the species alongside the North-West coast of France and South coasts of the UK (Philip, 1957). The large dispersal ability observed here supports the gene flow hypothesis. Conversely, a recent population size expansion from pre-glacial refugia could also account for such genetic structure (Estoup et al., 1996). This fact was supported by the current demographic analysis, indicating a recent expansion.

4.4. Demographic analysis

Based on AMOVA analysis results, we deduced that all hypothetical populations have similar genetic structures and do not vary significantly and as a result, consider them to be one. The molecular diversity indices also confirmed low genetic diversity. The Mismatch Distribution diagram presents a skewed unimodal pattern (Fig. 3) which can be interpreted as an indicator of the recent expansion in population size that could happen after a potential glacial bottleneck (Jenkins et al., 2018). The result of Tajima's *D* analysis ($p < 0.05$, -2.327) can also suggest that the population has recently experienced an expansion in its size and no potential mutation has been lost (Tajima, 1989). Thus, the 24 polymorphic sites across 240 samples that have been calculated for the species, could be relatively recent.

The combined data that have been derived from phylogenetic and demographic analyses based on COI, present a very low genetic diversity for the species that confirm the results of Estoup et al. (1996), Moreira et al. (2015), and Silva et al. (2020). Although our COI-based results are comparable to the former studies and are confirmed by them, it should be noted that COI marker has some limitations in regard to revealing genetic diversity amongst populations. While COI can be used as a suitable choice for barcoding and demonstrating diversity between species, using it for phylogeographical and population genetic studies may produce misleading results (Deagle et al., 2014; Hurst and Jiggins, 2005). The limited genetic diversity observed in our study may be influenced by using a single genetic marker with a relatively slow mutation rate compared to other markers. Although previous studies (e.g., Estoup et al., 1996; Moreira et al., 2015 and Silva et al., 2020) using various markers such as microsatellites and genomic analyses have produced similar results, it is crucial to conduct further investigations utilizing faster-evolving markers like the D-loop to enhance the reliability of the outcomes.

As mentioned before, the low level of species genetic diversity in a vast geographical area can occur due to the lack of a major barrier for gene flow and the species high dispersal ability (Woodard et al., 2015; Lecocq et al., 2016). The long flights of the queen bumblebee can make an adequate gene flow for keeping the genetic structure of the species homogenous (Lepais et al., 2010). Furthermore, considering the generalist nature of *B. terrestris* in both food plants and the environment (Chapman et al., 2003) and the high tolerance of different climatic conditions (Penado et al., 2016), the species can seek suitable habitats in a varied range of areas. These advantages enable the species to disperse and succeed in numerous habitats easily.

The high adaptation and colonization abilities of the species alongside the commercial trade in *B. terrestris* to different countries (Dafni et al., 2010) has led the species to get established as an alien species in regions where it is not native (Velthuis and van Doorn, 2006; Fontúrbel et al., 2021). The great ability of the species in colonization and recover their population has led to the successful recovery from a founder event in Tasmania and the colonization of the region with as little as three queens (Schmid-Hempel et al., 2007).

The high expansion rate and dispersal ability of the species are in concordance with the MMD diagram and Tajima's *D* results in the present study. According to our results, the species probably experienced a recent expansion in population size and could easily disperse in a vast area. The species may have suffered from a bottleneck in the last ice age and thus, a limited number of the individual survived in a glacial refugia which is the probable origin of the ancestral haplotype. After the last glacial maximum, the species could easily expand its size and repopulate suitable habitats in Eurasia. In this scenario, the expansion rate was probably faster than the rate of genetic mutation, leading to the observed shallow genetic differentiation. Although large populations generally show greater genetic diversity, the bumblebees showed that they can expand their size without gaining much genetic diversity when the habitat is suitable enough to host a big population (Maebe et al., 2019). While large populations may appear robust due to their sheer numbers, the demonstrated low genetic diversity in this study, if confirmed across the entire genome, renders the species highly vulnerable to future climate change. (Frankham, 2005; Zayed, 2009; Habel et al., 2014; Koch et al., 2017). In summary, climate change can disturb ecological processes and subject species to stress, particularly when they exhibit low genetic variation. These effects can have enduring implications for the adaptability and resilience of species when confronted with changing environments.

4.5. Climate change and species distribution modeling

Our SDMs reveal historical range fluctuations and project future northward shifts. During the Last Interglacial, the species covered Western Europe, parts of Turkey, and Southeast Asia. Shifting patterns continued through Heinrich Stadial and the subsequent 6400 years. In the Early Holocene, there was substantial expansion across Europe, Western Turkey, and a limited area south of the Caspian Sea. The Mid-Holocene saw significant range expansion. From the Late Holocene to the present, the pattern persisted, with continued expansion towards Eastern Eurasia. (Fig. 5 B, A). Our results confirm the findings of Dellicour et al. (2017) which illustrated the European distribution of five bumblebees including *B. terrestris* in current climate conditions and the Last Glacial Maximum. Their results suggest the same range expansion after the LGM that our model depicts. This expansion can also be indicative of the sudden expansion scenario from a glacial refugia which we reported in the demographic analyses based on COI sequence data.

Numerous studies have addressed the adverse impacts of climate change on bumblebee populations in North America and Europe. (Ploquin et al., 2013; Kerr et al., 2015). The seasonal cycle of the flowering plants can be altered by the climate change-induced temperature changes that affect bumblebees negatively as they are highly dependent on such plants (Ogilvie et al., 2017). The temperature rise causes the angiosperms to flower earlier than they used to but the bumblebees do not change their cycle as fast as the plants and these unsynchronized cycles may lead to a primary reduction in abundance and the gradual range shift to higher elevations (Pyke et al., 2016). The results of our model are in accordance with the mentioned studies as it illustrates a range shift towards higher latitudes in all three scenarios for the period 2070–2100 (Fig. 7). Based on our predictions, the species range will experience the most notable shift in the ssp585 scenario, which represents the worst-case scenario for greenhouse gas emissions by the end of the century. In this scenario, the species most suitable range covers most of Northwestern Europe and small parts of Russia, and suitability declines towards the southern parts of Eurasia. There are some fragmented patches of suitable habitats south of the Black Sea, Caucus, and South of the Caspian Sea (Fig. 7 C). This range shift is also apparent in the medium to the high end of the plausible future pathway (ssp370 scenario) and low end of the range of plausible future pathway (ssp126 scenario) but the area for highly suitable habitats seems to be vaster. Additionally, the patches in Eastern parts of Eurasia towards the Black Sea and Caspian Sea are more connected and cover more areas (Fig. 7 A, B). The map for the stability of suitable habitats through time also illustrates that the spots in the Eastern parts of Eurasia tend to lose stability over time and the most suitable areas are in Western Europe and narrow parts of North Africa, in addition to Southeast Asia (Fig. 6).

It has been noted that even considering the high dispersal ability of the *B. terrestris*, there will be some loss in their distribution

range. The existing gap between the speed of climate change and the bumblebee's ability to disperse to new habitats may exacerbate the effects of climate change on the species in the future (Sirosi-Delisle & Kerr, 2018).

5. Conclusion

In the present study, the phylogeographic and population genetics of the buff-tailed bumblebee population in the Eurasian and Caspian Sea region have been assessed, and its distribution in the past, present, and future climate conditions have been reconstructed. The analyses recognized one population throughout the study region and indicated low genetic differentiation between individuals from different localities. The SDM results confirm the expansion range of the species in the present. The demographic analyses suggested the possibility of a recent expansion as an explanation for the homogenous structure of the species that also demonstrates a high dispersal ability and could have spread easily and quickly. However, we should state that our results and hypotheses are entirely based on the COI mitochondrial marker, which may underrepresent the whole genetic diversity of a single population. Hence, we propose additional mitochondrial and nuclear genes to explore the diversity within the species more comprehensively.

CRediT authorship contribution statement

Rödder Dennis: Formal analysis, Writing – original draft, Writing – review & editing. **Saberi-Pirooz Reihane:** Formal analysis, Writing – original draft, Writing – review & editing. **Ranjbaran Yasaman:** Data curation, Formal analysis, Methodology, Writing – original draft, Writing – review & editing. **Ahmadzadeh Faraham:** Formal analysis, Methodology, Project administration, Supervision, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors have no relevant financial or non-financial interests to disclose.

Data availability

The data that has been used is confidential.

Data accessibility statement

-The sampling locations and the NCBI sequences' locations are presented in Table S1 as supplementary materials.
-DNA sequences will be deposited into NCBI Genbank upon acceptance.
-Climate data is available and MaxEnt input files will be archived and accessible upon acceptance.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.gecco.2023.e02775.

References

Ahmadzadeh, F., Shahrokhi, G., Saberi-Pirooz, R., Oladi, M., Taati, M., Poyarkov, N.A., Rödder, D., 2020. Alborz heritage: geographic distribution and genetic differentiation of the Iranian *Paradactylodon* (Amphibia: Hynobiidae). Amphib. Reptil. 41 (4), 519–534.
Aiello-Lammens, M.E., Boria, R.A., Radosavljevic, A., Vilela, B., Anderson, R.P., 2015. spThin: an R package for spatial thinning of species occurrence records for use in ecological niche models. Ecography 38, 541–545.
Akaike, H., 1974. A new look at the statistical model identification. IEEE Trans. Autom. Control 19 (6), 716–723.
Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J., 1990. Basic local alignment search tool. J. Mol. Biol. 215 (3), 403–410.
Amiri, N., Vaissi, S., Aghamir, F., Saberi-Pirooz, R., Rödder, D., Ebrahimi, E., Ahmadzadeh, F., 2021. Tracking climate change in the spatial distribution pattern and the phylogeographic structure of Hyrcanian wood frog, *Rana pseudodalmatina* (Anura: Ranidae). J. Zool. Syst. Evolut. Res. 59 (7), 1604–1619.
Baker, D.B., 1996. On a collection of humble-bees from northern Iran (Hymenoptera: Apoidea, Bombinae). Beitr. Zur. Èntomol. 46, 109–132.
Brown, J.L., Hill, D.J., Dolan, A.M., Carnaval, A.C., Haywood, A.M., 2018. PaleoClim, high spatial resolution paleoclimate surfaces for global land areas. Sci. data 5 (1), 1–9.
Cappellari, A., Mei, M., Lopresti, M., Cerretti, P., 2018. BumbleKey: an interactive key for the identification of bumblebees of Italy and Corsica (Hymenoptera, Apidae). ZooKeys 784), 127.
Chapman, R.E., Wang, J., Bourke, A.F.G., 2003. Genetic analysis of spatial foraging patterns and resource sharing in bumble bee pollinators. Mol. Ecol. 12 (10), 2801–2808.

Crandall, K.A., Clement, M., Posada, D., 2000. TCS: a computer program to estimate gene genealogies. Mol. Ecol. 9, 1657–1660.

Dafni, A., Kevan, P., Gross, C.L., Goka, K., 2010. *Bombus terrestris*, pollinator, invasive and pest: An assessment of problems associated with its widespread introductions for commercial purposes. Appl. Entomol. Zool. 45 (1), 101–113.

Deagle, B.E., Jarman, S.N., Coissac, E., Pompanon, F., Taberlet, P., 2014. DNA metabarcoding and the cytochrome c oxidase subunit I marker: not a perfect match. Biol. Lett. 10 (9), 20140052.

Dellicour, S., Kastally, C., Varela, S., Michez, D., Rasmont, P., Mardulyn, P., Lecocq, T., 2017. Ecological niche modelling and coalescent simulations to explore the recent geographical range history of five widespread bumblebee species in Europe. J. Biogeogr. 44 (1), 39–50.

Estoup, A., Solignac, M., Cornuet, J.M., Goudet, J., Scholl, A., 1996. Genetic differentiation of continental and island populations of *Bombus terrestris* (Hymenoptera: Apidae) in Europe. Mol. Ecol. 5 (1), 19–31.

Excoffier, L., Lischer, H.E., 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol. Ecol. Resour. 10 (3), 564–567.

Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek, R., 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol. Mar. Biol. Biotechnol. 3 (5), 294–299.

Fontúrbel, F.E., Murúa, M.M., Vieli, L., 2021. Invasion dynamics of the European bumblebee *Bombus terrestris* in the southern part of South America. Sci. Rep. 11 (1), 1–7.

Frankham, R., 2003. Genetics and conservation biology. Comptes Rendus Biol. 326, 22–29.

Frankham, R., 2005. Genetics and extinction. Biol. Conserv. 126 (2), 131–140.

Fu, Y.X., 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics 147 (2), 915–925.

Geue, J.C., Thomassen, H.A., 2020. Unraveling the habitat preferences of two closely related bumble bee species in Eastern Europe. Ecol. Evol. 10 (11), 4773–4790.

Ghisbain, G., Gérard, M., Wood, T.J., Hines, H.M., Michez, D., 2021. Expanding insect pollinators in the anthropocene. Biol. Rev. 96 (6), 2755–2770.

Goulson, D., Lye, G.C., Darvill, B., 2008. Decline and conservation of bumble bees. Annu. Rev. Entomol. 53, 191–208.

Habel, J.C., Husemann, M., Finger, A., Danley, P.D., Zachos, F.E., 2014. The relevance of time series in molecular ecology and conservation biology. Biol. Rev. 89 (2), 484–492.

Hewitt, G.M., 2004. Genetic consequences of climatic oscillations in the quaternary. Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci. 359 (1442), 183–195.

Hijmans, R.J., Cameron, S.E., Parra, J.L., Jones, P.G., Jarvis, A., 2005. Very high resolution interpolated climate surfaces for global land areas. Int. J. Climatol. A J. R. Meteorol. Soc. 25 (15), 1965–1978.

Hoang, D.T., Chernomor, O., Von Haeseler, A., Minh, B.Q., Vinh, L.S., 2018. UFBoot2: improving the ultrafast bootstrap approximation. Mol. Biol. Evol. 35 (2), 518–522.

Huelsenbeck, J.P., Ronquist, F., 2001. 'MRBAYES: Bayesian Inference of Phylogenetic Trees', Bioinformatics, 17. Oxford University Press, pp. 754–755.

Hurst, G.D., & Jiggins, F.M., 2005. Problems with mitochondrial DNA as a marker in population, phylogeographic and phylogenetic studies: the effects of inherited symbionts. *Proceedings of the Royal Society B: Biological Sciences, 272* (1572), 1525–1534.

Jenkins, T.L., Castilho, R., Stevens, J.R., 2018. Meta-analysis of northeast Atlantic marine taxa shows contrasting phylogeographic patterns following post-LG expansions. PeerJ 6, e5684.

Karger, D.N., Conrad, O., Böhner, J., Kawohl, T., Kreft, H., Soria-Auza, R.W., Zimmermann, N.E., Linder, P., Kessler, M., 2017. Climatologies at high resolution for the Earth land surface areas. Sci. Data 4 (1), 1–20.

Katoh, K., Misawa, K., Kuma, K.I., Miyata, T., 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Res. 30 (14), 3059–3066.

Keller, L.F., Waller, D.M., 2002. Inbreeding effects in wild populations. Trends Ecol. Evol. 17, 230–241.

Kerr, J.T., Pindar, A., Galpern, P., Packer, L., Potts, S.G., Roberts, S.M., Rasmont, P., Schweiger, O., Colla, S.R., Richardson, L.L., Wagner, D.L., 2015. Climate change impacts on bumblebees converge across continents. Science 349 (6244), 177–180.

Koch, J.B., Looney, C., Sheppard, W.S., Strange, J.P., 2017. Patterns of population genetic structure and diversity across bumble bee communities in the Pacific Northwest. Conserv. Genet. 18 (3), 507–520.

Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K., 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol. Biol. Evol. 35 (6), 1547.

Kwak, M.M., Velterop, O., van Andel, J., 1998. Pollen and gene flow in fragmented habitats. Appl. Veg. Sci. 1 (1), 37–54.

Lecocq, T., Coppée, A., Michez, D., Brasero, N., Rasplus, J.Y., Valterova, I., Rasmont, P., 2016. The alien's identity: consequences of taxonomic status for the international bumblebee trade regulations. Biol. Conserv. 195, 169–176.

Lepais, O., Darvill, B.E.N., O'connor, S., Osborne, J.L., Sanderson, R.A., Cussans, J., Goffe, L., Goulson, D., 2010. Estimation of bumblebee queen dispersal distances using sibship reconstruction method. Mol. Ecol. 19 (4), 819–831.

Leroux, S.J., Larrivée, M., Boucher-Lalonde, V., Hurford, A., Zuloaga, J., Kerr, J.T., Lutscher, F., 2013. Mechanistic models for the spatial spread of species under climate change. Ecol. Appl. 23 (4), 815–828.

Librado, P., Rozas, J., 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25 (11), 1451–1452.

Loarie, S.R., Duffy, P.B., Hamilton, H., Asner, G.P., Field, C.B., Ackerly, D.D., 2009. The velocity of climate change. Nature 462 (7276), 1052–1055.

Maebe, K., Karise, R., Meeus, I., Mänd, M., Smagghe, G., 2019. Level of genetic diversity in European bumblebees is not determined by local species abundance. Front. Genet. 10, 1262.

Maebe, K., Meeus, I., Ganne, M., De Meulemeester, T., Biesmeijer, K., Smagghe, G., 2015. Microsatellite analysis of museum specimens reveals historical differences in genetic diversity between declining and more stable Bombus species. PloS one 10 (6), e0127870.

Markert, J.A., Champlin, D.M., Gutjahr-Gobell, R., Grear, J.S., Kuhn, A., McGreevy, T.J., Jr, Roth, A., Bagley, M.J., Nacci, D.E., 2010. Population genetic diversity and fitness in multiple environments. BMC Evolut. Biol. 10, 205.

Monfared, A., Talebi, A.A., Tahmasbi, G., Williams, P.H., Ebrahimi, E., Taghavi, A., 2005. A survey of the localities and food-plants of the bumblebees of Iran (Hymenoptera: Apidae: Bombus). Èntomol. Gen. J. Gen. Appl. Entomol. 30 (4), 283.

Monfared, A., Talebi, A.A., Tahmasbi, G., Williams, P.H., Ebrahimi, E., Taghavi, A., 2007. A survey of the localities and food-plants of the bumblebees of Iran (Hymenoptera: Apidae: *Bombus*). Èntomol. Gen. J. Gen. Appl. Entomol. 30 (4), 283.

Monfared, A., Talebi, A.A., Tahmasbi, G., Ebrahimi, E., Biesmeijer, J., 2008. Bumblebee (Hymenoptera: Apidae) diversity and abundance in the Iranian Alborz Mountains. Zool. Middle East 44, 67–74.

Monfared, A., Talebi, A.A., Tahmasbi, G., Ebrahimi, E., Biesmeijer, J., 2009. Bumblebee diversity and abundance in the Iranian Alborz Mountains (Hymenoptera: Apidae). Zool. Middle East 46 (1), 83–94.

Moreira, A.S., Horgan, F.G., Murray, T.E., Kakouli-Duarte, T., 2015. Population genetic structure of Bombus terrestris in Europe: Isolation and genetic differentiation of Irish and British populations. Mol. Ecol. 24 (13), 3257–3268.

Naimi, B., Hamm, N.A., Groen, T.A., Skidmore, A.K., Toxopeus, A.G., 2014. Where is positional uncertainty a problem for species distribution modelling? Ecography 37 (2), 191–203.

Nguyen, L.T., Schmidt, H.A., Von Haeseler, A., Minh, B.Q., 2015. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol. Biol. Evol. 32 (1), 268–274.

Nylander, J.A.A. , 2004. MrModeltest v2. *Program distributed by the author*. Evolutionary Biology Centre, Uppsala University.

Ogilvie, J.E., Griffin, S.R., Gezon, Z.J., Inouye, B.D., Underwood, N., Inouye, D.W., Irwin, R.E., 2017. Interannual bumble bee abundance is driven by indirect climate effects on floral resource phenology. Ecol. Lett. 20 (12), 1507–1515.

Parmesan, C., Yohe, G., 2003. A globally coherent fingerprint of climate change impacts across natural systems. Nature 421 (6918), 37–42.

Parmesan, C., Ryrholm, N., Stefanescu, C., Hill, J.K., Thomas, C.D., Descimon, H., Huntley, B., Kaila, L., Kullberg, J., Tammaru, T., Tennent, W.J., 1999. Poleward shifts in geographical ranges of butterfly species associated with regional warming. Nature 399 (6736), 579–583.

Penado, A., Rebelo, H., Goulson, D., 2016. Spatial distribution modelling reveals climatically suitable areas for bumblebees in undersampled parts of the Iberian Peninsula. Insect Conserv. Divers. 9 (5), 391–401.

Philip, E.G., 1957. Migrating bumble bees. Entomol. ' Mon. Mag. 93, 104.

Phillips, S.J., Dudík, M., & Schapire, R.E. , 2004, July. A maximum entropy approach to species distribution modeling. In *Proceedings of the twenty-first international conference on Machine learning* (p. 83).

Phillips, S.J., Anderson, R.P., Schapire, R.E., 2006. Maximum entropy modeling of species geographic distributions. Ecol. Model. 190 (3-4), 231–259.

Phillips, S.J., Dudík, M., Elith, J., Graham, C.H., Lehmann, A., Leathwick, J., Ferrier, S., 2009. Sample selection bias and presence-only distribution models: implications for background and pseudo-absence data. Ecol. Appl. 19 (1), 181–197.

Pittoni, B., 1937. Eine Hummelausbeute aus dem Elburs-Gebirge (Iran). *Konowia*. Wien 16, 113–129.

Ploquin, E.F., Herrera, J.M., Obeso, J.R., 2013. Bumblebee community homogenization after uphill shifts in montane areas of northern Spain. Oecologia 173 (4), 1649–1660.

Popove, V.V. ,1967. The bees (Hymenoptera, Apidae) of Iran [in Russian]. - Trudy Zoologicheskogo Instituta, Akademiya Nauk Soyuza Sovetskikh Sotsialisticheskikh Respublik 43: 184–216.

Provan, J., Bennett, K.D., 2008. Phylogeographic insights into cryptic glacial refugia. Trends Ecol. Evol. 23 (10), 564–571.

Pyke, G.H., Thomson, J.D., Inouye, D.W., Miller, T.J., 2016. Effects of climate change on phenologies and distributions of bumble bees and the plants they visit. Ecosphere 7 (3), e01267.

Rambaut, A., Drummond, A.J., & Suchard, M. , 2007. Tracer v1. 6 ⟨http://beast⟩. bio. ed. ac. uk.

Rasmont, P., & Adamski, A. , 1995. [Corsican bumble-bees (Hymenoptera Apoidea, Bombinae)].[English]. *Gembloux Faunal Notes*.

Rasmont, P., Coppée, A., Michez, D., De Meulemeester, T., 2008. An overview of the *Bombus terrestris* (L. 1758) subspecies (Hymenoptera: Apidae). In: In Annales de la Société entomologique de France, Vol. 44. Taylor & Francis Group, pp. 243–250.

Rasmont, P., Ghisbain, G., & Terzo, M. , 2021. Bumblebees of Europe and neighbouring regions. *NAP Editions*.

Reinig, W.F. , 1939. Die Hummeln der Reisen von E. Pfeffer (1936) und E. Pfeffer und Dr. W. Foster (1937) in den Elburs. *Mitteilungen der Münchner Entomologischen Gesellschaft* 29: 145–148.

Rivers, M.C., Brummitt, N.A., Lughadha, E.N., Meagher, T.R., 2014. Do species conservation assessments capture genetic diversity? Glob. Ecol. Conserv. 2, 81–87.

Saberi-Pirooz, R., Rajabi-Maham, H., Ahmadzadeh, F., Kiabi, B.H., Javidkar, M., Carretero, M.A., 2021. Pleistocene climate fluctuations as the major driver of genetic diversity and distribution patterns of the Caspian green lizard, Lacerta strigata Eichwald, 1831. Ecol. Evol. 11 (11), 6927–6940.

Sambrook, J., Fritsch, E.F., Maniatis, T., 1989. Molecular cloning: a laboratory manual (No. Ed. 2). Cold spring harbor laboratory press.

Schmid-Hempel, P., Schmid-Hempel, R., Brunner, P.C., Seeman, O.D., Allen, G.R., 2007. Invasion success of the bumblebee, *Bombus terrestris*, despite a drastic genetic bottleneck. Heredity 99, 414–422.

Sepp, K., Mikk, M., Mänd, M., Truu, J., 2004. Bumblebee communities as an indicator for landscape monitoring in the agri-environmental programme. Landsc. Urban Plan. 67 (1-4), 173–183.

Silva, S.E., Seabra, S.G., Carvalheiro, L.G., Nunes, V.L., Marabuto, E., Mendes, R., Rodrigues, A.S., Pina-Martins, F., Yurtsever, S., Laurentino, T.G., Figueiredo, E., 2020. Population genomics of *Bombus terrestris* reveals high but unstructured genetic diversity in a potential glacial refugium. Biol. J. Linn. Soc. 129 (2), 259–272.

Skorikov, A.S., 1938. Zoogeographische Gestzmässigkeiten der Hummelfauna im Kaukasus, Iran und Anatolien (Hymenoptera, Bombinae). *Entomologicheskoe Obozrenie*, *27*, 145–151.

Tahmasbi, G.H., Taghavi, A., Ebrahimi, E., Talebi, A., Zarnegar, A., Monfared, A., 2008. Identification and distribution bumblebees (Hymenoptera: Apidae, *Bombus* spp.) in Central Elburz Mountains of Iran. Entomofauna 20, 265–280.

Tajima, F., 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123 (3), 585–595.

Velthuis, H.H., Van Doorn, A., 2006. A century of advances in bumblebee domestication and the economic and environmental aspects of its commercialization for pollination. Apidologie 37 (4), 421–451.

Widmer, A., Schmid-Hempel, P., Estoup, A., Scholl, A., 1998. Population genetic structure and colonization history of *Bombus terrestris* sl (Hymenoptera: Apidae) from the Canary Islands and Madeira. Heredity 81 (5), 563–572.

Williams, P.H., An, J., Brown, M.J., Carolan, J.C., Goulson, D., Huang, J., Ito, M., 2012a. Cryptic bumblebee species: consequences for conservation and the trade in greenhouse pollinators. PloS One 7 (3), e32992.

Williams, P.H., Brown, M.J.F., Carolan, J.C., An, J., Goulson, D., Aytekin, A.M., Xie, Z., 2012b. Unveiling cryptic species of the bumblebee subgenus Bombus s. str. worldwide with COI barcodes (Hymenoptera: Apidae). Syst. Biodivers. 10, 21–56.

Woodard, S.H., Lozier, J.D., Goulson, D., Williams, P.H., Strange, J.P., Jha, S., 2015. Molecular tools and bumble bees: revealing hidden details of ecology and evolution in a model system. Mol. Ecol. 24 (12), 2916–2936.

Zayed, A., 2009. Bee genetics and conservation. Apidologie 40, 237–262.

Yasaman Ranjbaran is primarily interested in the historical and contemporary processes that lead to speciation. This work represents her Master's thesis at the Shahid Beheshti University (Tehran/Iran) on the genetic diversity of Buff tailed Bumblebee. She and other authors collaborate on questions focused on evolutionary history and distribution of the species.