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Research Article

# Investigating the relationship between haplotype diversity of Asia minor spiny mouse (*Acomys cilicicus*) and environmental factors

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## Abstract

Asia Minor Spiny Mouse (*Acomys Cilicicus*) is one of the endemic spiny mouse species of Turkey. The species is vulnerable to environmental threats as conservation measures have not yet been determined. Understanding the genetic makeup of the species and its responses to ecological factors is, therefore, an important issue to develop applicable conservation measures. In the study, we aimed to identify the *Acomys cilicicus*' haplotype diversity for Cytochrome b (CYTB), Growth hormone receptor (GHR), and Recombination activating gene 2 (RAG2) genes and to explore spatial relationships between environmental factors and haplotype diversity of each gene. The spatial distribution pattern of haplotype diversity of genes was estimated using the Geographically weighted regression (GWR) model and Inverse Distance Weighted (IDW) interpolation, respectively. Moreover, the Monte Carlo permutation test was applied to reveal the relationship pattern between environmental predictors and haplotype diversity through local coefficient estimates. As a result, a logistic prediction map of the GWR model was obtained to indicate the distribution of haplotype diversity of genes. Outputs also showed considerable spatial variability in local coefficients estimates with the negative or positive association, and it was understood that the distribution pattern of haplotype diversity is delineated accordingly. In that context, it was concluded that local fluctuations of environmental conditions might negatively affect the haplotype diversity of genes, thus decreasing the species' adaptability to environmental changes. Outputs of the study are valuable

to support the conservation efforts of the target species and can be a guide for species with similar characteristics.

**Keywords:** *Acomys cilicicus*, environmental factors, haplotype diversity, geographically weighted regression, species conservation

## Introduction

Species conservation is one of the issues at the center of biodiversity conservation and provides significant contributions to biodiversity conservation. However, it needs much more than species conservation. Biodiversity is the variety of life on earth in all its forms and interactions. It also covers diversity at each biological level from genetic to ecosystem, and each component is tightly interconnected (Rao & Hodgkin, 2002). It is challenging to understand and protect the integrity of this complex system. It is, on the other hand, vital to the continuity of human beings (Rao & Hodgkin, 2002). In the face of today's enormous environmental problems such as pollution, climate change, and the destruction of habitats, biodiversity suffers irreparable damage at all levels. It is possible to reduce these destructions and ensure the sustainability of biodiversity. Conservation of biological diversity can be guaranteed by allocating time and resources for biodiversity research, raising global awareness in societies, and establishing sanctions on countries with biodiversity agreements.

Moreover, conservationists can identify the needs of biodiversity conservation, and seek to understand complex ecological relationships, thus offering roadmaps for biodiversity conservation. At this point, understanding the genetic characteristics of species emerges as one of the essential elements of biodiversity conservation (Fourcade et al., 2014). Genetic diversity is the building block of biodiversity. it expresses the compositional distinctiveness and fitness of species and thus determines the responses of species to environmental changes. In other words, genetic diversity is a marker of a species' ability to survive and cope with environmental changes. Moreover, it has been assumed that sustainable ecosystem functioning depends on the fitness of species of the ecosystems (Grime, 1997; Tilman et al., 1997). Therefore, understanding species' genetic characteristics and their relationship to environmental factors can significantly improve existing knowledge about the species and contribute to conservation efforts (Neel & Ellstrand, 2003). In this way, necessary precautions can be taken by predicting how the rare, threatened, and vulnerable species will be affected by changes in environmental conditions, and the long-term survival of the species is supported. With this point of view, it was aimed to identify the haplotype diversity of a spiny mouse, *Acomys cilicicus*, for CYTB, GHR, and RAG2 genes and explore the spatial relationship between environmental factors and the haplotype diversity of each gene. Acomys cilicicus is one of the narrow range endemic

spiny mouse species of Turkey. Little is known about it, so it was classified as data deficient (DD) by the IUCN in 2008 (Amori et al., 2008). Although it was later reported that it might be conspecific with a common and widespread species, *Acomys cahirinus*, studies have confirmed it is different (Amori et al., 2008; Çetintaş et al., 2017). In short, *Acomys cilicicus* is a narrowly distributed endemic species in the east of the Mediterranean region of Turkey and is vulnerable to environmental threats as conservation measures have not yet been determined. In this context, the findings of the study will enable us to understand the genetic characteristic of this vulnerable species and its responses to environmental factors, thus contributing to its conservation. On the other hand, since the CYTB, GHR and RAG2 genes are the genes that are widely used in mammalian studies and have published data, this study will allow the expansion of the published data and the comparison of the outputs. Moreover, the study will be a theoretical example of the conservation efforts of similar species. As it is known, small mammals perform many ecosystem services in their habitats that ensure the sustainability of the ecosystem. Their conservation, therefore, serves the sustainability of biodiversity. In order to achieve these goals of the study, geographically weighted regression (GWR) was used. GWR is a local regression model and obtains local estimates for modeled regression relationships (Brunsdon et al., 1996; Fotheringham et al., 2000; 2002; Shi et al., 2006). Thus, it enables ecologists to evaluate the relationships between wildlife and environmental factors.

## Materials and methods

### Study species

Asia Minor Spiny Mouse *Acomys cilicicus* is one of the endemic spiny mouse species of Turkey (Çetintaş et al., 2017). The species is a rodent described in the family of Muridae (Musser & Carleton, 2005). The specific feature of the species is that the back of its body is covered with coarse, inflexible spine-like hairs (Özüt et al., 2020; Özdemirel et al., 2022). The species is social and lives in groups. Like other mice, it is both carnivorous and herbivorous.

It is known that the species only occupies the area between Silifke and Erdemli counties of Mersin province on the southern coast of Turkey and has a very limited distribution here as well (Fig. 1A). The species is therefore defined as a narrow range of locally endemic species. Little is known about its ecology and habitat. However, field studies have shown that it mostly prefers shrubby vegetation with rocky ground, regions close to cultivated areas, and maquis as a habitat (Çetintaş et al., 2017).

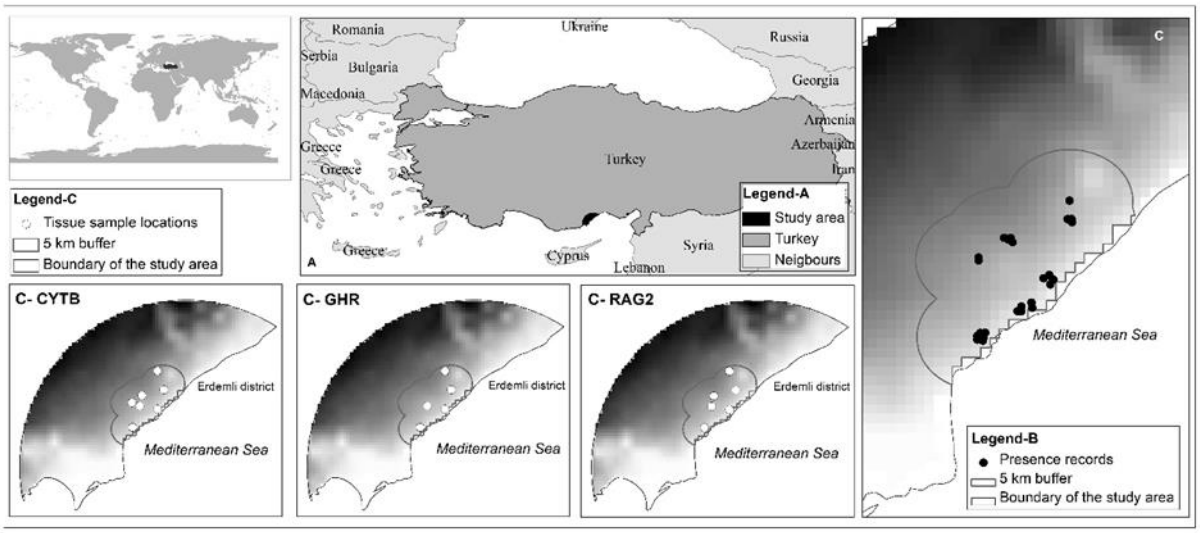


**Figure 1. (A)** Location of the study area; **(B)** Distribution of presence records; **(C)** Tissue sample locations.

In the study, the locations of literature records of the species and its close surroundings were trapped with Sherman-type traps. Accordingly, 3243 traps were set up in 39 localities, each with between 70 and 100 traps. Field studies were carried out between April 2013 and May 2016. As a result, individuals, i.e., species presence, were detected in 27 traps at 14 localities, and tissue samples from 18 of these 27 species presences were collected for the following DNA analyses (Çetintaş et al., 2017; Fig. 1B and C).

**Environmental Predictors**

Twenty-two environmental predictors were colligated to investigate their effects on the distribution of haplotype diversity. Of these, "17" are climatic predictors, "4" are topographic predictors, and "1" is the land cover predictor (Table 1). Climatic predictors are worldclim data and have a spatial resolution of 1 km (http://www.worldclim.org/). The digital elevation model (DEM) is Shuttle Radar Topography Mission (SRTM) data with 90 m. resolution. Aspect and slope layers were produced using standard topographic functions of GIS using dem. Both are in degree units. The aspect was used by separating it as the north and south aspects. The global land cover map was obtained from European Spatial Agency. The map was reprocessed using aspect, dem, and a digital hardcopy map of the local vegetation types. The resulting map includes nine land cover types of the area and is added to the analysis to figure out the impact of land cover types on the distribution of haplotype diversity. Eventually, all the environmental predictors mentioned above were resampled to the 1 km spatial resolution and checked for multicollinearity. Those with a correlation greater or lesser than the ±70 correlation coefficient threshold were eliminated and excluded from further analysis (Table 1).

**Identifying haplotype diversity and investigating the relationship between haplotype diversity and environmental factors**

Haplotypes of CYTB, GHR, and RAG2 genes were identified using liver tissue samples received in field studies. Tissue samples were collected from 7, 5, and 6 locations for CYTB, GHR, and RAG2 genes, respectively (Fig. 1C). Afterward, the following laboratory works were performed to determine haplotypes of genes. Firstly, total DNA extraction from ethanol-preserved liver tissue was carried out using the 5 Primer DNA Tissue kit, following the manufacturer's protocol, and examined through agarose gel electrophoresis. The CYTB, GHR, and RAG2 genes were amplified by PCR using an Eppendorf Mastercycler. PCR reactions were performed in 50 mL volumes (5 units Hot Start Taq polymerase, 5 µL 10 HyTest Ltd. PCR buffer, 0.2 µM dNTPs, 1, 5 µM MgCl2, approximately 100–200 ng template DNA, and 10 pmol of each primer, filled to 25 µL with water). Amplification of the entire CYTB gene was performed using primer set L14724 5'GATATGAAAAACCATCGTTG 3' (Irwin et al., 1991) and H15915 5'TCTCCATTTCTGGTTTACAAGAC3' (Irwin et al. 1991), GHR gene was performed using primer set GHR-D1 5'- TAGGAAGGAAAATTRGARGARGTNAA-3' and GHR-R1: 5'-AAGGCTANGGCATGATRTTRTT-3', after than primer was re-amplification by GHR-D2: 5'-GGAAAATTRGAGGAGGTGAAYACNATHTT-3' and GHR-R2: 5'-GATTTTGTTCAGTTGGTCRGTRCT- NAC-3' (Blanga-Kanfi et al., 2009) and RAG2 gene was performed using primer set RAG2-D1 5'-CGCTGCACAGAGAAAGACTT-3' and RAG2-R1: 5'-AAGGATTTCTTGGCAGGAGT-3', after than primer was re-amplification by RAG2-D2: 5'-TAYAGYCGAGGGAAAAGYATGGG-3' and RAG2-R2:5'-GACAAGTGGATGAGTGTGCGTd,TC-3' (Blanga-Kanfi et al., 2009). The following procedure was applied for all three genes. Accordingly, the thermocycler program was: one preliminary denaturation step at 95 °C for 3 min followed by 40 PCR cycles, each consisting of strand denaturation at 95 °C (1 min), annealing at 58 °C (30 sec), and primer extension at 72 °C (1.5 min). A final extension step was performed at 72 °C for 5 min. All sequences were created with abi format and converted to fasta format with Applied Biosystems Seqscape Software v.3.0. Sequence alignments were done with CLUSTAL W implemented in MEGA software (http://www.megasoftware.net). The number of haplotypes was calculated by DnaSP version 5 (Librado & Rozas, 2009). As a result, 4, 9, and 12 haplotypes were identified for CYTB, GHR, and RAG2 genes, respectively and haplotype diversity of the genes was calculated using the following haplotype diversity index (H).

$$H = \frac{N}{N-1}\left(1 - \sum_i x_i^2\right)$$

Where $x_i$ is the Haplotype frequency and $N$ is the sample size.

Subsequently, geographically weighted regression was applied to estimate the spatial pattern of haplotype diversity for the study area and explore the relationship between haplotype diversity and environmental factors. As it is known, environmental factors are not stationary. There is considerable spatial variation in environmental factors (Virgilio et al., 2014), and species groups respond to this variation. There is an explicit link between species distribution, composition, and environmental variation (Buckley & Jetz, 2008). This dependency of species-environment influences the genetic characteristics of populations (Heywood, 1991; Wang & Bradburd, 2014; Wu et al., 2016). Therefore, it is appropriate to consider the impact of spatial variation of environmental factors on the distribution of the spatial pattern of haplotype diversity. GWR model is a right choice in that context since it is a local regression model and considers the spatial variation of modeled relationships (Fotheringham et al., 2000; Fotheringham et al., 2002; Shi et al., 2006). It produces local estimates and calculates local $R^2$ values (Shi et al., 2006). In this way, it enabled us to identify the spatial distribution of haplotype diversity and figure out how the relationship between haplotype diversity of each gene and environmental predictors might vary geographically. GWR3 software was used to calculate estimated local coefficients and local $R^2$ values for haplotype diversity of genes. The software was released by the department of geography, University of Newcastle. In the study, local estimates were obtained associating haplotype diversity and 20 low correlated environmental predictors (Table 1), and then, these were used to identify spatial patterns of haplotype diversity applying IDW interpolation. IDW is a spatial analysis used to estimate unknown values from a set of points with known values. It is a mathematical process assuming closer values are more related than further values. Accordingly, it calculates unknown values giving greater weights to points closest to the prediction location, and the weights decrease as a distance function (Lu & Wong, 2008). Consequently, predicted models indicated the spatial pattern of haplotype diversity of the genes. These outputs were logistic predicted maps and range between 0 and 1.

**Table 1.** Environmental predictors included in Geographically weighted regression

| Environmental Predictor | Type | Source |
|---|---|---|
| *Climate Predictors* | | |
| Temperature annual range | | |
| Min Temperature of Coldest Month | | |
| Max Temperature of Warmest Month | | |
| **Precipitation of Coldest Quarter** | | |
| Precipitation of Driest Quarter | | |
| Precipitation of Warmest Quarter | | |
| **Precipitation of Wettest Quarter** | | |
| Precipitation Seasonality | Continuous | WorldClim database |
| Temperature Seasonality | | |
| Annual Precipitation | | |
| Mean Diurnal Range | | |
| Isothermality | | |
| Annual Mean Temperature | | |
| **Mean Temperature of Coldest Quarter** | | |
| Mean Temperature of Driest Quarter | | |
| **Mean Temperature of Warmest Quarter** | | |
| **Mean Temperature of Wettest Quarter** | | |
| *Topographic Predictors* | | |
| **Altitude** | Continuous | USGS |
| Slope | | Derived from DEM |
| Northern and Eastern Aspect | | |
| *Land Cover Predictor* | Categorical | European Spatial Agency |

Note: Environmental predictors written in bold are excluded from the further analysis due to the high correlation coefficient values.

Additionally, we explored the significance of non-stationarity in local coefficient estimates of environmental predictors for each gene. The Monte Carlo permutation test was used for this purpose. This test calculates p-values of the predictors by comparing the observed values of a test statistic with n-1 simulated ones and thus, provides to detect of significant non-stationarity in local coefficients of the predictors. On the other hand, the test also reveals the pattern of the relationship between environmental predictors and haplotype diversity through local coefficient estimates.

## Results

The estimated spatial pattern of haplotype diversity of genes was investigated through the haplotype diversity maps produced with IDW interpolation (Fig. 2). Accordingly, it can be said that, although the haplotype diversity patterns of the three genes match to a certain extent, different haplotype diversity patterns are mainly observed among them. It means that their haplotype diversity is not congruent throughout the study area (Fig. 2). As a result, CYTB showed high haplotype diversity in the northwest part of the area, while the haplotype diversity

decreased from the north-east to south direction. Likewise, GHR showed high haplotype diversity in the northwest of the area and low in the northeast. On the other hand, it has high haplotype diversity distributions the south of the area (Fig. 2). As can be seen, there is a small portion of congruence in the haplotype diversity pattern for the GHR and CYTB genes in the north-eastern and north-western parts of the area (Fig. 2). However, the haplotype diversity pattern of the RAG2 gene is entirely different from that of the CYTB gene. Unlike CYTB, the RAG2 gene has low haplotype diversity in the northwest and high in the northeast and south of the area. On the other hand, the haplotype diversity pattern of the RAG2 gene is congruent with the haplotype diversity pattern of the GHR gene in the southern part of the area (Fig. 2).

Results indicated that estimated coefficients of four, five, and four of the twenty environmental predictors are not constant across the study area for CYTB, GHR, and RAG2 genes, respectively, according to the spatial non-stationarity test of Monte Carlo permutation. It means that estimated regression coefficients of these environmental predictors displayed statistically significant non-stationarity ($p< 0.001$) (Fig. 2). In other words, there is significant spatial variation in the relationship between haplotype diversity of the genes and environmental predictors (Fig. 2). The environmental predictors indicated non-stationarity in their estimated coefficients are the minimum temperature of the coldest month, precipitation of warmest quarter, northern aspect and slope for CYTB gene, minimum temperature of the coldest month, annual precipitation, mean temperature of driest quarter, northern aspect, and slope for GHR gene and precipitation of warmest quarter, annual mean temperature, slope and diurnal range for RAG2 gene (Fig. 2).

The relationship between haplotype diversity of each gene and non-stationary environmental predictors was investigated by building maps using estimated coefficients with the predicted haplotype diversity. Accordingly, the relationship of haplotype diversity of CYTB gene with the minimum temperature of the coldest month and precipitation of the warmest quarter exhibited a similar spatial pattern, with negative associations in the north part of the area and positive associations in the south part of the area (Fig. 2A, B). In contrast to these two environmental predictors, the northern aspect indicated positive associations in the north part while displaying negative associations in the south part with haplotype diversity of the CYTB gene (Fig. 2C). Although the spatial distribution pattern of the slope's estimated coefficients looks the same as the spatial distribution pattern of the estimated coefficients of the northern aspect, the difference is that all the coefficients of the slope are positive across the area (Fig. 2D).

The estimated coefficients of the minimum temperature of the coldest month exhibited positive associations throughout the study area with the haplotype diversity of the GHR gene. However, the influence of the coefficients on haplotype diversity is smaller in the north part than in the south. It means an increase in the estimated coefficients of the minimum temperature of the coldest month from the north to the south direction (Fig. 2E). On the other hand, both the estimated coefficients of annual precipitation and mean temperature of the driest quarter displayed negative associations with the haplotype diversity of the GHR gene throughout the whole area, and also, the negative influence of these environmental predictors on haplotype diversity increased from north to south direction (Fig. 2F, G). Moreover, the estimated coefficients of the northern aspect exhibited a negative association with haplotype diversity in the south part, while this association was positive in the north (Fig. 2H). Unlike these predictors, the estimated slope coefficients indicated a positive influence on the haplotype diversity of the GHR gene in the whole area, and this positive influence on haplotype diversity increased from north to south direction (Fig. 2I).

The estimated coefficients of precipitation of the warmest quarter are negative throughout the area for haplotype diversity of RAG2 gene, and the negative influence of the predictor increased towards north to the south direction (Fig. 2J). Unlike the previous predictor, coefficients of annual mean temperature and diurnal range were estimated as positive across the entire area for haplotype diversity of the RAG2 gene. However, the influence of the coefficients is not the same everywhere and increased from the north to south direction (Fig. 2K, L).

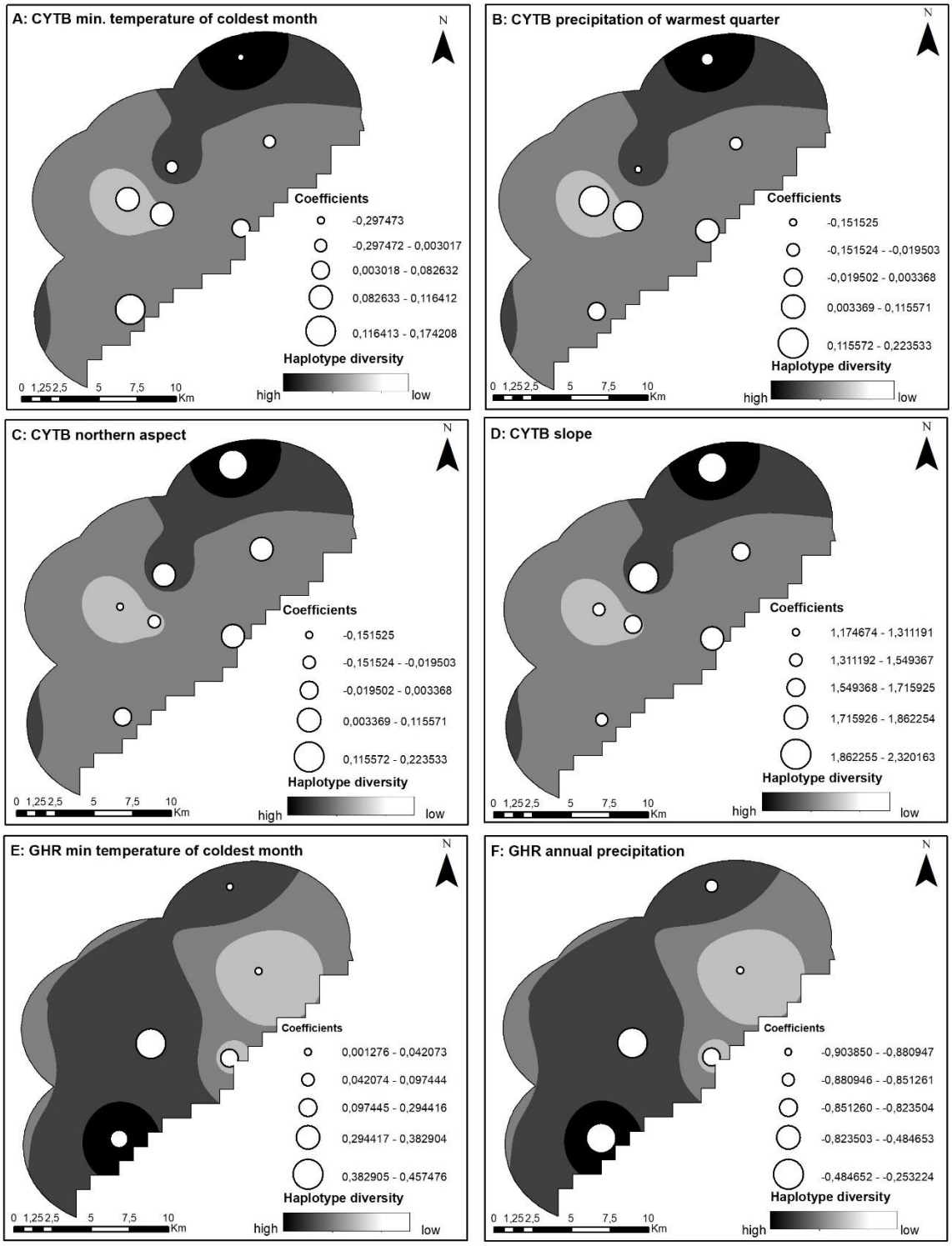


**Figure 2.** Maps showing spatial haplotype diversity pattern of genes and the spatial relationship between haplotype diversity of genes and environmental predictors through estimated coefficients; **(A, B, C, D)** pattern of CYTB with min temperature of the coldest month, precipitation of warmest quarter, northern aspect, slope; **(E, F)** pattern of GHR with min temperature of the coldest month, annual precipitation.

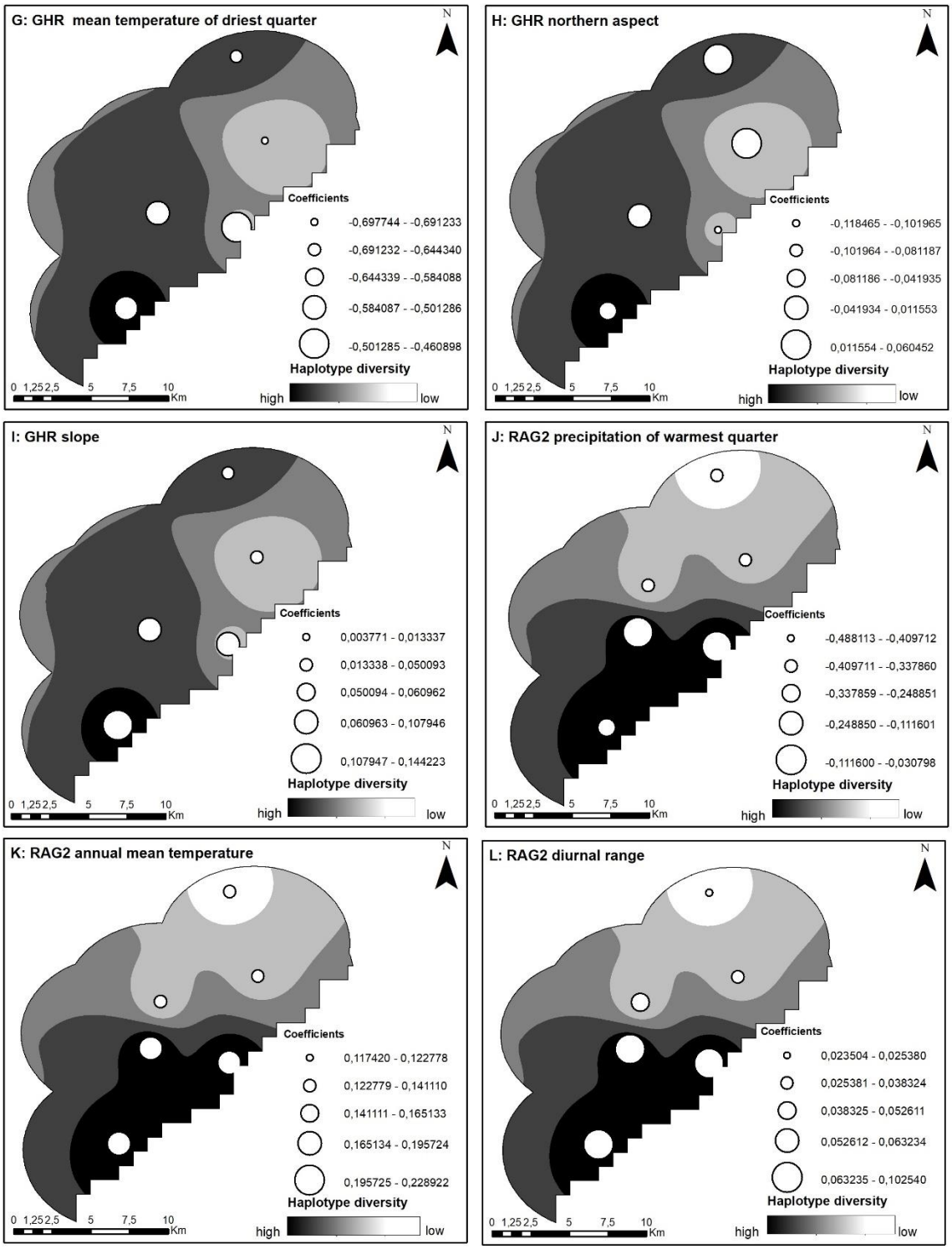


**Figure 2. cont.** Maps showing spatial haplotype diversity pattern of genes and the spatial relationship between haplotype diversity of genes and environmental predictors through estimated coefficients; **(G, H, I)** pattern of GHR with mean temperature of driest quarter, northern aspect, slope; **(J, K, L)** pattern of RAG2 with precipitation of warmest quarter, annual mean temperature, diurnal range.

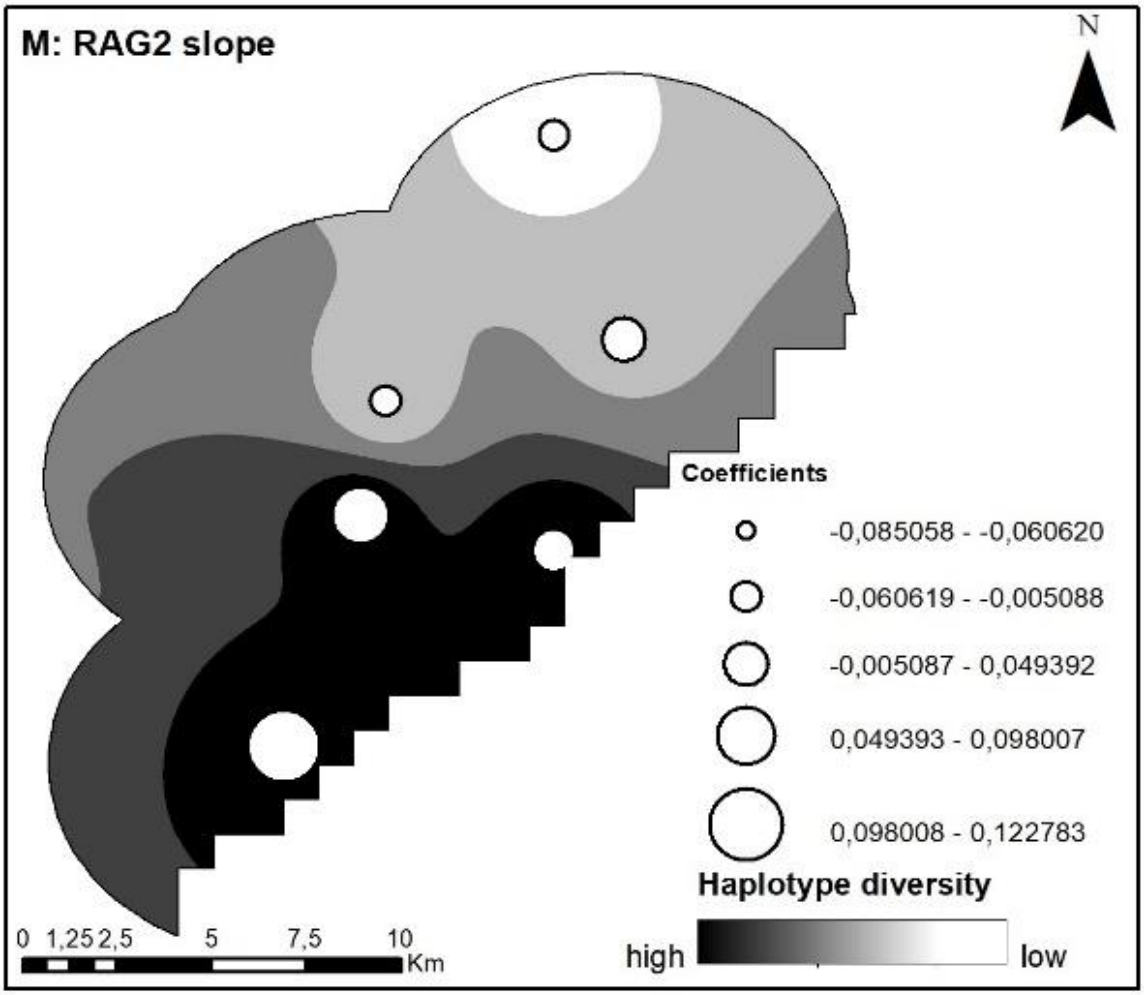


**Figure 2. cont.** Maps showing spatial haplotype diversity pattern of genes and the spatial relationship between haplotype diversity of genes and environmental predictors through estimated coefficients; **(M)** pattern of RAG2 with slope.

Moreover, the estimated slope coefficients displayed a negative association in the north part of the area while indicating a positive association in the south part (Fig. 2M).

## Discussion

Spatial haplotype diversity patterns of CYTB, GHR, and RAG2 genes were explored applying GWR and IDW interpolation sequentially, and spatial pattern maps of haplotype diversity were obtained for each gene. These maps showed the distribution pattern of haplotype diversity throughout the study area and enabled us to conceive genetic characteristics of the target species. It should be noted that the genetic features of a species determine its survival ability in different environmental conditions (Booy et al., 2000; Jump et al., 2009). It means that species with high genetic diversity can easily adapt to the rapid environmental changes and protect themselves from these changes, while species with low genetic diversity lack high adaptability

and are thus threatened with extinction (Barrett & Schluter, 2008; Prentis et al., 2008). In this context, understanding the genetic characteristics of a species and indicating the distribution of genetic diversity is extremely crucial to developing rational and applicable conservation strategies for vulnerable species. In the study, the target species is a local endemic species. As known, these species have low genetic diversity due to the higher rate of inbreeding and genetic drift (Oostermeijer et al., 2003; Frankham, 2005; Templeton & Read, 1994). Hence, the species has a weak adaptation ability to environmental changes and is at a higher risk of extinction. When assessing the situation of the target species, the resulting map of the spatial haplotype diversity pattern is a valuable output to decide on appropriate conservation action and provide knowledge for long-term viability and future planning of the species.

The spatial relationship between haplotype diversity of each gene and environmental predictors was also investigated in the study, and results indicated that there had been considerable spatial variability in local coefficient estimates with negative or positive associations. These are important to show the non-stationarity of the relationship between environmental predictors and haplotype diversity. However, evaluation with an ecologic perspective is needed to figure out the impact of environmental predictors on the spatial distribution of haplotype diversity. Accordingly, the study results clearly showed that local fluctuations in precipitation and temperature-related predictors affect the spatial distribution of haplotype diversity. Estimated local coefficients determine the direction (i.e., positive or negative) and magnitude of this impact. If there is a negative association between environmental predictors and haplotype diversity, changes (decrease or increase) in environmental predictors negatively affect haplotype diversity, consequently reducing haplotype diversity. However, if there is a positive association, then haplotype diversity increases at the rate of the estimated coefficient. In the study, negative associations were mainly observed between climate-related predictors and haplotype diversity. It means that haplotype diversity was negatively affected by changes in climate-related predictors. The equivalent of the result in real life may be that climate change may have an adverse effect on the genetic diversity of this vulnerable species and as a result, the species have more risk for lower adaptability and higher extinction. On the other hand, climate-related predictors are dynamic, then fluctuations in predictors are inevitable. The critical thing in this point is that small-scale changes should happen and spread over time. Thus, the species have time to adapt and a chance for survival. However, the rapid and devastating effects of climate change show that many species will not have time to adapt to climate change and will face the threat of extinction.

In contrast to the climate-related environmental predictors, the topographic predictors of the study area are not dynamic. It means they vary geographically but are constant through a specific time interval for an area. Results showed that there are non-stationary topographic predictors (slope and northern aspect), and the pattern of the relationship between spatially variable topographic predictors and haplotype diversity of each gene was not constant throughout the area. Ecologically speaking, the topographic features of the study area characterize the species' genetic features, and this impact is not the same everywhere. Therefore, populations of a species may have different genetic features from location to location within the distribution area of a species. Briefly, topographic differences enable higher genetic diversity for a species. This information is critical while planning to establish conservation strategies for vulnerable species because if local populations of species can be preserved and outbreeding among them is supported, the genetic diversity of the species increase and the strong adaptability of the species to adjust themselves to environmental changes may naturally develop.

## Conclusion

It is an unquestionable fact that genetic diversity is one of the main components of biodiversity. Nevertheless, it cannot find enough place in conservation studies. Many conservation practitioners mainly deal with conserving ecosystems, ecological communities, and species, whereas paying little attention to the conservation of genetic diversity. Incorporating conservation of genetic diversity into conservation strategies would provide more consistent and effective biodiversity conservation, especially for conservation features that need more conservation concern like restricted range, endemic, and highly threatened species. Knowledge of genetic diversity allows understanding of the strength of species and thus, gives insight into the survival of a population. The study was aimed to identify the haplotype diversity of CYTB, GHR, and RGA2 genes and understand the spatial relationship between environmental predictors and haplotype diversity. Findings presented detailed knowledge on the distribution of haplotype diversity of genes and the geographically varying relationship with the environmental predictors. They are the essential requirements to understand how genetic diversity is distributed through geographically varying environmental conditions and how the genetic characteristics respond to the changes in environmental conditions. Therefore, the outputs of the study may guide the development of strategic conservation measures and effective action plans for the protection of the species. In this context, the study may contribute current and future conservation of *Acomys cilicicus*.

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