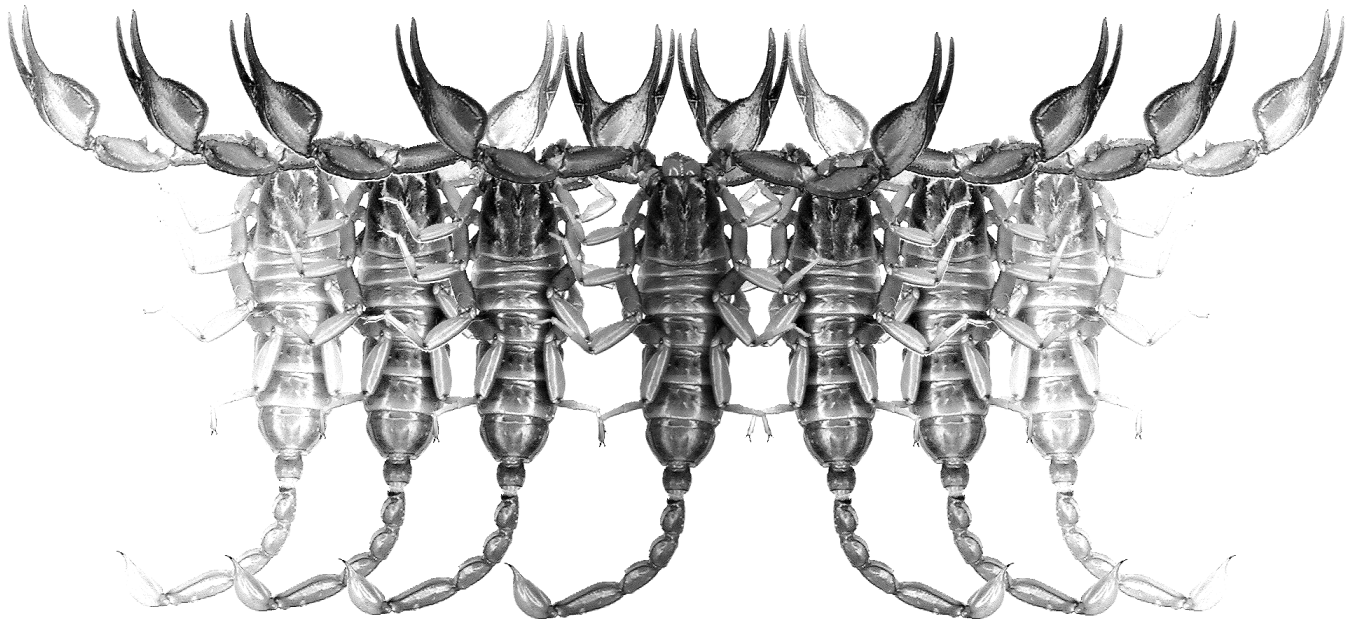


Euscorpius

Occasional Publications in Scorpiology



**Ecological Niche Modeling of Two Scorpion Species
Mesobuthus eupeus (C. L. Koch, 1839) and *M. phillipsii*
(Pocock, 1889) from the Iranian Plateau and Zagros
Region (Arachnida: Scorpiones)**

Omid Mirshamsi

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Occasional Publications in Scorpiology

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**Ecological niche modeling of two scorpion species
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(Arachnida: Scorpiones)**

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Summary

Ecological niche modeling has allowed several advances in the process of species delimitation. In the present study, I used this method to evaluate the climatic divergence between two scorpion species, *Mesobuthus eupeus* and *M. phillipsii*. The ecological niche models (ENMs) were created based on presence-only data with the maximum entropy method. The created models, results of spatial analysis (PCA and Hotelling discriminant), and an identity test suggested that the divergence between these two species is associated with significant divergence in their ecological niches. The results of this study provide additional support for the taxonomic validity the studied species.

Introduction

The species concept and the process of species delimitation have been a matter of controversy since the early days of systematic biology, and several new methods have been proposed, developed and compared to accelerate the process (Wiens & Penkrot, 2002). Among these methods, ecological factors should help delimit species, assuming each species has developed and occupied its own ecological niche (Kozak et al., 2008; Rissler & Apodaca, 2007; Kozak & Wiens, 2006; Phillips et al., 2006; Wiens, 2004).

The suite of methods variously called species distribution modeling, habitat modeling or ecological niche modeling (ENM) have been frequently applied to identify places suitable for the survival of populations of a species through identification of their environmental requirements (Soberón & Nakamura, 2009). ENM is a rich area of study that has seen tremendous growth in past years and is essential to diverse applications in ecology (DeVaney, 2008; Mingyang et al., 2008), conservation biology (Papes & Gaubert, 2007; Urbina-Cardona & Flores-Villa, 2009; Thorn et al., 2009), vector and disease control (Ayala et al., 2009; Chamaillé et al., 2010; Foley et al., 2010; Saupé et al., 2011), and evolution and systematics (Rissler & Apodaca, 2007; Raxworthy et al., 2007; Makowsky et al., 2010). These methods have allowed several advances in understanding the speciation process and geographic ecology of species, as well as ecological and evolutionary

determinants of spatial patterns of biological diversity (Peterson & Vieglais, 2001; Stockwell et al., 2006; Elith et al., 2006). Although the systematic application of ENM is well known, there has been little discussion of its application on species delimitation (Elith et al., 2006). A variety of methods exist for modeling the potential distribution of species based on occurrence data and digital maps of environmental variables (Elith et al., 2006). These models employ relationships between environmental variables and known species' occurrence localities to describe abiotic conditions within which populations can be maintained (Kozak & Wiens, 2006; Raxworthy et al., 2007).

The species *Mesobuthus eupeus* (C. L. Koch, 1839) occurs in the Palearctic region from eastern Anatolia to China (Fet, 1994; Karataş & Karataş, 2001, 2003; Teruel, 2000; Gromov, 2001; Qi et al., 2004; Shi et al., 2007). This species represents at least 14 morphological subspecies that can be distinguished on the basis of coloration, color patterns and carination (Fet & Lowe, 2000; Mirshamsi et al., 2010). Nevertheless, considerable debate exists regarding the nature of these morphological variations (Fet, 1994): Do the various *M. eupeus* subspecies simply represent different morphs of a single widely distributed species? Do the subspecies represent distinct evolutionary entities that could be considered as a separate species?

The results of recent studies based on mitochondrial sequence data and multivariate statistical analyses (Mirshamsi, 2010; Mirshamsi et al., 2010) clearly re-

vealed two divergent lineages within *M. eupeus* (~6% divergence between *COI* sequences). The geographic distributions of these two lineages are consistent with the proposal that the formation of Zagros Mountains in the south, and consequent uplifting of Alborz Mountains in the north, may have influenced their evolution. Therefore, this provided support for raising *M. e. phillipsii* (Pocock, 1889) to species level (Mirshamsi et al., 2010; Mirshamsi, 2010). The results of a detailed morphological analysis of this species also strongly support elevating the southwestern subspecies to species status (Mirshamsi et al., 2011).

In this study, I examined the association of climatic variation and geographic isolation between *M. eupeus* and *M. phillipsii*. I combined presence-only occurrence data, digital maps of climatic variation, and a maximum entropy method to estimate the potential distributions of these species in regard to their taxonomic status. Using this method, I also tested the hypothesis that historical and current geographic isolation between these species is a result of niche divergence.

Material and Methods

Occurrence data

Distributional data for *M. eupeus* and *M. phillipsii* were obtained in the field and from literature records that could be georeferenced (Pocock, 1899; Birula, 1903, 1905; Navidpour et al., 2008 a, b, c, d; Pirali-Khairabadi et al., 2009; Navidpour et al., 2010; Navidpour et al., 2011; Kovařík et al., 2011). Literature records were evaluated to check the species identification and localities, and all localities with uncertainty greater than 5 km were excluded from the analysis.

Ecological niche modeling and data analysis

Niche modeling requires two types of data: coordinates (georeferenced occurrence records) and GIS-based maps of the environmental variables (e.g. climatic variables such as temperature and precipitation) that are likely to affect the suitability of the environment for a given species (Kozak et al., 2008). I used the program Maxent ver. 3.3.2 to model the potential distribution of *M. eupeus* and *M. phillipsii*. Maxent combines occurrence records, random background points and environmental layers to provide a gridded model of potential distribution of the studied species using a statistical approach known as Maximum Entropy. Maxent was run using a subset of bioclimatic layers (out of 19) representing trends in temperature, precipitation, and seasonality with 30 arc second resolution (~1 km²) extracted from the WorldClim database (Hijmans et al., 2005; <http://www.worldclim.org>).

To avoid over-fitting problems which may result from inclusion of too many climatic variables, the environmental information from 1000 randomly generated geographic points across the study area was extracted using ArcMap. Correlation matrices were then created for all 19 climatic variables within each of the temperature and precipitation categories. A Pearson correlation coefficient of 0.75 (using PAST software, Hammer et al., 2001) showed that the variables were highly correlated (Rissler et al., 2006). For such correlated variables the more biologically informative variable was used to create niche models (Rissler & Apodaca, 2007). Finally, six temperature and five precipitation variables were selected and used to generate ENMs. The selected variables included: BIO1= annual mean temperature; BIO2 = mean diurnal temperature range; BIO3 = isothermality; BIO7 = annual range in temperature; BIO8 = mean temperature of wettest quarter of the year; BIO9 = mean temperature of driest quarter of the year; BIO15= precipitation of seasonality; BIO16= precipitation of wettest quarter of the year; BIO17= precipitation of driest quarter of the year; BIO18 = precipitation of warmest quarter of the year; BIO19= precipitation of coldest quarter of the year.

Models for targeted species were created using a total of 365 point localities. All Maxent runs were adjusted with a convergence threshold of 1.0E-5 with 1000 iterations and the regularization value was set to 0.1. I also chose the logistic output format, displaying suitability values from 0 (unsuitable) to 1 (optimal). Finally, I imported the resulting ASCII files into DIVA-GIS to visualize the models.

Evaluation of predictive models was conducted by splitting localities into 75% training and 25% test data partitions, and five replicates of random data partitions were performed (Phillips et al., 2006). Model validation was performed by calculating the area under the curve value (AUC), which reflects the model's ability to discriminate between presence records and random background points. AUC values range from 0.5 for models without any predictive ability to 1.0 for models with perfect predictive ability. Therefore, AUC values >0.9 considered to have 'very good', >0.8 'good' and >0.7 'useful' discrimination ability (Hawltischek et al., 2011).

Additional statistical analysis of modeling results was performed using ENMtools (Warren et al., 2010). Niche overlap of *M. eupeus* from Central Asia and eastern and northern Iran and of *M. phillipsii* from Iraq, Syria, southeast Turkey and southwest Iran was calculated using Schoener's *D* and the *I* statistics. Both of these indices range between 0 and 1, and values close

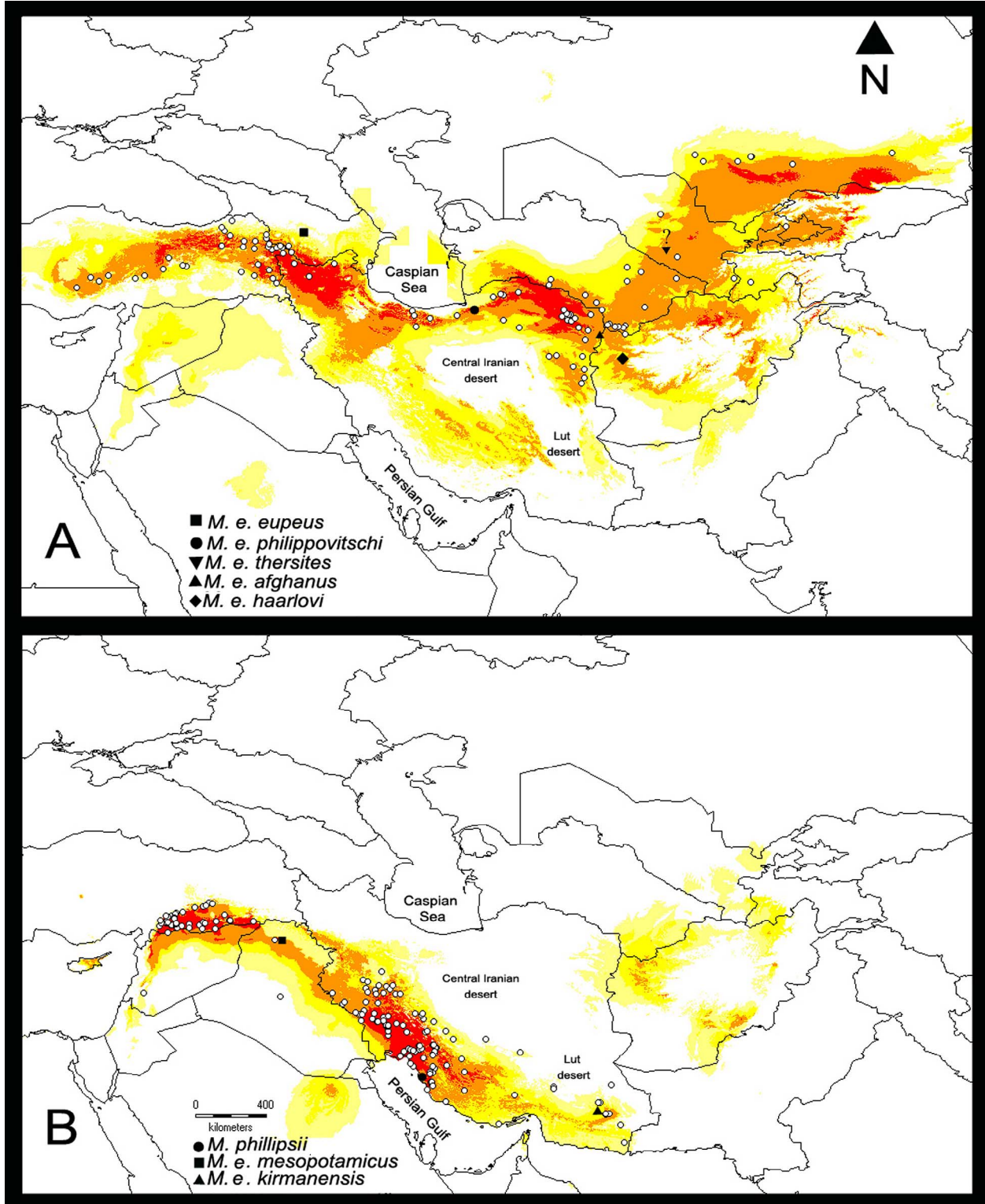


Figure 1: Ecological niche models constructed using Maxent. **(A)** *M. eupeus* niche model (AUC=0.966±0.012); the resulted ENM includes the type localities of five morphological subspecies, *M. e. eupeus*, *M. e. philippovitschi*, *M. e. afghanus*, *M. e. thersites* and *M. e. haarlovi*. **(B)** *M. phillipsii* niche model (AUC=0.977±0.013); the resulted ENM also includes the type localities of morphological subspecies *M. e. kirmanensis* and *M. e. mesopotamicus*. White circles indicate the occurrence points of each species.

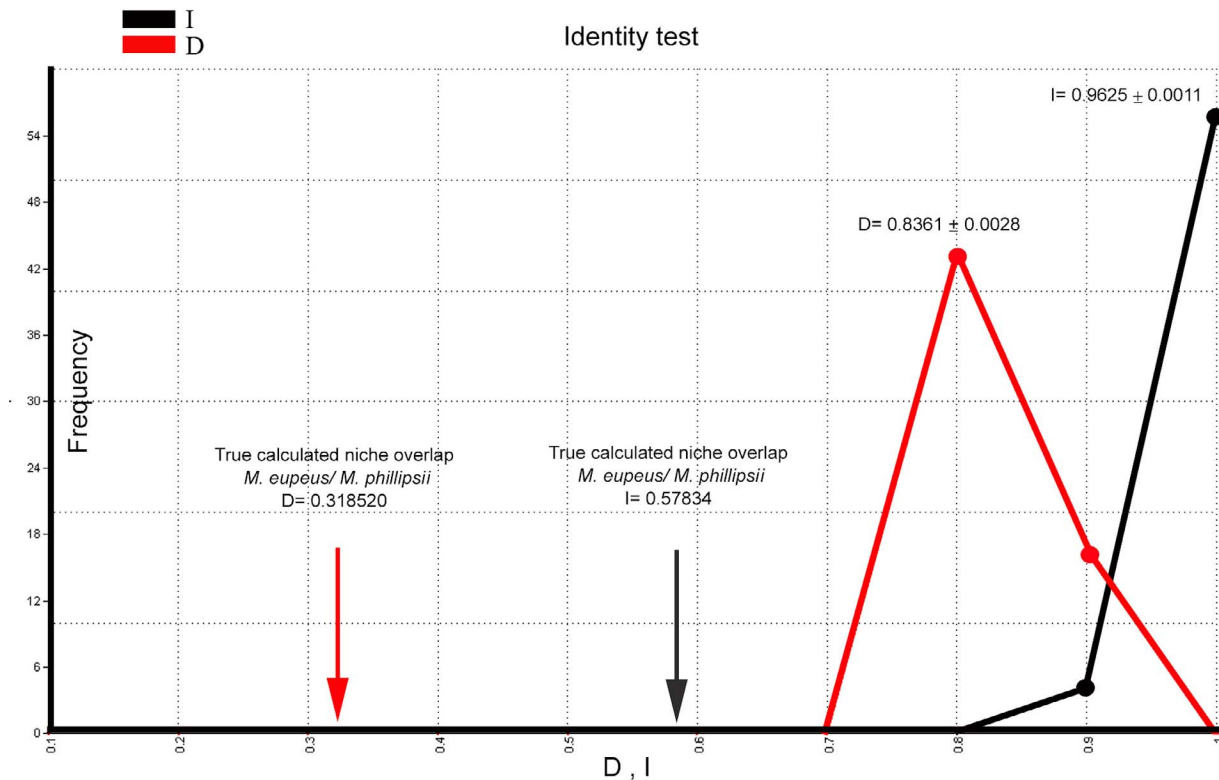


Figure 2: Result of the identity test performed by ENMtools. The red and black arrows indicate the result of ENMtools' niche overlap analysis representing the true calculated niche overlap (D and I). The red and black curves represent the niche overlap values created in the replicates of the identity test. The calculated overlap values (D and I) are outside the 99.9% confidence intervals of the identity test results and thus statistically significant.

to 1 explain high similarity between ENMs (Schoener, 1968). Also, the identity test included in ENMtools was performed. The niche identity test was used to determine whether the niche models generated for two species are identical or exhibit a statistically significant difference.

Finally, to evaluate the overall level of divergence in the ecological niches of *M. eupeus* and *M. phillipsii*, a principal components analysis (PCA) was conducted using the extracted values for each species. I then used a multivariate Discriminant/Hotelling test with PCA axis scores as dependent variables and lineage as the fixed factors to determine whether the observed separation in the ecological niche was statistically significant.

Results

In total, 176 and 189 point localities were used for generating ENMs of *M. eupeus* and *M. phillipsii*, respectively. Ecological niche models for *M. eupeus* and *M. phillipsii* are presented in Figs 1A & B. According to the estimated average AUC values, 0.966 ± 0.012 for *M. eupeus* and 0.977 ± 0.013 for *M. phillipsii*, the ability to differentiate presence from random background points for all generated models was larger than 0.9 and thus considered as "very good".

A heuristic estimate of relative contribution of the climatic factors to the MaxEnt models showed that for the distribution of *M. eupeus*, "mean temperature of wettest quarter of the year", "precipitation of coldest quarter of the year" and "precipitation of warmest quarter of the year" were the variables of highest importance with 27.9%, 25.5% and 12% of contribution, respectively. For *M. phillipsii*, "precipitation of warmest quarter of the year" was the variable of highest importance with 34.8% of contribution. For this species, "precipitation of coldest quarter of the year" and "mean temperature of wettest quarter of the year" was the second and third most important predictors with 31.2% and 9.3% of contribution, respectively.

According to the calculated niche overlap between *M. eupeus* and *M. phillipsii*, $I=0.5783$ and $D=0.3185$, the overlap between niche models is considered low. According to the result of identity test, which combines the occurrence data from two species into a common pool, the null hypothesis of niche identity was rejected. The results suggested that the estimated niche models for *M. eupeus* and *M. phillipsii* based on the climatic variables are significantly distinct ($D_{H0}=0.8373 \pm 0.002$ SE vs. $D_{H1}=0.3185$ and $I_{H0}=0.9641 \pm 0.0044$ vs. $I_{H1}=0.5783$) (Fig. 2).

The result of PCA based on 11 climatic variables indicated that the two species were distributed in divergent environmental spaces (Fig. 3). The first three components resulting from this analysis explained 40.95%, 21.87% and 17.82% of the total variation. The climatic variables BIO1, BIO2, BIO3, BIO8, BIO9, BIO15 and BIO19 have positive loadings on the first component while the climatic variables BIO2, BIO3 and BIO7 have positive loadings on the second component. The result of the Discriminant/Hotelling analysis based on PCA axis scores also revealed that the separation between ENMs was statistically significant ($P < 0.005$).

According to the model, climate suitable for *M. eupeus* ranges from Central Anatolia in the west to the east of Kazakhstan (Fig. 1A). The geographic distribution of this species appears to be limited between 28°N and 46°N. The distributional model includes the terra typica of the nominotypical subspecies *M. e. eupeus*, as well as *M. e. afghanus* (Pocock, 1889), *M. e. haarlovi* Vachon, 1958, *M. e. thersites* (C. L. Koch, 1839), and *M. e. philippovitschi* (Birula, 1905),

Moreover, on the basis of the model for *M. phillipsii*, suitable climate conditions for this species are distributed from southeast of Turkey to southeast of Iran (Fig. 1B). The distributional model includes the terra typica of *M. phillipsii*, as well as two other morphological subspecies, traditionally classified under *M. eupeus*: *M. e. kirmanensis* (Birula, 1900), and *M. e. mesopotamicus* (Penther, 1912).

Discussion

Species are essential units of study in biosystematic and evolutionary biology, and the precise delimitation of species, the process by which the boundaries of species are determined, is an increasingly important task of biosystematics (Wiens & Servedio, 2000; Wiens, 2007). In contrast to the major progress in the phylogenetic reconstruction process, there has been relatively little progress in the methodology of species delimitation process (Wiens, 2007). The use of ecological data and GIS-based analyses of climatic variables in determining the species boundaries, proposed by Raxworthy et al. (2007) and Rissler & Apodaca (2007), was a significant advance in species delimitation.

Traditionally, *M. eupeus* was known as one of the most widespread buthid scorpions in the Palearctic region. The wide geographic distribution of this species is accompanied by a large amount of morphological variation, which has been used to delimit a dozen subspecies (Farzanpay, 1987; Fet, 1994). Since these subspecies have mainly been described on the basis of inconclusive external characteristics, the traditional taxonomy of this taxon had been complicated and some authors believed that *M. eupeus* is a species complex (Fet et al., 2003).

Recently, the result of a molecular phylogenetic study of the *M. eupeus* species complex based on *COI* sequence data clearly indicated two distinctly divergent Northern (*M. eupeus*) and Southern (*M. phillipsii*) clades. The genetic divergence between these two lineages was nearly comparable to that observed between congeneric species [*i.e.* 6% mtDNA sequence divergence (Mirshamsi et al., 2010)]. These two clades also showed different morphometric features based on uni- and multivariate statistical analyses. Therefore, in order to decide whether the two clades warrant separate taxonomic designations, a detailed revision of diagnostic characteristics of this taxon and its morphological subspecies was recommended (Mirshamsi et al., 2010). Finally, the morphological and morphometric examination of different subspecies of the nominal *M. eupeus* clearly revealed that there are at least two valid species belonging to the *M. eupeus* species complex, including *M. eupeus* and *M. phillipsii* (Mirshamsi et al., 2011).

ENMs based on 365 occurrence records and 11 climatic variables clearly indicate that these two species differ in their ecological niches. Also, according to the results of the identity test, niche divergence between the studied species proved to be significant (Fig. 2). Therefore, the climatic envelope of *M. eupeus* differs from that of *M. phillipsii*. Moreover, the analysis of the spatial data (PCA) find that, in the genus *Mesobuthus*, closely related species occupy divergent ecological niches that show relatively little spatial overlap when projected in geographic space (Fig. 3).

Our results suggest that *M. eupeus* and *M. phillipsii* are parapatric species with a contact zone formed at the border of their distributional ranges, which corresponds to the Zagros Mountain system. According to the ENMs, suitable climate for *M. eupeus* mainly occurs in arid and semi-arid regions of Iranian Plateau, Central Asia, and Central Anatolia. Notably, there is no confirmed record of *M. eupeus* from Central Iranian and Lut deserts, which is also predicted as unsuitable in the MaxEnt model of *M. eupeus*. The model clearly shows that the western border of the central Iranian desert and eastern foothills of the Zagros Mountains may act as geographical barriers to dispersal of this species. According to the ENM and the type localities of traditional valid morphological subspecies, *M. eupeus* includes five morphological subspecies; *M. e. eupeus*, *M. e. afghanus*, *M. e. haarlovi*, *M. e. philippovitschi* and *M. e. thersites*. The subspecies *M. e. philippovitschi* has been synonymized with the nominotypical subspecies, *M. e. eupeus*, by Kovařík et al. (2011) (Fig. 1).

The data presented here also indicate that *M. phillipsii* mainly occurs in western foothills of Zagros Mountains towards the Tigris-Euphrate River drainage in Eastern Iraq. The model for *M. phillipsii* suggests that the niche of this species extends from Baluchistan in the southeastern Iranian Plateau, and westward to the Tigris-

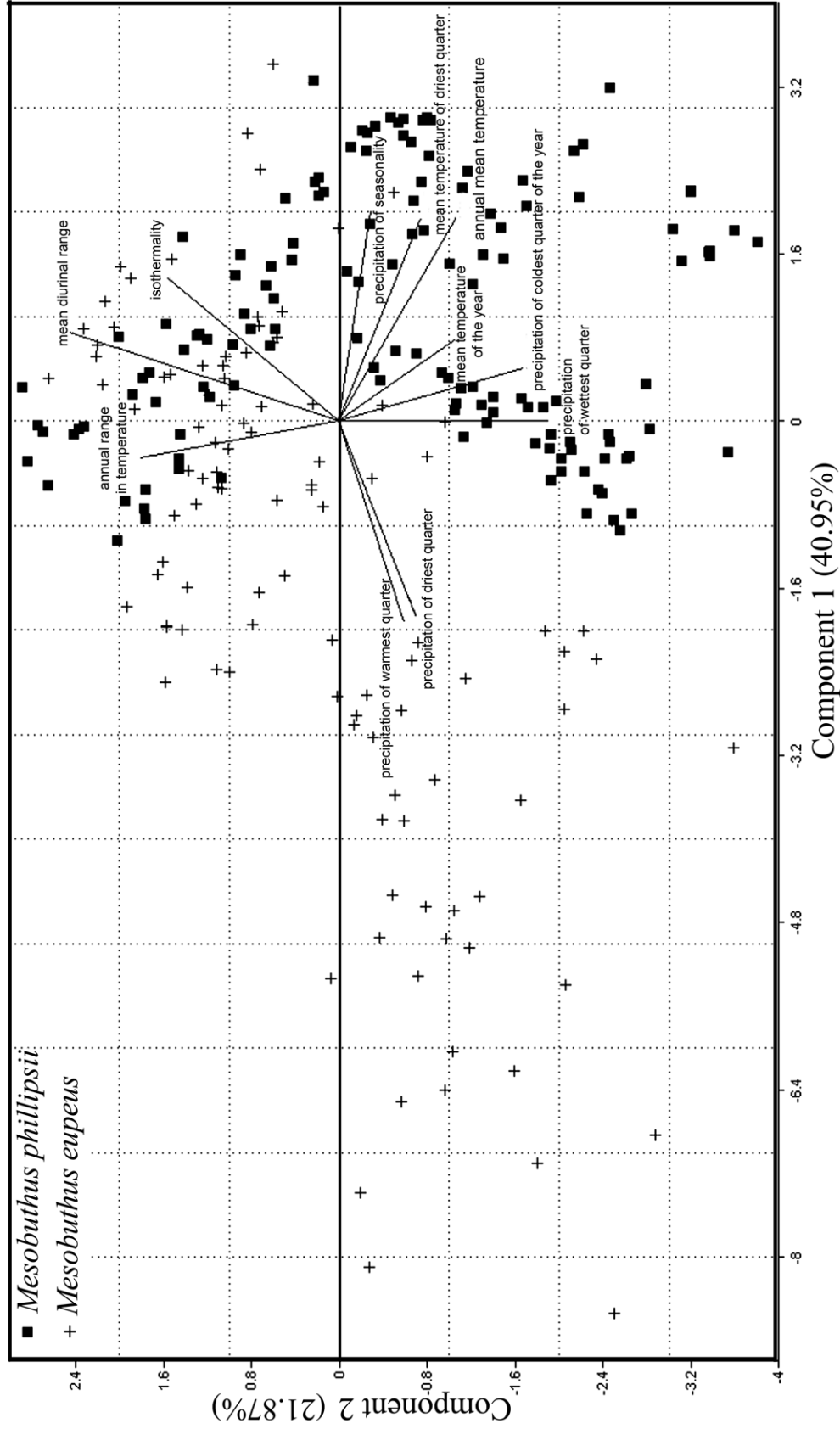


Figure 3: Principal components analysis (PCA) of *M. eupeus* (+) and *M. phillipsii* (■) based on extracted climatic values for each species. The x-axis (PCA₁) explains 40.95% of the variation, and the y-axis (PCA₂) explains 21.87%. Total variation explained by the first two principal components is 62.82%.

Euphrates River drainage in eastern Iraq. For this species, the southern and western foothills of Zagros Mountains might act as the main geographic barrier to its dispersal. Notably, according to the ENM, the range of *M. phillipsii* includes also the type localities of subspecies *M. e. kirmanensis* and *M. e. mesopotamicus* which were traditionally classified under the name of *M. eupeus*. The results of present study suggest that these two subspecies should be transferred to *M. phillipsii* (Fig. 1). The subspecies *M. e. mesopotamicus* has been synonymized with *M. phillipsii* (under *M. eupeus phillipsii*) by Kovařík et al. (2011) who did not find considerable difference between topotypical populations.

The ENMs predicted by the maximum entropy method and presence-only occurrence data represents the realized niche, because correlations among the species distribution and the environmental factors are obtained through the presence data (Sillero, 2011). The actual geographic distribution is a modification of fundamental niche and could be defined according to the complex interaction of the realized environment, historical and biotic factors, as well as the fundamental niche. Because in presence-only niche modeling, we look for all the habitats suitable for the species, we cannot distinguish between really occupied and unoccupied habitats as the true absence data are not included in the models. This is perhaps the reason for the existence of false-positive predicted areas in the resulting geographic distribution of both species. The results of analyses of spatial data all support the conclusion that the genetic and morphological divergences between the studied species are associated with significant divergence in the ecological niche of *M. eupeus* and *M. phillipsii*.

According to the results of the current study, the present-day distribution of *M. eupeus* and *M. phillipsii* may have been affected by topographic barriers such as the Zagros orogeny. A model of vicariant speciation by the Zagros orogeny is consistent with the divergence time estimated based on mtDNA sequences (*i.e.* 4.60–3.15 mya) (Mirshamsi et al., 2010) and the distribution models of these species presented here. Based on this model, the evolution and speciation of these species and other terrestrial faunas in the Iranian Plateau have been influenced by uplifting of Zagros Mountains, which occurred in the late Tertiary approximately 10–5 mya (Macey et al., 1998; Gök et al., 2003). In mid-Pliocene (5–3 mya), the Zagros orogeny was intensified as the Arabian plate rifted from Africa, leading to sinking and formation of the Central Iranian and Lut deserts. These geologic processes may have acted as the main vicariant events that partitioned *Mesobuthus* into two species; *M. eupeus* inhabiting northern regions of the Iranian Plateau, and *M. phillipsii* which became adapted to the southern regions of the Iranian Plateau, western foothills

of the Zagros mountains and the Tigris-Euphrates River drainage in eastern Iraq.

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