Significant Divergence and Conservatism in the Niche Evolution of the Eurasian Green Woodpecker Complex (Aves, Picidae)

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A B S T R A C T

Aims Integrating ecological niche models (ENMs) into phylogeographic studies, enables identifying the evolutionary processes and can be used to confirm species delimitation, particularly in species complex. The current study examined the divergence of two lineages of Eurasian green woodpecker complex (*Picus viridis* and *P. innominatus*), which were recently genetically proposed to the species level, using ecological niche modeling (ENM).
Materials & Methods The ENMs were built using 1346 occurrence points, and environmental layers including 19 bioclimatic variables as well as elevation and land cover for past and present timescales. Niche identity, background tests and principal component analyses (PCA) were then performed to compare their ecological niches and explain ecological differentiation.
Findings Results of identity and background tests and PCA revealed the lineages occupy unique and distinctive niches, and supported recently proposed genetically distinct species. Regarding the environmental variables, PCA results revealed that precipitation has a more important role in the separation of *P. viridis* and *P. innominatus*.
Conclusion This study, in addition to provide an ecological support for recent molecular classifications, revealed niche conservatism had a more important role in the evolution of the two lineages of Eurasian green woodpecker complex.

Keywords Ecological Niche Modeling; Niche Conservatism; Divergent Lineages; Eurasian Green Woodpecker Complex

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C I T A T I O N L I N K S

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Introduction

The two most important aims of phylogeography are identifying spatial distribution of genetic lineages and determining the mechanisms responsible for this spatial distribution[1]. Taking account of the environmental components in the occupied area is necessary to understanding the processes [2]. While niche is a key concept in ecology and evolution, assessing ecological niche conservatism and divergence helps biologists to identify such processes and can be used to confirm species delimitation [3, 4]. Integrating ecological niche models (ENMs) into phylogeographic studies enables the study of the role of ecology in the processes of species diversification [5].

In ecological concept, it is assumed that species can be understood as groups of individuals occupying the same niche or adaptive zone. In other words, each species has formed its own particular niche and so ecological factors should help delimit species [6, 7]. The tendency of species to maintain aspects of their fundamental niche over time is called niche conservatism [3], and it is believed that niche conservatism may prompt lineage divergence in allopatric speciation by hindering gene flow between populations [5]. Furthermore, inferences of niche conservatism or divergence additionally can aid in prioritizing conservation strategies [8].

Ecological niche modeling (ENM), which is based on the ecological criterion, has been suggested as a powerful tool to investigate niche divergence and conservatism. This relatively new method may be used for several diverse aims, including taxonomic decisions, speciation mechanisms and conservation planning [3]. Many studies used the ENM approach to delimit species complexes and to identify the mechanisms of speciation. For example, two taxonomically problematic groups of Phelsuma day geckos were investigated by Raxworthy *et al.* [4], using integrated ENM with genetic and morphological data to evaluate species limits. Despite relatively modest levels of morphological and genetic divergence, they found parapatric ecological niche models and divergent ecological niches among closely related species. They also elevated some subspecies to species rank [4]. In another study, the taxonomic status of two genetically and morphologically suggested species (*Eremias strauchi strauchi* and *E. s. kopetdaghica*) which have allopatric distribution was evaluated using ENM to confirm the hypothesis that they are two distinct species. The results confirmed the niche differentiation and added new insights into the taxonomic distinction between them [9]. The Eurasian green woodpecker complex (*Picus viridis*) is a widespread, resident bird breeding across boreal and temperate forest regions in the western Palearctic region (Figure 1) [10].
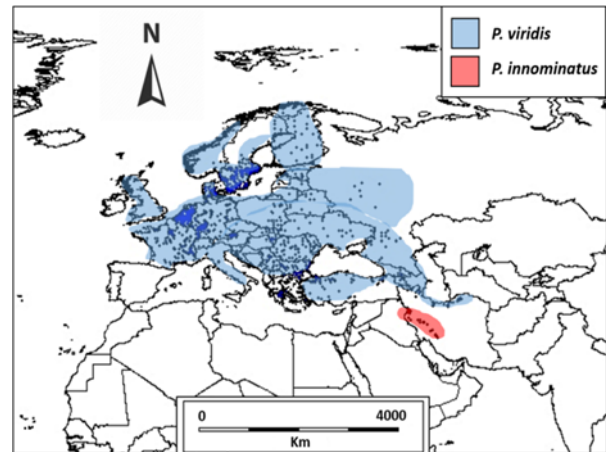

Figure 1) Current species' distribution map (colored areas) and occurrence points (dots) for two members of Eurasian green woodpecker complex

The study of some mitochondrial and nuclear loci within this species complex (with no Iranian samples) led to the proposal that the complex be divided into three allopatric/parapatric lineages distributed in North Africa (*P. vaillantii*), the Iberian Peninsula (*P. sharpei*) and Europe (*P. viridis*), respectively [11]. In other recent studies (which included Iranian samples), four distinct species, *P. viridis*, *P. sharpei*, *P. vaillantii*, and *P. innominatus,* have been suggested [12, 13]. It has been discussed that both morphological and genetic differences involve only subtle variations and are insufficient to recognize *P. v. innominatus*, which has allopatric distribution in the Zagros Mountains (W and SW Iran) as a separate species [14].

Objective

In this study, the current distribution of the two proposed nominal species, *P. viridis* and *P. innominatus* was analyzed using the ENM approach to examine the recently-mentioned potential species across the western Palearctic and the Zagros Mountains. Moreover, as the present-day pattern of species distribution

reflects the historic climatic [15], by projecting the niche models onto historic (Last interglacial and Last glacial maximum) and present climate scenarios, how the distribution of these two taxa has changed over time was also examined.

Materials and Methods

Study area

The study was performed in the western Palearctic region (Figure 1). As recent studies suggested two species within the Eurasian green woodpecker complex, prior to niche modeling, a geographic range was estimated separately for each lineage and a polygon was drawn around each, suggesting the species' localities, creating a range for the species.

Occurrence points and environmental data

In the present study, occurrence records from both our own field survey and from the Global Biodiversity Information Facility online database (GBIF, www. gbif.org) across these regions were collected. A total of 1346 georeferenced occurrences (1294 for *P. viridis* and 52 for *P. innominatus*) were included in this study.

To characterize the ecological niches for the present time, 19 bioclimatic layers were downloaded from WorldClim. Elevation and land cover layers were downloaded from WebMap and GlobCover websites, respectively. All highly inter-correlated ($r \geq 0.75$) bioclime variables were discarded. Ultimately, eight bioclime layers (BIO_01= Annual mean temperature, BIO_02= Mean diurnal range, BIO_04= Temperature seasonality, BIO_08= Mean temperature of wettest quarter, BIO_12= Annual precipitation, BIO_14= Precipitation of driest month, BIO_15= Precipitation seasonality, and BIO_18= Precipitation of warmest quarter), as well as land cover and elevation in the next steps were used. Additionally, the potential distributions of the members were estimated during the Last Interglacial (LIG) as well as the Last Glacial Maximum (LGM) for the Community Climate System Model (CCSM) [16]. In the current study, the land cover layer for these two time periods did not use.

Modeling

Among the available tools for ENM, the maximum entropy approach is one of the most widely used for predicting species distributions and is currently available in MaxEnt software [17]. This software builds models with presence-

only occurrences and a set of environmental variables and generates habitat suitability models [18]. In this study, the replication method (100 replicates) and subsampling were used for model validation and to reduce errors that may occur from the random splitting of data into test (25%) and training (75%) subsets using MaxEnt 3.4.1. The Jackknife test and percent variable contribution were utilized and model performance was tested by considering the area under the curve (AUC) of the receiver operating characteristics (ROC) plots [19].

Niche equivalency and similarity tests

To calculate niche similarity, the average model obtained from MaxEnt output models was used in ENMTools 1.4.4 [20]. The niche overlap test makes multiple pairwise comparisons between the ENMs of taxa to determine their observed similarity using Schoener's D [21] and modified Hellinger's I [20] of niche overlap. To test whether the ENMs produced by different taxa were equivalent to one another, the identity test in ENMTools with 100 pseudoreplicates was also used [20]. Also, the background test in ENMTools was carried out, which compares the actual ENM of one taxon to an ENM generated from random background points within the other taxon's distribution, and vice versa [22].

Principal component analysis (PCA)

In the present study, PCA was conducted to obtain the principal components from environmental variables. Thus, pixel values for each of the selected environmental layers were extracted at each occurrence point site using openModeller, and then the biplot analysis was performed in R 3.5.2.

Findings

Figure 2 shows the ecological niche models for all three timescales. According to their AUC values (range 0.889 to 0.996; Table 1), models showed "good" to "very good" ability to predict observed distributions based on the classification of Swets.

The predicted current distribution of the members was found to be in agreement with the known range of these species. It should be noted that under present bioclimatic conditions, the model overpredicted the distribution of *P. viridis* in Iberia. This is most likely an effect of competitive interaction, because a sister species (Iberian green woodpecker) is distributed in Iberia [13]. However, suitability maps suggest that each

member has a unique environmental niche. The three most important variables based on MaxEnt jackknife values as well as more important variables and their contribution (%) to the MaxEnt models appear in Table 1.

ENM comparison produced low values for Hellinger's I and Schoener's D (0.45 and 0.29, respectively) and revealed a restricted niche overlap in geographic space as expected from their allopatric distribution. Observed niche overlaps fell below the identity values of randomly-generated pseudoreplicate data in the niche identity test (Figure 3a), and the estimated environmental niches were significantly distinct (p<0.01). Figure 3b shows the results of the background test. In this test, actual niche overlap scores fell out the 99% confidence intervals of the null distribution. Results showed niches are significantly (p<0.01, both I and D) more similar than expected in both directions.

According to the PCA results, 60.7% of the variance was explained by the first two principal components (PCs). PC1 explained 44% of the variance, and PC2 explained 16.7% of the variance. Precipitation of driest month (Bio_14), precipitation seasonality (Bio_15), precipitation of warmest quarter (Bio_18) for PC1, mean temperature of wettest quarter (Bio_08), and temperature seasonality (Bio_04) for PC2 had the highest contributions. Altogether, there is clear separation of the taxa of the *P. viridis-innominatus* complex in the space defined by PC1 and PC2 (Figure 4).
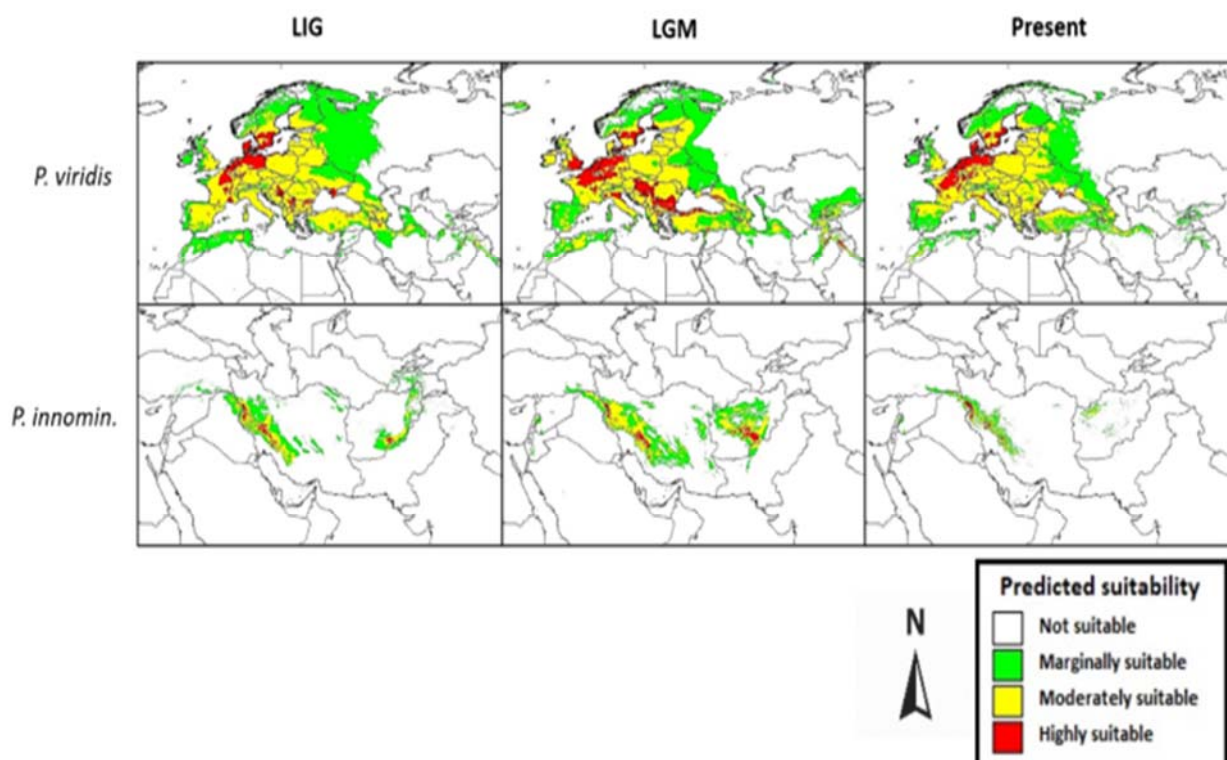

Figure 2) Ecological niche models (ENMs) plotted in geographic space for two members of Eurasian green woodpecker complex under LIG, LGM, and present

Table 1) Amount of area under the curve (AUC) of the receiver operating characteristics (ROC) plots, the estimation of relative contributions of the three most important environmental variables to the MaxEnt model and the results of the jackknife test of relative importance variables

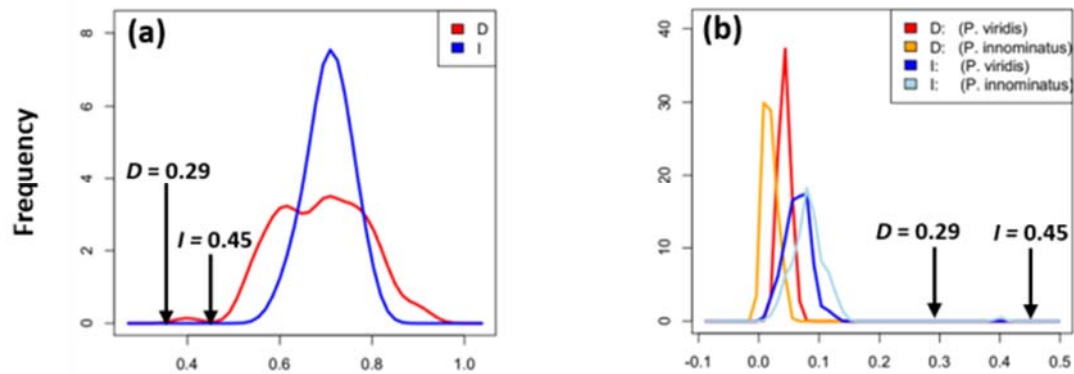| Taxa	*P. innominatus*	*P. viridis*
Training AUC	0.996	0.891
Test AUC	0.995	0.889
More important variables and their contribution (%)	Bio_14; Cover; Elevation 31.54; 29.48; 27.95	Bio_14; Bio_04; Bio_01 47.63; 30.13; 11.78
Jackknife	Bio_08; Elevation; Bio_15	Bio_01; Bio_14; Bio_12

Figure 3) Result of the identity (a) and background tests (b); The histograms illustrate distributions of (Schoener's D and modified Hellinger's I) with overlapping scores from pseudoreplicates; Arrows represent niche overlap values.
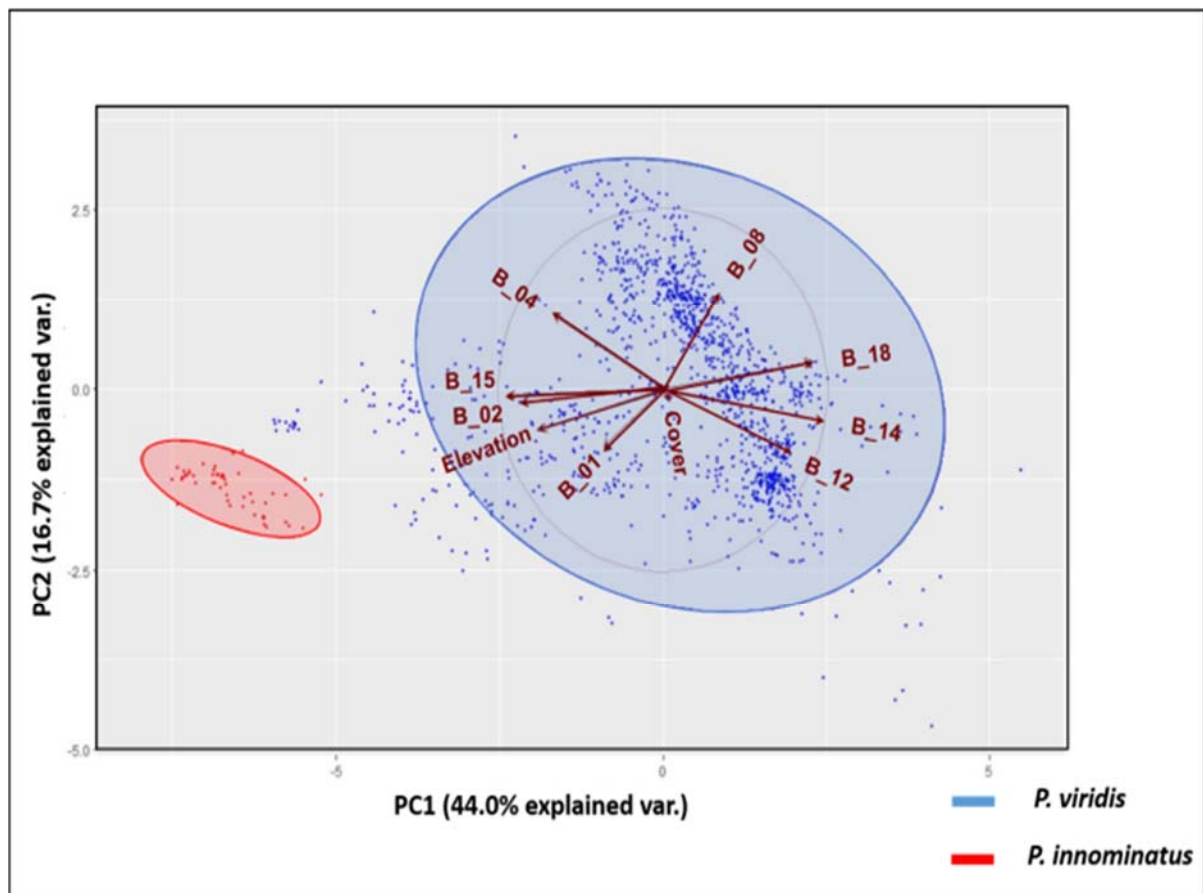

Figure 4) Visualization of principal components analyses (PCA) using selected bioclim variables as well as vegetation cover and altitude for two members of Eurasian green woodpecker complex; In this biplot, vectors indicate the direction and strength of each environmental variable to the overall distribution, and a colored, normal probability (95%) ellipse for each group is drawn to match point color.

Discussion

Distinct lineages with conserved niches

According to the ecological species concept proposed by Van Valen and Andersson, individuals occupying the identical niche or adaptive zone form a single species [6, 7]. In the current study, the results of identity and background tests revealed the lineages occupy unique and distinctive niches, and supported recently proposed genetically distinct lineages in the Eurasian green woodpecker complex. Moreover, although species delimitation is often supported by findings of divergent ecological niche space [4], findings of the present study show niche conservatism across these lineages. A recent study has shown that Eurasian green woodpecker complex has recently evolved [13], and so, adaptive niche diversification appears to play a relatively minor role in specification and evolutionary

divergence. Previous studies demonstrated insignificant importance of niche adaptation in evolutionary divergence in recently evolved lineages [3, 23]. Contrary to niche divergence, species exhibiting niche conservatism strategy must either risk extinction or disperse to new habitats in response to environmental changes [24]. Since *P. innominatus* faces further problems due to geographical isolation (Zagros Mountains), it seems to need more conservation efforts than *P. viridis*.

Evolutionary timescales

As shown in Figure 2, the current study uncovered two different patterns of range shifts for these two species during the three compared timescales: 1) *P. viridis*, which is located in the northern Palearctic region, had experienced maximum range expansion during LIG, while the eastern range had decreasing and/or southern range increasing during LGM, and 2) *P. innominatus*, which experienced an expansion and then a significant contraction during the LGM and the present timescale, respectively. Many studies show the Holarctic temperate and boreal arboreal species have experienced northern range contraction and/or southern range expansion during glacial periods, as a response to cold temperatures and aridification, but expanded their ranges and colonized areas to the north during warmer and wetter interglacials [25, 26]. These shifts can primarily be linked to vegetation changes in response to climate change, with a dominance of forest-adapted and open vegetation species during warm and cold periods, respectively [27]. Furthermore, it is believed that longitudinal range shifts in the western Palearctic are created by vegetation changes in response to a strong west-east moisture gradient [28].

Previous studies demonstrated that *P. viridis* experienced a range contraction during the LGM and post-glacial expansion events associated with multiple refugia /single large refugium, located in southern Europe, Anatolia and the Caucasus/Caspian region [11, 12]. The findings of this study agree with the contraction and expansion events, however, they are not entirely congruent in case of the location of refugium during the LGM. It should be noted that the slight differences might be due to accounting for *P. viridis* and *P. innominatus* as two distinct species or applying an extra layer of elevation or ignoring permafrost extension and coastline differences in this study.

The Zagros Mountains, as habitat for *P. innominatus*, is located in lower latitudes and surrounded by unsuitably dry and warm clime, and so the LGM and present periods probably promoted and demoted the suitable habitat by decreasing and increasing the temperature of contiguous areas, respectively. Recently, several species have been described in the Zagros Mountains, uncovering SW and W Iran as an important region of endemism with a deep history of radiations [29].

PCA analysis

PCA results of the present study are congruent with a recent molecular study [12] that supported the clear separation of *P. innominatus* from *P. viridis*. Regarding the environmental variables, PCA results revealed that precipitation has a more important role in the separation of *P. viridis* and *P. innominatus*, probably due to the Zagros Mountains, as habitat for *P. innominatus*, has lower precipitation than the Europe continent which *P. viridis* occupies. It is demonstrated that the fluctuation of woodpecker population is indirectly related to precipitation by impact on the structure and composition of forest ecosystems [30, 31].

Conclusion

The present study revealed a significant niche distinction between *P. viridis* and *P. innominatus* and approved the taxonomic suggestion of Perktas *et al.* that both subspecies can be promoted to the species level [12]. In addition, niche conservatism had more important role in the evolutionary divergence of the two lineages of Eurasian green woodpecker complex than niche divergence. Hence, the current study shows stability in the environmental niche across evolutionary timescales, which might facilitate allopatric speciation, generating present-day patterns of biodiversity.

Ethical Permissions: The case was not found by the authors.
Conflict of Interest: The authors have no conflict of interest.
Authors' Contribution: Mehdi Elahi (First author), Original researcher (40%); Mansour Aliabadian (Second author), Introduction/Discussion author (25%); Seyed Mahmoud Ghasempouri (Third

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