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Climatic adaptation explains responses to Pleistocene oscillations and diversification in European vipers

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Abstract

Aim: Allopatric speciation is the primary mode of diversification in the Mediterranean Basin. However, the contribution of climatic adaptation during this process is contradictory. In this work, we investigate the eco-evolutionary processes that drove diversification in this region, using European vipers as a case study. We describe the climatic requirements of different lineages to compare their responses to the Pleistocene climatic oscillations and tackle the evolutionary mechanisms underlying their diversification.

Location: Eurasia and North Africa.

Taxon: European vipers (genus *Vipera*).

Methods: We used ecological niche modelling (ENM) to identify the climatic requirements of 24 *Vipera* lineages and infer past range dynamics associated with their diversification during the Pleistocene. To test whether climatic niches varied across lineages, we calculated the phylogenetic signal of different climatic variables and examined the relationship with phylogenetic relatedness. To investigate climatic niche evolution and test for phylogenetic niche conservatism (PNC), we quantified pairwise niche overlap in sister phylogenetic units under a 3D hypervolume approach.

Results: ENM identified temperature annual range, precipitation of wettest month and precipitation of driest quarter as the most important climatic variables related to the distribution of most lineages, validating *Pelias* clade as cold-adapted, and *Vipera* 1 and *Vipera* 2 as warm-adapted clades. Projections to past conditions varied among clades, with *Pelias* and *Vipera* 1 having more similar responses, while *Vipera* 2 exhibited greater variability. We found significant phylogenetic signal in one temperature-related and two humidity-related climatic variables and detected high complexity in ecological niche evolution across the phylogeny, both rejecting the hypothesis of PNC.

Main Conclusions: Climatic adaptation played a significant role in driving diversification among European vipers. Cold-adapted and warm-adapted lineages presented similar climatic requirements and remarkable responses to Pleistocene stages, resulting in an intricate pattern of niche divergence along the phylogeny that favours local adaptation rather than PNC.

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KEYWORDS

ecological niche modelling, Mediterranean Basin, n -dimensional hypervolume, niche overlap, phylogenetic comparative methods, *Vipera*

1 | INTRODUCTION

Understanding how ecological factors promote species diversification is a fundamental topic in ecology and evolutionary biology (Coyne & Orr, 2004; Travis et al., 2013). The role of niche divergence and niche conservatism during speciation can impact species persistence and colonization of new geographical areas (Rato et al., 2015; Vaissi & Rezaei, 2022). Allopatric speciation, the most common mode of diversification, arises from genetic differences between isolated populations, promoting the gradual acquisition of divergent characters (Coyne & Orr, 2004). Differences in environmental conditions may promote ecological niche divergence (Castro-Insua et al., 2018), while retention of ancestral niche affinities may result from ecological constraints or behavioural responses, leading to phylogenetic niche conservatism (PNC; Wiens & Graham, 2005).

The Mediterranean Basin is ideal to investigate the role of niche evolution in species diversification due to its high biodiversity (Myers et al., 2000) resulting from complex palaeogeographical processes since the early Miocene (Duggen et al., 2003; Hewitt, 2011). Different phylogenetic studies have linked patterns of interspecific diversification in Mediterranean sister taxa to vicariance processes following the formation of the Strait of Gibraltar, 5.33Mya (Akin et al., 2010; Mendes et al., 2017). Likewise, consistent patterns of intraspecific genetic structure detected in Mediterranean terrestrial fauna have been related to geographical processes (e.g. range shifts, population fragmentation) occurred during the climatic oscillations of the Pliocene (5.3–2.6Mya) and Pleistocene (2.6–0.2Mya) (Hewitt, 2011; Liz et al., 2021).

Contrasted ecological requirements among taxa have shaped allopatric processes during periods of climate change (Hewitt, 2004). During cold periods, many warm-adapted species retracted their distributions to lower latitudes and lower elevations (e.g. *Bufo bufo*, Garcia-Porta et al., 2012; *Natrix* sp. Kindler et al., 2013; *Coracias garrulus*, Kiss et al., 2020), while cold-adapted species expanded their ranges following the increase of suitable habitats (e.g. *Rana iberica*, Sousa-Guedes et al., 2020; *Iberolacerta monticola*, Sousa-Guedes et al., 2020; *Alopex lagopus*, Dalén et al., 2007). The processes affecting species ranges and diversification dynamics are often more complex and involve other parameters. In the last decade, exceptions to this general pattern of climate-driven 'expansion-contraction' have been studied, and revealed that geographical position and topographic features of the distribution range may influence species' responses. For instance, in coastal and insular species, demographic and range expansions have been associated with the glacial increase of climatically suitable coastal lowlands (Salvi et al., 2014). Similarly, a reduction in the aridity of the Sahara Desert during the last glacial period might have determined a demographic and range expansion

of the warm-adapted snake *Malpolon monspessulanus* in North Africa (Machado et al., 2021).

Recent studies tackling the ecological perspective of diversification processes in the Mediterranean region have identified varying patterns of niche evolution. By focusing on the identification and comparison of realized climatic niches (i.e. as described by temperature and precipitation variables), several studies found evidence of climatic adaptation (i.e. niche divergence between sister lineages) at the interspecific level (e.g. in *Lacerta* and *Timon*, Ahmadzadeh et al., 2013, 2016; Enríquez-Urzelai et al., 2022) and described the occurrence of geographical processes in the absence of climatic adaptation (i.e. niche similarity between sister lineages) during intraspecific diversification (e.g. *Alytes obstetricans*, Maia-Carvalho et al., 2018; *Daboia mauritanica*, Martínez-Freiría et al., 2017). PNC has been progressively reinforced as a common mechanism in the evolution of Mediterranean vertebrates. Relatively fast and recent events, such as responses to climatic oscillations during the Pleistocene and Holocene (Martínez-Freiría et al., 2017; Rato et al., 2015) or contemporary invasions and colonizations (Rödger & Lötters, 2009), seem significantly governed by niche conservatism, while previous diversification events are dominated by niche divergence (Ahmadzadeh et al., 2016; Castro-Insua et al., 2018). Nonetheless, some studies contradicted this pattern, showing significant niche shifts among recently diverged taxa and claiming that niche divergence, rather than conservatism, is predominant in shaping species evolution (e.g. Anadón et al., 2015). Understanding whether ecological divergence constitutes the primary force driving speciation or not will shed light on the mechanisms that underlie diversification processes in the Mediterranean biodiversity hotspot.

European vipers (Family Viperidae, genus *Vipera*) are a promising model to investigate the evolutionary processes shaping Mediterranean biodiversity. They present ectothermic physiology and low dispersal ability compared with other vertebrates (Dezetter et al., 2022; Martínez-Freiría et al., 2010), which makes them highly susceptible to climatic change (Brito et al., 2011; Martínez-Freiría et al., 2015). According to Freitas et al. (2020), the genus is composed of three main parapatric clades (*Pelias*, *Vipera* 1 and *Vipera* 2; Figure 1a) comprising 27 lineages, that diversified around the Mediterranean Basin and adjacent areas (Sindaco et al., 2013). The geographical location of their distributional ranges (Sindaco et al., 2013), together with assessments of environmental correlates derived from ecological modelling (Martínez-Freiría et al., 2015, 2020), suggest that European vipers occupy distinct climatic niches. The *Pelias* clade includes lineages with Euro-Siberian affinity that are considered cold-adapted, while the clades *Vipera* 1 and *Vipera* 2 include lineages with Mediterranean affinity are considered warm adapted. Regional studies on selected species and/or clades have shed light on the evolutionary history of European vipers (Chamorro

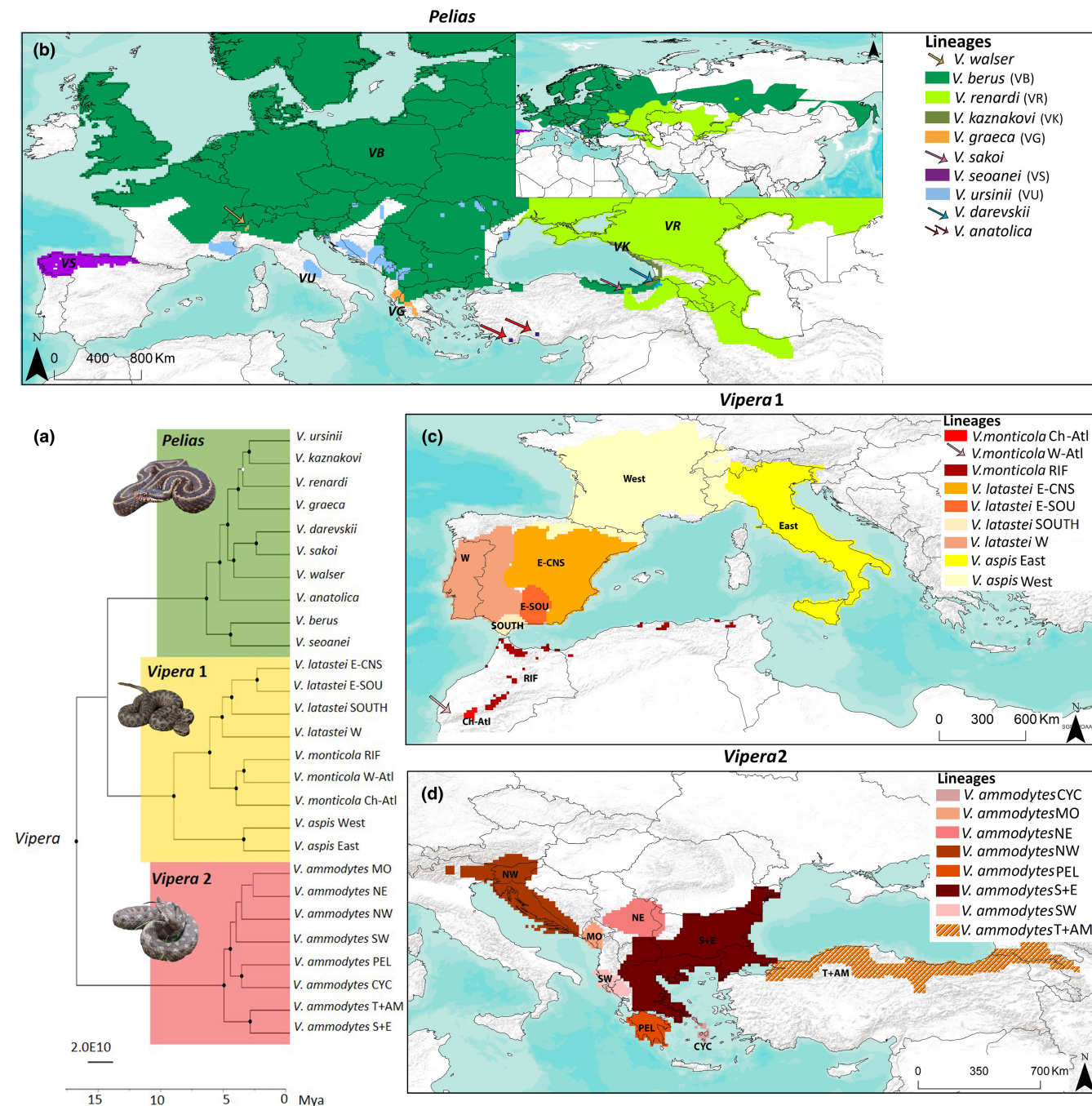


FIGURE 1 (a) Dated phylogenetic tree showing the main mtDNA clades and lineages of European vipers, adapted from Freitas et al. (2020). (b)–(d) Distribution maps for the lineages of the *Pelias*, *Vipera 1* and *Vipera 2* clades, respectively, adapted from Martínez-Freiria et al. (2020). Black and light-grey dots represent posterior probabilities higher than 0.95 and between 0.9 and 0.95, respectively.

et al., 2021; Mebert, Jagar, et al., 2015; Ursenbacher et al., 2008), as well as on the role of climate during diversification. However, to date, no comprehensive study investigated the ecological dynamics underlying the evolutionary history of the entire genus *Vipera*.

In this work, we hypothesize that distribution and diversification patterns in European vipers involve climatic niche specialization of major clades and the lineages within them. If ancestral climatic niche specialization has driven the evolutionary biogeography of European vipers, we predict that (a) a pattern of niche conservatism would be

identifiable across the phylogeny (i.e. PNC), where older lineages would be climatically more divergent than recent ones. If PNC is a dominant pattern during recent lineage diversification, we expect that (b) lineages within each of the three major clades should share similar ecological requirements, that is cold requirements for *Pelias* and warm requirements for *Vipera 1* and *Vipera 2*, and (c) the members of each clade should present congruent responses to the cold/warm periods of the Pleistocene (e.g. cold-adapted lineages would have experienced range expansions during the cold periods of the

Pleistocene, whereas warm-adapted ones would have retracted their distributional ranges). Deviations from these predictions would suggest that climatic adaptation occurred independently from the phylogenetic relationships among taxa, playing a role in the diversification of particular lineages. Using ecological niche modelling (ENM), phylogenetic comparative methods and niche overlap analyses, we aim to (1) describe the current climatic requirements of different viper lineages, (2) compare their responses to the Pleistocene climatic oscillations and (3) understand the evolutionary mechanisms that underlie such patterns.

2 | MATERIALS AND METHODS

2.1 | Model system

The genus *Vipera* is a monophyletic group of small- to medium-sized viviparous, venomous snakes. A recent phylogenetic study based on mtDNA identified three main parapatric clades within the genus (Freitas et al., 2020): *Pelias*, *Vipera* 1, and *Vipera* 2 (Figure 1a). *Pelias* is the most diverse clade, with about 10 Pliocene lineages (Freitas et al., 2020). These vipers mainly inhabit dense scrublands and heathlands with an Atlantic and Subarctic climate, across the northern temperate and boreal regions (Figure 1b), and are thus considered cold adapted (Lourdais et al., 2013; Martínez-Freiría et al., 2015). The *Vipera* 1 clade includes three species, *Vipera aspis*, *Vipera latastei* and *Vipera monticola*, for a total of nine Pliocene lineages (Freitas et al., 2020; Martínez-Freiría et al., 2020, 2021). Members of this clade mostly inhabit bushy and/or rocky environments with Atlantic and Mediterranean climates in the Western Mediterranean region (Figure 1c) and are considered warm adapted (Martínez-Freiría et al., 2020). The *Vipera* 2 clade is represented by the *Vipera ammodytes-transcaucasiana* species complex, and includes eight Pliocene lineages (Freitas et al., 2020; Ursenbacher et al., 2008). These vipers mostly occupy open rocky environments in the East Mediterranean region up to Asia Minor (Figure 1d), inhabit Mediterranean climate areas and are considered warm adapted (Dyugmedzhiev et al., 2021; Tomović, 2015).

2.2 | Occurrence data

We compiled a total of 10,720 occurrence records at 10-min resolution (~20×20 km) for all the Pliocene lineages of European vipers following the phylogenetic inference from Freitas et al. (2020). Occurrence data were gathered from existing bibliography (Appendix S1), online repository (GBIF, 2020, www.gbif.org), museum collections and fieldwork campaigns carried out by the authors and collaborators. Due to their restricted distributions (see Figure 1) and low sample sizes ($n < 2$), three lineages (i.e. *V. anatolica*, *V. monticola* W-Atl and *Vipera sakoi*) were not further considered. To reduce spatial biases of occurrence data in ENM (Merow et al., 2013; Sillero et al., 2021), we spatially rarefied occurrence records per lineage

considering the climatic heterogeneity of the whole distribution area of the genus (Appendix S2.1).

2.3 | Climatic variables

In all, 19 bioclimatic variables for current times (1979–2013) at 10-arc minutes (~20×20 km) were downloaded from PaleoClim (Brown et al., 2018). To avoid collinearity in the predictors used to train ecological models, spatial correlation among variables was tested, and only six low correlated variables ($R < 0.7$) were retained (Sillero et al., 2021). Variables were selected considering their biological relevance for the study purpose (see Freitas et al., 2018; Martínez-Freiría et al., 2015, 2020). For past conditions, the same six climatic variables were downloaded for the Last Glacial Maximum (LGM~21 ka) and the Last Interglacial (LIG; ~130 ka). LGM and LIG variables accounted for one general circulation model (the NCAR-CCSM, PMIPP5; Otto-Bliesner et al., 2006).

To perform analyses of phylogenetic signal on current conditions (see below), the same six bioclimatic variables selected for the ENMs were used. The average monthly environmental conditions for each lineage were calculated using the 'Sample' function from the Spatial Analyst extension of ArcGIS (ESRI, 2016) (Table S2.2).

To perform niche overlap analysis, all 19 bioclimatic variables were considered and the average monthly environmental conditions for each lineage were calculated, with the same method described above.

2.4 | Ecological niche models

To identify climatic requirements and Pleistocene climatic responses of viper lineages, we calculated ENMs using the Maximum Entropy algorithm implemented in MAXENT 3.4.1 (Phillips et al., 2006). To avoid spatial biases in the background data (Sillero et al., 2021), the study area used for training models was defined according to the extension of the gathered distribution, after a calibration process (Appendix S2.2).

Occurrence records and climatic variables were imported to Maxent, where 30 bootstrap replicates were run with random seed, using 70% of the records for training and 30% for testing the models. For *Vipera walsler*, because of its restricted distribution and the low number of occurrence records available ($n=7$), only 10 model replicates were run using the leave-one-out technique (Pearson et al., 2007). Model parameterization was optimized for each lineage (Merow et al., 2013) using the *ENMeval* R package (Muscarella et al., 2014), which considered a combination of different 'feature class' and 'regularization multiplier' parameters, and models were evaluated using Akaike's information criterion (Table S2.3).

Model replicates were developed in a training area under current conditions and then projected to the entire study area for current and past climatic conditions (LGM and LIG). All model replicates and respective projections were used to derive average models and

projections. Average projections were assessed using standard deviation maps, and the environmental similarity of projecting variables in relation to training variables was assessed through multivariate environmental similarity surface analyses (Elith et al., 2011). Model performance was evaluated using the area under the curve (AUC) of the receiving operator characteristics plot. The importance of each climatic variable for describing the distribution of each lineage was determined by its average percentage of contribution to the model across replicates.

To quantify the potentially suitable area for each lineage in each time period, continuous predictions in cloglog probability were imported into ArcMAP 10.5 (ESRI, 2016) and converted to binary predictions (presence/absence) using the maximum training sensitivity plus specificity threshold (Martínez-Freiría et al., 2020). Finally, to assess lineages' range dynamics over time, the binary LGM and LIG predictions were standardized for the size of the distributions compared to present times as [(historical time suitable pixels – current time suitable pixels)/current time suitable pixels] and plotted in terms of gain or loss of suitable area.

2.5 | Phylogenetic signal analyses

To test whether the climatic niche varied across lineages in accordance with their phylogenetic relatedness, we measured the strength of phylogenetic signal for each climatic variable considered. Specifically, we estimated Blomberg's K (Blomberg et al., 2003) and evaluated its significance using a randomization procedure, as implemented in the *phylosig* function of the *phytools* R-package (Revell, 2012). Blomberg's K has an expected value of 1 under a Brownian motion (BM) model of evolution, and it ranges from 0 to ∞ . Values of K lower or higher than one imply that closely related species are, respectively, more different or more similar than expected under BM (Blomberg et al., 2003). A multivariate phylogenetic signal test, based on 1000 random permutations using the *physignal* function of the *geomorph* R-package (Adams, 2014), was also performed on the combined dataset including all environmental variables together.

2.6 | Niche overlap analyses

To investigate macro-evolutionary variation of the climatic niche and test for PNC, we used a three-dimensional hypervolume approach to test for pairwise niche-overlap in sister phylogenetic units (Blonder et al., 2014). Comparisons tested niche overlap between evolutionary units that resulted from major splits along the phylogeny (Martínez-Freiría et al., 2020). To take phylogenetic reconstruction uncertainty into account, alternative relationships resulting from statistically non-significant nodes (i.e. with a posterior probability lower than 0.95) were also tested.

The first three components of a global principal component analysis (PCA) on the climatic data for each lineage were used to

estimate the hypervolumes. All analyses were performed in R with the 'hypervolume' package (Blonder et al., 2014), using a Silverman bandwidth estimator and a set of 200 random points to sample the kernel density to build hypervolumes.

To quantify climatic niche overlap, the Sørensen index (K) and the overlap index (OI) were used (Martínez-Freiría et al., 2020). These statistics quantify predicted niche similarity, and they both range from 0 (no overlap) to 1 (identical niche). The OI relates the observed and maximum values of K , and is preferred when niches present different sizes (Simó-Riudalbas et al., 2018). To test the significance of the overlap, a randomization procedure similar to that applied by Warren et al. (2008) was used. By comparing the produced K s and OIs, we tested whether the observed niche overlap was more different than the overlap that would be obtained by merging occurrences randomly. This procedure was performed 499 times to generate the null distribution and to evaluate the significance of each overlap.

3 | RESULTS

3.1 | Ecological niche models

All models exhibited high values of AUC for both training (avg. AUC > 0.79) and test datasets (avg. AUC > 0.75), and low standard deviations (avg. SD < 0.03), indicating high model accuracy (Table S2.3). Overall, the probabilistic models predicted suitable areas consistent with the known distribution of each lineage (Appendix S3).

Temperature annual range (TAR), precipitation of wettest month (PWM) and precipitation of driest quarter (PDQ) were the most important variables related to the distribution of most of the lineages, whereas mean temperature of coldest quarter (TCQ), mean temperature of warmest quarter (TWQ) and precipitation of warmest quarter (PWQ) resulted to be important only for some lineages (Table S4.1). Overall, TAR and PDQ were more important for most of the lineages of the *Pelias* clade, while PWM was more important for most of the lineages of *Vipera* 1 and *Vipera* 2 clades (Figure 2; Table S4.1).

The average profiles of the response curves suggested consistency among groups (Figure 2; Table S2.2). While most *Pelias* lineages inhabit cold areas, *Vipera* 1 and *Vipera* 2 lineages mainly occur in areas with moderate to high temperatures. Additionally, most lineages are found in areas with moderate to high values of PWM, except for *V. ammodytes* CYC, which occupies drier areas. Regarding the PDQ, similar response curves among clades are detected, still with some exceptions. In *Vipera* 2, for example, *V. ammodytes* NE and PEL occupy areas with different values of precipitation. While the first occurs in more humid habitats, the second can be found in drier environments. The same pattern is also observed in *Vipera* 1 and *Pelias*, with *V. aspis* West (*Vipera* 1), *V. renardi*, *V. berus* and *V. kaznakovi* (*Pelias*) inhabiting more humid places compared with *V. latastei* (*Vipera* 1), *V. darevskii* and *V. walser* (*Pelias*). The PWQ, instead, resulted to be important only for *V. ursinii* (*Pelias*) and *V. ammodytes* S+E (*Vipera* 2). The average profiles of the two response curves of

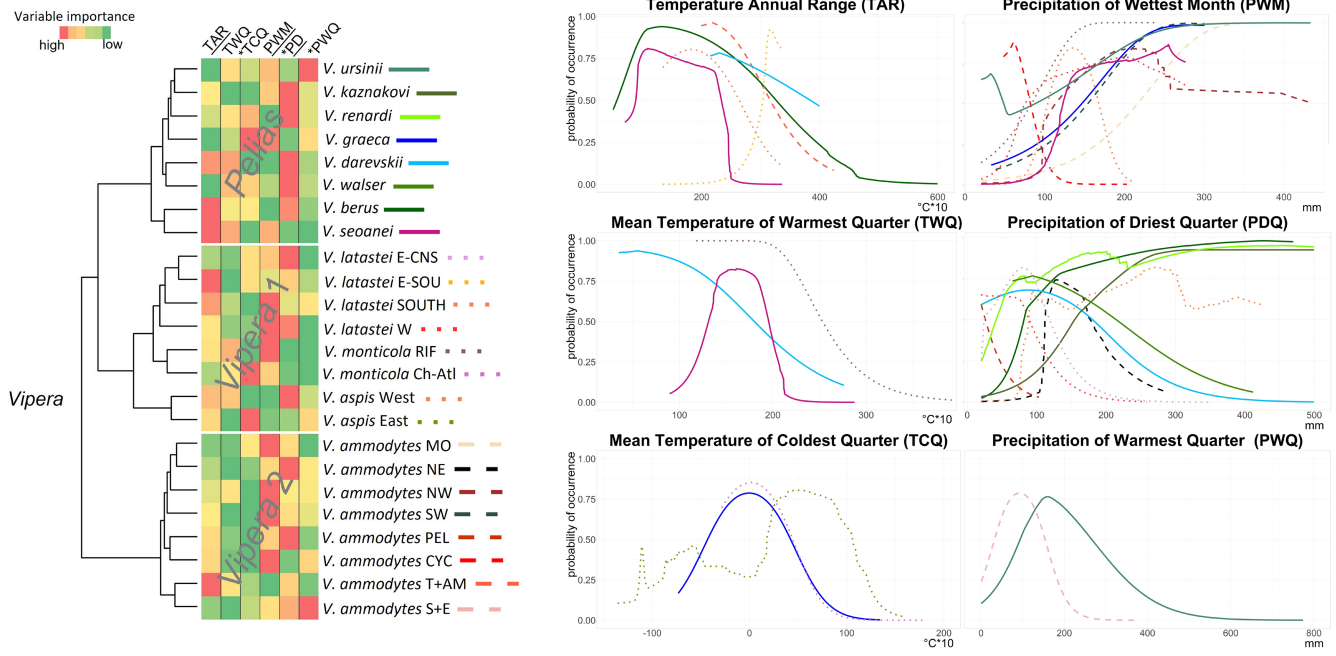


FIGURE 2 Importance of variables in the ecological models derived for 24 lineages of European vipers and average response curves profiles. The most important variables are underlined, while the variables with significant phylogenetic signal ($p < 0.05$) are marked with an asterisk. Phylogenetic relationships and clade membership are represented. Nodes support values correspond to the phylogenetic tree in Figure 1a. PDQ, precipitation of driest quarter (mm); PWM, precipitation of wettest month (mm); PWQ, precipitation of warmest quarter (mm); TAR, temperature annual range ($^{\circ}\text{C} \cdot 10$); TCQ, mean temperature of coldest quarter ($^{\circ}\text{C} \cdot 10$); TWQ, mean temperature of warmest quarter ($^{\circ}\text{C} \cdot 10$).

these lineages appear to be quite similar, although *V. ursinii* occupies a higher and wider range compared with the lineages belonging to *Vipera 2*.

Projections to past conditions were variable between clades (Appendix S3). *Pelias* and *Vipera 1* clades E showed more similar responses to climatic oscillations, while *Vipera 2* had more variable responses (Figure 3). For instance, half of the lineages in *Vipera 2* (i.e. *V. ammodytes* NW, SW, MO and PEL) experienced an expansion of their range in the LIG, while for the same period the other half (i.e. *V. ammodytes* S + E, T + AM, NE and CYC) experienced a reduction of suitable areas. By contrast, all lineages in *Vipera 1* and *Pelias* (with the only exception of *V. graeca*) suffered a range decrease during the LIG (Figure 3). During the LGM, the responses of all the lineages were more variable, with extensive variability within clades.

Analysing lineage responses in detail, distinct patterns of range variation over time emerge within clades (Figure S4.1). Within (1) *Pelias*, contractions are noticeable during both the LGM and LIG in *V. berus*, *V. darevskii* and *V. renardi*, while expansions are detected during the LGM in *V. graeca*, *V. seoanei*, *V. ursinii* and *V. walseri*. A third pattern can be identified for *V. kaznakovi*, where a range decrease likely occurred in the LGM, while a less marked fluctuation is noticeable in the LIG. Within (2) *Vipera 1*, contractions in the LGM and LIG are noticeable in *V. latastei* E-CNS, *V. latastei* E-SOU and both *V. aspis* lineages, and range expansions during the LGM are noticeable in both *V. monticola* lineages and *V. latastei* SOUTH and W., and within (3) *Vipera 2*, *V. ammodytes* CYC, PEL and SW showed a range

increase during the LGM period, while *V. ammodytes* NE, S + E and T + AM presented a reduction in both periods.

3.2 | Phylogenetic signal

We found significant phylogenetic signal in three climatic variables (TCQ, PDQ and PWQ) (Table S4.2). According to Blomberg's *K* values, which were all considerably lower than one, lineages are more different than expected under a BM model of evolution for one temperature-related (TCQavg) and two humidity-related variables (PDQavg, PWQavg). The corresponding multivariate phylogenetic signal was also significant with $K = 0.538$ (p -value = 0.005).

3.3 | Niche divergence

The first three components of the PCA accounted for almost 80% of the climatic variance across lineage niches (Table S4.3). In niche overlap analyses, the degree of hypervolume intersection varied extensively across pairs of compared groups, ranging from null ($n = 0$) to considerably high values ($n = 193.225$). Almost all pairwise comparisons suggested statistically significant overlap between pairs ($p < 0.05$), with *K* and *OI* lower than expected under the null hypothesis, indicating that lineages exhibit different niches despite the variable overlap present between them. In most of the comparisons, niche volumes presented different sizes; therefore, we focus on the

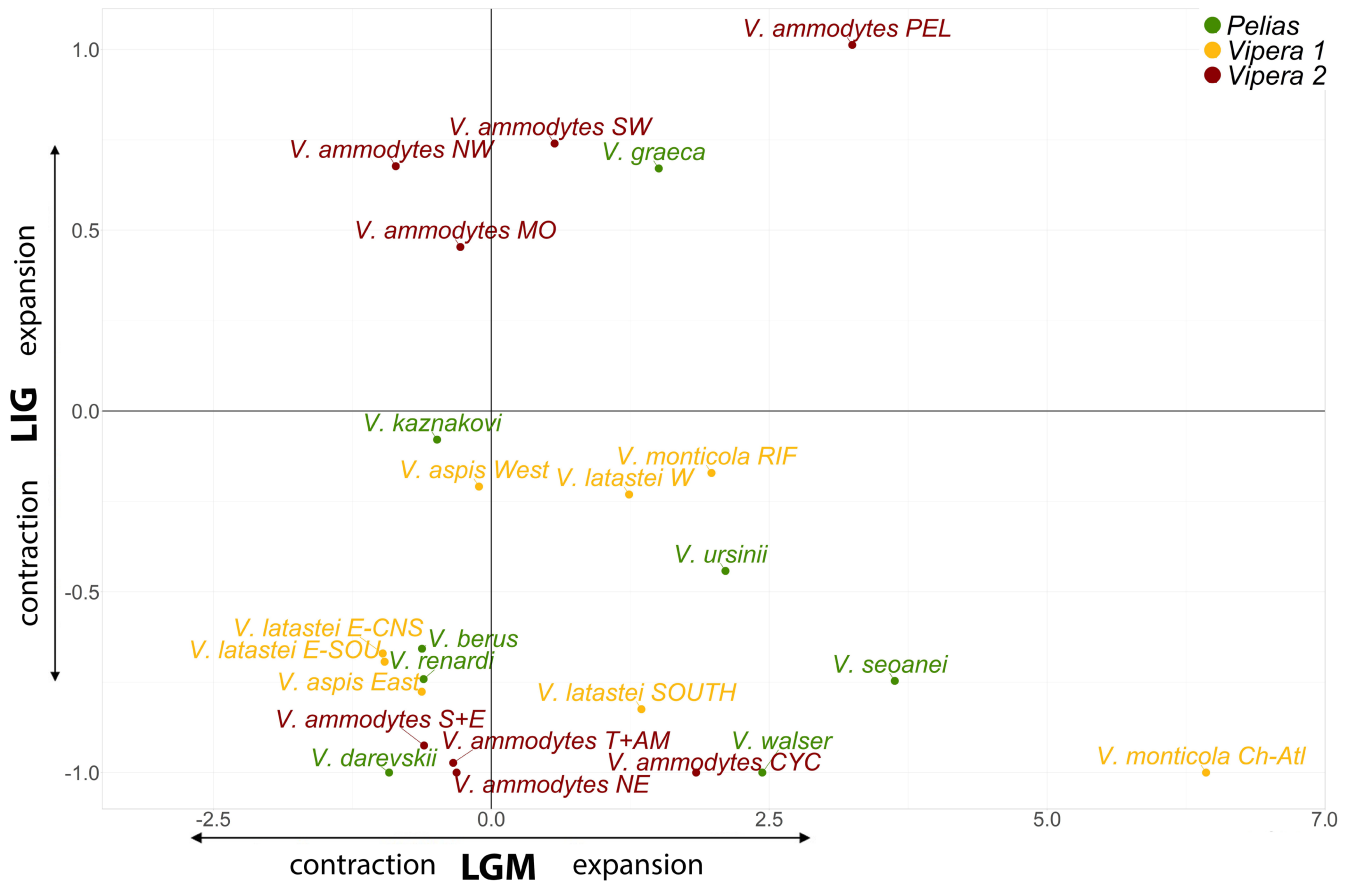


FIGURE 3 Summary plot of the Last Glacial Maximum (LGM, represented on the X-axis) and Last Interglacial (LIG, represented on the Y-axis) for each lineage.

values of niche overlap as measured by the OI (Table S4.4). OI values ranged from low to high ($0.0 < OI < 0.887$), with a complex pattern of variation across the phylogeny, where the oldest splits showed a moderate overlap ($0.488 < OI < 0.739$), early divergent events within each clade had moderate to high overlap ($0.305 < OI < 0.877$) and the most recent lineages varied from low (e.g. *V. berus* vs *V. seoanei*, *V. ammodytes* CYC vs *V. ammodytes* PEL), through moderate (e.g. *V. aspis* East vs *V. aspis* West, *V. ammodytes* NE vs *V. ammodytes* MO) and to high overlap (e.g. *V. kaznakovi* vs *V. ursinii*, *V. monticola* RIF vs *V. monticola* Ch-Atl, *V. ammodytes* MO vs *V. ammodytes* SW) (Table S4.4; Figure 4).

4 | DISCUSSION

In the Mediterranean Basin, several studies confirmed a pattern of PNC among different taxa, with old-diversified taxa being climatically more divergent than more recent ones (Ahmadzadeh et al., 2016; Rato et al., 2015). In this study, we combined ENMs, phylogenetic comparative methods and niche overlap analyses to investigate for the first time how climatic niche evolution or conservatism has shaped European vipers' diversity. In line with our original hypothesis, the ENMs show adaptation of main lineages within each examined clade to specific climatic conditions based on their geographical

origin. Lineages belonging to the *Pelias* clade can be considered cold adapted, while those within *Vipera* 1 and *Vipera* 2 clades are generally warm adapted (Dyugmedzhiev et al., 2021; Lourdais et al., 2013; Martínez-Freiría et al., 2020). However, closely related lineages responded differently to the Pleistocene climatic oscillations, suggesting that other factors have defined their recent range dynamics. Furthermore, phylogenetic signal deviated from the BM expectation for some climatic variables, suggesting adaptation to some environmental conditions. These results are in line with the complex pattern of niche overlap across the phylogeny. Together, these results do not support the hypothesis of PNC, but rather favour a scenario of local adaptation as main driver of niche diversification.

4.1 | Climatic requirements of European vipers

Climatic conditions have a dominant effect on the distributions of ectotherms (Deutsch et al., 2008; Parmesan, 2006) and we show that this effect is prominent in European vipers. ENMs identified specific climatic requirements linked to the distribution of different lineages, which are generally consistent with our initial hypothesis of *Pelias* being cold adapted, while *Vipera* 1 and *Vipera* 2 are warm adapted. Lineages of Mediterranean origin (i.e. within *Vipera* 1 and *Vipera* 2 clades) tend to occur in warmer and dryer habitats as compared with

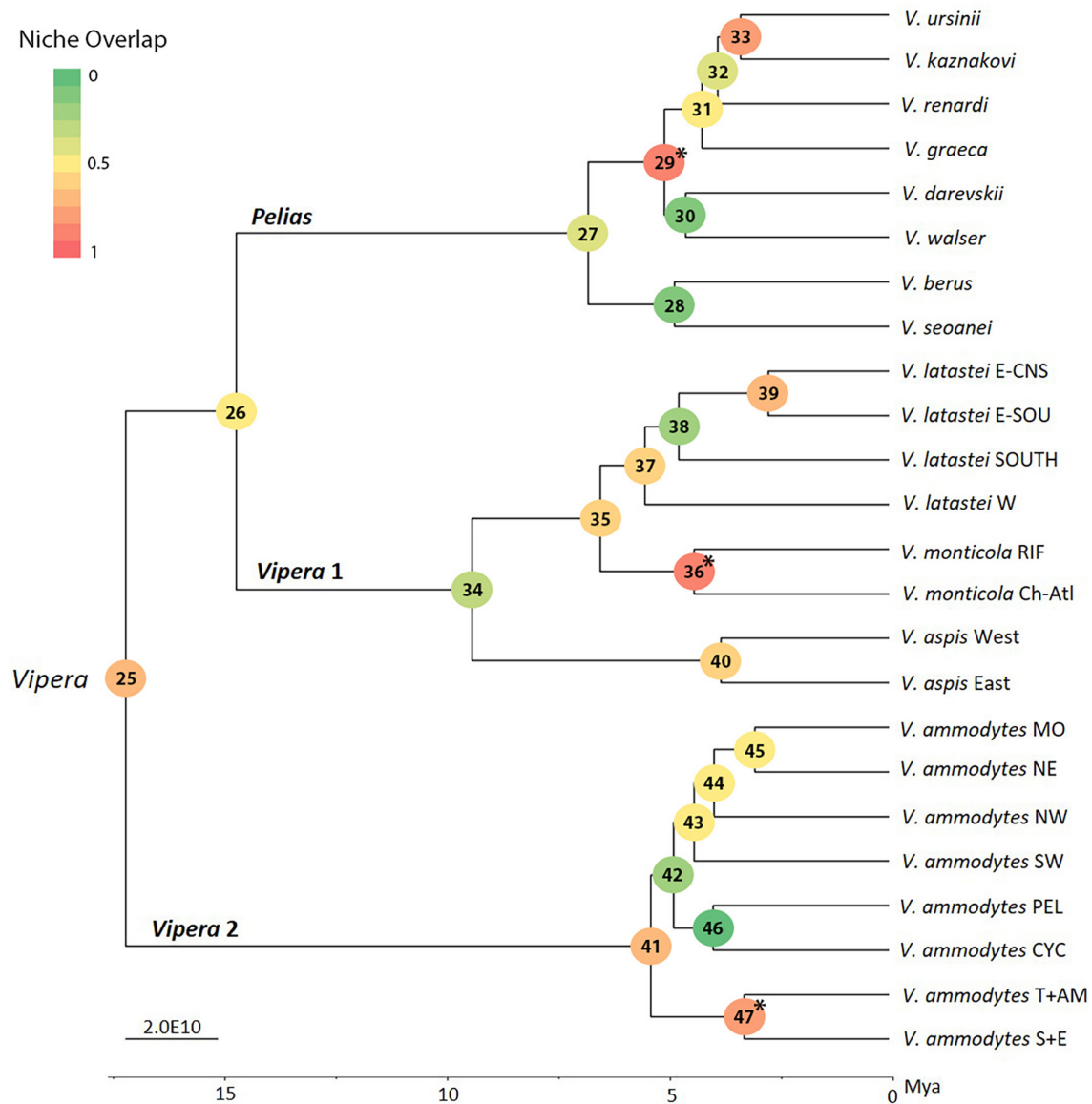


FIGURE 4 Niche overlap values (overlap index, OI) as represented by coloured circles in the nodes of the dated phylogenetic tree of European vipers. Non-significant values ($p > 0.05$) are marked with an asterisk. See [Table S4.4](#) for all niche overlap results and a detailed description of all overlap comparisons conducted. Nodes support values correspond to the phylogenetic tree in [Figure 1a](#).

Euro-Siberian ones (*Pelias* clade), which are rather associated with colder and more humid habitats. For instance, most *Pelias* lineages exhibited higher suitability in areas with lower temperatures and/or higher levels of precipitation. By contrast, the occurrence of *Vipera* 1 lineages as *V. latastei* SOUTH, *V. latastei* E-SOU and *V. monticola* RIF, and *Vipera* 2 lineages as *V. ammodytes* S+E and *V. ammodytes* CYC was positively correlated with moderate to high temperatures and low to moderate precipitation levels ([Figure 2](#)).

Despite this general trend, some warm-adapted lineages exhibit specific temperature correlates, making them climatically more similar to members of the *Pelias* clade. This is the case of the warm-adapted *V. monticola* Ch-Atl (*Vipera* 1), which occurs across similar temperature ranges (low range in TCQ, [Figure 2](#)) as the cold-adapted *V. graeca* (*Pelias*). Both lineages are restricted to high elevation

mountain ranges (*V. monticola* Ch-Atl in the central High Atlas of Morocco; *V. graeca* in the Pindos Mountains in the south-western Balkans), and exhibit some phenotypic traits (e.g. small body size, ectotherm-based diet) typical of montane environments (Freitas et al., 2018; Mizsei et al., 2017). Similarly, *V. monticola* RIF shares climatic correlates comparable to lineages belonging to the *Pelias* clade. Like *V. darevskii*, this lineage distribution was negatively correlated with the temperature the warmest quarter ([Figure 2](#)), although it exhibits a higher range of variation. *Vipera monticola* RIF is the lineage with the widest distribution in North Africa, and, like *V. darevskii*, it occurs mostly across mountain ranges (Martínez-Freiria et al., 2020; Mebert, Göçmen, et al., 2015). Adaptation to mountain environments thus appears as the major factor explaining the specific temperature correlates of some warm-adapted lineages.

Besides temperature, precipitation variables are important correlates of ectotherm distribution, due to their influence on thermoregulation (Rozen-Rechels et al., 2019) and water balance (Dezetter et al., 2021). These physiological constraints are likely behind the particular response to precipitation gradients of some lineages regardless of their putative warm-adapted condition. This likely occurs in *V. aspis* West (*Vipera* 1), which is distributed across a wide range of precipitation values, avoiding dry summers (PDQ, Figure 2) and, thus, its response seems closer to cold-adapted taxa. The response of this lineage to precipitation has been already indicated as intermediate between cold- and warm-adapted taxa across regional and local scales (Chamorro et al., 2021; Martínez-Freiria et al., 2008). Several eco-physiological works on this species highlighted its dependence to water, especially during pregnancy (Dupoué et al., 2015; Lourdais et al., 2017), as well as its ability to behaviourally select air hygrometry (i.e. 'hydroregulation', Dezetter et al., 2022). The strong water dependency of this mesic lineage appears to be a key aspect explaining the specific precipitation correlates found by ENMs. Similarly, the warm-adapted *Vipera* 2 lineages *V. ammodytes* MO, *V. ammodytes* NW and *V. ammodytes* SW prefer environments with high levels of precipitation (PWM). This suggests that, as in *V. aspis* West, water plays an important role in the physiology of these lineages, and physiological studies should be implemented to address its magnitude.

Remarkably, within the *Pelias* clade, *V. ursinii* exhibits a pattern of high suitability at both low and high precipitation levels (PWM, Figure 2). This species occurs across two main habitats located at distinct altitudinal ranges: the warm and dry lowland steppe grasslands and the montane subalpine-alpine meadows (Nilson & Andren, 2001). Past range shifts promoted by contractions of suitable habitat during the Pleistocene (Ferchaud et al., 2012) and recent population extinction mediated by anthropogenic drainage in the Carpathian Basin (Vörös et al., 2022) explain this species' particular range, thus being behind the precipitation correlates revealed by ENMs.

4.2 | Range dynamics during the Pleistocene

Pleistocene climatic oscillations promoted range shifts in Mediterranean biodiversity, with glacial and interglacial periods differently influencing species with distinct climatic requirements (Hewitt, 2004, 2011). We expected cold- and warm-adapted lineages to respond in opposite ways to the same Pleistocene period: the cold-adapted *Pelias* lineages were expected to retract their ranges, while the warm-adapted *Vipera* 1 and 2 were expected to expand them. Conversely, in cold periods, *Pelias* lineages were expected to expand their ranges, while the warm-adapted were expected to reduce them. ENM-based palaeo-projections of the distributions of European vipers during the Pleistocene only partially concurred with these predictions (Figure 3).

For the cold-adapted *Pelias* clade, our findings confirmed range decrease during the LIG (Figure 3), in line with previous studies that addressed the genetic structure of these taxa and/

or considered palaeo-climatic scenarios (Ferchaud et al., 2012; Martínez-Freiria et al., 2015). Still, we found variable responses in the LGM, with about half of the lineages (*V. berus*, *V. renardi*, *V. kaznakovi*, *V. darevskii*) showing a marked decrease in suitable areas (Figure 3). Expansion of ice sheets, permafrost (Hughes et al., 2015) and aridity (Zhang & Li, 2022) likely prevented the persistence of cold-adapted European vipers in northern and eastern regions. While other cold-adapted species probably persisted in the proximity of high mountain systems like the Alps or the Carpathians (Pinceel et al., 2005), our spatial projections show that *V. berus* shifted its distribution southwards, and *V. renardi*, *V. kaznakovi* and *V. darevskii* maintained the core areas with higher suitability around the Black Sea (Appendix S3). These latitudinal range shifts produced an overall reduction in the distributional range of these taxa during the LGM.

Although considered warm adapted, the members of *Vipera* 1 clade exhibited remarkable deviations from our predictions. All lineages reduced their distribution during interglacial periods and, additionally, *V. latastei* W, *V. latastei* SOUTH, *V. monticola* RIF and *V. monticola* Ch-Atl presented an increased range during the glacial phase (Figure 3; Appendix S3). These lineages mostly inhabit mountain ranges at mid-latitudes and our models show a relationship with lower temperature and higher precipitation (Figure 2). Cold environment adaptation can be inferred by the geographical position of their ranges and the reported climatic correlates, explaining the pattern found during the Pleistocene oscillations (see Martínez-Freiria et al., 2020). We are currently experiencing a warm period, where the same areas that appeared to be unsuitable in past interglacial stages for some warm-adapted *Vipera* 1 lineages seem to remain unsuitable nowadays (Brito et al., 2011; Martínez-Freiria et al., 2013). As aridity increases, humid conditions should be gradually pulled up in altitude, promoting population reduction and isolation in mountain tops (Martínez-Freiria et al., 2013; Sinervo et al., 2010).

ENM projections identified different responses for the *Vipera* 2 lineages during the Pleistocene climatic oscillations, which are rarely consistent with our initial expectation of warm-adapted clade (Appendix S3). Only *V. ammodytes* MO and *V. ammodytes* NW responded as expected, expanding their range during the warm interglacial periods and reducing their suitable areas during the cold glacial stages. The remaining lineages, instead, experienced expansions during the LGM (*V. ammodytes* SW, PEL and CYC) or retractions during the LIG (*V. ammodytes* S+E, T+AM, NE, CYC) (Figure 3). The highly variable responses observed for the lineages of this clade, mostly distributed in the Aegean archipelago, could reflect the intricate palaeo-tectonic and -climatic history of the region during the Pleistocene. Besides the large variation of landmass extension and configuration over the Pleistocene as a result of tectonic and volcanic events (Triantis & Mylonas, 2009), cold periods led to a drop of sea level, increasing landmass extension in archipelagos as the Aegean (Perissoratis & Conispoliatis, 2003). Consequently, lineages inhabiting islands or coastal regions as *V. ammodytes* SW, PEL and CYC were likely able to expand to new areas during the LGM (Appendix S3).

On the other hand, the reduction in precipitation levels during the LIG might have been highly restrictive for some *V. ammodytes* lineages, which from our analyses appear to be dependent on precipitation rather than temperature (Figure 2; Table S4.1). The remarkable lack of suitability identified for the lineages *V. ammodytes* S + E and CYC in the LIG could be explained by such increase in aridity during this period, as recent studies have underlined the importance of water stress (Dezetter et al., 2021, 2022). Yet, this could also reflect limitations related to our correlative approach, which neglects potential ecological shifts that could have happened recently (Maiorano et al., 2013) or biotic factors, such as interspecific competition, which could have limited species range shifts, as it happens in present times (Chamorro et al., 2021; Monney, 1996).

4.3 | Complex patterns of niche evolution

Our analyses reveal a complex pattern of ecological niche evolution in European vipers, with a general lack of niche equivalence in the majority of phylogeny nodes (Table S4.4) and variable levels of niche overlap between lineage pairs, without a straightforward relationship to age since divergence (Figure 4). Overall, these evidences reject the hypothesis of PNC as a major pattern governing the European vipers' diversification. This is in agreement with the results derived from phylogenetic signal analyses, which suggest adaptive evolution across some climatic variables.

During the first diversification of the genus (early Miocene; Freitas et al., 2020), our analyses indicate a moderate overlap and thus partially divergent niches between the three major clades. The Miocene was characterized by a gradual global cooling, ice sheet growth, regional aridification and expansion of grasslands at the expense of forests (Steinhorsdottir et al., 2021). These climatic conditions likely acted as selective pressures across landscapes, causing divergent evolutionary responses and environmental tolerances across different groups (Ahmadzadeh et al., 2013; Vaissi & Rezaei, 2022). Fossil records of *Vipera* ancestors in the early Miocene support a first establishment of the three clades in distinct landmasses located in the north (*Pelias*), southwest (*Vipera* 1) and southeast (*Vipera* 2) of Europe (Szyndlar & Rage, 1999). The northern establishment of the *Pelias* clade could have been favoured by an eco-physiological shift towards colder climatic requirements. *Vipera* 1 and *Vipera* 2, instead, being located southwards, experienced similar climatic conditions, which explains the moderate overlap and relatively reduced niche divergence in the oldest diversification levels.

In the early events of diversification within the different clades, we found moderate to high niche overlap, indicating a low climatic divergence and a major role of geographical factors in species diversification. This pattern was reported for the first splits within *Vipera* 1 (Martínez-Freiría et al., 2020) and may apply to other clades. For instance, within *Pelias* the first event of lineage divergence has been associated with the formation of a Strait between Eastern Mediterranean and the Black Sea Basin in the Late Miocene, while the diversification between the *V. renardi-ursinii*

group and *V. kaznakovi* during the Pliocene is attributed to the temporary isolation of the Caucasus from Asia Minor (Zinenko et al., 2015). Remarkably, the different groups within this subclade are specialized in distinct habitat types (Cesaroli et al., 2021; Ghielmi et al., 2016; Mebert, Jagar et al., 2015): while *V. ursinii-V. renardi* are typically considered meadow vipers, *V. walsler-V. darevskii* are instead adapted to rocky habitats. Together with the moderate niche divergence reported here, this suggests that habitat specialization, rather than climatic adaptation, guided the evolutionary history of this subclade.

Low niche overlap was instead recorded in some of the more recent splits, including extremely low values in *V. berus* and *V. seoanei* (*Pelias*; Table S4.4). While *V. berus* is a widespread generalist with a broad, flexible niche adapted to boreal/temperate climates, *V. seoanei* is restricted to northern Iberia (Martínez-Freiría et al., 2015; Nilson & Andren, 1997). Combined genetic and ecological inferences on the evolutionary history of *V. seoanei* indicated that, after its divergence from *V. berus* about 5 Mya, the ancestor of *V. seoanei* underwent important bottleneck events in the Pleistocene interglacial periods, experiencing a population expansion in the LGM (Martínez-Freiría et al., 2015). This expansion could have been mediated by a rapid niche shift to an Atlantic climate, which allowed the species to thrive in the Iberian Peninsula. Further support of the species adaptation to the particular environments in the Iberian Peninsula can be found in its type of reproduction (aestival spermatogenesis and two potential mating periods), which is typical in warm-adapted *V. aspis* and *V. latastei*, and exceptional in cold-adapted *Pelias* vipers (Nilson & Andren, 1997; Vázquez & Martínez-Freiría, 2020).

A similar scenario of rapid niche shift as a consequence of local adaptation to drier environments has already been suggested to be responsible for the divergence between *V. latastei* E and *V. latastei* SOUTH, allowing the former to further diversify across the Iberia Peninsula (Martínez-Freiría et al., 2020). Likewise, cases of climatic adaptation could have taken place also in the Balkan Peninsula. In *Vipera* 2 clade, *V. ammodytes* PEL and *V. ammodytes* CYC present highly diverse climatic niches without overlap (Figure 4). The origin of these lineages is likely associated with the separation between the Peloponnese, the central Cyclades islands and the Greek mainland during the early Pliocene (Dermitzakis, 1990), which isolated populations of *V. ammodytes* in different regions (Ursenbacher et al., 2008). Subsequently, *V. ammodytes* CYC remained isolated on different islands during periods of high sea level and evolved separately (Ursenbacher et al., 2008), likely adapting to different climatic conditions, thus explaining the drier requirements of this lineage compared with the mainland lineages, and the highly marked ecological niche divergence observed.

5 | CONCLUSIONS

Our integrative study shows that climatic adaptation primarily drove diversification in the genus *Vipera*, resulting in a complex pattern of niche divergence along the group's phylogeny. Our approach

contributes to understand evolutionary and ecological dynamics shaping the Mediterranean biodiversity hotspot. The integration of complementary methods, such as multi-locus phylogenomic analyses (Dinis et al., 2019), and the application of mechanistic modelling considering eco-physiological tolerances of distinct taxa could corroborate the climatic specialization patterns we detected, and could allow an in-depth investigation of range dynamics (Evans et al., 2015). Finally, our insights into the ecological factors that most contributed to past species range dynamics could aid the development of tools for the conservation of species highly dependent on humid conditions for hydro-regulation (Rozen-Rechels et al., 2019), and therefore more threatened by future drought events (Dezetter et al., 2021).

AUTHOR CONTRIBUTIONS

NL, AK and FMF conceived the ideas; NL and FMF collected the data with additional material from collaborators; NL analysed the data and wrote the manuscript with assistance from FMF, AK and OL.

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CONFLICT OF INTEREST STATEMENT


The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Occurrence data are available in Zenodo (<https://doi.org/10.5281/zenodo.8126268>).

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BIOSKETCH

Nahla Lucchini is a PhD student at CIBIO-InBIO (University of Porto) investigating the diversification of European vipers to assess potential responses of Mediterranean diversity to anthropogenic change. The authors are part of the PhenEvol research group (<https://cibio.up.pt/en/groups/phenotypic-evolution-phenevol/>) and are interested in understanding the processes related to the evolution of phenotypic diversity.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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