The tale of the black viper: distribution and bioclimatic niche modelling of melanistic *Vipera aspis* in Italy

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**Abstract.** For decades, the evolutionary role of melanism in reptiles has been highly debated. According to the thermal melanism hypothesis, melanistic phenotypes should provide thermal advantages, thus positively impacting various biological aspects of these individuals. Nevertheless, these benefits seem to be countered by environmental constraints and predatory pressure. Here, we mapped for the first time the distribution of the melanistic phenotypes in the highly polymorphic asp viper (*Vipera aspis*). We focused our research effort on the Italian peninsula, where this species reaches its highest level of taxonomic diversity with three currently described subspecies. Furthermore, we investigated via bioclimatic niche modelling, the influence of a wide array of bioclimatic variables on the distribution of melanism in Italian asp vipers. In general, our results seem to support the implications of the thermal melanism hypothesis, highlighting the central influence of mean annual temperature and elevation on the geographic distribution of melanistic *V. aspis*. At the finest scale, our analyses have highlighted a distinction in bioclimatic niches among the three assessed subspecies. However, further fine-scale investigations are needed in order to exclude the potential influence of latitude and elevation on the observed intersubspecific bioclimatic niche segregation pattern.

**Keywords.** Bioclimatic model, habitat suitability, MaxEnt, Mediterranean, melanism, snake, *Vipera aspis*.

**INTRODUCTION**

In snakes, polychromatism is a widespread phenomenon (Wolf and Werner, 1994; Pizzatto and Dubey, 2012; Ruane et al., 2018; de Avila et al., 2019; Regnet et al., 2022) and its related functions have recently been investigated (Allen et al., 2013; Cox and Davis Rabosky, 2013). It is generally believed that dorsal colouration in snakes may have various functions (Allen et al., 2013) such as anti-predatory purpose, expressed as both camouflage...
and aposematism (Madsen, 1987; Santos et al., 2018; Cyr and Kodandaramaiah, 2019), advantage in thermoregulation (Monney et al., 1995; Bittner et al., 2002; Clusella-Trullas et al., 2007b), and role in intraspecific communication and interactions (Shine, 1993; Brooks et al., 2022).

Intraspecific polymorphism is generally considered an evolutionary advantage, linked to a greater range of adaptive possibilities towards perturbative events, such as climate or habitat variations (Forsman et al., 2008; Pizzatto and Dubey, 2012; Forsman, 2016). Within intraspecific polychromatism, less frequent forms may occur compared to the standard chromatic range of a given species, which can be indicated as chromatic anomalies.

Among chromatic anomalies in wild snakes, melanism is certainly amongst the most well-known and widespread (e.g., Andrén and Nilson, 1981, Bittner et al., 2002; Lorrioux et al., 2008; Castella et al., 2013; Benito et al., 2022; Kalogiannis, 2021; Bruni et al., 2022). Melanism is the condition whereby individuals are characterised by darker pigmentation either as intraspecific polymorphism or as consistent variation between closely related taxa (Majerus, 1988; True, 2003; Clusella-Trullas et al., 2007b; Pernetta and Reading, 2009). Melanism can occur not only as an anomalous condition but also as a common form in some taxa (e.g., in Hierophis viridiflavus carbonarius; see Storniolo et al., 2023).

In reptiles, the darkening of the skin colour is mainly determined by a surplus in the production (or dispersion) of pigment by melanophores and, although environmental factors can contribute to physiological (e.g., in lizards) and ontogenetic colour variations, the condition is genetically determined (Sherbrooke and Frost, 1989; Clusella-Trullas et al., 2007b). Although various hypotheses have been formulated, the factors that determine the expression and maintenance of melanism in some individuals or taxa remain to be better understood (Clusella-Trullas et al., 2007b; San-Jose and Roulin, 2018). In ectothermic animals, melanism is considered an advantageous condition with regards to thermoregulation, in fact dark colours reflect less light, absorbing a greater amount of energy. According to the thermal melanism hypothesis, in areas with low temperatures, melanistic individuals are advantaged because they warm-up faster and maintain optimal body temperatures more easily (Jong et al., 1996; Clusella-Trullas et al., 2007b; Martínez-Freiría et al., 2020). Furthermore, melanistic phenotypes appear to be less susceptible to the damaging effects of UV rays (Fu et al. 2022). Nonetheless, the extent of this phenomenon and the molecular mechanisms underlying it in ectotherms are still debated (see Chang and Zheng, 2003; Cox et al. 2013; Reguera et al., 2014; Jin et al. 2020; Senczuk et al. 2021). Moreover, they can achieve greater body size, wider activity cycle, better body condition and a higher fitness than their clearest conspecifics (Huay and Kingsolver, 1989; Luiselli, 1992; Capula and Luiselli, 1994; Monney et al., 1996; Tanaka, 2009). On the other hand, melanism also has disadvantages such as lower camouflage which is reflected in a higher rate of predation, lower efficiency as predators and other stress-related problems (Gibson and Falls, 1979; Andrén and Nilson, 1981; Pérez-Tris et al., 2004; San-Jose et al., 2008).

The asp viper Vipera aspis (Linnaeus, 1758) is a polytypic and highly polymorphic species for which four subspecies are currently recognised: Vipera aspis aspis (Linnaeus, 1758), Vipera aspis francisciredi Laurenti, 1768, Vipera aspis hugyi Schinz, 1833 and Vipera aspis zinnikeri Kramer, 1958 (Speybroeck et al., 2016; Di Nicola et al., 2021; Sindaco and Razzetti, 2021).

Vipera a. aspis is widespread in a territory that includes the Italian, French and Swiss western Alps and a large part of France with the exclusion of the northern areas and those around the Pyrenees (Zuffi, 2002; Ursenbacher et al., 2006). Its dorsal pattern is usually formed by two transverse series of dorsal dark bars of variable thickness, which can extend up to the sides, in which a further series of rounded spots can run (De Smedt, 2006). The ground colour can be grey, reddish, brown or yellowish. A separate discussion can be made on the highly distinctive V. a. aspis alpine populations (formerly known as ssp. atra. Meisner, 1820; see Ursenbacher et al., 2006; Golay et al., 2008; Barbanera et al., 2009 for the synonymy of the two subspecies). These present a highly variable dorsal pattern (Mebert et al., 2011; Dubey et al., 2015), which can be made up of transverse bars, a marked dorsal zig-zag line or as a series of large round-ed blotches more or less fused together or totally absent in the patternless individuals (Mebert et al., 2011; Tessa, 2016) (Fig. S1A).

Vipera a. francisciredi is widespread from central Italy to the central-eastern Italian Alps, including Elba Island, with some populations beyond the borders in Switzerland and Slovenia (Kumar, 2009; Grano et al., 2017; Di Nicola et al., 2021). Vipera a. francisciredi is characterized by a dorsal pattern similar to V. a. aspis, mainly consisting of four transverse series of dark bars offset from each other, two on the back and two on the sides (Bruno, 1985; De Smedt, 2006). The ground colour can be brown, grey, reddish, or yellowish (Fig. S1B).

Vipera a. hugyi is endemic to southern Italy, being distributed in central and southern Campania, Apulia and Basilicata (excluding its northernmost portions), Calabria, Sicily and on Montecristo Island, where it was introduced in historical times (Masseti and Zuffi, 2011; Di Nicola et al., 2021). Vipera a. hugyi is a highly distinc-
tive subspecies, characterized by a pattern consisting of a vertebral series of rounded blotches more or less in contact with each other and often surrounded by a dark margin (Zuffi et al., 2011). On the flanks there are small dark circles, offset from the dorsal blotches. The ground colour ranges from light grey to brown and patternless individuals are known (Di Nicola and Faraone, 2020) (Fig. S1C).

_Vipera a. zinnikeri_ Kramer, 1958 – the only subspecies absent in our study area (i.e., Italy) –, has a geographic range that includes the Pyrenees and the contiguous territories of France and Spain (Ursenbacher et al., 2006). This subspecies usually has a dorsal pattern characterized by a marked zig-zag band of variable thickness, which may expand to a broad vertebral band (Geniez, 2015).

In the asp viper, melanism is a fairly recurrent mutation, which appears with different frequency in the four subspecies. In _V. a. aspis_, melanism appears very frequent in the Alpine populations (Bruno, 1976; Broenniman et al., 2014; Castella et al., 2013; Muri et al., 2015) but rare elsewhere (De Smedt, 2006). The melanistic specimen records for _V. a. zinnikeri_ are not infrequent (Pottier, 2001; Rivera et al., 2001; Baena and Oliveras, 2015; Garcia-Roa and Carbonell, 2020), as well as for _V. a. francisciredi_, for which they are located exclusively in the regions of central Italy (Bruno, 1976; Di Nicola et al., 2019, 2021; Borjanni and Paolino, 2020). As for _V. a. hugyi_, melanism has been found in only a few cases, located in the Calabria region (Brodmann, 1987; Di Nicola and Meier, 2013).

In any case, scientific literature on the presence and distribution of melanistic _V. aspis_ in Italy is scarce, and limited to localized individuals or populations (see Bruno, 1976; Monney et al., 1996; Di Nicola and Meier, 2013; Borjanni and Paolino, 2020). Since Italy is a key territory for _V. aspis_, hosting the highest intraspecific diversity for the taxon, the present research aimed to fill such a lack of information by collecting all possible records of melanistic _V. aspis_ and using them to produce both a real and potential distribution for these individuals.

Accordingly, the main objectives of the present study were: (1) to describe the current knowledge on the geographical distribution of melanism among the Italian populations of _V. aspis_, mapping the occurrence of the three subspecies present in the study area; (2) to assess and analyse the altitudinal distribution of melanistic individuals and compare the results obtained for each of the three subspecies; (3) to test the relationship between the onset of melanism and some climatic parameters by implementing distribution models and assessing their link with the detected geographic ranges and subspecific identity. Based on the literature available on the asp viper (Castella et al., 2013; Broennimmann et al., 2014; Muri et al., 2015; Martínez-Freiría et al., 2020), we expect to detect a relationship between the onset of melanism and bioclimatic and altitudinal variables in the study area, and to record a similar pattern in the three subspecies.

**MATERIALS AND METHODS**

**Eligibility criteria for melanistic individuals**

For the creation of the dataset of melanistic individuals, privately collected data and third-party records (see next section) were used.

In snakes, melanism is a highly variable condition and its expression can vary in intensity, also depending on the part of the body affected (Lorioux et al., 2008; Bruni et al., 2022). Without the availability of photographs taken in a targeted and standardized way, a trait that was verifiable in all the different photos was needed to define the melanistic individuals and evaluate their suitability for the dataset. For this purpose, the overall colouration of dorsal and cephalic scales was chosen, with the following two forms considered eligible:

- Highly dark individuals (tending towards black), with the background colouring presenting very dark reddish-brown tones, with respect to which the dark pattern is only scarcely visible and not very distinguishable (or becomes so only in some light conditions such as under direct sunlight) (Fig. 1A, B);
- Individuals with a uniformly black dorsal colouration – which eventually leaves space only for tiny and sporadic lighter spots –, in which the dorsal pattern is not visible (Fig. 1C).

A distinction between uniformly black individuals and highly dark individuals was not made because the different lighting of the photos would not have guaranteed sufficient precision in this type of evaluation (i.e., a highly dark individual underexposed may appear completely black).

On the other hand, individuals with dark grey or anthracite dorsal background colour and sufficiently distinguishable dark pattern (Fig. S2A, B) were not included in the dataset, as well as individuals in which the dark pattern is anomalously expanded but a lighter background colour is still present and clearly distinguishable (Fig. S2C). The latter was excluded because, with the photographic material available, it would not have been possible to quantify the expansion of the dark pattern and therefore establish a threshold beyond which individuals would no longer be considered eligible.

Finally, individuals in pre-shedding condition were excluded as this is a life phase that is not always suitable for evaluating colouration.
Information on the distribution of melanistic *Vipera aspis* in Italy was obtained from observations carried out by three of the authors (MRDN, NB and LL) during herpetological surveys or hiking carried out from July 2011 to August 2022 (32% of the whole dataset), and combined with data received from collaborators and citizen science, updated until May 2023 (68% of the whole dataset). Moreover, a review of both scientific and popular bibliographic sources was undertaken, along with interactions with expert herpetologists and museum curators. The goal was to enhance understanding of the actual distribution of melanistic individuals in Italy, bridging any gaps between the sample and the documented real distribution. No bibliographic or museum records outside the regions already covered by the authors’ dataset have been recorded. Furthermore, the few records deemed ineligible due to imprecise coordinates or data unreliability were located in regions already included in the dataset.

Each snake found by the authors was identified on morphological and geographical basis (see Di Nicola, 2019; Di Nicola et al., 2021, 2022); the date, time, coordinates, and altitude were recorded, and a photo of the dorsal pattern was taken. As for the records from third parties, the data was obtained through direct requests to collaborators or surveyors following consultation of citizen science platforms (i.e., Inaturalist.it, Ornitho.it and Observation.org) and social networks such as Flickr.com, and the Facebook group “Identificazione Anfibi e Rettili” (https://www.facebook.com/groups/283231695476830), administered by MRDN. Only the records provided with coordinates with an accuracy of at least 200 m and accompanied by a photo with sufficient resolution were considered. The altitudes of third-party records were obtained using the Google Earth Software (ver. 9.194.0.0).

The records were used to compile the Italian distribution map of melanistic *V. aspis* individuals, following the subspecies spatial division indicated by Barbanera et al. (2009), and an ETRS89 / ETRS-LAEA 10×10 km grid covering the Italian territory. The altitudinal values of melanistic individuals of *V. aspis* individuals were compared with the relative species’ values in Italy. The latter were obtained from the dataset provided by the Global Biodiversity Information Facility network, selecting only the directly observed (non-museum) records, equipped with photos, with a coordinate accuracy of at least 180 m (GBIF.org, 2023). Upon extraction of the altitudinal values for all individuals, normality and homoscedasticity of the data were tested using Shapiro-Wilk and Levene’s test, respectively. As the normality assumption was not met, statistical differences were compared using a Wilcoxon rank sum test. All analyses were performed in R software version 4.3.1 (R Core Team, 2023).

**Bioclimatic niche models**

The bioclimatic niche occupied by melanistic individuals of *V. aspis* was modelled using the correlative

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**Fig. 1.** Examples of melanistic *Vipera aspis* individuals from Italy. A) Highly dark male *V. a. aspis* from Piedmont; B) Highly dark female *V. a. francisciredi* from Lazio; C) Uniformly black female *V. a. hugyi* from Calabria. Photo credit: Matteo R. Di Nicola.
presence-background software MaxEnt 3.4.1. (Phillips et al., 2017). Only occurrence records with precise coordinates (n = 83) were used for this purpose. A first model was computed using all occurrence records and a mask of the known areal of the species in Italy as background. Then, the records were divided among V. aspis aspis (n = 36), V. aspis francisciredi (n = 36), and V. aspis hugyi (n = 11). Despite the low number of occurrences, these records should constitute a sufficient sample size to produce accurate predictions of species suitability, especially given the restricted extent of the study area (van Proosdij et al., 2015; Stoa et al., 2019). Nonetheless, care was taken in order to minimize model overfitting by checking the data for spatial autocorrelation, avoid multicollinearity between predictor variables, and reducing models’ complexity (Boria et al., 2014). As each subspecies occupies a distinct geographic region within the Italian peninsula (Barbanera et al., 2009), three additional models were computed, each using a customized background mask representing the area available to the relative subspecies. Finally, in order to test the effect of background selection on model performance and predictions, minimum bounding polygons were drawn from each subspecies occurrence records and used to draw background points for the relative models. The predictor variables used for modelling were downloaded from the WorldClim 2.1 database (Fick and Hijmans, 2017) at a 30 arc-sec spatial resolution. These contained 19 bioclimatic layers (bio1-19), elevation, and monthly solar radiation (srad01-12). Slope aspect (exposition) was derived from elevation. As aspect is a circular rather than continuous variable, it was split into four classes: North (0°-45° and 315°-360°), East (45°-135°), South (135°-225°) and West (225°-315°). Additionally, a land cover raster was obtained from the Global Land Cover National Mapping Organizations (GLCNMO v3, available at https://globalmaps.github.io/glcno.html, accessed on 20 August 2023). Values for all 34 layers were extracted at each presence location, and pairwise Pearson’s correlation coefficients were calculated. In order to minimize multicollinearity among predictors, a single variable was retained from each correlation group (| r | > 0.75). The selected variable was the one deemed most ecologically relevant for the distribution of the species. As high positive correlation was observed among solar radiation values during the period of activity of the species (May-October), an average was calculated (srad05-10) and used as predictor in the models. Upon selection, the variables used for modelling were: mean annual temperature (bio1), mean diurnal temperature range (bio2), annual precipitation (bio12), precipitation of the coldest quarter (bio19), average solar radiation of the month of April (srad04), average solar radiation of the period May-October (srad05-10), land cover (lc) and slope exposition (exp). As melanistic individuals of V. aspis are often linked with high altitude environments, we also tested the effect of using elevation (elev), in place of mean annual temperature, to model suitability, given the high negative correlation (r < -0.9) between the two. Accordingly, four model combinations were tested for V. aspis and for each subspecies: i) only climatic variables (n = 6); ii) climatic variables plus land cover (n = 7); iii) climatic variables plus land cover and slope exposition (n = 8); and iv) climatic variables plus land cover and slope exposition, but using elevation instead of bio1 (n = 8). For each model combination, 10 bootstrap replicates were computed in MaxEnt (default settings, logistic output), each randomly selecting 70% of records for model training and 30% for testing. Permutation importance was used to estimate the relative contribution of each predictor variable to the models. Model performance was evaluated based on the area under the receiver operating curve (AUC), a measure of discrimination between presence and background points (Fielding and Bell, 1997). Additionally, omission rates (OR) were inspected to evaluate model overfitting. These express the proportion of records predicted to fall outside the area defined as suitable by the model, based on various theoretical thresholds. For the purpose of this study, the 10th percentile training presence omission rate (OR10) was adopted (Boria et al., 2014). The relative OR10 logistic threshold was also used to set the minimum suitability threshold to MaxEnt continuous suitability outputs. As the output maps for different model combinations did not differ greatly from each other, an average model was calculated for V. aspis and each of its subspecies, applying the average OR10 threshold for minimal suitability. These ensemble models were used for final representation. All analyses were performed in R 4.3.1 (R Core Team, 2023) and QGIS 3.14 (QGIS Development Team, 2020).

**Principal component analysis**

A principal component analysis (PCA) was computed in order to explore potential differences between the bioclimatic niches occupied by melanistic individuals of Vipera aspis aspis, V. a. francisciredi, and V. a. hugyi. To do so, the presence and background points used to compute the MaxEnt models were plotted across the two first principal component dimensions, showing their variation along the continuous predictor variables used for modelling (see the “Bioclimatic Niche Modeling” section). All analyses were performed in R 4.3.1 using the packages ‘FactoMineR’ (Lê et al., 2008), ‘factoextra’ (Kassambara and Mundt, 2020) and ‘ggplot2’ (Wickham, 2016).
RESULTS

Distribution

A total of 124 eligible observations of melanistic *Vipera aspis* were collected (40 through personal surveys, 84 through collaborators and citizen science), carried out between 1984 and 2023, of which 90% (n = 112) were from 2010 onwards (Table S1). The altitudinal range of the records covered from 515 to 2212 meters above sea level (mean value = 1533 m a.s.l.; Fig. S3 A).

At the subspecific level, 50 observations of melanistic *V. a. aspis* (five from Aosta Valley and 45 from Piedmont; altitudinal range = 954-2212 m a.s.l., mean value = 1630 m a.s.l.); 58 observations of melanistic *V. a. francisciredi* (35 from Abruzzo, 19 from Lazio, one from Marche, two from Molise and one from Umbria; altitudinal range = 515-2200 m a.s.l., mean value = 1505 m a.s.l.); 16 observations of melanistic *V. a. hugyi* (four from Basilicata, 10 from Calabria and two from Campania; altitudinal range = 645-1783 m a.s.l., mean value = 1333 m a.s.l.) were collected (Fig. 2; Fig. S3A-C).

The 124 observations fall into 57 ETRS89 / ETRS-LAEA 10×10 km squares (Fig. S4); 24 squares concern *V. a. aspis*, 22 *V. a. francisciredi* and 11 *V. a. hugyi*. There was a significant difference (Wilcoxon's W = 13979, p-value < 0.001) between the altitudinal range of melanistic individuals (n = 124; this study) and that of *V. aspis* individuals from the whole species (n = 451; GBIF.org, 2023). On average, melanistic individuals were encountered at a higher altitude (Mean = 1533 m a.s.l.) and showed a more restricted distributional range (Min = 515 m; Max = 2212 m; St. Dev. = 323 m) compared to other individuals (Mean = 976 m a.s.l.; Min = 0 m; Max = 2816 m; St. Dev. = 665 m) (Fig. S5).

Bioclimatic niche models

All models computed showed robust statistical performances (see Table S1). Mean annual temperature (bio1) was the most influential predictor for all models (42.9-84.1% contribution). Given the high correlation between bio1 and elevation (r < -0.9), the latter was the most important predictor for models that did not contain bio1 (24.8-85.1% contribution). The models for *Vipera aspis* were also influenced by the mean solar radiation of the month of April (srad04; 3.5-9.5%) and precipitation of the coldest quarter (bio19; 1.7-6.6%), whereas other variables showed low overall contribution (<5%). The same pattern was observed for model combinations computed for *V. a. aspis* in NE Italy, with srad04 (12.2-16.8%) followed by bio19 (4.2-13.3%). Contrarily, models for *V. a. francisciredi* in Central Italy were most influenced by the mean solar radiation of the period May-October (srad05-10; 24.2-32.3%) and mean annual precipitation (bio12; 4.3-11.6%) rather than srad04 (0.5-1.3%) or other variables. Finally, model combinations computed for *V. a. hugyi* in Southern Italy showed low overall contribution of solar radiation (0-4.0%) and were instead most influenced by mean diurnal temperature range (bio2; 17.2-56.7%) and bio19 (4.9-27.1%). These models also showed highest contribution of land cover (3.2-7.2%) and slope exposition (3.7-4.1%) compared to the previous models.

Areas predicted as suitable for melanistic individuals of *V. aspis* were in agreement across the four model combinations and also between the ensemble model and the independent models computed.
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for each subspecies (Fig. 3A, B). The average model shows areas predicted as suitable for the species by all model combinations, using the relative OR10 as threshold for minimum suitability for each subspecies (Table S2). The model predicts high suitability across three main areas, respectively in Northern, Central and Southern Italy: in Northern Italy (involving Piedmont and secondarily Val d’Aosta), the western sector of the central Alps (Lepontine and Pennine Alps), and, with less involvement, the eastern portions of the Graian and Cottian Alps in the Western Alps; in Central Italy (mainly involving Abruzzo, but also Marche, Umbria, Lazio, Molise and Campania), the central Apennines, where the Abruzzo Apennines are mainly involved, including in the North the southern portion of the Umbria-Marche Apennines and, to the south, the northern portion of the Southern Apennines (specifically, the Samnite Apennines); in Southern Italy (involving Calabria and a small part of Southern Basilicata), the Southern Apennines involving the Lucanian Apennines and the Calabrian Apennines.

It is important to highlight that the models presented in Fig. 3 are produced using few occurrence records localized over a large available background. As such, they depict a conservative projection of bioclimatic suitability, intended to highlight areas sharing very similar environmental conditions to those where melanistic *V. aspis* individuals were recorded. Vice versa, restricting the modelling background to areas directly surrounding the presence records produced significantly larger predictions of bioclimatic suitability, which include the Northern Apennines and Eastern Alps (Fig. S6). However, projections based on restricted backgrounds are strongly influenced by environmental variables and features being limited to the range encountered during training. The treatment of variables outside their training ranges is likely to have a strong effect on predicted suitability, so predictions in those areas should be treated with strong caution (Elith et al., 2011. For these reasons, we focused our discussion on the most conservative models for the purposes of this study.

Fig. 3. Predicted bioclimatic suitability for melanistic individuals of *Vipera aspis* in Italy. A) Suitability models computed independently for subspecies *V. a. aspis*, *V. a. francisciredi* and *V. a. hugyi* within their relative geographic ranges (according to Barbanera et al., 2009). B) Ensemble model computed using all occurrence records together and the complete Italian range as background. The outputs shown represent the average of four model combinations, each replicated 10 times (see materials and methods for details). Warmer colours indicate higher suitability, the background falls below the minimum suitability threshold for each model (see Table S2).
The results of the principal component analysis (PCA) are shown in Fig. 4. The first two PCA dimensions explain 42.2% (Dim1) and 31.1% (Dim2) of the variation observed in the data. Mean annual temperature (bio1), elevation (elev), and annual precipitation (bio12) contribute mostly to the variation along the first dimension, whereas solar radiation (srad04 and srad05-10) and diurnal temperature range (bio2) influence most of the variation along the second dimension. Precipitation of the coldest quarter (bio19) shows little contribution. The distribution of the background points used for the MaxEnt models shows a clear distinction in the bioclimatic environments available to the three subspecies of *Vipera aspis* in Italy (Fig. 4A). Nonetheless, the presence records of melanistic individuals used for the analyses occur within similar bioclimatic conditions, shared by the background environments. On a finer scale, a distinction is present between the bioclimatic niche occupied by melanistic individuals of the three subspecies, mostly along the second dimension of the PCA (Fig. 4B). However, due to the contribution of solar radiation and diurnal temperature range to Dim2, this difference could be attributed to the latitudinal gradient rather than a specialization towards different niches.

**DISCUSSION**

*Vipera aspis* can be considered as one of the most geographically widespread snakes in Italy (Zuffi, 2006), but despite that, melanistic individuals of this species appear to be characterised by a very limited and fragmented distribution (Bruno et al., 1985; Grano et al., 2017).

For elusive and not always easily contactable animals such as vipers, there is always the risk of underestimation in sampling. Nevertheless, considering the multiple channels exploited for data acquisition and the quantity of *V. aspis* records examined from each area in Italy, we believe that the mapping of melanistic individuals here presented does not deviate significantly from the actual distribution of these snakes.

According to our distributional data, melanistic individuals of *V. aspis* seems to be restricted to alpine and temperate bioclimatic regions along the Alps and the southern Apennines, with no evidence of melanistic individuals within the Mediterranean bioclimatic region (Attorre et al., 2007) (Fig. 2). These results are in strong agreement with the outputs of our bioclimatic niche modelling analyses (Fig. 3A, B). However, the overall species model highlighted a few areas of modest suitability that currently lack formal records of melanistic individuals of *V. aspis*. In particular, low but still noticeable suitability was found in proximity of Mount Etna, a small portion of the Ligurian Apennines and within a restricted area of the southern part of the Tuscan-Emilian Apennines (Fig. 3B). These areas are highly explored from a herpetological point of view (e.g., Zuffi, 1984; Bassini et al., 1991; Turrisi and Vaccaro, 2001, 2004), and so far, no melanistic individuals of *V. aspis* have been formally found. Even if this phenomenon may be the product of research biases, correlated with the cryptic nature of the taxon, mechanisms such as predation pressure, genetic
isolation, and biographic pattern may explain the lack of melanistic phenotypes of *V. aspis* within the suitable areas highlighted by our models. These potential explanations become even more relevant when considering the broader suitability zones provided by the less conservative model shown in Fig. S6.

In particular, populations of *V. a. hugyi* from Sicily and Southern Apulia represent an old clade which diverged from the rest of the subspecies around 1.5–1.2 Mya and poorly contributed to the expansion of the taxon in the southern part of the Italian peninsula during the Pleistocene (Barbanera et al., 2009). On the other hand, populations of *V. a. hugyi* from the rest of its geographic range were subject to more complex patterns of gene flow which involved introgression with *V. a. francisciredi* (Barbanera et al., 2009). The melanistic phenotype in the latter populations could have independently arisen via random mutation or, it may be the product of historic introgression with *V. a. francisciredi* as found in other species (McRobie et al., 2019; Storniolo et al., 2023).

Therefore, the absence of melanistic individuals of *V. a. hugyi* in the suitable areas highlighted by the model may be the consequence of the lack of the phenotype among the highly divergent and genetically isolated populations of southern Apulia and Sicily.

Moreover, modest suitability was highlighted in the proximity of the Lombardy Prealps, part of the Leopontine Alps and a small sector of the Rhaetian Alps in north-western Lombardy (Fig. 3B). Interestingly, anomalously dark grey individuals, characterised by the presence of visible dorsal pattern and high concentration of melanin (*sen* su Fânaru et al., 2022), have been observed in the highlighted areas (MRDN and AVP, pers. comm.) (Fig. S2). This geographic region is likely to represent a transition zone between *V. a. aspis* and *V. a. francisciredi* (Ursenbacher et al., 2006), but formal genetic studies from this area are currently lacking (Barbanera et al., 2009), thus individuals from north-western Lombardy are formally ascribed to the subspecies *francisciredi* (Ursenbacher et al., 2006; Di Nicola et al., 2021). As the melanistic phenotype is very common in the alpine populations of the nominal subspecies (Mebert et al., 2011), the presence of partially melanistic individuals of *V. a. francisciredi* in the area may be the result of genetic introgression between the two subspecies. Another possibility involves the sudden increase of random mutations and interactions between genes correlated with pigment regulation in the focal populations, such as the pro-opiomelanocortin gene (POMC) (Ducrest et al., 2014). It is possible to speculate that partial melanism may have been then maintained at low frequency within these populations via weak selection due to environmental-related advantages (Martínez-Freiría et al., 2020).

In this regards, our bioclimatic niche modelling analyses highlighted a general correlation between the distributional pattern of melanistic *V. aspis* and various bioclimatic variables (Table S2). Specifically, the strong influence of mean annual temperature and elevation seems to support the implications of the thermal melanism hypothesis (Watt, 1968; Clusella-Trullas et al., 2007b). According to the latter, melanistic individuals should be in fact favoured in cooler environments thanks to their ability to thermoregulate in sub-optimal thermal conditions. The adaptive role of melanicm in reptiles, and in particular in snakes, has been highly debated. While some studies have highlighted physiological advantages correlated with melanism (Luiselli, 1993; Bittner et al., 2002; Clusella-Trullas et al., 2007a; Tanaka, 2009; Moreno Azócar et al., 2020; Hantak et al., 2022), others failed to find any related evidence of selection (San-Jose et al., 2008; Matthews et al., 2016; Bury et al., 2020, 2022; Fânaru et al., 2022). Within European vipers, the benefits associated with a melanistic pigmentation appear to be context-dependent with a high variability in terms of observed advantages across populations (Madsen and Stille, 1988; Luiselli, 1992; Monney et al., 1995, 1996; Strugariu and Zamfirescu, 2011; Broennimann et al., 2014; Lucchini et al., 2020). Our models indicated temperature and elevation as main predictors of suitability for melanistic *V. aspis* individuals in Italy. However, differences were observed among the three subspecies in their response to other predictor variables, which could be the consequence of bioclimatic niche specialization.

When annual average temperature was excluded in the models, altitude represented the most important predictor for the distribution of melanistic individuals of *V. aspis*, apart from *V. a. hugyi*. Melanistic individuals were found, on average, at significantly higher elevations than non-melanistic individuals (Fig. S5), nevertheless, both general and subspecies’ altitudinal averages of melanistic *V. aspis* were found below the treeline within both the Alps and the Apennines (Bonanomi et al., 2020; André et al., 2023). This was further corroborated by the high ratio of melanistic individuals found within – or at the margins of – forested areas (authors, pers. comm.) and by the high suitability in forested areas highlighted by our models (Fig. S7). Similar results were shown by Muri and colleagues (2015), with substantial difference in micro-habitat choice between melanistic and non-melanistic *V. aspis* individuals, with the former exploiting environments characterised by higher vegetation productivity. Exploitation of different microhabitats between non-melanistic and melanistic individuals is likely due to the ability of the latter to inhabit areas where their lower skin reflectance may represent an ecological advantage, such as forested
areas characterised by low solar radiations (Borgianni and Paolino, 2020; Martínez-Freiría et al., 2020). Due to the less cryptic nature of melanistic individuals, inhabiting forested areas may reduce predator detection probability, especially in the case of avian taxa (Andrén and Nilson, 1981; Monney et al., 1996; Tanaka, 2009). On the other hand, phenotypes such as the blotched or the concolor ones seem to be more advantageous in open areas characterised by low vegetation coverage (Castella et al., 2013; Dubey et al., 2015; Tessa, 2016; Pizzigalli et al., 2020).

At a finer scale, melanistic individuals from the three analysed subspecies seems to be characterised by three quite distinct bioclimatic niches (Fig. 4). Surprisingly, the breadth of the nominal subspecies’ bioclimatic niche resulted wider compared to the others despite a quite restricted geographic distribution (Zuffi, 2006; Di Nicola et al., 2021). This seems to showcase the ability of melanistic V. a. aspis to adapt to a wide variety of heterogeneous bioclimatic conditions (Brodmann et al., 1987). On the other extreme, melanism in V. a. hugyi appears to be correlated with a very narrow set of bioclimatic and environmental conditions. While the thermal melanism hypothesis implicates a positive correlation between skin reflectance and solar radiations (Clusella-Trullas et al., 2008), melanistic individuals of the latter subspecies seem to follow an opposite trend, with their bioclimatic niche being characterised by the highest levels of solar radiations among the investigated subspecies (Fig. 4). Melanistic pigmentation and relative low reflectance should provide a stronger protection against UV rays exposure compared to lighter pigmentation, thus shielding internal organs from UV-related damages (Clusella-Trullas et al., 2008; Goldenberg et al., 2021). According to the Gloder’s rule, individuals characterised by high level of melanism should be favoured in warm and humid environments, due to better protection from solar radiations and pathogens (Delhey, 2017). Melanistic individuals of V. a. hugyi have been observed in forested areas, where levels of humidity are likely to be higher compared to other habitats inhabited by this subspecies (Brodmann, 1987; Di Nicola and Meier, 2013). Moreover, at low latitudes, solar radiations rather than air temperature may represent a better predictor for skin reflectance (Clusella-Trullas et al., 2008). Therefore, the observed correlation between the assessed bioclimatic variables and melanistic individuals of V. a. hugyi seems to agree, at least in part, with the implications of the Gloder’s rule.

Nevertheless, it is critical to point out that this apparent bioclimatic niche partitioning among the assessed subspecies may be, at least in part, an artefact derived by changes in environmental conditions caused by latitudinal gradients rather than pure differences in bioclimatic niches (Andersen et al., 2022). A good amount of the variation in the PCA analysis is in fact primarily correlated with solar radiation and daily temperature variation, which are both factors highly affected by latitude. Thus, while melanistic individuals from the three subspecies may have similar general bioclimatic requirements (Fig. 4), the observed niche partitioning could be the results of differences in the available bioclimatic conditions at different latitudes.

In the same way, some of the observed similarities among the relative bioclimatic niches may be caused by the homogenizing effect of altitude on bioclimatic conditions (Körner, 2007). Therefore, due to the complex interplay between the above-described factors, our results at the subspecies level must be taken with caution. Further studies aimed at characterising the microhabitats of melanistic individuals of V. aspis ssp. are thus needed to test for our observed differences in inter-subspecific niche partitioning.

In conclusion, this study reported, for the first time, an accurate geographic distribution of melanistic individuals of Vipera aspis in Italy. Moreover, on a broad scale, our bioclimatic niche modelling results for melanistic V. aspis seems to agree with the insights provided by the thermal melanism hypothesis. On the other hand, finer scale analyses of bioclimatic niche partitioning between the three subspecies of V. aspis portrayed a discrete level of differentiation, although confounding factors may overestimate the real extent of bioclimatic niche differentiation.

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SUPPLEMENTARY MATERIAL

Supplementary material associated with this article can be found at <http://www-9.unipv.it/webshi/appendix/index.html> manuscript number 15271.

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