

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

MATTEO R. DI NICOLA<sup>1,2,3,\*</sup>, FRANCESCO P. FARAONE<sup>4</sup>, ANDREA V. POZZI<sup>5,6</sup>, NICOLÒ BORGIANNI<sup>7</sup>, LORENZO LADDAGA<sup>8</sup>, JEAN-LOU M. C. DORNE<sup>9</sup>, GIANMARCO MINUTI<sup>10</sup>

<sup>1</sup> Faculty of Veterinary Medicine, Department of Pathobiology, Pharmacology and Zoological Medicine, Wildlife Health Ghent, Ghent University, Merelbeke, Belgium

<sup>2</sup> Unit of Dermatology and Cosmetology, IRCCS San Raffaele Hospital, Via Olgettina 60, 20132 Milan, Italy

<sup>3</sup> Asociación Herpetológica Española, Apartado de correos 191, 28911 Leganés, Madrid, Spain

<sup>4</sup> Dipartimento Scienze e Tecnologie Biologiche, Chimiche e Farmaceutiche, University of Palermo, Via Archirafi 18, 90123 Palermo, Italy

<sup>5</sup> Molecular Ecology and Evolution Group, School of Environmental and Natural Sciences, Bangor University, Bangor, Wales, UK

<sup>6</sup> Evolutionary Ecology Group, Department of Zoology, University of Cambridge, Cambridge, UK

<sup>7</sup> Rewilding Apennines ETS, Gioia dei Marsi (AQ), Italy

<sup>8</sup> Società di Scienze Naturali del Verbano Cusio Ossola, Museo di Scienze Naturali, Collegio Mellerio Rosmini, Domodossola, Italy

<sup>9</sup> Methodology and Scientific Support Unit, European Food Safety Authority (EFSA), Via Carlo Magno 1A, Parma 43126, Italy

<sup>10</sup> Department of Biology, Ecology & Biodiversity Research Unit, Vrije Universiteit Brussels, Brussels, Belgium

\*Corresponding author. Email: matteodinicola86@libero.it

Submitted on: 2023, 15<sup>th</sup> November; revised on: 2024, 7<sup>th</sup> March; accepted on: 2024, 3<sup>rd</sup> May

Editor: Stefano Scali

**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; Cyriac 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., Andr n and Nilson, 1981, Bittner et al., 2002; Lorioux 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; Mart nez-Freir a 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 (Huey 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; Andr n and Nilson, 1981; P rez-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; Ursembacher 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 Ursembacher 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 rounded 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; Broennimann 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; García-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; Borgianni 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; Borgianni 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; Broennimann 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.



**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.

### Distribution

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 com-

bined 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

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; Støa 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 ( $0^{\circ}$ - $45^{\circ}$  and  $315^{\circ}$ - $360^{\circ}$ ), East ( $45^{\circ}$ - $135^{\circ}$ ), South ( $135^{\circ}$ - $225^{\circ}$ ) and West ( $225^{\circ}$ - $315^{\circ}$ ). Additionally, a land cover raster was obtained from the Global Land Cover National Mapping Organizations (GLCNMO v3, available at <https://globalmaps.github.io/glcnm.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 10<sup>th</sup> 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*

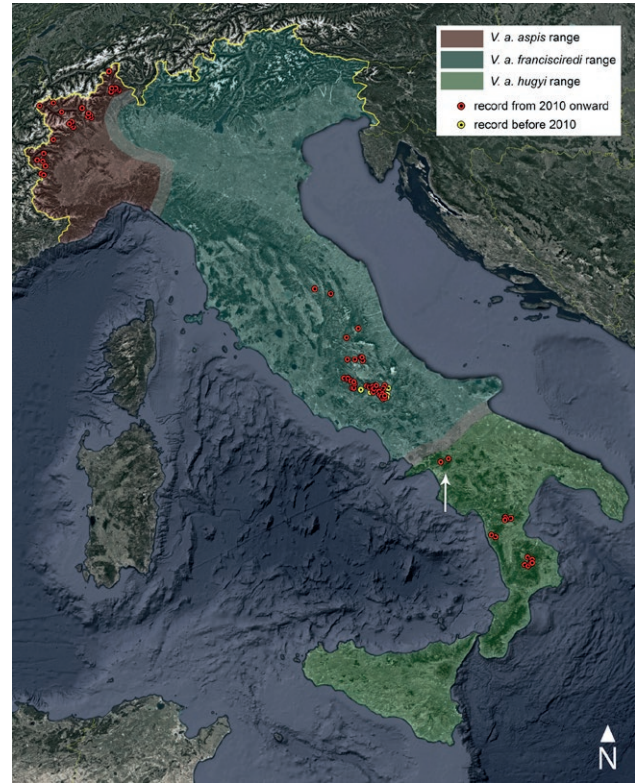
A total of 124 eligible observations of melanistic *Vipera aspis* were collected (40 through personal surveys, 84 through collaborators and citizen science), carried out between 1984 and 2023, of which 90% ( $n = 112$ ) were from 2010 onwards (Table S1). The altitudinal range of the records covered from 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*

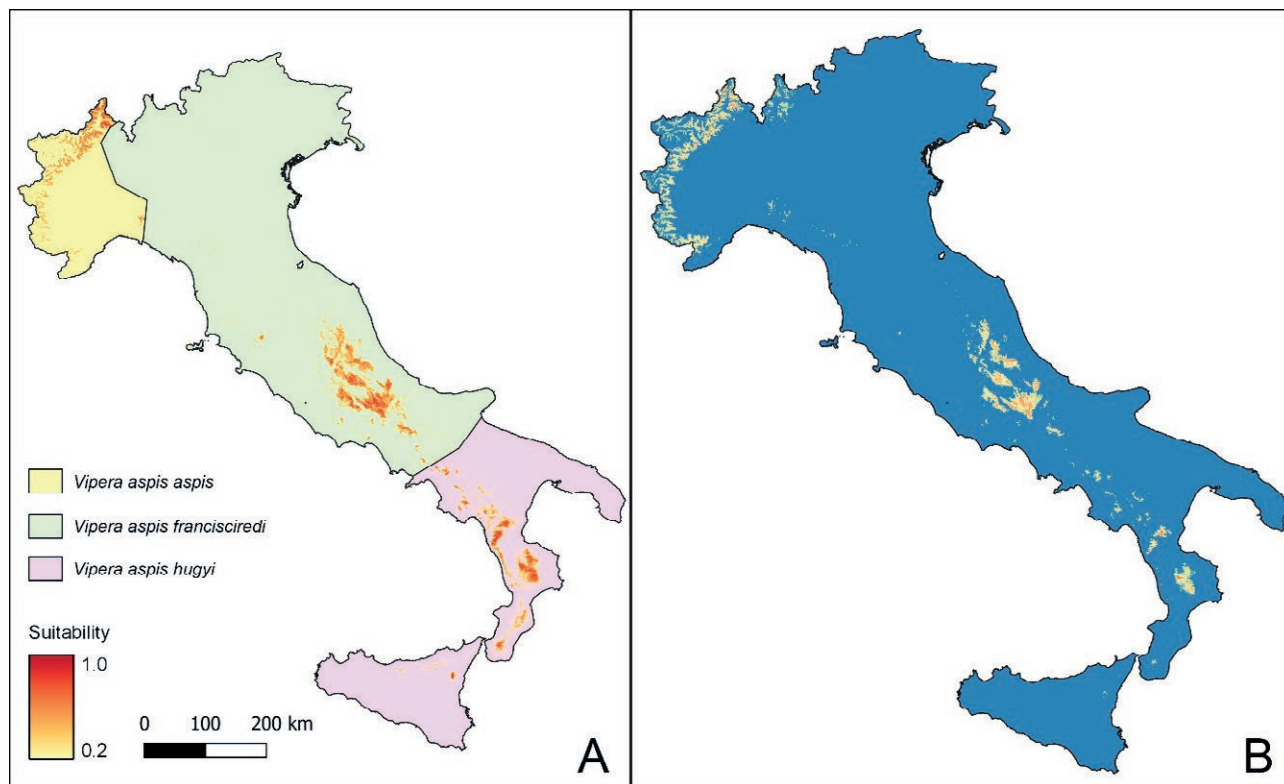
All models computed showed robust statistical performances (see Table S1). Mean annual temperature (bio1) was the most influential predictor for all models (42.9-84.1% contribution). Given the high correlation between bio1 and elevation ( $r < -0.9$ ), the latter was the most important predictor for 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,



**Fig. 2.** Map of Italy with the 124 verified observations of melanistic *Vipera aspis* (the positioning of each point is slightly approximated for conservation reasons). The approximative subspecies range is in agreement with Barbanera et al. (2009). Two records indicated by a white arrow come from an area (i.e., Monti Picentini, Campania) of possible intergradation between the subspecies *hugyi* and *francisciredi*, where non-melanistic individuals recurrently have dorsal patterns that can be defined as intermediate between those of the two taxa (MRDN personal obs.). Map credits: Google Earth. Data SIO, NOAA, U.S. Navy, NGA, GEBCO. Image Landsat / Copernicus (modified).

models for *V. a. francisciredi* in Central Italy were mostly 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.

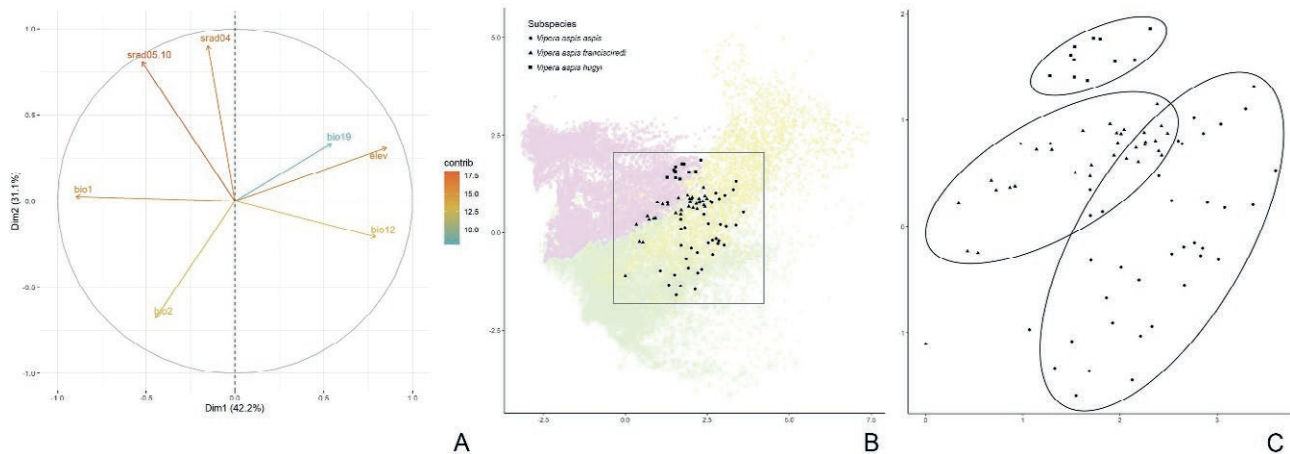
Areas predicted as suitable for melanistic individuals of *V. aspis* were in agreement across the four model combinations and also between the ensemble model and the independent models computed



**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).

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. 4.** Principal component analysis of melanistic individuals of *Vipera aspis* in Italy. A) Bioclimatic variables used for the analysis, defined by their contribution to the first two dimensions (warmer colours indicate higher contribution). B) Distribution of the background (coloured points) and occurrence records (black points) represented in bidimensional environmental space, divided by subspecies (different colours and shapes). C) Focus on the inset of the panel B, showing only the occurrence records (ellipses indicate the 95% confidence interval).

### Principal component analysis

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; Turrise 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 Lepontine 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 (*sensu* Fănaru 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-Freiria 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 melanisms 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; Fănaru 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 microhabitat 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.

#### ACKNOWLEDGEMENTS

The authors would like to thank all the people who have kindly provided us with their finding data and photos of melanistic *Vipera aspis* from Italy: Angela Tomei, Angelina Iannarelli, Bruno Di Cecco, Carlo Zanetta, Chiara Santini, Daniel Li Veli, Daniele Seglie, Domenico Belcastro, Emanuele Lotti, Ezio Giuliano, Fabrizio Bernardi, Fabrizio Moglia, Federica Gaydou, Francesco Di Toro, Francesco Raffaele, Gaetano De Persiis, Giandomenico La Barbera, Gianluca Congi, Gianni Liguori, Giulia Tessa, Luciano Caporale, Maria Felicia Coppola, Mario Marconi, Maurizio Caniglia, Maurizio D'Amico, Maurizio Lo Fiego, Mauro Saltarelli, Michela Mastrella, Mimmo Ippolito, Mirko Galuppi, Pasquale Buonpane, Raffaele Ferretti, Sergio De Santis, Tiziano Fioriti and Valter Rossotti. Author's records were carried out in field surveys authorized by the following permits: MATTM reg. 0012442, 13.06.2017; ISPRA Prot. 25913, 26.5.2017; Parco Nazionale d'Abruzzo, Lazio e Molise Prot. N.

0005299/2017, 26/06/2017; MATTM reg. 0083124, 16/10/2020; Prot. ISPRA 46387, 12/10/2020; MITE reg. 0024526, 28.2.2022; Prot. ISPRA 0009384/2022 del 23.02.2022; Parco Nazionale d'Abruzzo, Lazio e Molise Prot. N. 0004651/2022, 07/04/2022. AVP is supported by the Natural Environment Research Council grant number NE/S007164/1.

#### SUPPLEMENTARY MATERIAL

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

#### REFERENCES

- Allen, W.L., Baddeley, R., Scott-Samuel, N.E., Cuthill, I.C. (2013): The evolution and function of pattern diversity in snakes. *Behav. Ecol.* **24**: 1237-1250.
- Andersen, D., Litvinchuk, S.N., Jang, H.J., Jiang, J., Koo, K.S., Maslova, I., Kim, D., Jang, Y., Borzée, A. (2022): Incorporation of latitude-adjusted bioclimatic variables increases accuracy in species distribution models. *Ecol. Model.* **469**: 109986.
- André, G., Lavergne, S., Carcaillet, C. (2023): Unsuspected prevalence of *Pinus cembra* in the high-elevation sky islands of the western Alps. *Plant Ecol.* **224**: 865-873.
- Andrén, C., Nilson, G. (1981): Reproductive success and risk of predation in normal and melanistic colour morphs of the adder, *Vipera berus*. *Biol. J. Linn. Soc.* **15**: 235-246.
- Attorre, F., Alfo', M., De Sanctis, M., Francesconi, F., Bruno, F. (2007): Comparison of interpolation methods for mapping climatic and bioclimatic variables at regional scale. *Int. J. Climatol.* **27**: 1825-1843.
- Baena, O., Oliveras, I. (2015): Nou cas de melanisme en *Vipera aspis* (Linnaeus, 1758) al massís del Montseny (Vallès Oriental; Catalunya). *Butll. Soc. Catalana d'Herpetologia* **22**: 29-30.
- Barbanera, F., Zuffi, M.A.L., Guerrini, M., Gentili, A., Tofanelli, S., Fasola, M., Dini, F. (2009): Molecular phylogeography of the asp viper *Vipera aspis* (Linnaeus, 1758) in Italy: Evidence for introgressive hybridization and mitochondrial DNA capture. *Mol. Phylogenet. Evol.* **52**: 103-114.
- Bassini, E., Bruno, S., Mazzei, P., Stagni, G. (1991): I serpenti dell'Emilia-Romagna e in particolare della provincia di Bologna (Italia settentrionale). *Mediterranea. Serie de Estudios Biológicos*, N. 13 (1991); Pp. 27-66.
- Benito, M., Pérez-Salerno, A., Gómez, S., Albero, L. (2022): New cases of melanism in *Chalcides striatus*, *Coronella austriaca*, and *Vipera seoanei* from Burgos, northern Spain. *Herpetol. Notes* **15**: 687-689.
- Bittner, T.D., King, R.B., Kerfin, J.M. (2002): Effects of body size and melanism on the thermal biology of garter snakes (*Thamnophis sirtalis*). *Copeia* **2002**: 477-482.
- Bonanomi, G., Zotti, M., Mogavero, V., Cesarano, G., Saulino, L., Rita, A., Tesei, G., Allegranza, M., Saracino, A., Allevato, E. (2020): Climatic and anthropogenic factors explain the variability of *Fagus sylvatica* treeline elevation in fifteen mountain groups across the Apennines. *For. Ecosyst.* **7**: 5.
- Borgianni, N., Paolino, G. (2020): Melanism in *Vipera aspis francisciredi* (Laurenti, 1768) (Reptilia Serpentes) in the Lazio Region (Parco Naturale Regionale dei Monti Simbruini), Italy. *Biodivers. J.* **11**: 615-618.
- Boria, R.A., Olson, L.E., Goodman, S.M., Anderson, R.P. (2014): Spatial filtering to reduce sampling bias can improve the performance of ecological niche models. *Ecol. Model.* **275**: 73-77.
- Brodmann, P. (1987): Die Giftschlangen Europas und die Gattung *Vipera* in Afrika und Asien. Bern, Kümmerly u. Frey.
- Broennimann, O., Ursenbacher, S., Meyer, A., Golay, P., Monney, J.-C., Schmocker, H., Guisan, A., Dubey, S. (2014): Influence of climate on the presence of colour polymorphism in two montane reptile species. *Biol. Lett.* **10**: 20140638.
- Brooks, J., Rohrer, J., Beck, D.D. (2022): Color Variation, Tail Banding, and Sexual Dichromatism in Washington Populations of Northern Pacific Rattlesnakes, *Crotalus oreganus*. *Herpetologica* **78**: 192-200.
- Bruni, G., Di Nicola, M.R., Banfi, F., Faraone, F.P. (2022): Distribution and characterization of melanism in Grass Snakes from Italy. *Nat. Sicil.* **46**: 41-48.
- Bruno, S. (1976): L'ornamentazione della *Vipera aspis* (L., 1758) in Italia (Serpentes Viperidae) (Studi sulla fauna erpetologica italiana. XXI). *Atti Soc. ital. Sci. Nat. Mus. Civ. Stor. Nat. Milano* **117**: 165-194.
- Bruno, S. (1985): Le vipere d'Italia e d'Europa. Edagricole, Bologna.
- Bury, S., Kolanek, A., Chylarecki, P., Najbar, B., Kurek, K., Mazgajski, T.D. (2022): Climatic conditions and prevalence of melanistic snakes—contrasting effects of warm springs and mild winters. *Int. J. Biometeorol.* **66**: 1329-1338.
- Bury, S., Mazgajski, T.D., Najbar, B., Zając, B., Kurek, K. (2020): Melanism, body size, and sex ratio in snakes—new data on the grass snake (*Natrix natrix*) and synthesis. *Sci. Nat.* **107**: 22.

- Capula, M., Luiselli, L. (1994): Reproductive strategies in alpine adders, *Vipera berus*. The black females bear more often. *Acta Oecol.* **15**: 207-214.
- Castella, B., Golay, J., Monney, J. -C., Golay, P., Mebert, K., Dubey, S. (2013): Melanism, body condition and elevational distribution in the asp viper. *J. Zool.* **290**: 273-280.
- Chang, C., Zheng, R. (2003): Effects of ultraviolet B on epidermal morphology, shedding, lipid peroxide, and antioxidant enzymes in Cope's rat snake (*Elaphe taeniura*). *J. Photoch. Photobio. B* **72**: 79-85.
- Clusella Trullas, S., Terblanche, J.S., Van Wyk, J.H., Spotila, J.R. (2007a): Low repeatability of preferred body temperature in four species of Cordylid lizards: Temporal variation and implications for adaptive significance. *Evol. Ecol.* **21**: 63-79.
- Clusella Trullas, S., Van Wyk, J.H., Spotila, J.R. (2007b): Thermal melanism in ectotherms. *J. Therm. Biol.* **32**: 235-245.
- Clusella-Trullas, S., Terblanche, J.S., Blackburn, T.M., Chown, S.L. (2008): Testing the thermal melanism hypothesis: a macrophysiological approach. *Funct. Ecol.* **22**: 232-238.
- Cox, C.L., Davis Rabosky, A.R. (2013): Spatial and Temporal Drivers of Phenotypic Diversity in Polymorphic Snakes. *Am. Nat.* **182**: E40-E57.
- Cox, C.L., Rabosky, A.R.D., Chippindale, P.T. (2013): Sequence variation in the Mc1r gene for a group of polymorphic snakes. *Gene* **513**: 282-286.
- Cyriac, V.P., Kodandaramaiah, U. (2019): Conspicuous colours reduce predation rates in fossorial uropeltid snakes. *PeerJ* **7**: e7508.
- De Avila, F., Oliveira, J., De Oliveira, M., Borges-Martins, M., Valiati, V.H., Tozetti, A. (2019): Does color polymorphism affect the predation risk on *Phalotris lemniscatus* (Duméril, Bibron and Duméril, 1854) (Serpentes, Dipsadidae)? *Acta Herpetol.* **14**: 57-63.
- De Smedt, J. (2006): *The Vipers of Europe*. Halblech, JDS Verlag.
- Delhey, K. (2017): Gloger's rule. *Curr. Biol.* **27**: R689-R691.
- Di Nicola, M.R. (2019): A revised dichotomous key to the snakes of Italy (Reptilia, Squamata, Serpentes), according to recent systematic updates. *Zootaxa* **4686**: 294-296.
- Di Nicola, M.R., Cavigioli, L., Luiselli, L., Andreone, F. (2019): *Anfibi & Rettili d'Italia*. Edizioni Belvedere, Latina.
- Di Nicola, M.R., Cavigioli, L., Luiselli, L., Andreone, F. (2021): *Anfibi & Rettili d'Italia*. Edizione aggiornata. Edizioni Belvedere, Latina.
- Di Nicola, M.R., Faraone, F.P. (2020): *Vipera aspis hugyi* (Southern Italian Asp). *Coloration. Herpetol. Rev.* **51**: 631.
- Di Nicola, M.R., Faraone, F.P., Zabbia, T. (2022): An updated dichotomous key to the snakes of Europe. *Basic Appl. Herpetol.* **36**: 47-64.
- Di Nicola, M.R., Meier, G.J. (2013): *Vipera aspis hugyi* (Southern Italian asp) melanism. *Herpetol. Rev.* **44**: 698.
- Dubey, S., Zwahlen, V., Mebert, K., Monney, J.-C., Golay, P., Ott, T., Durand, T., Thiery, G., Kaiser, L., Geser, S.N., Ursenbacher, S. (2015): Diversifying selection and color-biased dispersal in the asp viper. *BMC Evol. Biol.* **15**: 99.
- Ducrest, A.-L., Ursenbacher, S., Golay, P., Monney, J.-C., Mebert, K., Roulin, A., Dubey, S. (2014): Pro-opiomelanocortin gene and melanin-based colour polymorphism in a reptile: Colour Polymorphism. *Biol. J. Linn. Soc. Lond.* **111**: 160-168.
- Elith, J., Phillips, S.J., Hastie, T., Dudík, M., Chee, Y.E., Yates, C.J. (2011): A statistical explanation of MaxEnt for ecologists: Statistical explanation of MaxEnt. *Divers. Distrib.* **17**: 43-57.
- Fănar, G., Telea, A.E., Gherghel, I., Melenciuc, R. (2022): Melanism in the grass snake *Natrix natrix* (Linnaeus, 1758) from the Danube Delta Biosphere Reserve, Romania. *Herpetozoa* **35**: 257-263.
- Fick, S.E., Hijmans, R.J. (2017): WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. *Int. J. Climatol.* **37**: 4302-4315.
- Fielding, A.H., Bell, J.F. (1997): A review of methods for the assessment of prediction errors in conservation presence/absence models. *Envir. Conserv.* **24**: 38-49.
- Forsman, A. (2016): Is colour polymorphism advantageous to populations and species? *Mol. Ecol.* **25**: 2693-2698.
- Forsman, A., Ahnesjö, J., Caesar, S., Karlsson, M. (2008): A model of ecological and evolutionary consequences of color polymorphism. *Ecology* **89**: 34-40.
- Fu, T.-T., Sun, Y.-B., Gao, W., Long, C.-B., Yang, C.-H., Yang, X.-W., Zhang, Y., Lan, X.-Q., Huang, S., Jin, J.-Q., Murphy, R.W., Zhang, Y., Lai, R., Hillis, D.M., Zhang, Y.-P., Che, J. (2022): The highest-elevation frog provides insights into mechanisms and evolution of defenses against high UV radiation. *Proc. Natl. Acad. Sci. U.S.A.* **119**: e2212406119.
- García-Roa, R.G., Carbonell, G. (2020): The dark side of *Vipera aspis*: a case of melanism in the Iberian Peninsula. *Bol. Asoc. Herpetol. Esp.* **31**: 34-36.
- GBIF.org (2023): GBIF Occurrence Download <https://doi.org/10.15468/dl.3nht55>.
- Geniez, P. (2015): *Serpents d'Europe, d'Afrique du Nord et du Moyen-Orient*. Paris, Delachaux et Niestlé.
- Gibson, A.R., Falls, B. (1979): Thermal biology of the common garter snake *Thamnophis sirtalis* (L.): II. The effects of melanism. *Oecologia* **43**: 99-109.

- Golay, P., Monney, J.-C., Conelli, A., Durand, T., Thiery, G., Zuffi, M.A.L., Ursenbacher, S. (2008): Systematics of the Swiss asp vipers: some implications for the European *Vipera aspis* (Linnaeus, 1758) complex (Serpentes: Viperidae) - A tribute to Eugen Kramer. *Amphibia-Reptilia* **29**: 71-83.
- Goldenberg, J., D'Alba, L., Bisschop, K., Vanthournout, B., Shawkey, M.D. (2021): Substrate thermal properties influence ventral brightness evolution in ectotherms. *Commun. Biol.* **4**: 26.
- Grano, M., Meier, G., Cattaneo, C. (2017): Vipere Italiane. Gli ultimi studi sulla sistematica, l'ecologia e la storia naturale. Aicurzio, Castel Negrino.
- Hantak, M.M., Guralnick, R.P., Cameron, A.C., Griffing, A.H., Harrington, S.M., Weinell, J.L., Paluh, D.J. (2022): Colour scales with climate in North American ratsnakes: a test of the thermal melanism hypothesis using community science images. *Biol. Lett.* **18**: 20220403.
- Huey, R.B., Kingsolver, J.G. (1989): Evolution of thermal sensitivity of ectotherm performance. *Trends Ecol. Evol.* **4**: 131-135.
- Jin, Y., Tong, H., Shao, G., Li, J., Lv, Y., Wo, Y., Brown, R.P., Fu, C. (2020): Dorsal Pigmentation and Its Association with Functional Variation in MC1R in a Lizard from Different Elevations on the Qinghai-Tibetan Plateau. *Genome Biol. Evol.* **12**: 2303-2313.
- Jong, P.W.D., Gussekloo, S.W.S., Brakefield, P.M. (1996): Differences in Thermal Balance, Body Temperature and Activity Between Non-Melanic and Melanic Two-Spot Ladybird Beetles (*Adalia bipunctata*) Under Controlled Conditions. *J. Exp. Biol.* **199**: 2655-2666.
- Kalogiannis, S. (2021): Cases of melanism in *Dolichophis caspius* (Gmelin, 1789) (Squamata: Colubridae) from Greece and a new distribution record. *Parnassiana Archives* **9**: 19-22.
- Kassambara, A., Mundt, F. (2020): Factoextra: Extract and Visualize the Results of Multivariate Data Analyses. R Package Version 1.0.7.
- Körner, C. (2007): The use of 'altitude' in ecological research. *Trends Ecol. Evol.* **22**: 569-574.
- Kumar, B. (2009): Nove najdbe laškega gada (*Vipera aspis*) v Sloveniji. *Natura Sloveniae* **11**: 59-63.
- Lê, S., Josse, J., Husson, F. (2008): FactoMineR : An R Package for Multivariate Analysis. *J. Stat. Soft.* **25**.
- Lorioux, S., Bonnet, X., Brischoux, F., De Crignis, M. (2008): Is melanism adaptive in sea kraits? *Amphibia-Reptilia* **29**: 1-5.
- Lucchini, N., Kaliontzopoulou, A., Val, G.A., Martínez-Freiría, F. (2020): Sources of intraspecific morphological variation in *Vipera seoanei*: allometry, sex, and colour phenotype. *Amphibia-Reptilia* **42**: 1-16.
- Luiselli, L. (1992): Reproductive Success in Melanistic Adders: A New Hypothesis and Some Considerations on Andrén and Nilson's (1981) Suggestions. *Oikos* **64**: 601.
- Luiselli, L. (1993): The ecological role of color polymorphism in male adders *Vipera berus*: testing the hypotheses. *Rev. Ecol.* **48**: 49-56.
- Madsen, T. (1987): Are Juvenile Grass Snakes, *Natrix natrix*, Aposematically Coloured? *Oikos* **48**: 265.
- Madsen, T., Stille, B. (1988): The Effect of Size Dependent Mortality on Colour Morphs in Male Adders, *Vipera berus*. *Oikos* **52**: 73.
- Majerus, M. (1998): Melanism: evolution in action. Oxford university press, Oxford, New York, Tokyo.
- Martínez-Freiría, F., Toyama, K.S., Freitas, I., Kaliontzopoulou, A. (2020): Thermal melanism explains macroevolutionary variation of dorsal pigmentation in Eurasian vipers. *Sci. Rep.* **10**: 16122.
- Masseti, M., Zuffi, M.A.L. (2011): On the origin of the asp viper *Vipera aspis hugyi* Schinz, 1833, on the island of Montecristo, Northern Tyrrhenian Sea (Tuscan archipelago, Italy). *Herpetol. Bull.* **117**: 1-9.
- Matthews, G., Goulet, C.T., Delhey, K., Chapple, D.G. (2016): The effect of skin reflectance on thermal traits in a small heliothermic ectotherm. *J. Therm. Biol.* **60**: 109-124.
- McRobie, H.R., Moncrief, N.D., Mundy, N.I. (2019): Multiple origins of melanism in two species of North American tree squirrel (*Sciurus*). *BMC Evol. Biol.* **19**: 140.
- Mebert, K., Zwahlen, V., Golay, P., Thierry, D., Ursenbacher, S. (2011): Ungewöhnlich hoher Farb-Polymorphismus in alpinen Aspisvipern in Frankreich - Zufall oder natürliche Selektion? *Elaphe* **1**: 13-19.
- Monney, J.-C., Luiselli, L., Capula, M. (1995): Correlates of melanism in a population of adders (*Vipera berus*) from the Swiss Alps and comparisons with other alpine populations. *Amphibia-Reptilia* **16**: 323-330.
- Monney, J.-C., Luiselli, L., Capula, M. (1996): Taille et mélanisme chez *Vipera aspis* dans les Préalpes suisses et en Italie centrale et comparaison avec différentes populations alpines de *Vipera berus*. *Rev. Suisse Zool.* **103**: 81-100.
- Moreno Azócar, D.L., Nayan, A.A., Perotti, M.G., Cruz, F.B. (2020): How and when melanic coloration is an advantage for lizards: the case of three closely-related species of *Liolaemus*. *Zoology* **141**: 125774.
- Muri, D., Schuerch, J., Trim, N., Golay, J., Baillifard, A., El Taher, A., Dubey, S. (2015): Thermoregulation and microhabitat choice in the polymorphic asp viper (*Vipera aspis*). *J. Therm. Biol.* **53**: 107-112.
- Pérez-Tris, J., Bensch, S., Carbonell, R., Helbig, A.J., Tellera, J.L. (2004): Historical diversification of migration patterns in a passerine bird. *Evolution* **58**: 1819.

- Pernetta, A.P., Reading, C.J. (2009): Observations of two melanistic smooth snakes (*Coronella austriaca*) from Dorset, United Kingdom. *Acta Herpetol.* **4**: 109-112.
- Phillips, S.J., Anderson, R.P., Dudík, M., Schapire, R.E., Blair, M.E. (2017): Opening the black box: an open-source release of Maxent. *Ecography* **40**: 887-893.
- Pizzatto, L., Dubey, S. (2012): Colour-polymorphic snake species are older. *Biol. J. Linn. Soc.* **107**: 210-218.
- Pizzigalli, C., Banfi, F., Ficetola, G.F., Falaschi, M., Mangiacotti, M., Sacchi, R., Zuffi, M.A.L., Scali, S. (2020): Eco-geographical determinants of the evolution of ornamentation in vipers. *Biol. J. Linn. Soc.* **130**: 345-358.
- Pottier, G. (2001): Notes sur trois cas de mélanisme chez *Vipera aspis zinnikeri* Kramer, 1958 (Ophidia, Viperidae) dans les Hautes-Pyrénées (France). *Bull. Soc. Herpétol. Fr.* 49-53.
- QGIS Development Team (2020): QGIS Geographic Information System. Open Source Geospatial Foundation Project, <http://qgis.osgeo.org>.
- R Core Team (2023): R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria, <https://www.R-project.org>.
- Regnet, R.A., Quintela, F.M., Roedder, D., Loebmann, D. (2022): Attributes of the population structure and ventral polychromatism of *Helicops infrataeniatus* Jan, 1865 (Serpentes, Colubridae, Dipsadinae) in subtropical Brazil. *North-West. J. Zool.* **18**: 161-167.
- Reguera, S., Zamora-Camacho, F. J., & Moreno-Rueda, G. (2014): The lizard *Psammotromus algirus* (Squamata: Lacertidae) is darker at high altitudes. *Biol. J. Linn. Soc.* **112**: 132-141.
- Rivera, X., Arribas, O., Martí, F. (2001): Anomalías pigmentarias en las especies de reptiles presentes en la Península Ibérica, Islas Baleares y Canarias. *Butll. Soc. Catalana d'Herpetologia* **15**: 76-88.
- Ruane, S., Myers, E.A., Lo, K., Yuen, S., Welt, R.S., Juman, M., Futterman, I., Nussbaum, R.A., Schneider, G., Burbrink, F.T., Raxworthy, C.J. (2018): Unrecognized species diversity and new insights into colour pattern polymorphism within the widespread Malagasy snake *Mimophis* (Serpentes: Lamprophiidae). *Syst. Biodivers.* **16**: 229-244.
- San-Jose, L.M., Gonzalez-Jimenez, V., Fitze, S. (2008): Frequency and phenotypic differences of a melanistic and normally colored common lizard, *Lacerta (Zootoca) vivipara* of the Southern Pyrenees (Spain). *Herp. Rev.* **39**: 422-425.
- San-Jose, L.M., Roulin, A. (2018): Toward Understanding the Repeated Occurrence of Associations between Melanin-Based Coloration and Multiple Phenotypes. *Am. Nat.* **192**: 111-130.
- Santos, X., Azor, J.S., Cortés, S., Rodríguez, E., Larios, J., Pleguezuelos, J.M. (2018): Ecological significance of dorsal polymorphism in a Batesian mimic snake. *Curr. Zool.* **64**: 745-753.
- Senczuk, G., Gramolini, L., Avella, I., Mori, E., Menchetti, M., Aloise, G., Castiglia, R. (2021): No association between candidate genes for color determination and color phenotype in *Hierophis viridiflavus*, and characterization of a contact zone. *J. Zool. Syst. Evol. Res.* **59**: 748-759.
- Sherbrooke, W.C., Frost, S.K. (1989): Integumental chromatophores of a color-change, thermoregulating lizard, *Phrynosoma modestum* (Iguanidae; Reptilia). *Am. Mus. Novit.* **2943**: 1-14.
- Shine, R.G. (1993): Sexual dimorphism in snakes. In: *Snakes: Ecology and Behavior*, p. 49-86. Seigel, R.A., Collins, J.T, Eds, McGraw-Hill, New York.
- Sindaco, R., Razzetti, E. (2021): An updated check-list of Italian amphibians and reptiles. *Nat. Hist. Sci.* **8**: 35-46.
- Speybroeck, J., Beukema, W., Bok, B., Van Der Voort, J., Velikov, I. (2016): Field guide to the amphibians & Reptiles of Britain and Europe. Bloomsbury, London, Oxford, New York, New Dehli, Sydney.
- Støa, B., Halvorsen, R., Stokland, J.N., Gusarov, V.I. (2019): How much is enough? Influence of number of presence observations on the performance of species distribution models. *Sommerfeltia* **39**: 1-28.
- Storniolo, F., Mangiacotti, M., Zuffi, M.A.L., Scali, S., Sacchi, R. (2023): Large scale phenotypic characterisation of *Hierophis viridiflavus* (Squamata: Serpentes): climatic and environmental drivers suggest the role of evolutionary processes in a polymorphic species. *Evol. Ecol.* **37**: 419-434.
- Strugariu, A., Zamfirescu, Ş.R. (2011): Population characteristics of the adder (*Vipera berus berus*) in the Northern Romanian Carpathians with emphasis on colour polymorphism: is melanism always adaptive in vipers? *Anim. Biol.* **61**: 457-468.
- Tanaka, K. (2009): Does the Thermal Advantage of Melanism Produce Size Differences in Color-dimorphic Snakes? *Zool. Sci.* **26**: 698-703.
- Tessa, G. (2016): Preliminary data on distribution of a rare dorsal pattern in *Vipera aspis aspis* (Ophidia: Viperidae) in the Gran Paradiso National Park. In: *Atti XI Congresso Nazionale Della Societas Herpetologica Italica*, pp. 22-25. Menegon, M., Rodriguez-Prieto, A., Deflorian, M.C., Eds, Ianieri Edizioni, Pescara.
- True, J.R. (2003): Insect melanism: the molecules matter. *Trends Ecol. Evol.* **18**: 640-647.
- Turrisi, G.F., Vaccaro, A. (2001): Distribuzione altitudinale di anfibi e rettili sul monte Etna (Sicilia orientale). *Pianura*, **13**: 335-338.

- Turrise, G.F., Vaccaro, A. (2004): Anfibi e rettili del Monte Etna (Sicilia orientale). *Boll. Accad. Gioenia Sci. Nat* **36**: 5-103.
- Ursenbacher, S., Conelli, A., Golay, P., Monney, J.-C., Zuffi, M.A.L., Thiery, G., Durand, T., Fumagalli, L. (2006): Phylogeography of the asp viper (*Vipera aspis*) inferred from mitochondrial DNA sequence data: Evidence for multiple Mediterranean refugial areas. *Mol. Phylogenet. Evol.* **38**: 546-552.
- van Proosdij, A.S.J., Sosef, M.S.M., Wieringa, J.J., Raes, N. (2015): Minimum required number of specimen records to develop accurate species distribution models. *Ecography* **39**: 542-552.
- Watt, W.B. (1968): Adaptive Significance of Pigment Polymorphisms in *Colias* Butterflies. I. Variation of Melanin Pigment in Relation to Thermoregulation. *Evolution* **22**: 437.
- Wickham, H. (2016): *ggplot2: elegant graphics for data analysis*. Switzerland, Springer.
- Wolf, M., Werner, Y.L. (1994): The striped colour pattern and striped/non-striped polymorphism in snakes (Reptilia: Ophidia). *Biol. Rev.* **69**: 599-610.
- Zuffi, M.A.L. (1984): Cenni sulla distribuzione dell'ofidofauna in un ambiente tipo dell'Appennino pavese: fasce preferenziali e loro condizioni ecologiche. *Natura, Milano* **75**: 65-68.
- Zuffi, M.A.L. (2002): A critique of the systematic position of the asp viper subspecies *Vipera aspis aspis* (Linnaeus, 1758), *Vipera aspis atra* Meisner, 1820, *Vipera aspis francisciredi* Laurenti, 1768, *Vipera aspis hugyi* Schinz, 1833 and *Vipera aspis zinnikeri* Kramer, 1958. *Amphibia-Reptilia* **23**: 191-213.
- Zuffi, M.A.L. (2006): *Vipera aspis* (Linnaeus, 1758). In: *Atlante Degli Anfibi e Dei Rettili d'Italia*, pp. 594-599. Sindaco, R., Doria, G., Razzetti, E., Bernini, F., Eds, Edizioni Polistampa, Firenze.
- Zuffi, M.A.L., Gentili, A., Luiselli, L. (2011): *Vipera aspis* (Linnaeus, 1758). In: *Fauna d'Italia - Vol. XLV - Reptilia*, pp. 608-617. Corti, C., Capula, M., Luiselli, L., Razzetti, E., Sindaco, R., Eds, Calderini, Bologna.