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GIACOMO BRUNI, MATTEO RICCARDO DI NICOLA, FEDERICO BANFI
& FRANCESCO PAOLO FARAONE

DISTRIBUTION AND CHARACTERIZATION OF MELANISM IN GRASS SNAKES FROM ITALY

SUMMARY

In snakes, melanism can improve thermoregulation efficiency, reproductive success and provide additional protection to internal organs from solar radiation. For these reasons, such conditions could be beneficial for animals living in both cold and warm environments. For widely distributed species of grass snake (genus *Natrix*), several single encounters of melanic individuals are reported in the literature. In the period 2015-2021 a significant number of unpublished observations of melanic *Natrix helvetica* and *N. natrix* were collected on the Italian territory and integrated with bibliographic data. The present work illustrates the chromatic variation within melanic individuals and investigates their distribution. Within the chromatic variability of melanic individuals, two main forms of melanism were detected in *N. helvetica*. They show an almost parapatric distribution throughout the national territory which raises interesting questions about their potential adaptive advantages in different ecological and climatic contexts.

Key words: *Natrix*, thermoregulation, chromatic variation, ecology.

RIASSUNTO

Distribuzione e caratterizzazione del melanismo nelle natrix dal collare in Italia. Nei serpenti, il melanismo può incrementare l'efficienza della termoregolazione, il successo riproduttivo e costituire una protezione aggiuntiva per gli organi interni nei confronti della radiazione solare. Per queste ragioni, questa condizione può essere vantaggiosa per animali che vivono sia in ambienti freddi, sia caldi. Per le specie ad ampia distribuzione appartenenti al genere *Natrix*, sono riportati vari casi di melanismo, per la maggior parte riferiti a osservazioni di singoli individui. Durante il periodo compreso fra il 2015 e il 2021 è stato raccolto un numero significativo di osservazioni inedite di individui melanici di *Natrix helvetica* e *N. natrix* all'interno del territorio italiano, integrandole con dati bibliografici. In questo contributo viene descritta la variazione cromatica all'interno del campione di natrix melaniche e analizzata la loro distribuzione. Sono state individuate due principali varianti

cromatiche nel campione di individui melanici di *N. helvetica*. Esse mostrano una distribuzione quasi paraptrica lungo il territorio nazionale, la quale pone alcuni interessanti spunti sui potenziali vantaggi del melanismo in differenti contesti ecologici e climatici.

Parole chiave: *Natrix*, termoregolazione, variazione cromatica, ecologia.

INTRODUCTION

Several cases of chromatic anomalies are known for the grass snakes *Natrix natrix* and *N. helvetica*, concerning albino, leucistic and mostly melanic individuals (e.g., KABISCH, 1974; BRUNO, 1984; JANDZÍK, 2004; GVOZDENOVIC & SCHWEIGER, 2014; BRUNI, 2017). Melanism consists in an abnormally high production or over-dispersion of melanin pigments (BECHTEL, 1978) making the animal entirely, or almost entirely black (ZUFFI, 2008). In snakes, melanism can occur in two different forms, via an expansion of black areas which can cover all the skin surface or through a diffuse darkening of the background color which allows to distinguish the markings of the typical pattern in certain conditions (BOULENGER, 1913). The dark or black coloration may represent an advantage in thermoregulation (GIBSON & FALLS, 1979) even at low temperatures (ZUFFI, 2008), in reproductive success (CAPULA & LUISELLI, 1994) and in protection of internal organs from solar radiation (BECHTEL, 1978). In Italy, reports of individuals with dark/black coloration are present in the literature but they usually refer to observations of single individuals (LANZA, 1983; SCALI *et al.*, 2011; LUNGI *et al.*, 2019). *Natrix natrix* and *N. helvetica* are considered polytypic, with several subspecies currently recognized (see FRITZ & SCHMIDTLER, 2020). Regarding Italy, the following taxa are present on the national territory: *N. helvetica cetti* Gené, 1839, endemic to Sardinia; *N. helvetica sicula* (Cuvier, 1829), distributed in Sicily and in peninsular Italy except for the north-eastern portion; *N. natrix* ssp. distributed in E Veneto, NE Emilia-Romagna and Friuli-Venezia Giulia (SCHULTZE *et al.*, 2020; ASZTALOS *et al.*, 2021; DI NICOLA *et al.*, 2021). In the present study a significant number of observations of melanic grass snakes was collected throughout the entire Italian Peninsula, Sicily and Sardinia, to evaluate the presence of different types of melanism and their distributional patterns. This is the first extensive study on melanism occurrence in grass snakes at a national level.

MATERIALS AND METHODS

The data were collected in the period 2015-2021 from field observations made by the authors and from online data obtained through the mailing list of

the *Societas Herpetologica Italica*, from the platforms of Citizen Science, Ornitho.it and iNaturalist, and from social networks (mainly via the Facebook groups “Identificazione Anfibi e Rettili” and “Fauna Siciliana”). Other records were obtained from the scientific literature. The resulting dataset contains all coordinates and localities for all sources. Whenever localities were mentioned and clearly described but GPS coordinates were not provided, that information was tracked and obtained by Google Earth Pro software (version 7.3). Such extrapolated coordinates were not used in the extraction of elevation data for our analyses. When available, information was collected on the characteristics of the individual, such as sex and measurements. Snake images were examined to obtain information on the coloration in order to distinguish the different degrees of melanism. Within the chromatic variability of melanistic individuals we have identified two main forms of melanism (Fig. 1). Reference can be made to the *picturata* color morph (see JAN, 1864; HECHT, 1930; MERTENS, 1947), which consists of an intense black coloration with a variable amount of pale dots scattered over the entire surface of the body, the iris is



Fig. 1 — Examples of *picturata* (A-B) and *charcoal* (C-D) melanistic forms in *Natrix helvetica*: dorsal view of a *N. b. sicula* from Emilia-Romagna (A); head detail of a *N. b. sicula* from Tuscany (B); dorsal view of a *N. b. cetti* from Sardinia (C); head detail of a *N. b. sicula* from Sicily (D).

dark, the ventral side usually shows a variable white color at or near the throat and anterior portion of the belly, the transition from black to pale parts is mostly sharp. The second type of melanism, hereinafter referred to as charcoal, is characterized by an overall dark anthracite/bluish color, the dorsum is generally unpatterned except in the lower parts of the sides, where the normal pattern made of black bars down the sides is visible. The iris is generally grey; on the ventral part the usual white parts generally show a bluish color. The transition from dark to pale parts is shaded.

Discrimination at specific level was made on the basis of the known distribution of the two species in Italy (KINDLER *et al.*, 2017; SCHULTZE *et al.*, 2020; Di NICOLA *et al.*, 2021) and supported by morphological characteristics (SCALI *et al.*, 2011).

The collected observations were first imported in the Quantum Geographic Information System (QGIS 3.16) software (QGIS.ORG, 2021), then altitude data was extracted on those points with a resolution of 25 m from the raster layers of the European Copernicus Digital Elevation Model (EU-DEM) (COPERNICUS, 2016). The comparison of the altitudinal distribution between melanic morphs was carried out at an intraspecific level, considering only observations of *N. helvetica sicula*. *Natrix h. cetti* was excluded for its morpho-ecological distinctiveness (VANNI & CIMMARUTA, 2011). Prior to analyses, the altitudinal data for each color morph was checked for normality and according to the results (see below) we used the non-parametric Mann-Whitney U test.

RESULTS

A total of 232 records of melanic grass snakes come from new observations and bibliographic data (Fig. 2A). Of these records, 20 refer to observations from more than one individual (e.g., more than one melanic snake in the same basking site or a few meters from each other). The melanic forms *picturata* (86 observations) and *charcoal* (82 observations) show an almost parapatric distribution (Fig. 2B) with few exceptions. The *picturata* morph appears present exclusively northwards from the province of Salerno, occurring along the Apennines and the Alps in *N. helvetica sicula*, and in the Po Plain for *N. natrix*. The *charcoal* morph appears widely present in *Natrix helvetica sicula* in Sicily and southern Italy, south of the province of Salerno with sporadic records in central and northern Italy (Abruzzo, Emilia-Romagna, Tuscany and Lombardy). This morph is also present in the rare Sardinian grass snake *N. helvetica cetti* (see also LUNGHI *et al.*, 2016). The *charcoal* form was also found in *N. natrix* of Veneto in the Po Plain and perhaps could also

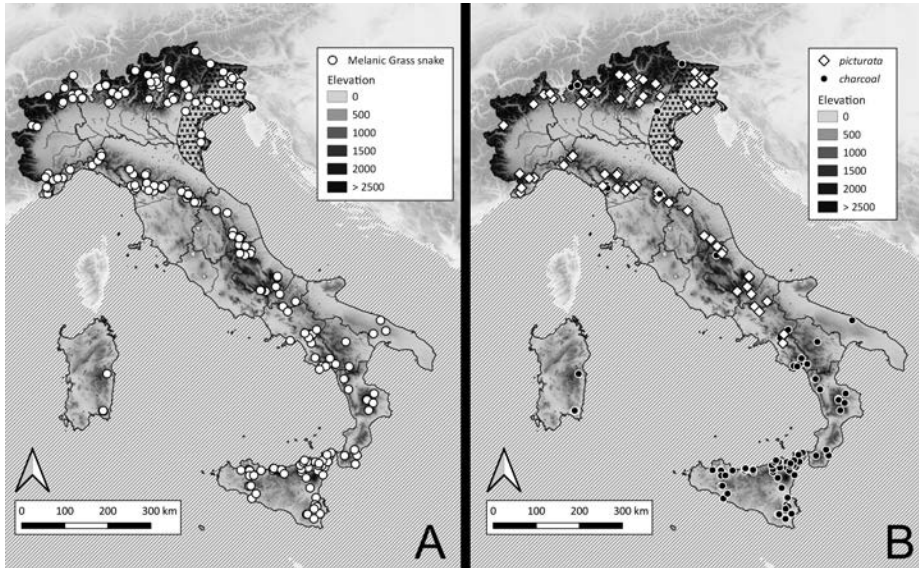


Fig. 2 — Distribution maps of melanic Grass snakes in Italy: total records of melanic Grass snakes (A); records of *picturata* and charcoal forms (B). The area covered by the “x” pattern represents the *Natrix natrix* range.

be found in the Alps (transition area between *N. natrix* and *N. helvetica*). The two melanistic forms are found in sympatry in the province of Salerno. The altitudes relative to the *picturata* and charcoal records have, respectively, the following descriptive statistic values [mean \pm SD (min-max) n]: 735.5 ± 367.5 (48-1775) 70; 520.9 ± 438.8 (8-2275) 73. The *picturata* observations show on average a significantly higher elevation than those of charcoal ($U = 1638.5$; $P < 0.001$) (Fig. 3).

DISCUSSION

The *picturata* and charcoal melanistic forms show a distinct distribution pattern in *N. b. sicula*. The first one is almost limited to Apennine and Alpine reliefs (in the sense of higher and lower altitudes), while the second seems to have a wider ecology and it is mainly found at low altitudes. The southern limit of the *picturata* form is located in the province of Salerno (Campania) where the two melanistic types can be found in sympatry. This area can be considered also as the northern limit for the high density of the charcoal form, for which only few observations (12.2%) occur northward from the Salerno

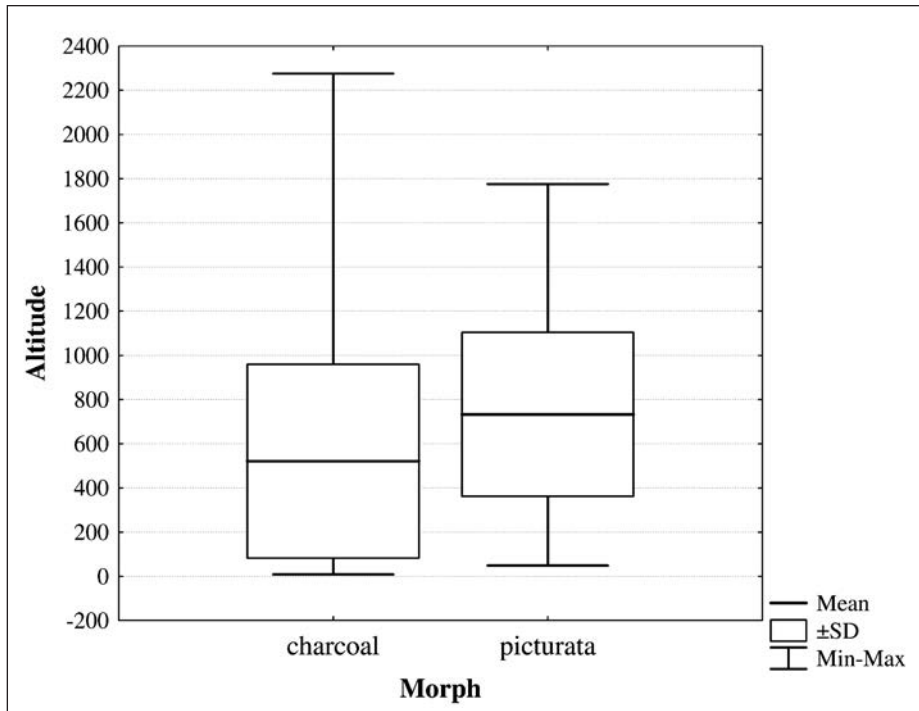


Fig. 3 — Box plots describing the altitudinal distribution of the picturata and charcoal morphs records.

province. The area where only the charcoal morph occurs roughly corresponds to a significant variation in mtDNA, as the populations of Calabria and Sicily form a highly distinct monophyletic clade (SCHULTZE *et al.*, 2020). However, this mtDNA difference is not reflected at the nuDNA level (SCHULTZE *et al.*, 2020). Furthermore, the distinctiveness of the Calabrian and Sicilian populations is known in relation to morphometry (MANGIACOTTI & SCALI, 2010), pholidosis and coloration (HECHT, 1930; MERTENS, 1947; LANZA, 1983). The high frequency of dark colored grass snakes in Sicily has already been highlighted in several studies (BRUNO, 1970, 1980, 1984; FARAONE *et al.*, 2010; GENIEZ, 2018). The diffusion of the charcoal morph mainly affects the populations of the northern areas of the island where, locally, it is often the prevailing color pattern (BRUNO, 1970; FARAONE *et al.*, 2010; Faraone, *unpub. data*). The distribution pattern of the two melanic forms in *N. b. sicula* could be related to several physiological benefits. In the form picturata the black coloration can overcome thermoregulation difficulties in cold environments in the mountains of Central and Northern Italy (GIBSON

& FALLS, 1979; ZUFFI, 2008). This hypothesis is also supported by its distribution at high altitude. The charcoal form, on the other hand, may be related to the benefits of solar radiation protection in warmer habitats (BECHTEL, 1978). These hypotheses are currently being studied to evaluate their correlations with environmental variables.

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Addresses of the authors — G. BRUNI, Viale Palmiro Togliatti - 50019 Sesto Fiorentino (Florence, I); M.R. DI NICOLA, I.R.C.C.S. San Raffaele Hospital, Unit of Dermatology, Via Olgettina 60, 20132 Milan (I); F. BANFI, Laboratory of Functional Morphology, Department of Biology, University of Antwerp, Universiteitsplein 1 - 2610 Wilrijk (B); F.P. FARAONE, Viale Regione Siciliana S.E. - 90129 Palermo (I).