

## Variability in the dorsal pattern of the Sardinian grass snake (*Natrix natrix cetti*) with notes on its ecology

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**Abstract.** The Sardinian grass snake (*Natrix natrix cetti*) is a Critically Endangered snake endemic to Sardinia (Italy), for which information is still scarce. In the present work, we report information obtained from 36 observations of *N. n. cetti* performed in different areas of the Island. Three different colorations were mainly observed and darker snakes were in general males and big adults; the only juvenile found showed a complete different dorsal colouration. Snakes were observed active during day-time and often far from the aquatic habitats.

**Keywords.** Melanism, abundance, mimicry, predator avoidance, island.

The Sardinian grass snake (*Natrix natrix cetti*) is a Critically Endangered snake endemic to Sardinia, for which we still have very limited information (European Reptile & Amphibians Specialist Group, 1996; Vanni and Cimmaruta, 2010). A recent phylogenetic analysis performed on the *Natrix natrix* complex grouped the Sardinian grass snake together with the Corsican subspecies (*N. n. corsa*) into a distinct genetic group, which was likely promoted by their isolation occurred during glaciations (Fritz et al., 2012; Kindler et al., 2013). According to the most recent phylogenetic study, the western grass snakes belong to a new clade called *Natrix helvetica* (Kindler et al., 2017); however, in this study no samples from Sardinia have been analysed. Its elusiveness could be one of the causes of the few data available on its distribution, ecol-

ogy and biology (Lunghi et al., 2016; Lunghi et al., 2018; Vanni and Cimmaruta, 2010). It is known that *N. n. cetti* can exploit different environments, from dry rocky areas to wetlands, rivers and even caves, and that the species is relatively widespread on the Island (Capula et al., 1994; de Pous et al., 2012; Lanza 1986; Mulargia et al., 2018; Salvi and Bombi, 2010). However, the presence of the Viperine snake (*Natrix maura*), as potential competitor, seems to affect its distribution (Stefani 1983; Vanni and Cimmaruta 2010).

Adults of *N. n. cetti* differ morphologically from the continental subspecies because of the lack of the typical light "collar", and the smaller size (Speybroeck et al., 2016; Vanni and Cimmaruta, 2010). Stefani (1983) reports for *N. n. cetti* a typical light greyish background

colour, a characteristic that should be of help in distinguishing the Sardinian grass snake from the Corsican one, being the latter characterised by a dark greenish background. Recently, abundant (i.e., dorsum characterized by enlarged dark stripes) and melanotic (i.e., nearly completely black coloration) individuals of *N. n. cetti* were observed (Lunghi et al., 2016), thus increasing the variability of the dorsal colouration known for this subspecies. Furthermore, as far as we know, no information exists on juveniles.

Here we report observations of *Natrix natrix cetti* gathered during three years fieldwork. All captured snakes were measured, and information on dorsal pattern and habitat were recorded. The observation of one juvenile is also reported.

From 2016 to 2018, we conducted herpetological field observations focusing on Eastern and Southern Sardinia (see Table 1; coordinates are not reported for species safeguard, see Lunghi et al., 2019). Repeated linear transects, based on VES (Visual Encounter Survey), were carried out. The surveys were performed by day (9 a.m. – 5 p.m.)

throughout the year, but the most in spring (Jan-Mar = 9.1%; Apr-Jun = 70.6%; Jul-Sep = 14.4%; Oct-Dec = 5.9%; total surveys = 153). For each snake we recorded: locality, elevation and time of the observation, habitat typology and dorsal background colour pattern (greyish, greenish-brownish, melanotic) (Fig. 1). Captured snakes were measured (total length) and sexed. Total length was measured photographing the snakes on a plasticised millimetre paper; snakes' length was extrapolated using the program ImageJ. Sex was assessed combining visual inspection of the morphology of the snake (proportion of the head and tail) and number of sub-caudal scales (Vanni and Cimmaruta, 2010). Head pattern was used for individually snake recognition (Sacchi et al., 2016; Vaughan, 1999). A Generalized Linear Model (R software with nlme package; Pinheiro et al., 2016; R Development Core Team 2018) was used to assess whether snake coloration is related to sex, elevation, time of survey and total length (TL). We used colouration as dependent variable, while sex, TL, time of survey and elevation as independent variables; year and transect identity as random factors. To use col-



**Fig. 1.** The four different dorsal colourations of *Natrix natrix cetti* reported in this study: A) greyish, B) greenish-brownish, C) black, D) the juvenile. (Photos A and B by M. Di Nicola; C by E. Lunghi; D by S. Giachello).

**Table 1.** Data of the captured individuals of *Natrix natrix cetti*: sex, total length (TL), dorsal background colour (1 = greyish, 2 = greenish-brownish, 3 = melanotic), elevation (m a.s.l.), time at which snakes were observed (24h), province, year, transect identity and the mountain complex. \*individuals captured twice.

Sex	TL (cm)	Colour	Elevation	Time	Province	Year	Transect	Mountain
Female	65.7	2	341	16:15	Carbonia-Iglesias	2017	Barega1	Barega
Female	44	1	474	14:32	Cagliari	2017	Cagliari3	Sette Fratelli
Female	72.1	2	515	10:30	Cagliari	2016	Cagliari2	Sette Fratelli
Female	74	1	534	10:08	Cagliari	2018	Cagliari2	Sette Fratelli
Female	53.1	1	535	11:10	Cagliari	2017	Cagliari2	Sette Fratelli
Female	62.3	2	539	12:00	Cagliari	2017	Cagliari2	Sette Fratelli
Female	61.8	2	541	14:01	Cagliari	2017	Cagliari2	Sette Fratelli
Male	54.3	2	633	11:02	Cagliari	2017	Cagliari2	Sette Fratelli
Female	59	2	634	10:36	Cagliari	2017	Cagliari2	Sette Fratelli
*Female	63	2	638	16:21	Cagliari	2017	Cagliari2	Sette Fratelli
Female	57.9	2	670	15:45	Cagliari	2016	Cagliari2	Sette Fratelli
Male	64.7	2	689	14:05	Cagliari	2017	Cagliari2	Sette Fratelli
Male	60.1	3	706	13:45	Cagliari	2016	Cagliari2	Sette Fratelli
Female	58.8	2	730	16:42	Cagliari	2016	Cagliari2	Sette Fratelli
*Female	63	2	736	16:40	Cagliari	2016	Cagliari2	Sette Fratelli
Female	63	1	792	15:58	Cagliari	2018	Cagliari2	Sette Fratelli
Male	46.4	2	830	15:50	Cagliari	2017	Cagliari2	Sette Fratelli
Female	59.8	2	863	13:20	Cagliari	2017	Cagliari3	Sette Fratelli
Male	55.5	1	1029	14:45	Nuoro	2018	M_albo3	Monte Albo

ouration as dependent variable, we ascribed an ascending order to the different colourations, going from the lightest (greyish = 1) to the darkest (greenish = 2) (Table 1). In this analysis we excluded the juvenile, the single melanotic individual, and, for the individual captured twice (see below) only the first observation.

In total we performed 153 surveys on 3 different areas (Barega, no. of transects = 1, total surveys = 5; Monte Albo, no. of transects = 11, total surveys = 102; Sette Fratelli, no. of transects = 2, total surveys = 46) and we observed 19 *Natrix natrix cetti*; seventeen were adults. Most of the observations (17) were performed on the Sette Fratelli Mountain and only an adult female was captured twice. The greenish-brownish ( $n = 12$ ) coloration resulted to be the most common, followed by the greyish ( $n = 5$ ) and the melanotic ( $n = 1$ ) (Fig. 1A-C). The observed juvenile (TL = 22 cm) showed a complete different dorsal colouration: the background colour was black, the stripes white and a white collar was also present (Fig. 1D). Snakes were observed at an elevation between 341 and 1029 m a.s.l. Two individuals were found in small temporary water bodies, one in a mine, while the others 15 in rocky areas sometimes even more than 1 km far from the nearest water body.

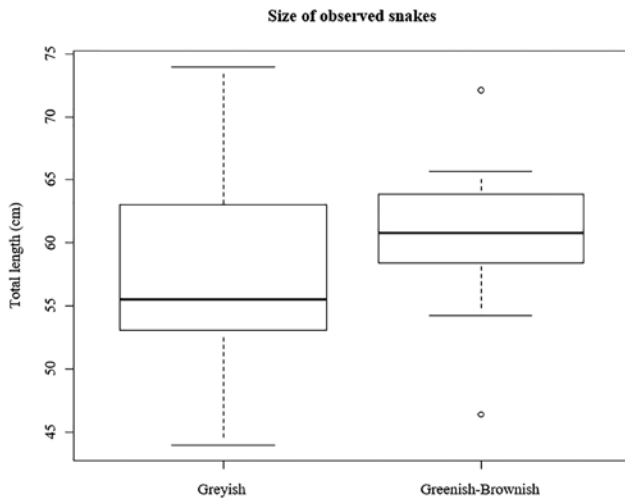
Snakes' coloration significantly correlated only with total length ( $F_{1,8} = 8.94$ ,  $P = 0.017$ ); the darker (greenish)

coloration was most frequently observed in the longer snakes (Fig. 2). No significant effect was observed for other variables (sex,  $F_{1,8} = 2.00$ ,  $P = 0.195$ ; elevation,  $F_{1,8} = 2.90$ ,  $P = 0.127$ ; time of survey,  $F_{1,8} = 1.69$ ,  $P = 0.229$ ).

During our study, a female with a wounded eye was captured twice, the first on May 2016 and the second in April 2017; in both occasions this female was active at about the same time (16:41 and 16:21 respectively). This individual was recaptured almost 90 m far from the site of first encounter (difference in altitude 98 m).

The Sardinian grass snake is one of the least studied Italian species. Elusiveness, rather than its potential rarity, could be the reasons for the few existing studies on this species (Vanni and Cimmaruta, 2010); indeed, although being made of just 19 records, our dataset is one of the richest available. The dorsal coloration of *Natrix natrix cetti* seems to be more variable than previously reported; indeed, most of our observations highlighted the high frequency of the greenish-brownish coloration, a characteristic that should be typical of the Corsican grass snake (Stefani, 1983).

Because of our dataset paucity, we cannot provide any assumptions on the causes supporting the high pattern differentiation observed in this subspecies (but see also Lunghi et al., 2016). Further studies are needed to assess whether factors are influencing the observed vari-



**Fig. 2.** Boxplots indicating differences in *Natrix natrix cetti* body size (total length) per dorsal coloration (only for greyish and greenish background colorations). Individuals used here are the same used in the GLM analysis (see Table 1). Dark bar inside boxes represents the median, boxes represent the range between 1<sup>st</sup> and 3<sup>rd</sup> quartile, whiskers represent the variability outside the upper/lower quartile (box), circles represent outliers.

ability and, if darker coloration may be beneficial for snakes' longevity (Castella et al., 2013; Clusella Trullas et al., 2007; Fulgione et al., 2015; Stevens et al., 2009; Zuffi 2008). The juvenile highlighted ontogenetic variability in dorsal coloration in *N. n. cetti*.

Our study agrees with Stefani (1983) and Lanza (1986) considering *N. n. cetti* not strictly related to water bodies. This likely allows the snake to exploit further habitats, increasing prey availability and lowering competition with *Natrix maura* (Lunghi et al., 2018; Stefani, 1983; Vanni and Cimmaruta, 2010). Our study was carried out in day-time (see also Lanza, 1986) but we cannot exclude nocturnal activity as reported by Capula et al. (1994).

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#### REFERENCES

- Capula, M., Rugiero, L., Luiselli, L. (1994): Ecological observations on the Sardinian grass snake, *Natrix natrix cetti*. *Amphibia-Reptilia* **15**: 221-227.
- 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.
- Clusella Trullas, S., van Wyk, J.H., Spotila, J.R. (2007): Thermal melanism in ectotherms. *J. Therm. Biol.* **32**: 235-245.
- de Pous, P., Speybroeck, J., Bogaerts, S., Pasmans, F., Beukema, W. (2012): A contribution to the atlas of the terrestrial herpetofauna of Sardinia. *Herpetol. Notes* **5**: 391-405.
- European Reptile & Amphibians Specialist Group (1996): *Natrix natrix* ssp. *cetti*. The IUCN Red List of Threatened Species **1996**: e.T14364A4436077.
- Fritz, U., Corti, C., Päckert, M. (2012): Mitochondrial DNA sequences suggest unexpected phylogenetic position of Corso-Sardinian grass snakes (*Natrix cetti*) and do not support their species status, with notes on phylogeography and subspecies delineation of grass snakes. *Org. Divers. Evol.* **12**: 71-80.
- Fulgione, D., Lega, C., Trapanese, M., Buglione, M. (2015): Genetic factors implied in melanin-based coloration of the Italian wall lizard. *J. Zool.* **296**: 278-295.
- Kindler, C., Böhme, W., Corti, C., Gvoždík, V., Jablonski, D., Jandzik, D., Metallinou, M., Široký, P., Fritz, U. (2013): Mitochondrial phylogeography, contact zones and taxonomy of grass snakes (*Natrix natrix*, *N. megalocephala*). *Zool. Scr.* **42**: 458-472.
- Kindler, C., Chèvre, M., Ursenbacher, S., Böhme, W., Hille, A., Jablonski, D., Vamberger, M., Fritz, U. (2017): Hybridization patterns in two contact zones of grass snakes reveal a new Central European snake species. *Sci. Rep.* **7**: 7378.
- Lanza, B. (1986): I Rettili e gli Anfibi. In: L'ambiente naturale in Sardegna (Elementi di base per la conoscenza e la gestione del territorio), pp. 289-321. Camarda, I., Falchi, S. Nudda, G., Eds, Carlo Delfino Editore, Sassari.
- Lunghi, E., Corti, C., Manenti, R., Ficetola, G.F. (2019): Consider species specialism when publishing datasets. *Nat. Ecol. Evol.* **3**: 319.
- Lunghi, E., Deschandol, F., Cornago, L., Cogoni, R. (2016): Dark coloration in Sardinian grass snakes (*Natrix natrix cetti*). *Herpetol. Bull.* **137**: 28-29.
- Lunghi, E., Mascia, C., Mulargia, M., Corti, C. (2018): Is the Sardinian grass snake (*Natrix natrix cetti*) an active hunter in underground environments? *Spixiana* **41**: 160.
- Mulargia, M., Corti, C., Lunghi, E. (2018): The herpetofauna of the Monte Albo, Sardinia (Italy). *Russ. J. Herpetol.* **25**: 172-176.

- Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., Team, R.C. (2016): nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-128.
- R Development Core Team (2018): R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org/>. [accessed 28 September 2018]
- Sacchi, R., Scali, S., Mangiacotti, M., Sannolo, M., Zuffi, M.A.L. (2016): Digital identification and analysis. In: Reptile Ecology and Conservation, pp. 59-72. Kenneth Dodd, C.J., Eds, Oxford University Press, Oxford.
- Salvi, D., Bombi, P. (2010): Reptiles of Sardinia: updating the knowledge on their distribution. *Acta Herpetol.* **5**: 161-177.
- 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.
- Stefani, R. (1983): La Natrice del Cetti. *Biogeographia* **8**: 745-755.
- Stevens, M., Winney, I.S., Cantor, A., Graham, J. (2009): Outline and surface disruption in animal camouflage. *Proc. R. Soc. Biol. Sci. Ser. B* **276**: 781-781.
- Vanni, S., Cimmaruta, R. (2010): *Natrix cetti* Gené, 1839. In: Fauna d'Italia. Reptilia, pp. 538-545. Corti, C., Capula, M., Luiselli, L., Razzetti, E. Sindaco, R., Eds, Edizioni Calderini de Il Sole 24 Ore Editoria Specializzata S.r.l., Bologna.
- Vaughan, R. (1999): Provisional results from study of facial features as a means of individual identification in *Natrix natrix*. *Br. Herpetol. Soc. Bull.* **68**: 39-46.
- Zuffi, M.A.L. (2008): Colour pattern variation in populations of the European Whip snake, *Hierophis viridiflavus*: does geography explain everything? *Amphibia-Reptilia* **29**: 229-233.