## An anomalous phenotype of the Italian Aesculapian snake Zamenis lineatus from Mt. Etna, Sicily

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Zamenis lineatus (Camerano, 1891) is a semi-arboreal Colubrid snake (Harrington et al., 2018) endemic to Italy where it occurs in southern Lazio, Campania, Calabria, Molise, Basilicata as well as in Sicily (Di Nicola, 2019).

The body of adult *Z. lineatus* is usually grey, olive, beige or brown with four dark stripes, two dorsally and two laterally. The head is characterised by a dark band starting from the post-ocular and terminating on first labial corresponding to the angle of the jaw (Di Nicola, 2019), but possibly the most notable trait of *Z. lineatus* is the copper red or orange iris, a diagnostic feature that is generally used to distinguish it from the Aesculapian snake *Zamenis longissimus* (Laurenti, 1768) (Salvi et al., 2017). Individuals of *Z. longissimus* presenting aberrant colour patterns are known (Cattaneo, 2015), and dark coloured individuals are found regularly in Primorsko, Bulgaria (Speybroeck et al., 2016) but no colour anomalies of *Z. lineatus* have been published. The authors and other volunteers of the Sicily Wildlife Fund (SWF - a Wildlife protection NGO) communicate here their discovery of individuals of *Z. lineatus* presenting an anomalous phenotype in Sicily.

The Sicily Wildlife Fund is a non-profit NGO with the objectives of monitoring and conserving wildlife in eastern Sicily. As part of its mission, recovery facilities are provided for animals rescued in urban and suburban areas by the local population and institutions. In 2010, a citizen brought to SWF three *Z. lineatus* found stuck in a water tank on his property located at Etna under Bronte municipality (Catania); these individuals showed a peculiar colour, appearing overall grey and lacking the typical red iris. These animals were underfed and thus they were kept under observation, fed and rehydrated until fully recovered. After one week, as per our animal management protocol, these individuals were then returned to their original wild area.



**Figure 1.** A rescued adult and a juvenile *Zamensis lineatus* showing abnormal coloration – **A.** Full body coloration and pattern of the adult as it looks on a substrate, **B.** Full body coloration and pattern of the juvenile as it looks on a substrate, **C.** Close-up of the head of the adult, **D.** Close-up of the head of the juvenile

In the following years, there have been surveys of the area, leading to other sightings of similar individuals of *Z. lineatus* confirming their presence in the wild. Unfortunately, only starting from 2020 has SWF implemented a cataloguing protocol for rescued individuals (in the field or when admitted at the wildlife centre); for this reason we cannot exclude the possibility that these animals are the same ones that were observed previously. Here we present only the data for an adult and a juvenile observed in 2020.

The adult (SVL = 89.6 cm, weight = 275 g) and juvenile (SVL = 26.8 cm, weight = 14 g) were found on two different occasions in September 2020 (Fig. 1). The area surveyed is a sparse forest over 1000 m a.s.l. on Mt. Etna, presenting leaf litter on volcanic soil. We have not indicated the exact position of these snakes in order to discourage poaching activity. Instead, the precise GPS coordinates have been uploaded to the ORNITHO platform (https://www.ornitho.it/) which is managed by many wildlife conservations agencies and scientific bodies (including the Societas Herpetologica Italica - the Italian Herpetological Society). The users of this platform are vetted routinely and consequently we have full trust in this system. The GPS data can be requested through the ORNITHO platform or directly by contact with the corresponding author of this paper.

Both individuals have been identified as *Z. lineatus* following Di Nicola (2019). The body of the adult was grey (Fig. 1A) and lacked the red-coloured iris, showing a clearly grey iris instead (Fig. 1C) while the colour aberration is even more pronounced in the juvenile. This individual preserves the chequered pattern of the body but completely lacks any other colour other than shades of grey and black (Fig. 1B) including the eyes which are grey (Fig. 1D). As with *Z. longissimus* (Cattaneo, 2015), we consider these individuals to be a variation of the normal colour pattern of *Z. lineatus*. This might be due to the absence or malfunctioning of xanthophores for the body and erythrophores for the eyes, but further studies are needed to confirm this suggestion.

The distribution of ectotherms is critically limited by temperature and exposure to the sun. For this reason, it has been suggested that individuals showing a darker body coloration, compared to lighter coloured individuals, may have an adaptive advantage by warming more quickly during basking, especially at higher altitudes, in what has been called the thermal melanism hypothesis (Fernando Martínez-Freiría et al., 2020; Clusella-Trullas et al., 2007). However, it has also been proposed that a darker colour might make these snakes stand out on lighter backgrounds, thus increasing predation risk (Clusella-Trullas et al., 2008). In the case of the individuals presented here those with a darker colour pattern might have an advantage in thermoregulation while still blending well with the background of the black-dark brown volcanic soil. The observations between the period of 2010-2020, the presence of an adult and a juvenile in the area surveyed, and the correspondence of the dark coloration with the dark soil of the habitat, all suggest that the individuals reported here are part of a population of Z. lineatus that has genetically fixed the anomalous colour pattern, thanks to the ecological advantages it may provide. Nevertheless, individual Z. lineatus with a normal colour have been observed in the same area, leaving our hypothesis in need for further surveys to establish i) What is the percentage of individuals presenting the colour anomaly over the whole *Z. lineatus* population in the area, and ii) Whether the two different phenotypes are somehow separated spatially or ecologically (i.e. do they occupy a different niches?).

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