Axanthism in *Emys orbicularis hellenica* (Valenciennes, 1832) (Testudines: Emydidae) from Piedmont, northern Italy

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The European pond Turtle *Emys orbicularis* (Linnaeus, 1758) is a polytypic and polymorphic species with a western palearctic distribution for which several subspecies were described based on molecular and morphological differences (Lenk et al., 1999; Fritz, 2003). In Italy, three subspecies are currently recognised: *E. o. ingauna* Jesu, Piombo, Salvidio, Lamagni, Ortale & Genta, 2004 in Liguria, *E. o. galloitalica* Fritz, 1995 along the Tyrrhenian coast and *E. o. hellenica* (Valenciennes, 1832) on the Adriatic one (Zuffi et al., 2011).

In the latter, the coloration is typically characterised by a black carapace with yellow elements (spots in males and lines organised in radial pattern in females) and a yellow plastron. Yellow areas are also present on soft parts like specks on limbs and also on the head in females. Iris coloration is usually white or yellow in males (Zuffi et al., 2011), sometimes reddish (R. Cavalcante pers. obs.), while females typically possess a yellow iris (R. Cavalcante pers. obs.). As in other Mediterranean subspecies, *E. o. hellenica* hatchlings present a well-defined yellow pattern on the marginal scutes of the carapace and the plastron is covered for at least two-thirds by a black central blotch, leaving yellow coloration only on the lateral rim (Fritz et al., 2006).

Given that *E. o. hellenica* is at risk of local extinction in Piedmont (Zuffi et al., 2011, Seglie & Cavalcante, 2016), the Conservation Centre "Centro Emys Piemonte" has been implemented in 2016, provided with an outdoor breeding facility. The main activity of the Centre was to rescue adult individuals in the Po and Orba natural reserves to create breeding groups, which would then provide 3-4 years old individuals to be reintroduced in restored habitats. All of these operations were authorised by the Italian Ministry of Environment, Land and Sea (Protocol number 0015025/PNM-28/07/2015). A molecular characterisation was carried on rescued individuals by Ana Rodriguez Prieto at Museo delle Scienze (MUSE) in Trento, which ascribed all the turtles to the *hellenica* subspecies. The outdoor breeding facility is designed to allow the collection of the eggs right after the deposition for artificial incubation. However, not all the nests are easy to spot, and some litters are discovered only at the time of hatching.

On 3 September 2017 during an inspection of these nests, in an area where three females and one male were kept, two out of twenty-nine hatchlings were found to display an anomalous coloration. In these individuals, portions of the carapace, plastron and soft parts that are typically yellow present a whitish-greyish coloration (Fig. 1A), and the iris is dark brown (Fig 2A). This particular coloration was never observed before in wild individuals or in other hatchlings born in the Centre (10 in 2016, 38 in 2017 and 44 in 2018).

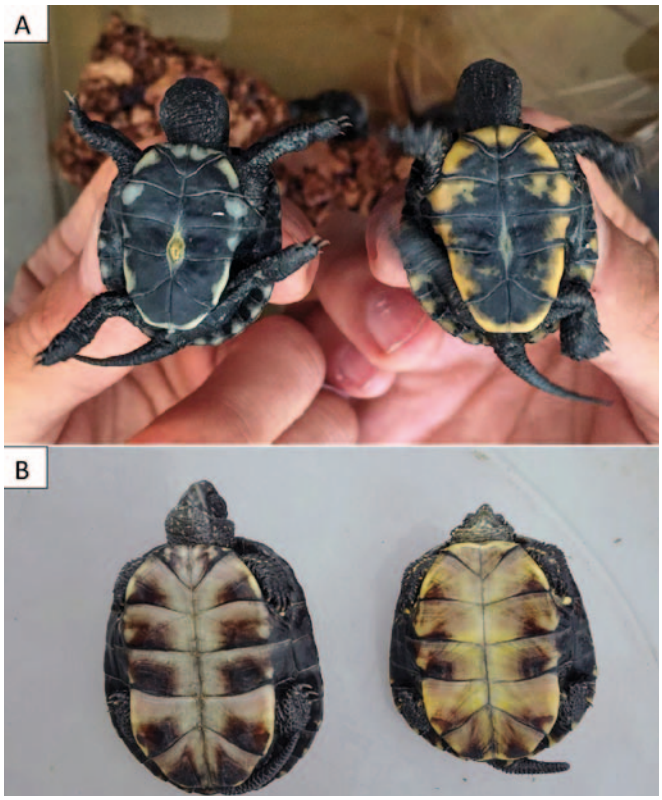

Figure 1. Comparison of the plastron of an axanthic individual (left) with the one of a normal coloured individual (right): **(A)** hatchlings in September 2017; **(B)** one-year old juveniles in September 2018

Yellow, orange and red coloration in reptiles are produced via the interaction between xanthophores and the underlying iridophores (Cooper & Greenberg, 1992; Morrison et al., 1995; Steffen & McGraw, 2009). Xanthophores contain pteridines like xanthopterin, sepiapterin and riboflavin inside pterinosomes and carotenoids like carotenes and xanthophylls in carotenoid vesicles (Obika & Bagnara, 1964; Watt, 1964; Bagnara & Hadley, 1973; Morrison et al., 1995). Iridophores contain guanine crystals responsible for structural coloration given

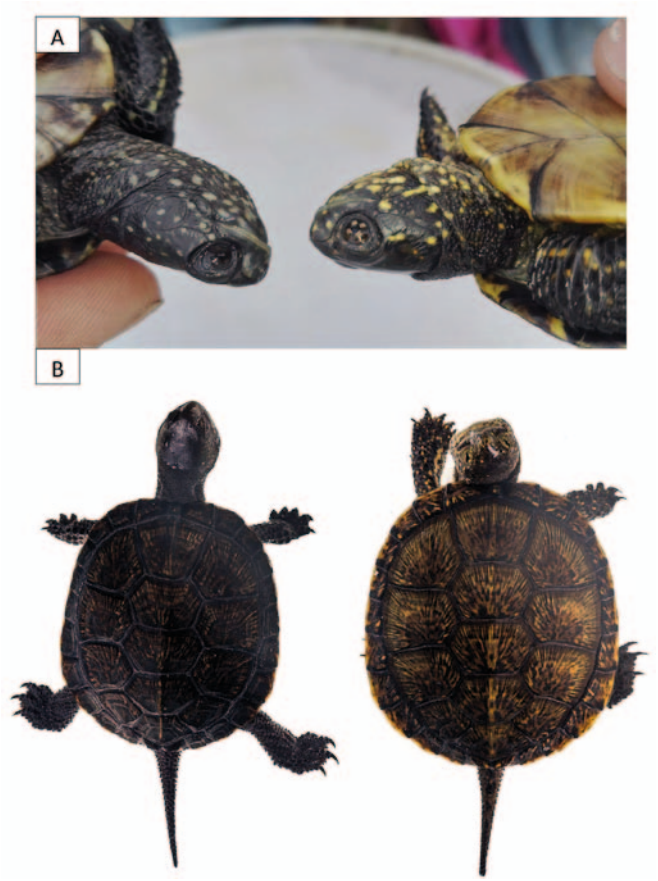

Figure 2. Comparison between one-year old axanthic (left) and normal coloured (right) individuals: **(A)** coloration of head and eyes; **(B)** coloration of the carapace

by light reflecting properties which depends on platelets' size and disposition (Bagnara, 1966). Regarding pigments within xanthophores, pteridine pigments have a major role in reptiles, while carotenoids seem to be less important (Olsson et al., 2008; Steffen & McGraw, 2009) and sometimes to be even absent (Kikuchi & Pfennig, 2012; Olsson et al., 2013). While carotenoids are assimilated from the diet (Olson & Owens, 1998), pteridine pigments are endogenously synthesised from purines (Watt, 1967). Colour aberration for which a reduction or absence of yellow occurs is known as axanthism, and it can be generated through a lack or non-functionality of xanthophores and iridophores (Jablonski et al., 2014).

Considering that the pale coloration was maintained by both individuals in their first year of life, whilst becoming slightly yellow (Figs. 1B and 2B), we assume that xanthophores are present and that this mild "yellowing" may be caused through carotenoids sequestered from the diet, since they have been also fed with KOI beauty First (Tetra®), food pellets which contain β-carotene. Therefore, it is probable that the observed axanthism involves primarily iridophores or some metabolic defects in pteridine production (Olsson et al., 2013).

In literature, axanthism and other chromatic anomalies were never reported before in the European pond Turtle. Axanthism seems also rare in general in testudines, since only cases of albinism (e.g. Türkozan & Durmuş, 2001; Sönmez & Özdilek, 2011), leucism (e.g. Erickson & Kaefer, 2015), melanism (e.g. Yabe, 1994; Gronke et al., 2006), hypomelanism (e.g. Turner, 2011; Di Giuseppe et al., 2014) and amelanism (e.g. Martìnez Silvestre & Soler, 2001) are currently published.

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