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SHORT NOTE

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Variability and hybridisation in the introduced pond slider turtle *Trachemys scripta* in Romania

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ABSTRACT - A qualitative survey of the chromatic and morphological variability of the pond slider turtle *Trachemys scripta* was undertaken in the parks of ten cities in Romania. Large variability was observed, melanistic individuals and intra-specific hybrids were found, and the characteristics of some specimens were such as to imply possible hybridisation with other species. The large morphological variability of the introduced populations of *T. scripta* indicates multiple geographical origins from the native range and very likely hybridisation. These factors contribute to a great diversity in the introduced populations and may influence their capacity for acclimatisation in the new range.

INTRODUCTION

The pond slider turtle *Trachemys scripta* is a North American species that has been disseminated across the world by the pet trade (Rhodin et al., 2017; Uetz et al., 2022). The constant release of unwanted pet turtles has resulted in innumerable concentrations of such animals, many of which have become established and even started to reproduce in their new environments. This species has elicited concern, being listed as one of the '100 of the World's Worst Invasive Species' (GISD, 2021) and of the 'Invasive Alien Species of Union Concern' (European Commission, 2017) - although "little is known of their impact on indigenous ecosystems" (GISD, 2021). Pond sliders are considered invasive or at least potentially so because "Their omnivorous diet and ability to adapt to various habitats, gives them great potential for impacting indigenous habitats" (GISD, 2021). In Romania, the species has been widely released (see, for the distribution, Sos, 2007; Stănescu et al., 2017; Cioflec, 2017-2021; Iftime & Iftime, 2021 and other sources quoted therein), but reproduction has so far only been documented from urban parks in four cities (Cioflec, 2013; Matei & Tudor, 2014; Iftime & Iftime, 2021). However, successful production of hatchlings does not necessarily reflect a healthily reproducing population, as *T. scripta* has a temperature-dependant sex determination mechanism and outside of a certain temperature bracket single-sex clutches may be produced (Wibbels et al., 1998). Nevertheless, the species is long-lived and so, at least in theory, populations can grow for some time before they become self-limiting - or adapt to find optimal egg-laying conditions. The ecological impact of *T. scripta* is in fact little-known for a species so widely claimed to be invasive (see above, GSID, 2021) and studies claiming negative interaction with native European species, especially *Emys orbicularis*, have been met by results to the contrary (see discussion in Iftime & Iftime, 2021). In this context, the diversity of the introduced stock, reflected in the variability of the introduced population, is of paramount importance, as it provides the material for adaptation and selection in the introduced population, impacting the probability of establishment. To document this, we observed and photographed the chromatic and morphological variation in as many specimens of *Trachemys* as possible in several parks in the cities of Bucharest, Mogoşoaia, Craiova, Râmnicu-Vâlcea, Constanța, Ploiești, Buzău, Târgoviște, Pitești (over the warm seasons of 2020 and 2021) and the thermal resort of Băile Felix (older data, as of 2014). The identification of morphs and possible hybrids of *T. scripta* was based on the descriptions presented in several publications that are quoted in the Obervations and Discussion section below.

OBSERVATIONS & DISCUSSION

We found *Trachemys scripta* to be present in all the parks we visited (see Supplementary Material, Table S1). A number of definite forms were commonly observed: the subspecifically defined *T. s. scripta* (Fig. 1A) and *T. s. elegans* (Fig. 1B); *T. s. troosti* (Fig. 1C) which nowadays is relegated to the status of a natural intergrade between the two above-mentioned valid subspecies (Parham et al., 2020); specimens very similar to the 'southern intergrades' between *T. s. scripta* and *T. s. elegans* (Fig. 1D); 'anthropogenic hybrids' described by Parham et al., 2020 (Fig. 2A) (see also Cioflec, 2017-2021; Iftime & Iftime, 2021). Melanistic specimens were also found (Figs. 2 B & C), as well as some showing morphological similarities to *Trachemys decussata* (Gray, 1831), a native of Cuba (Uetz et al., 2022) (Iftime & Iftime, pers. obs. - Fig. 2D [compare with *T. decussata angusta* (Barbour & Carr, 1940) as illustrated by Rhodin et al. 2017, p. 64], and Fig. 3A). Some specimens show more or less marked morphological similarity to *Trachemys gaigeae* (Hertweg, 1939), native to USA-Mexico border area (Uetz et al., 2022) (Fig. 3B), and *T. decorata* (Barbour & Carr, 1940), native to Hispaniola (Uetz et al., 2022) (Fig. 3C). A specimen with features of both *T. scripta* and *Pseudemys* (*floridana*) *peninsularis*, possibly an intergeneric hybrid, was

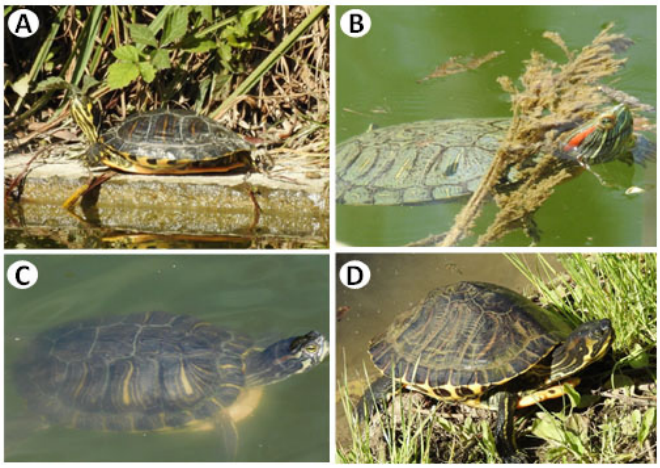

Figure 1. Turtles from various parks in Bucharest, Romania - **A.** Typical *Trachemys scripta scripta*, from lake in Tineretului Park, **B.** Typical *Trachemys scripta elegans*, from lake in Tineretului Park, **C.** '*Trachemys scripta troosti*', from lake in Titan Park, **D.** Probable *Trachemys scripta scripta* - *Trachemys scripta elegans* 'southern intergrade' from lake in State Circus Park

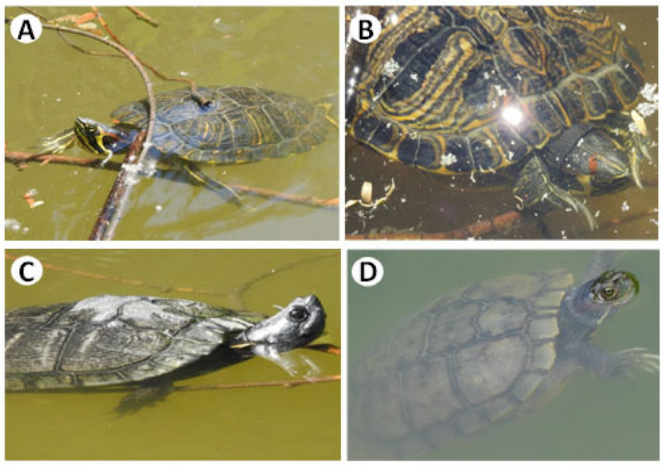

Figure 2. Turtles from the lake in Titan Park, Bucharest, Romania - **A.** Probable *Trachemys scripta scripta* - *Trachemys scripta elegans* anthropogenic hybrid, **B.** *Trachemys scripta* with incipient melanism, **C.** Fully melanistic *Trachemys scripta*, **D.** Probable *Trachemys scripta* - *Trachemys decussata* hybrid

also found (Figs. 4 A & B).

Our observations show a large variability in the introduced *T. scripta* population, including various degrees of melanism. Melanistic individuals are common in *T. scripta*, especially elderly males (McCoy, 1968; Lovich et al., 1990), in both native populations (McCoy, 1968; Lovich et al., 1990; Smith et al., 2016) and introduced ones (Böhm, 2013; Martins et al., 2014; Jablonski et al., 2016). The process of melanisation is hormonally driven (Lovich et al., 1990); both aging (Lovich et al., 1990) and thermal influences (Rowe et al., 2016) appear to be involved. Melanisation can be permanent or transient (Lovich et al., 1990); it goes through various stages with associated colouration patterns (McCoy, 1968; Lovich et al., 1990; Tucker et al., 1995). Melanisation was linked with behavioural particularities, which may, however, be associated rather with age and/or hormonal changes than with the co-occurring melanisation per se (see discussion in Garstka et al., 1991; Tucker et al., 1995; Stone et al., 2015). In our samples, melanism was seen in adult but not necessarily senescent specimens, mostly in males, but also in a small number of females. Both *T. s. scripta* and *T. s. elegans* appear to undergo melanisation. All stages were seen, from incipient melanisation (Fig. 2B) to all-black individuals (Fig. 2C).

Some of the melanisation stages in *T. scripta* are partly convergent with the known pattern of colouration (including melanism) in *T. decussata* (Barbour & Carr, 1940), hence the risk of misidentifying melanistic *T. scripta* as *T. decussata* (Poch et al., 2020). The well-documented hybridisation of *T. scripta* and *T. decussata* (Parham et al., 2013) also confuses the issue. Moreover, diagnostic traits between these and also other *Trachemys* species are often quantitative, difficult to follow, and not always consistent (see, e.g. Barbour & Carr 1940; Seidel, 1988). Many *Trachemys* interspecific hybrids appear to be fertile, and in the complicated context where current species delineation does not follow the objective reproductive incompatibility criterion, the issue of the relationships among putative *Trachemys* species is considered "poorly resolved" (Seidel & Ernst, 2017). In these circumstances, it may be legitimate to advance the hypothesis that some of the morphologically *decussata*-like *T. scripta* specimens observed in Bucharest are of a probable *T. scripta* x *T. decussata* hybrid origin. Indeed, some show a short, rounded snout, relatively large eyes and flat head, relatively flat carapace profile, and traces of radial wrinkling on carapace plates (e.g. Fig. 2D; Fig. 3A). When found individually, and much more so when co-occurring, they are indicative of *T. decussata*, according to some authors (cf. Barbour & Carr, 1940; Seidel, 1988). It is also worth mentioning that a *T. d. angusta* photographed on Grand Cayman Island (Rhodin et al., 2017) may well be a hybrid, showing even less of the typical *T. decussata* traits than the specimen in our Fig. 2D; Grand Cayman is well-known for hybridisation between the two species (Parham et al., 2013, and literature quoted therein). Again, melanistic *decussata*-like *Trachemys* in Bucharest survive the cold season by hibernating, which is not documented for *T. decussata*, and is strongly indicative of the hybrid condition (see also the discussion in Poch et al., 2020).

Other possible hybrids are also present. One specimen found in Piteşti (Fig. 3B) suggests, through the cephalic pattern (fragmented right temporal stripe, intense vermiculation pattern on head) a *T. scripta elegans* x *T. gaigeae* hybrid (cf. Stuart & Ward, 2009; Parham et al., 2020). Also, one 'odd' Bucharest specimen suggests, by its morphological features (sharp, conic snout; muted, yellowish temporal stripe; curved but quite short forelimb claws in a male - cf. Barbour & Carr, 1940; Seidel & Inchaustegui Miranda, 1984), a possible introgression from *T. decorata* (Fig. 3C). Another such peculiar specimen (Figs. 4 A & B) may even be an intergeneric hybrid, as it shows a head and carapace pattern reminiscent of both *T. scripta* and *P.* (*floridana*) *peninsularis*. *T. scripta* x *Pseudemys* hybridisation has been reliably demonstrated (Soler et al., 2015) and such hybrids are occasionally found in the reptile

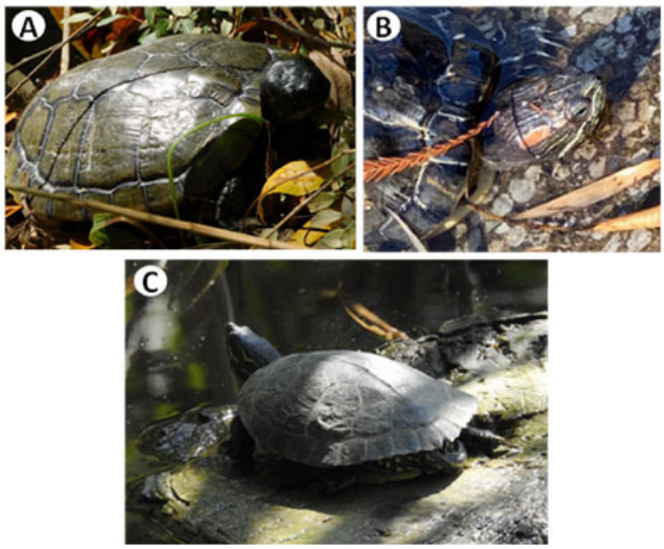

Figure 3. Turtles from various city parks in Romania - **A.** Probable *Trachemys scripta* - *Trachemys decussata* hybrid, Bucharest, from lake in Titan Park, **B.** Probable *Trachemys scripta* - *Trachemys gaigeae* hybrid, Pitești, from lake in Expo Park, **C.** Possible *Trachemys scripta* - *Trachemys decorata* introgressive hybrid, Bucharest, from lake in State Circus Park

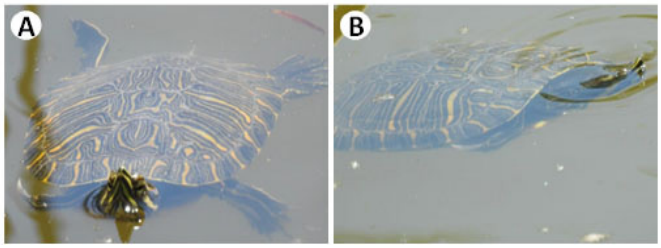

Figure 4. Possible *Trachemys scripta* - *Pseudemys* (*floridana*) *peninsularis* hybrid, Bucharest, lake in Titan Park, Romania (A & B are different views of the same specimen)

trade - see for example *T. scripta* x *P.* (*floridana*) *peninsularis* (Underground reptiles, undated) and *T. scripta* x *P. concinna* (The Turtle source, undated). The specimen pictured in the last source is quite similar to Figure 4 in its mix of features, but the 'Romanian' one is somewhat more reminiscent of *P.* (*floridana*) *peninsularis*, though we cannot altogether exclude the possibility that it has *P. concinna* ancestry. Such hybrids may have been sold in Romania as well, though not necessarily under the correct label, and therefore could be discarded into urban lakes; similarly, 'cooter/slider' (i.e. *Pseudemys-Trachemys*) hybrids were found in the London area (Langton et al., 2011).

Besides supposed interspecific hybridisation, there is definite evidence of intraspecific hybridisation/intergrading, with numerous specimens displaying features of either 'northern' *T. s. scripta* - *T. s. elegans* intergrades ('*T. s. troosti*'), 'southern' *T. s. scripta* - *T. s. elegans* intergrades, or anthropogenic hybrids between the same subspecies (cf. morphological indications in Parham et al., 2020). Such diversity is indicative of both widespread collection of *T. scripta* individuals from the native range in the USA and hybridisation in captive-breeding facilities (or even post-release), and suggests great morphological, and presumably genetic, diversity in the introduced population.

To date, the most widespread forms remain the classical subspecies, *T. s. scripta* and *T. s. elegans* (the former now more widespread than the latter, at least in our sampling). It is apparent that the greatest diversity of turtle forms is in Bucharest (Table 1S), where there is considerable variation in diversity between water bodies, followed by Craiova. This probably reflects the intensity of pet trade, which we expect to vary in approximate proportion to city size and general intensity of commerce. The variability in Bucharest would appear to be accounted for by some parks (e.g. Titan Park) being preferred as release sites and perhaps also more favourable to the survival of the new arrivals.

We conclude that introduced populations of *T. scripta* are quite varied in morphology, indicating multiple geographical origins from the native range and very likely hybridisation. These factors contribute to a great diversity in the introduced populations, which may influence their capacity for acclimatisation in the new range. Such diversity may be an asset for a population under selective pressure and the melanism observed in this survey may be a case in point. Conversely, hybridisation with tropical species may be a hindrance in a temperate environment, nevertheless the supposedly hybrid specimens appear to have survived through some winters at least. Further study may establish whether some morphs become better established in the new environment than others and if so then the nature of the selective advantages that have led to this may be revealed.

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