Changes in dorsal spot pattern in adult *Salamandra salamandra* (Linnaeus, 1758)

Mark-recapture techniques for the individual recognition of animals are commonly used in population studies of amphibians. The supposed longer-term constancy of the individual colorpattern represents the basis for a widely accepted non-invasive technique for individual recognition in amphibian species in which the dorsal or ventral pattern varies among individuals (Donelly et al. 1994; Carafa & Boni 2004; Plăiașu et al. 2005; Biancardi & DiCerbo 2010; Gollmann & Gollmann 2011; Courtois et al. 2013; Leeb et al. 2013). Thus, the conspicuous dorsal pattern of yellow to orange spots on black background of Fire Salamanders, *Salamandra salamandra* (Linnaeus, 1758), is considered a suitable identification tool in mark-recapture studies (e.g., Carafa & Boni 2004; Bar-David et al. 2007; Schmidt et al. 2014). The authors of the present note used this approach in their research on hibernation and sexual dimorphism in the colorpattern of Fire Salamanders in Slovakia (Balogova & Uhrin 2014, 2015). In the present study, minor, however unexpected, changes in the shape of spots of adult *S. salamandra* observed during their ontogeny are reported.

Salamanders were repeatedly captured from November 2011 to March 2014 in an artificial damp gallery near the village of Tichá Voda (eastern Slovakia; 48°46.2′N, 20°36.3′E; 855 m a. s. l.; 49 individually recognized specimens, 24 males, 15 females, 10 juveniles) and from December 2011 to April 2014 in Bobačka Cave (eastern Slovakia; 48°46.9′N, 20°06.3′E; 811 m a. s. l.; 106 individually recognized specimens, 47 males, 51 females, 8 juveniles). Each individual’s dorsal side was photographed and coded according to a method based on unique spot markings (Opatrný 1983).

From 49 identified individuals in the gallery near the village of Tichá Voda, 37 (75.5 %) were recaptured [number of recaptures > 0] and 27 (55.1 %) more than once [number of recaptures > 1]. In seven salamanders (14.3 %), individual changes in dorsal coloration were recorded, with 301 days (3 March to 28 December, 2012) representing the shortest interval leading to the observed modification. In Bobačka Cave, from 106 identified individuals 42 (39.6 %) were recaptured [number of recaptures > 0], 23 (21.7 %) more than once [number of recaptures > 1]. In three salamanders (2.8 %), dorsal pattern changes were detected, with 307 days (9 March, 2012 to 9 January, 2013) representing the shortest interval leading to the observed modification. The gradual alteration of a complex circular spot (Fig. 1, individual no. VIII) was recorded during two years (639 days). Generally, two types of changes were observed: (1) reduction of the spot area, and (2) spot splitting (Fig. 1).

Recognition of individual animals using photo documentation has great advantages; e.g., it does not involve stress and risk of infection as do invasive techniques, such as applying PIT (Passive Integrated Transponder) tags, toe clipping or branding (Plăiașu et al. 2005; Waye 2013). In the present study, minor changes in the dorsal pattern were seen already as early as at the age of about ten months when compared to earlier developmental stages.

The growth velocity of Fire Salamanders decreases at about the age of two years and varies between individuals depending on external ecological factors (Baruš & Ošlava 1992). For instance, in one salamander, an increase by 19.0 mm in total length over five years was observed, whereas another one gained just 3.0 mm over five years and three months. The authors assume that the velocity of dorsal pattern alteration is correlated with individual growth velocity. Thus, future studies should try to find out whether or not changes in dorsal spots are more rapid and pronounced in juvenile individuals than in adults because of the faster growth rate of the former. Several observations raised doubts regarding pattern constancy. Bogaerts (2002) reported colorpattern modifications during ontogenesis in *S. salamandra* which were however, declared to be of minor significance in sexually mature adults and would not impede individual identification (Thiesmeier 2004). Beukema (2011) provided evidence on the ontogenetic development of the dorsal pattern in *Salamandra corsica* Sav, 1838,
Fig. 1 (this and opposite page): Ontogenetic changes in the shape of yellow dorsal spots of recaptured individuals of *Salamandra salamandra* (LINNAEUS, 1758).

Brightness differences between identical spots photographed at different dates are caused by different light conditions in the field.

Individuals I – VII from Tichá Voda artificial damp gallery, VIII – X from Bobačka Cave.

I. 1a, 1b – 3 March, 2012; 2a, 2b – 1 March, 2013 (364 days).
II. 1a – 3 March, 2012; 2a – 28 December, 2012 (301 days).
III. 1a – 14 November, 2011; 2a – 30 December, 2013 (778 days).
IV. 1a – 14 November, 2011; 2a – 30 December, 2013 (778 days).
V. 1a – 14 November, 2011; 2a – 28 December, 2012 (411 days).
VI. 1a – 14 November, 2011; 2a – 1 March, 2013 (474 days).
VII. 1a – 20 December, 2011; 2a – 30 December, 2013 (742 days).

VIII. 1a – 9 March, 2012; 2a – 9 January, 2013 (307 days); 3a – 7 December, 2013 (333 days).
IX. 1a, 1b, 1c – 4 January, 2012; 2a, 2b, 2c – 21 December, 2013 (718 days).
X. 1a, 1b, 1c, 1d – 4 January, 2012; 2a, 2b, 2c, 2d – 21 December, 2013 (718 days).
recording a significant increase in the number of spots, which changed from a more rounded to irregular shape with increasing snout vent length. This could have been caused by the splitting of coherent larger spots into smaller spots, as was recorded in the present study. BEUKEMA (2011) furthermore drew attention to long-term studies and possible identification errors that may arise from his observation. WAYE (2013) observed marked ontogenetic alterations in the color pattern of Ambystoma tigrinum (GREEN, 1825) which may complicate not only individual identification but also sub-specific classification of Tiger Salamanders. Furthermore, the reliability of photo documentation for individual identification was critically reviewed in some frog species, e.g., *Litoria genimaculata* (HORST, 1883) and Bombina variegata (LINNAEUS, 1758) (KENYON et al. 2009; GOLLMANN & GOLLMANN 2011).

In conclusion, the authors suggest that in long-term studies of Salamandra salamandra photo documentation should be combined with another method of individual recognition. The non-invasive method proposed for *S. salamandra* by SUKALO et al. (2013) could be suitable. In a modified approach, the unique individual alphanumeric code would be formed by the number of openings on the yellow surface of the parotid glands only, exclusive of the openings on the potentially unstable yellow areas along the dorsum and tail.

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Moroccan herpetofauna distribution updates including a DNA barcoding approach

The herpetofauna of the kingdom of Morocco is among the richest and most diverse in the Maghreb and Western Europe. This fact is clearly related with the considerable topological variation of the region, with the Rif and Atlas Mountains dividing the country into climatically different zones.

The first step towards any ecological, conservation or modeling approaches concerning this rich diversity is to develop accurate distribution datasets. BONS & GENiEZ (1996) presented a detailed assessment of the known diversity at that time. However, various researchers have since then presented data indicating extension of known ranges for many species (e.g., GUZMAN et al. 2007; HARRIS et al. 2008, 2010; BARNESTEIN et al. 2010; BARATA et al. 2011; BEUKEMA et al. 2013; DAMAS-MOREIRA et al. 2014), and it is clear that current records are still limited, especially in the eastern region (BARATA et al. 2011; BEUKEMA et al. 2013). At the same time, modeling approaches have been employed, which may help to highlight regions in need of further prospection (DEPOUS et al. 2010). In particular, in an extensive review of the distribution and biogeography of Moroccan amphibians (BEUKEMA et al. 2013), models indicating regions of high probability of occurrence were presented for all species along with greatly improved distribution maps. New records for amphibians can therefore both be compared to these models and can be informative in determining whether distribution maps are stabilizing for such well-studied groups. Additionally, they can also be used to draw a parallel scenario in groups known to be much harder to locate, such as fossorial species or snakes.

The accuracy of distribution maps relies on species being correctly identified. This is not always simple – many forms of the Moroccan herpetofauna have recently been identified as species complexes (e.g., RATO et al. 2012), and several of these are "cryptic" implying that identification is made using molecular markers. This includes forms of the Podarcis vaucheri (BOULENGER, 1905) complex (PINHO et al. 2007), or lineages within various geckos including Quedenfeldtia (BARATA et al. 2012), Ptyodactylus (PERERA & HARRIS 2010) and Stenodactylus (METALLINOU et al. 2012). Other species can be difficult to identify when only juveniles or tadpoles are collected. Additionally, many groups such as snakes are often widely sampled as road-killed animals, and in some cases, identifying remains to the species level is again difficult. In these cases the use of a DNA “barcoding” approach (HEBERT & GREGORY 2005) can be useful.

In the present study, the authors compiled records of two expeditions to Morocco over a combined five week period in spring 2013 and 2014. Sampling covered a wide range of southern and eastern Morocco, and was done in complement to a recent survey of northern and central regions (DAMAS-MOREIRA et al. 2014). In total, 138 localities were sampled and 53 species recorded. GPS coordinates and a detailed list of species per locality are given in Table 1. Photos of most animals are available on request from the authors.