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Photo-identification of horseshoe whip snakes (*Hemorrhois hippocrepis*, Linnaeus, 1758) by a semi-automatic procedure applied to wildlife management

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Photo-identification is an increasingly used method for the study of animal populations. Natural marks such as coloration or scale pattern to identify individuals provide an inexpensive and less invasive alternative to conventional tagging methods. Photo-identification has previously been used to distinguish individual snakes, usually by comparing the pileus region. Nevertheless, this method is seldom used in capture-recapture studies. We show the effectiveness of photo-identification in snakes using specific software for individual recognition applied to a wildlife control study of horseshoe whip snakes. Photos were analysed with Automatic Photo Identification Suite (APHIS), which allowed us to compare the variability of head scale patterns surrounding the parietal shields instead of the traditional method of using large scale groups of the pileus. APHIS correctly identified 100 % of recaptures of snakes. Although further studies are needed, the variability of the surrounding scales of the pileus region seems a robust method to identify and differentiate individuals.

Keywords: Photo-identification, capture-recapture, scale patterns, invasive snake, wildlife control

The management of natural populations relies on robust estimates of demographic parameters such as population size, individual survival probability or per-capita fertility (Williams, Nichols & Conroy, 2002). These estimates can be obtained by the monitoring of individuals' fates through capture-mark-recapture (CMR) techniques (Southwood & Henderson, 2009). A common assumption in CMR techniques is that marks do not harm the animal or influence its fate, and marks are not lost; for practicality, the marking method should not be expensive to ensure that a large number of animals can be identified. In this respect, reptiles, and Squamata in particular, constitute a challenge (Ferner, 1979; Fitch, 1987). The continuous growth and the complete skin moult render most external tagging methods temporary for lizards and snakes. Moreover, external marks can affect the behaviour, growth or probability of recapture of marked animals, and can impair survival (Murray & Fuller, 2000). For example, snakes were traditionally marked by freeze branding or scale-clipping (Lewke & Stroud, 1974; Spellerberg, 1977). These marks had a variable permanence (Shine et al., 1988) and could affect behaviour of the animals (Weary, 1969). The use of medical cautery units (Winne et al., 2006) and of subcutaneous Passive Integrated Transponder (PIT) tags provided an alternative to the previous marking methods (Jemison et al., 1995; Christy et al., 2010; Oldham et al., 2016). PIT tags have near 100 % reliability if correctly implanted (Gibbons & Andrews, 2004) but the implantation of the device in small species can be complicated and, when many animals have to be marked, expensive.

With the advent of digital photography, attention has focused on using natural marks such as spots, scale patterns or colouration for individual recognition of reptiles (Sacchi et al., 2010; Rotger et al., 2016) as successfully done on other taxa (Katona & Whitehead, 1981; Van Tienhoven et al., 2007; Díaz-Calafat et al., 2018). Photo-identification techniques have the advantage of reducing handling time (Reisser et al., 2008; Gardiner et al., 2014; Sannolo et al., 2016), and in some cases, to preclude physical capture (Gatto et al., 2018). The scale pattern of individual snakes as unique was recognised in the last century, but manual techniques of comparison were time-consuming and limited the study to a small number of individuals. Nowadays, visual identification of individual snakes is based on the colouration pattern (Albu et al., 2008) or more commonly by the scale pattern of the pileus region (i.e. apicals, canthals, intercanthals, parafrontals, frontal, parietals and interparietals) (Benson, 1999; Stoyanov & Tzankov, 2017; Bauwens, Claus & Mergeay, 2018). However, the variability of these few large scales is limited, constraining the number of distinguishable identities. Here, we describe an alternative and more variable region of snake head scalation and illustrate a semi-automatic procedure (APHIS; Moya et al., 2015) used to successfully identify individuals of horseshoe whip snakes, *Hemorrhois*

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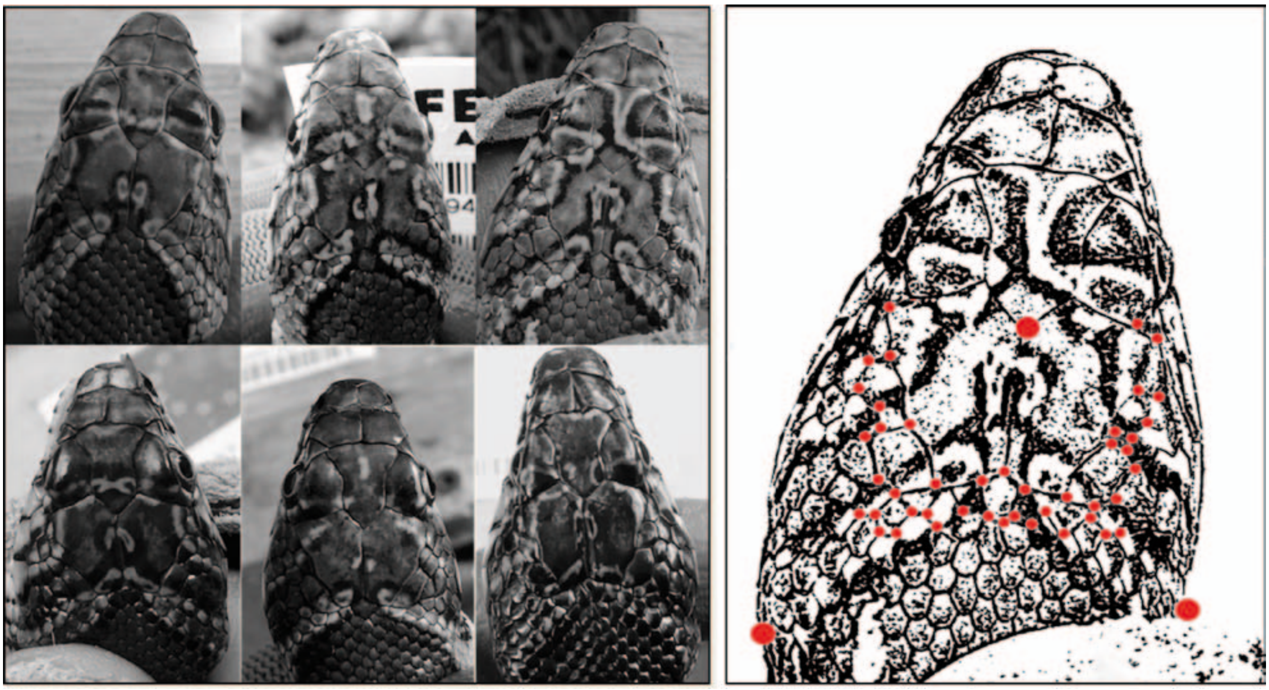

Figure 1. On the left, some photos of the head of horseshoe whip snake (*H. hipocrepis*) individuals where one can observe the scale pattern of the pileus region. On the right, picture showing the 3 reference points used (big dots) and the placement of the other points marking the intersections between scales.

hippocrepis. To our knowledge, this is the first time that a computer aided procedure has been used to identify snakes and may constitute an alternative to PIT tagging and scale marking.

The horseshoe whip snake is a long and slender-bodied colubrid, with a total length of up to 1800 mm, and no sexual dimorphism in body size. Populations are distributed in warm Mediterranean habitats of North Africa (northern Tunisia, Algeria and most regions of Morocco), and the eastern and southern half of the Iberian Peninsula. The invasive species was first reported on the island of Ibiza (Balearic archipelago, Spain) in 2003 inside Iberian olive trees (Álvarez et al., 2010). At present, this alien species represents one of the main threats to the native biota of Ibiza and several control campaigns have been carried out by the autonomic government. In 2018, a capture-mark-recapture study was conducted with the aim to quantify snake detectability and dispersal distance to optimise the control campaign. The study took place between the 17th of September and the 9th of November in 2018, where 50 traps were installed in five ha of a study area. Every three to five days, traps were checked at midday and all snakes trapped during the study period were photographed (head picture) with a digital camera, and marked with PIT tags to validate the photo-identification methodology. Finally, each snake was released near the trap in which it was captured.

Images were analysed using the I3S procedure in software APHIS (Moya et al., 2015), in which we delimit an area of interest with three reference points, then we use several additional landmarks inside this delimited area (in this study, at pre-defined scale intersections) to draw the scale pattern (see Van Tienhoven et al., 2007). The three reference points that we selected discarded many of the large scales of the head (pileus region) commonly used in photo-identification, after we found that this region varied little (Supplementary Material; Fig. S1), and we included the first row of scales posterior to the two parietal scales for its more variable pattern. As a first reference point, we used the intersection of the frontal scale and the two parietals. The second and third reference points were the most distal scales of the left and right diagonal row below the parietals (Fig 1). Once the three reference points were marked, we placed 30–50 spots on scale intersections within this area of interest. These additional points define the pattern of scales of the area that will be used as a 'fingerprint' by APHIS (Fig. 1). An initial photo of each individual snake was entered into APHIS before any comparisons could be made. These photos comprise the repository folder that APHIS uses as a reference when matching new photos. Additional photos entered into APHIS will then contrast the resulting patterns with those in the repository and present users with a ranking of possible matches sorted by a score. APHIS presents a ranking of matches of all the individuals saved in the repository (up to 100 images). However, we considered that APHIS successfully matched two photos of the same individual if the match was within the top 10 candidate images (Gatto et al., 2018). After the analysis, we inspected the images identified as possible scored matches by APHIS and confirmed as either a recapture or a new individual. Photos were then saved in the APHIS repository, allowing APHIS to accumulate multiple photos for each individual when they are recaptured over time. This results in multiple photos of the same individual that may be used as comparison photos for future analysis. In order to avoid duplicates of the same individual in the scored matches list, APHIS only shows the best scored photo of each individual in comparison with the new processed image.

A total of 26 captures were made during the study period, of which seven were recaptures from five different individuals. These seven recaptures were the photos that APHIS matched. All images were correctly matched (100 %) and in 85.7 % of cases, the match was within the first 10 ranked images proposed by APHIS (see Supplementary Material, Table S1). The average score was 0.366 and rankings for all pictures are out of 19 snakes (see Supplementary Material, Fig. S2). Scores were very tight indicating that defined patterns with points of two different images of the same individual are very similar to each other, although pictures were taken at different times (see Gatto et al., 2018). To sum up, head scale patterns in snakes provide a powerful tool to identify individuals and this method may be extended many species of snakes that combined with the use of specific software allow to optimise the identification process. The possibility to handle batches of images makes APHIS a good candidate but other software can be considered for the same purpose (see Sacchi et al., 2016).

In conclusion, photo-identification proved to be a reliable and non-invasive method to identify snakes based on scale patterns. Although this study has its limitations due to the small sample size, and further studies are needed to test correctly the reliability and accuracy of APHIS identifying individuals, it is a first step in the use of photo-identification software as a main identification method in snakes. Further development is needed and conventional methods may be still more reliable, especially when identifying many individuals. However, we show that scale patterns display potential as a robust identification method that should be considered when field studies are conducted; it is cheaper and less invasive than conventional PIT tag methods, in addition to involving minimal animal handling and null negative effects.

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