## Semi-automated photo-identification of Bahamian Racers (*Cubophis vudii vudii*)

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**Abstract.** Photo-identification is a non-invasive option for mark-recapture. Here, we tested the effectiveness of APHIS, a semi-automated photo-identification software, to distinguish between individual Bahamian Racers (*Cubophis vudii vudii*) on the island of Eleuthera, The Bahamas. Over 10 months, we photographed 50 Bahamian Racers. We first identified individuals by manually comparing colouration and scale patterns in the pileus and labial regions. Next, we used APHIS to identify recaptured individuals after manually identifying the locations of intersections of the scales in the pileus and labial regions. In addition, we assessed whether images taken with a hand-held camera or by a smart phone affected the accuracy of APHIS. All recaptured snakes were correctly identified using APHIS from both camera or phone images as validated by our manually derived results. We conclude that APHIS is an effective tool for photo-identification in snakes.

Keywords. APHIS, 13S, Mark-recapture, non-invasive, Snake, Dipsadidae, Colubridae, The Bahamas.

Mark-recapture studies require individuals to be reliably identified upon repeat encounters. This enables researchers to monitor specific individuals over time and this can allow for the estimation of ecological relevant information such as growth or survival rates (Pradel, 1996; Besbeas et al., 2002). In snakes, long-term marking is typically achieved by scale clipping (Brown and Parker, 1976), branding (Winne et al., 2006), passive integrated transponder tags (PIT tags) (Gibbons and Andrews, 2004), and/or visible implant elastomers (VIE) (Hutchens et al., 2008; Major et al., 2020). As each method has different advantages and disadvantages, researchers must critically assess which method is most appropriate for their study. Branding and scale clipping is generally inexpensive but can leave lasting physical damage (Weary, 1969; Brown and Parker, 1976). In contrast, PIT tags or VIE are highly reliable but are associated with considerable financial cost (Gibbons and Andrews, 2004; Major et al., 2020) and can be unsuitable for smaller individuals (Gibbons and Andrews, 2004). An alternative method that has the benefit of being both non-invasive and relatively inexpensive is the use of photo-identification (Sacchi et al., 2016).

Photo-ID has been successfully applied to numerous snake species (e.g., Carlström and Edelstam, 1946; Vaughan, 1999; Creer, 2005; Bauwens et al., 2018; Lunghi et al., 2019). Photos can be processed manually but this can be time consuming. In contrast, pattern recognition software offers a fast and robust approach to compare patterns in photos and distinguish between individual animals (Sacchi et al., 2010). One such pattern recognition software is the Automated Photo-Identification Suite (APHIS), developed by Moya et al. (2015). APHIS enables users to choose between two image matching

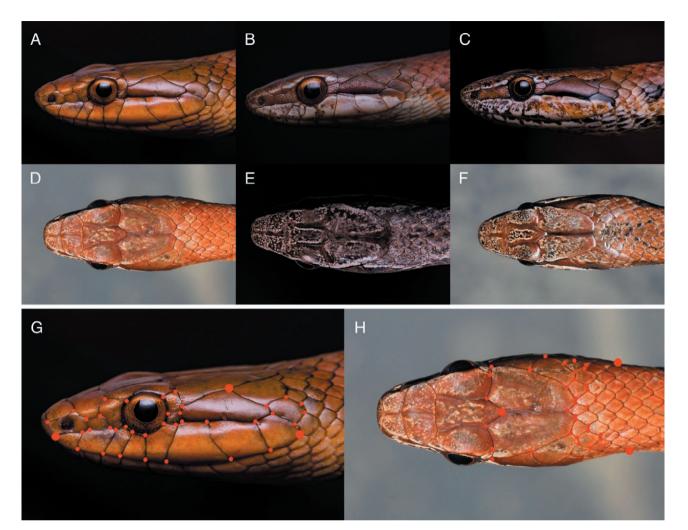
methods; the Image Template Matching, (ITM) a pixelbased colour comparison, and the Spots Pattern Matching (SPM) procedure that compares user-defined spot patterns. We selected to use APHIS because images can be processed in batches due to the independence of the manual pre-processing and automated photo-matching which allows substantial time saving (see Mova et al., 2015). APHIS also creates log files that can be used to track the analyses and allow for successive examinations. In addition, APHIS offers the use of two image matching methods. Initially, we wanted to compare both matching methods but decided to discard the ITM method because of quality issues in some of our images and, in addition, we were concerned about potential colour changes of the snakes over the course of the study. So far, APHIS has been used to differentiate between individual horseshoe whip snakes (*Hemorrhois hippocrepis*) (Rotger et al., 2019) but it has not yet been applied to other snake species. Here, we tested the efficacy of the Spots Pattern Matching (SPM) procedure implemented in APHIS (see Moya et al., 2015 for details) to identify individual Bahamian Racers (Cubophis vudii vudii). Specifically, we investigated (1) whether APHIS was able to accurately identify individuals based on scale patterns in the pileus and labial regions, and (2) if image quality influenced the successful identification.

Bahamian Racers are colubrid snakes endemic to the eastern parts of the Great Bahama Bank (Henderson and Powell, 2009). These opportunistic snakes feed on a wide variety of vertebrate prey (Hoefer et al., 2020; 2021), are diurnally active and frequently encountered around human settlements.

On opportunistic encounters with Bahamian Racers, we observed that head colouration and scalation was notably variable, particularly in the labial and pileus region (Fig. 1). Thus, we selected these areas for photo-ID. We did not use colour patterns as there are several examples of ontogenetic colour change in snakes (Creer, 2005; Lunghi et al., 2019). Consequently, we used only head scalation for identification purposes, as it is generally considered to be robust throughout a snakes' life (Bauwens et al., 2018). Upon capture, we measured snout-vent length (SVL) and tail length (TL) to the nearest millimetre using a flexible measuring tape, weighed the snake to the nearest gram using a weighing scale (DAPHA DWS Weighing Scale) and determined the sex via probing. Using a Nikon D3300 DSLR camera and Sigma 105 mm 1:2.8 DG Macro HSM EX lens in combination with an external flash and flash diffuser, we took photos of the pileus and the right and left labial regions. We assumed that both labial regions were identical for the use in APHIS and so we combined them when comparing to the pileus region. We initially identified recaptures via visual examination of colour, scale patterns, unique scarring and scale counts. We selected APHIS for our recapture matching analyses because pictures can be processed in batches due to the independence of the manual pre-processing and automated photo-matching, which allows for great time savings (Moya et al., 2015). In addition, APHIS creates log files that can be used to track the analyses and allow for successive examinations.

From August 2019 to June 2020, we opportunistically captured a total of 50 Bahamian Racers including 11 recaptures from five unique individuals. All snakes were found in small shrubs or leaf litter close to walking paths and buildings, which is likely the result of a detection bias due to the opportunistic nature of sampling. For the recaptures, we used 10 pileus, 10 right labial, 9 left labial images. In addition, we used 11 iPhone 7 images of a selection of the same recaptures (4 pileus, 4 right labial, 3 left labial) to compare to the DSLR photos and assess the usability of phone quality images in APHIS. The angle and composition for DSLR and phone images were consistent across photos, but overall image quality and resolution differed (DSLR: 300 PPI 6000 x 4000 px, iPhone 7: 72 PPI 4032 x 3024 px). For each snake, we marked between 35 to 45 reference points on the corners of the scales (Fig. 1) and analysed the images using the I<sup>3</sup>S procedure in an automated process described by Moya et al. (2015). We considered a successful match of a recapture when APHIS suggested the correct images for an individual within the top 10 candidates (i.e., top 20% of photos) (Gatto et al., 2018) and a top match when it was the first suggestion in the list. The candidate list created in APHIS is based on a similarity score where images that differ very little, i.e., photos of the same snake, produce a low score and are ranked at the top. Images that differ considerably result in a high score and are ranked at the lower end of the list. The similarity score represents the difference between photos in the relative distances of the reference points.

When comparing the combined labial and pileus regions, we found that all of the labial and pileus images were correctly identified as recaptures, with 100% (19/19) of the labial photos and 90% (9/10) of the images in the pileus region resulting in a top match, making either area suitable to use in APHIS. When only using photos collected via the DSLR camera (n = 29) across all head regions combined, recaptures were correctly identified 100% (29/29) of the time and 97% (28/29) were suggested as top matches. Images of recaptures taken with an iPhone 7 were successfully identified 100% (11/11) of the time when compared to the DSLR database photos, with 82% (9/11) of top match sugges-



**Fig. 1.** Photographs of three different Bahamian Racers: (A) - C) shows the labial region; (D) - F) shows the pileus region. (G) and (H) shows an example of the reference points selected within APHIS marking the intersection of the scales in the labial and pileus region, respectively.

tions. When the top match was incorrectly identified, the difference in score between the incorrect first candidate and the correct candidate was marginal. Even when the two images that were compared differed substantially in resolution and composition, APHIS was able to successfully match recaptures. In addition, we did not observe any colour or scale pattern changes in neither the labial nor the pileus region of any individual Bahamian Racer over the course of this study (duration between captures ranging from 6 days to 216 days, Figs. S1 and S2). However, 10 months likely only provides a glimpse into the lives of these snakes and, furthermore, all the recaptured individuals were adult snakes and thus ontogenetic changes in colouration or scalation cannot be dismissed.

In conclusion, we were able to use APHIS to correctly identify and successfully match all our recaptured Bahamian Racers. The software provided accurate results even when the image quality differed substantially between the photos compared and the recaptures were up to seven months apart. The semi-automated analysis resulted in high matching probability when using images taken from a smartphone, which is likely more accessible in the field than a dedicated camera. The use of pattern recognition software in recapture studies allows for short handling times in the field and only requires taking a photograph. This likely increases the time available for searching and recording new individuals, thus enhancing data collection. Particularly for studies on snakes, where detectability of animals can be quite low, maximising the time to find snakes could enable researchers to gather more crucial information for many of these understudied species. Even though this study has its limitations due to low sample sizes, we provide

further support for the use of APHIS to effectively distinguish between individual animals.

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## SUPPLEMENTARY MATERIAL

Supplementary material associated with this article can be found at <a href="http://www.unipv.it/webshi/appendix">http://www.unipv.it/webshi/appendix</a>> manuscript number 11502.

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