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Using chin spot pattern to differentiate individual *Masticophis lateralis* (Hallowell, 1853) on the Pacific Coast of the United States

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Individual identification of animals is often critical when investigating their natural history (Baumgartner, 1940; Stonehouse, 1978; Lebreton et al., 1992; Caughley and Gunn, 1996; Silvy et al., 2020). Researchers have used a variety of techniques to mark reptiles, including painting, tattoos, branding, toe-clipping, scale clipping, sewing beads into the skin or tail, and other methods (Ferner, 2007; Kellner et al., 2017; Silvy et al., 2020). Specific criteria for marking techniques were listed by Ferner (2007) which included: the recipient should be free from stress and pain, the marking should be clearly identifiable, indefinite, easily readable, functional on different size specimens, functional in the lab and field, and be cost effective. In our experience, the three most common techniques for marking snakes are, ventral scale clipping (Blanchard and Finster, 1933; Brown and Parker, 1976), branding (Weary, 1969), and PIT (passive integrated transponder) tags (Keck, 1994). We found that scale clipping and PIT tags were both practicable and effective methods to mark many snake species we worked with in Central California. However, both methods require permitting approvals that include species and technique-specific training, which may not be available to all researchers (Silvy et al., 2020; pers. obs.). More recently, researchers have used natural markings as a means to identify individuals (Wilson and McMahon, 2006; Baker and Allain, 2020; Clapman et al., 2020; Silvy et al., 2020; Couffer, 2022). It is important to note, however, that some researchers have found error rates that may result in misidentifications for some species (Sacchi et al., 2010; Rocha et al., 2013; Jones et al., 2020), and mock trials may be required to determine error rates for the species in question (Kellner et al., 2017)

While conducting demographic studies on populations of the California whipsnake (*Masticophis lateralis*), we trapped snakes to determine species' site use from 2017-2022. Snakes received an individual scale clip, and we inserted a subdermal PIT tag in appropriately sized adult snakes. We also took photos to determine the phenotypic range of the two California whipsnake subspecies: Chaparral whipsnake (*M. l. lateralis*) and Alameda whipsnake (*M. l. euryxanthus*), following Riemer (1954), and the ranges presented by Jennings (1983) and Richmond et al. (2016).

During analysis of these photos, we suspected that the spot pattern on the ventral portion of the head (chin) was likely unique for each individual, and may be consistent over time (from juvenile stage to adult stage). The serendipitous discovery of this natural, non-invasive method appeared to be effective and meets all of the criteria suggested by Ferner (2007) for marking individual snakes.

Between 2017 and 2022 we collected 64 *M. l. euryxanthus* in Alameda and Contra Costa County, California. We photographed eight regions of the body of each snake. Photographs of the ventral portion of the chin and anterior portion of the head of each whipsnake were then compared to all previous captures at the same site, over consecutive years. One of the 64 snakes was a recapture from a previous year. Photos of the recaptured snake were collected from the ventral portion of the chin for comparison (Figs. 1 and 2). Ventral chin spots tend to slightly increase in size and darken over time, but spot arrangement did not appear to change. However, some level of colouration change was observed during shed cycles, which may partially obscure chin patterns.

An individual juvenile male that was collected on 12 June 2020 measured 620 mm total length and weighed 23 g. Subsequently, an adult individual was captured on 14

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May 2022, 23 months later, and measured 992 mm and weighed 66 g, an increase in length and weight of 160% and 287%, respectively. We compared chin patterns and noted an identical pattern. This identification was followed up by an examination of a scale clipping shadow, indicating that the recaptured snake was the same individual. The individual snake retained a chin spotting pattern that was identical over the two-year period despite substantial changes in growth (Figs. 1 and 2).

To further support our visual estimate of similarity, we digitised and rasterised the spot pattern and overlaid the photographic images with the digitised image (Fig. 3). No new spots appeared, and no original spots were lost. Carlström and Edelstram (1946) and Baker and Allain (2020) found similar results with grass snakes (*Natrix natrix*) and barred grass snakes (*N. helvetica*), respectively. Baker and Allain (2020) used ventral colouration and dark patterns to differentiate among nearly 700 snakes, up to 4.5 years after initial capture.

Most populations of *M. l. euryxanthus* are relatively small, which facilitates using ventral chin patterning as an effective and easy method to determine individual identity. Bolger et al. (2011) suggested using software like WILD-ID (https://github.com/ConservationInternational/Wild.ID), or Individual Identification System (I^3S Spot 4.02; www.reijns.com/i3s), used by Love et al. (2018) when the numbers of photographs increase. These software programs use several reference locations and analyse the distribution and size of spots on the animal's surface. Numerous options for free, online image-pattern software are available to aid in analysis of larger data sets.

Passive integrated transponder tags and scale clipping remain effective means of marking snakes. However, we found that scale clipping can be obscured by regeneration of ventral scutes, particularly on small snakes. Our anecdotal observations suggest that ventral scale clipping on small snakes may last for one season, or possibly two, but is not permanent. Further, numerous projects exist that are guided by resource agency permitting that may allow capture and translocation, but not permanent marking. In these cases, the handler can collect photos of the chin and anterior portion of

Figure 1. Photographic image of a juvenile male *Masticophis lateralis euryxanthus* showing chin spotting pattern at the time of initial capture, 12 June 2020. Photograph by Amanda Colombo Murphy.

Figure 2. Photographic image of an adult male *Masticophis lateralis euryxanthus* showing chin spotting pattern 2 years after initial capture, 14 May 2022. Photograph by Amanda Colombo Murphy.

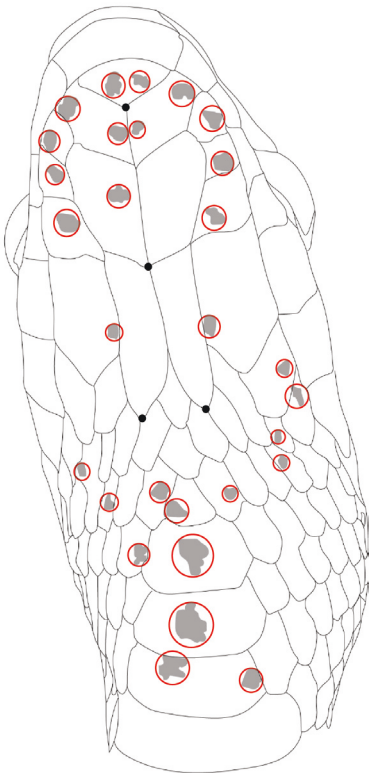

Figure 3. Rasterised and digitised pattern of the chin spotting (upper labials omitted) of a male *Masticophis lateralis euryxanthus* collected in Alameda County, California, USA. Black dots represent reference points for computer program analysis. Red circles are spot annotations used to differentiate individuals in I³S computer program.

the snake's ventral surface to determine individual identification. Ideally, snakes are positioned such that the head and neck are held horizontal against a contrasting substrate, with the head facing away from the photographer; the photographer then collects the image of the chin and neck region.

We feel confident that this method will work for both subspecies of *M. lateralis* and suspect that this method may work for other species of *Masticophis* that we have worked with that have chin and anterior ventral patterning (i.e., *M. fuliginosus, M. flagellum*, etc.). Our work reported here suggests that a non-invasive method for identifying individuals of *M. lateralis* may be effective, long lasting, and avoids invasive techniques.

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