

Verification of Natural Marking for Individual Identification Using a Duplex Marking Approach in Ijima's Sea Snakes, *Emydocephalus ijimae* (Reptilia: Elapidae)

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The mark and recapture method for free-ranging animals provides valuable information in ecological studies. Recently, natural marking has become more frequently used for individual identification, but it almost inevitably induces problems associated with the corroboration of individual specificity and the persistence of the given markings. We employed a duplex natural marking approach to resolve this problem over a four-year field study of a banded hydrophine sea snake and tested the effectiveness of this approach in corroborating the accuracy of individual identification. We conducted monthly field surveys in southwestern Japanese waters and photographed the patterns of the last five bands on each captured sea snake. We converted the band patterns into profile codes with five sections (one section corresponding to each band), according to the scale configurations involved in the bands. We considered the bilateral band patterns as a duplex set of natural markings for individual identification and checked their accuracy mutually. We looked at 593 photos of recorded snakes and recognized 179 unique profile codes on both the left and right sides, 96 of which were recorded more than once on both side. A particular code for the left side was always accompanied by a particular code on the right side in the same combination. It is certain that the 593 recorded snakes consisted of 179 snakes and their recaptures. The perfect correspondence between the left and right side profile codes throughout the four years showed the high individual uniqueness and persistence of each pattern. This study also showed that the duplex natural marking approach is effective in verifying the accurate individual identification. The duplex natural marking approach can be applied to various animals to justify the usage of a given natural marker for individual identification, without the aid of combined artificial markings. The duplex method itself can be a combination of the first five bands and the next five bands on the same side in a single photo, or a combination of some patterns on the head and those on the body.

Key words: Natural marker, Mark-recapture, Population ecology, Ryukyu Islands, Sea snake.

BACKGROUND

The mark and recapture method for free-ranging animals is an effective tool in ecological studies, providing information about the growth, age, survivorship, movements, and other life history traits of a given animal. In reptiles, individuals have been identified by scale clipping, branding, tattooing, subcutaneous elastomer injections, passive integrated transponder (PIT) tags, painting, and toe-clipping (Ferner and Plummer 2016). However, artificial marking may impose stress on the animal and/or may affect their behavior or survivorship (Murray and Fuller 2000).

In recent years, a method for individual identification based on natural marking, which utilizes individually unique natural features, has been developed for various groups of animals, including mammals (Hiby et al. 2009), reptiles (Schofield et al. 2008; Treilibs et al. 2016; Bauwens et al. 2018), amphibians (Caorsi et al. 2012), fish (Speed et al. 2007), and arthropods (MacDiarmid et al. 2005). The natural marking method targets indigenous features, such as body surface patterns (Sreekar et al. 2013), scale arrangement (Gardiner et al. 2014; Gatto et al. 2018), ornament shapes (MacDiarmid et al. 2005), and iris pattern (Rocha et al. 2013). Many of these natural markers are based on the visual recognition of a particular body part; thus, they can be recorded in photos (generally referred to as photo identification). In typical cases, photo identification is conducted without catching the target animals, making it far less invasive than other identification methods. Even in cases involving temporary capture, photo identification encompasses a few other advantages simultaneously, such as being relatively inexpensive (as it only requires a digital camera and computer), requiring only basic expertise to manage recorded images, and allowing for the identification of a large number of individuals (Sacchi et al. 2016).

One serious concern of natural marking, in contrast to artificial marking, is how we can corroborate individual specificity of certain features recorded. If a pattern is not unique to a single individual, the accuracy of individual identification is violated. Regrettably, corroborating individual uniqueness is theoretically difficult: even if we confirm that 100 individuals show 100 unique patterns, the pattern of the 101st individual may be identical to one of the previous 100 individuals. Also, one must ensure that a particular pattern that is seemingly unique to a single individual does not change over time, at least not in a manner that would confuse their identity with that of another individual. One effective resolution of these problems is the use of artificial marking together with natural marking

to verify the accuracy of the latter in a pilot study (Schofield et al. 2008; Caorsi et al. 2012; Bauwens et al. 2018). However, such a combined approach is difficult to conduct for animals that are negatively affected by artificial marking treatments like clipping toes or scales, tagging, or other possible treatments. Another possible resolution of this issue may be duplex individual identification, which involves using two sets of natural markers and mutually verifying results of the identifications. The duplex marking approach allows the researcher to verify the accuracy of the individual identification based on one set of natural markers, using the second markers to verify the first and vice versa. When captured snakes are individually identified by two independent natural markers, they are expected to be identical only when each the markings in each of the two sets is unique to an individual and not changed over time. In contrast, a simplex marking system, using only one set of marks, does not allow the researcher to falsify possible incorrect individual identifications, even if the single marking system seems intuitively accurate. Here we present an example of duplex verification without the aid of artificial markers using a hydrophine sea snake, *Emydocephalus ijimae*, as a model species.

Emydocephalus ijimae is a true sea snake that inhabits the shallow coral reefs of the East China Sea (Takahashi 1984; Rasmussen and Ineich 2010). This species is cream and black banded from the neck to the tip of the tail, and the shape of the cream bands appears to vary across individuals. The species is known to be highly sedentary, with a high recapture rate (Masunaga and Ota 2003). Altogether, these characteristics make this sea snake a suitable species for testing the effectiveness of natural marking for individual identification. We thus attempted a mark and recapture study of *E. ijimae* using band pattern as a natural marker and verified its accuracy and stability by treating the patterns on the left and right sides as duplex sets of markings.

MATERIALS AND METHODS

Study sites and sampling

A field survey was conducted in two neighboring sites (Urunosachi, 26°13'25"N, 127°17'37"E and Ama, 26°13'27"N, 127°17'27"E) in the southern area off the Zamami Island of the Kerama Islands, Central Ryukyus, Japan (Fig. 1). The two sites are approximately 500 m apart, and separated by a gap in the coral reef with a bare sandy bottom that spreads more than 100 m in width. Mark and recapture surveys were conducted almost every month from March 2016 to January 2020.

In each survey, researchers searched for snakes by slowly swimming with the aid of a snorkel. When a sea snake was found, it was caught and temporarily carried to land, at which time the animal’s sex was determined by counting the number of subcaudals, following Masunaga (2002). Snout-vent length (SVL) was measured using a tape measure. Tail patterns on both sides were photographed using a digital camera at resolutions between 72 and 314 dpi (Tough TG-4, Tough TG-6, OLYMPUS, Tokyo). Because the sea snakes’ tails are flattened vertically, the photo images can be treated as two-dimensional without any problem related to the photographing angle. After this data was collected, the snakes were released back to their original points of capture.

Determination of the profile code from photograph as a natural marker

In each photo, the band pattern on the tail was transformed via visual inspection into a profile code. In *E. ijimae*, the scales on the body and lateral sides of the tail are large, and each scale is usually either entirely cream or black. Black scales are dominant, and the head and tail tip are mostly black, such that a clump of cream scales can be recognized as a band against the black background. A transverse cream band usually consists

of two to three successive scales along the body axis, and usually five to seven (though in some rare cases, up to nine) scale rows along the dorso-ventral axis (Fig. 2).

We defined the profile code of a band as follows: 1) each scale was classified as either “cream” or “black” according to its tone; when a single scale was conflated by a cream part and a black part, it was assigned to cream or black according to the dominant color (> 50%) in the area; 2) when cream scales piled in three or more successive rows, we regarded them as a band; 3) the band nearest to the tail tip was defined as the first band, and then the bands were numbered toward the head and recorded up to the fifth band. With these rules, the number of successive cream scales in a single row within a single band was noted, and a series of these digits from the dorsal-most row to the ventral-most row was defined as the code of that band. For example, when a cream band consisted of 2, 3, 2, 2, and 3 scales from the dorsal to ventral sides, its code was expressed as “23223”, and we referred to this as the “partial code” of that band. Partial codes were combined with hyphens from the first to the fifth bands, comprising a profile code for that individual snake. The coding system requires caution when there is an insertion of a scale row from the tail side to the head side within a single cream band. When a large scale is followed by two small scales, the large scale is regarded as a component

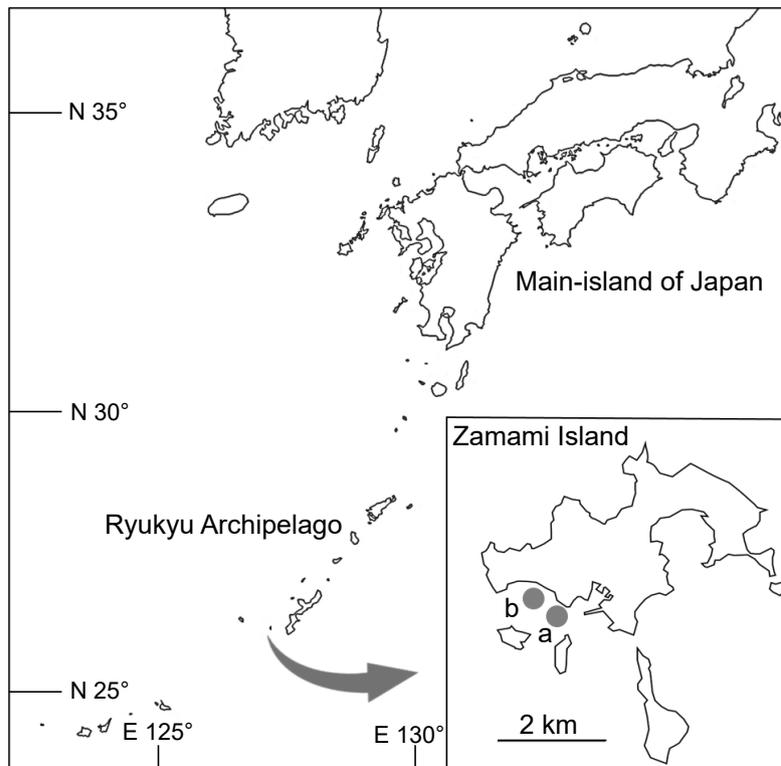


Fig. 1. Maps showing the location of Zamami Island and the study sites: (a) Urunusachi and (b) Ama.

of both rows and counted twice. When a new row is inserted between two rows and the sizes of the involved scales are not apparently different, the inserted scale is judged as an independent row (Fig. 3). On the basis of these definitions, the profile code of the band pattern shown in figure 2, for example, is expressed as “12323-12233-12232-122222-223232”. The band patterns on both sides of all captured snakes were transformed into profile codes in this manner. The profile codes of all recorded snakes for the left and right sides were compiled into two independent data sets.

We also recorded cream flecks posterior to the fifth band, which often existed on black spaces independent from any cream band as much smaller, irregularly-shaped cream marks within a single scale. Because of their irregular shapes and sizes, we treated their number as the number of scales upon which one

or more cream flecks are recognized. We recorded the number and position of the fleck-bearing scales and used this information in individual identification as supplementary visual markings.

RESULTS

Profile code variation

A total of 593 snakes were captured from March 2016 to January 2020. Of these snakes, 392 were captured in Urunosachi and 201 were captured in Ama. A maximum of 17 snakes were captured in a single day. No snakes had damaged tails and we could determine the profile codes on both sides based on the photographic records. Among the 593 snake photos of



Fig. 2. An example of the tail banding pattern of *Emydocephalus ijimae*, which was converted into the profile code “12323-12233-12232-122222-223232”, according to the scale configurations within the cream bands. See the text for details concerning the conversion to the code.

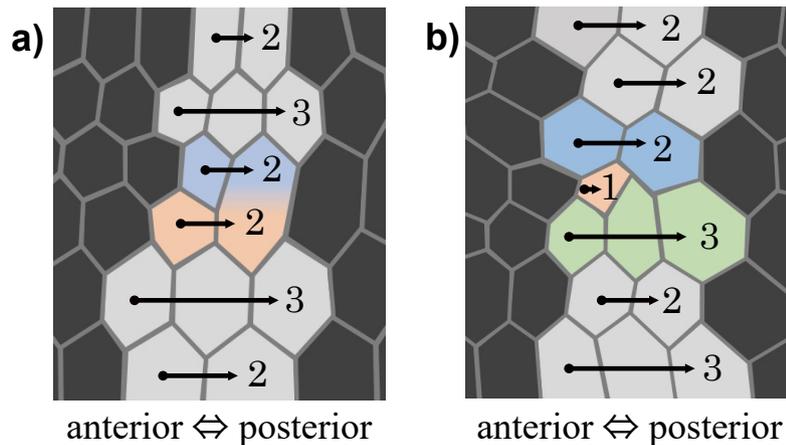


Fig. 3. The present coding system when there is an insertion of a scale row from the posterior to the anterior within a single cream band. a) When a large scale is followed by two small scales, the large scale is counted twice and the code for this example is “232232”. b) When a new row is inserted between two rows, the inserted scale is judged as an independent row and the code for this example is “2221323”.

the first band on the left side, 63 different partial codes were recognized, indicating numerous overlaps in the partial codes across the photos. When we included up to the second band on the left side (*i.e.*, the accumulative partial code from the first to the second bands), 151 codes were recognized. When we included up to the third and fourth bands, 174 and 179 codes, respectively, were recognized; however, there was no further increase up to the fifth band (179 codes). Results were similar for the right sides: when we accumulated partial codes up to the first to fifth bands, the number of codes was 61, 156, 177, 179, and 179, respectively, and thus, 179 codes was the peak, with no further increase upon incorporation of the fifth band.

Amongst the left side photos obtained throughout the study period, 96 out of the 179 profile codes appeared more than once; similarly, 96 profile codes were recorded more than once amongst the right side photos. In these repeatedly-recorded profile codes, a particular left side code was always accompanied by a particular right side code, and they never appeared in different combinations. For instance, a unique left side profile code (23232-22232-22221-1222233-22232222) was recorded five times on 19 March 2016, 18 August 2016, 9 December 2016, 3 May 2017, and 7 July 2017, and this was accompanied with a unique right side profile code (21212-22121-212312-122222-122232) on all five occasions, and the latter code was never recorded on other days. The one-to-one correspondence between the left side code and right side code was true for the remaining 95 profile codes that were recorded more than once. Furthermore, snakes captured at the same time, and thus evidently were different individuals, never exhibited the same profile code. Snake photos with the same profile code were always recorded on subsequent captures as being the same sex, and their SVL was never smaller, within the range of measurement error, but rather was always just as large or became larger over time. The profile codes that appeared more than once were recorded 2–21 times, with a mean of 5.3 times ($n = 96$). The time durations from the first to the last records of the same profile codes were 1–1373 days, with a mean of 454.3 days ($n = 96$).

Within the 179 recorded profile codes, several particular partial codes for each band appeared more frequently than others. Of the 63 partial codes for the first band on the left side, 12221, 12121, and 12222 appeared in 16 (8.9%), 12 (6.7%), and 9 (5.0%) out of the 179 codes, respectively. Several partial codes in the second to fifth bands also occurred frequently on the left side, with the most frequent codes occupying 17.3%, 11.7%, 14.0%, and 7.8% of the 179 profile codes, respectively. The same was true for the right side, with the most frequent codes in each of the first to fifth bands

occupying 10.1%, 10.1%, 11.7%, 15.1%, and 6.1% of the 179 profile codes, respectively.

Cream flecks were recognized in the photos of almost all snakes (in 589 out of the 593 photos of the left side and 591 out of the 593 photos of the right side). With respect to their correspondence with the profile code, a cream fleck was usually recognized in the same position in a snake's photos with a particular profile code which was recorded more than once. In other words, photos with the same profile code usually had fleck(s) in the same position on the tail. However, photos with 18 patterns of profile codes were exceptions. In these snakes' photos, one or more flecks were not recognized in a photo from a previous record, but appeared in the photos of a snake with the same profile code in the following records. This addition of cream flecks was only recognized in juveniles or semi-adults whose SVL was less than 507 mm. Among all 593 snake photos, the number of cream flecks on one side of individuals less than 500 mm in SVL tended to be smaller than those in individuals as large as or larger than 500 mm in SVL. More specifically, for the left side, the number of cream flecks was 2–27 (mean: 8.6; $n = 76$) among those with SVL < 500 mm and 0–37 (mean: 12.7; $n = 103$) among those with SVL \geq 500 mm. Similarly, on the right side, the number of cream flecks was 0–35 (mean: 8.6; $n = 76$) among those with SVL < 500 mm and 1–50 (mean: 11.9; $n = 103$) among those with SVL \geq 500 mm. These differences in fleck numbers between the two size classes were statistically significant for both sides of the tail (Welch's *t*-test, left side; $t = 3.838$, $P < 0.001$, right side; $t = 2.845$, $P < 0.005$).

DISCUSSION

Validation of individual uniqueness and band pattern stability

The numbers of accumulative partial codes from the first band to the fifth band in the left side system plateaued at 179, and 96 out of the 179 profile codes appeared multiple times. The same was true for the right side—the codes plateaued at 179, and 96 profile codes appeared multiple times. Each of the 96 repeatedly-recorded profile codes on the left side always appeared with a particular right side code in the same combinations. These results strongly suggest that the 593 snakes recorded over the four years of this study consisted of 179 individuals and their recaptures. This is supported by additional circumstantial evidence that snakes with the same profile code were always of the same sex and their body sizes did not apparently

decrease, but rather became gradually larger over time. The complete absence of different combinations of particular profile codes on both sides suggests that each profile code was unique to each of the putative 179 snakes upon analysis of all of the first to fifth bands.

Our results also support the high stability or persistence of each particular profile code over time. Many species of snakes are known to exhibit ontogenetic changes in color pattern (Burghardt 1978). If the scale configurations in a band in *E. ijimae* changed ontogenetically, this would violate accurate individual identification (Sacchi et al. 2016). If we assume that some individuals had changed their profile code during the survey period, some profile codes of one side would be expected to be recorded with more than one profile code on the other side in different combinations, unless the changes in profile codes of both sides always occurred simultaneously. However, this was not the case in our data, strongly suggesting that the profile code seldom changed over time. The mean time duration from the first to the last records of recaptured snakes was 454.0 days ($n = 96$), and the longest time interval

was 1,373 days (3 years and 9 months) with 15 putative recaptures. This result supports the idea that particular codes persist for a long time in both adult and young snakes (Fig. 4).

It is worth considering how many individuals we could potentially discriminate using the present profile code system. Several particular partial codes for each band appeared at high frequencies and would cause an occurrence of individuals with identical profile codes. Nonetheless, the emergence probability of an individual with the most frequent partial codes at all five bands on the left side is 1.98^{-5} which is multiplied by the most frequent value of each partial code, under the assumption of the independent nature of partial codes among the bands. Actually, we can find the probability that any two snakes do not share an identical partial code by calculating the sum over the square of the frequency of each recorded partial code in this sample (*i.e.*, 179 snakes), expressed as $1 - \sum_{i=1} P_i^2$, where P_i is the frequency of *i*th recorded partial code. This equation is equivalent to Simpson's diversity index (D). Actual values for the second term in this equation ($\sum_{i=1} P_i^2$) for

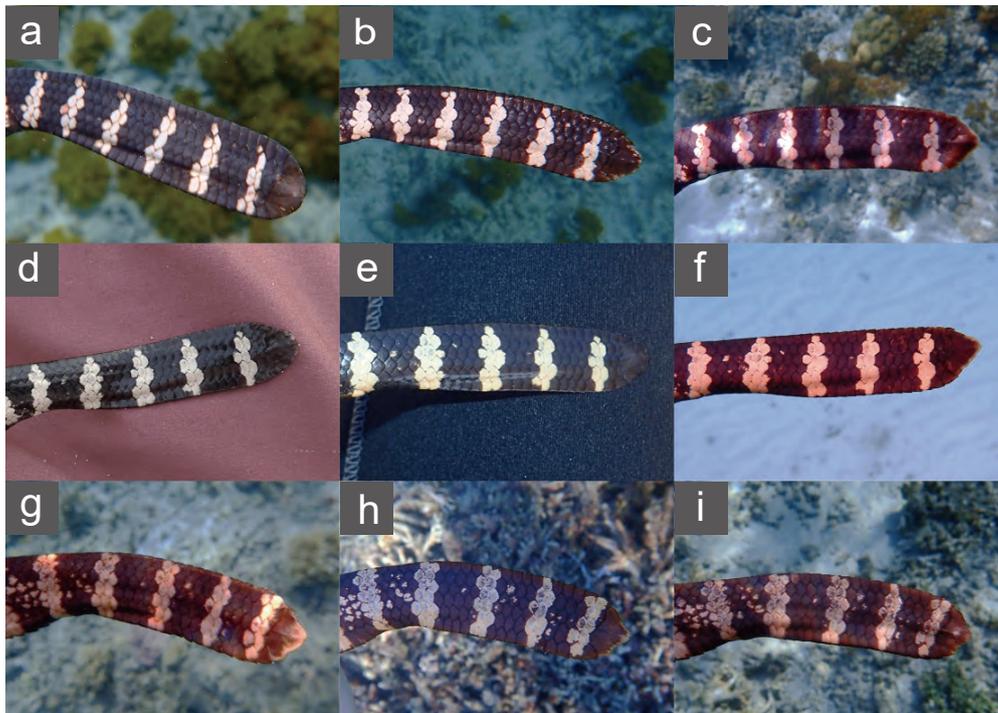


Fig. 4. Photographs of the left side of the tails of three recaptured snakes. (a) A juvenile male with 338 mm in SVL and the profile code 1212-12222-122121-112222-112221, recorded on 15 March 2018, (b) The snake's recapture 159 days later, with 460 mm in SVL on 21 August 2018 and more cream flecks, and (c) the snake's additional recapture a further 373 days later, with 612 mm in SVL on 29 August 2019 and no additional increase in the number of flecks. (d) A juvenile male with 415 mm in SVL and the profile code 2221-1222-123222-123232-1222322, recorded on 22 August 2018. (e) The snake's recapture 58 days later with 437 mm in SVL on 19 October 2018 with enlarged flecks, and (f) another recapture a further 268 days later, with 551 mm in SVL on 14 July 2019 and no additional change in the flecks. (g) A semi-adult female with 499 mm in SVL and the profile code 21221-12221-222222-222232-23223322, recorded on 7 July 2017. (h) The snake's recapture 527 days later with 636 mm in SVL on 16 December 2018 and more flecks, and (i) another recapture a further 404 days later, with 691 mm in SVL on 24 January 2020 and no additional change in the flecks.

the first to fifth bands on the left side were 0.031, 0.059, 0.040, 0.044, and 0.016, respectively, and the probability that any two snakes are not identical is “ $1-5.08^{-8}$ ”. Then, the probability that any two snakes with an identical profile code are not included in n snakes is obtained by “ $(1-5.08^{-8})^n$ ”. According to this formula, the probability that identical snakes are not included among 10,000 snakes ($n = 10,000$) is still 0.9995. In reality, the assumption of independence among the five bands is not guaranteed, but it is supposed that thousands of snakes would be distinguished without any overlap using the profile code system on one side, and importantly, it can be verified by comparing the profile code for the other side.

The condition of cream flecks is not necessarily constant over time. A larger number of flecks were found in larger-sized than in smaller-sized individuals, suggesting the emergence of new flecks with their growth. If we assume that individual identification using the profile code is reliable, it is evident from our recapture data that the cream flecks had newly emerged with growth in several snakes (Fig. 4). However, these increases in the number of cream flecks were mostly recognized in snakes whose SVLs were less than 500 mm, and the conditions of the flecks were largely stable in adult snakes. Collation of profile codes with past records is not an easy task because the code system is long and complicated, however, if desired, the pattern of cream flecks can be used supplementarily in narrowing candidates of past records in individual identification via visual inspection, especially for adult snakes.

CONCLUSIONS

In conclusion, we demonstrated that the scale configuration involved in the cream bands, as expressed by the profile code, was useful as a means of natural marking for individual identification in *E. ijimae*. The duplex usage of profile codes (on both sides) allowed us to corroborate the strict uniqueness and high stability of the profile code without the need for any artificial markings. This effective approach does not necessarily require bilateral markers. For example, it can be a combination of the first five bands and the next five bands on the same side in a single photo taken by camera trapping, or in case of other animals where tail defects will occur, it may be a combination of some patterns on the head and those on the body.

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