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Photographic Identification of Green Turtles (*Chelonia mydas*) at Redang Island, Malaysia

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Details of life history traits of animals are often obtained through mark-recapture projects that identify individuals. For sea turtles, the most common approach is to attach metal tags to the trailing edge of the flippers. Through recaptures of marked individuals, valuable information such as the reproductive biology, movement patterns, habitat use and population size estimates may be obtained (Godley *et al*. 2008; Mansfield 2006; van de Merwe *et al*. 2009). However, this identification method is not foolproof. Apart from tag loss, which causes bias in sea turtle studies such as overestimation of the number of nesting females in a nesting season; underestimation of the number of clutches deposited by individual turtles and incorrect inter-nesting data (Bjorndal *et al*. 1996; Liew & Chan 2001), flipper tagging is an invasive method that may cause disturbance and discomfort to sea turtles (Shanker *et al*. 2003). Passive Integrated Transponder (PIT) tags that are injected into muscle have a higher retention rate than metal flipper tags (Fontaine *et al*. 1987; van Dam & Diez 1999), but require a scanner to record the unique ID.

An alternative method for recognizing individual turtles is photographic identification (photo-ID). Compared to conventional tagging, this method of visual identification has advantages such as animals are not physically captured or hurt and the chances of stressing the animal are reduced (Schofield *et al*. 2008). Moreover, with the advent of digital technology, studies of various taxa are turning to photo-identification as an alternative method of identification. This method has been used in green-eyed tree frogs (Kenyon *et al*. 2009), painted crayfish (Frisch & Hobbs 2007), dolphins (Currey & Rowe 2008; Weir *et al*. 2008), whalesharks (Brooks *et al*. 2010), cheetahs (Kelly 2001), small-scaled skinks (Gebauer 2009), elephants (Ardovini *et al*. 2008) and polar bears (Anderson *et al*. 2007). For sea turtles, this unique, natural feature is found on the head.

In 1986, McDonald & Dutton (1996) reported that the pineal "pink" spots of nesting leatherbacks were recognizable over time. The method was reinvestigated by
Buonantony (2008) and De Zeeuw *et al*. (2010). Subsequently, photo-ID utilizing the facial scute patterns has been explored in other species of sea turtles: hawksbill, green and loggerhead turtles (Chassagneux *et al*. 2013; Feliz *et al*. 2013; Jean *et al*. 2010; Lloyd *et al*. 2012; Reisser *et al*. 2008; Schofield *et al*. 2008). There are two main assumptions underlying the application of photo-ID: i) the distinct characteristic of each individual is different and ii) the distinct characteristics are stable over time.

We used photo-ID to recognize individual green turtles at Chagar Hutang, Malaysia (Fig. 1). This nesting beach was declared a turtle sanctuary in 2005 and public access is now restricted. During the 2010 (May-June), 2013 (March-July), and 2014 (March-May) nesting seasons, we photographed the left and right facial profiles of nesting females after they had finished oviposition on the beach. Sand was first cleared from the head so that the scute patterns could be seen clearly. With a flashlight shining from above or sideways, both sides of the facial profile (left and right) were photographed using a digital camera (SONY DSC-T2-8.1 Megapixels or Olympus XZ-1-10 Megapixels). The profile pictures were taken at a distance of approximately 20 cm at a 90° angle to reduce distortions caused by inclination. Flash photography was not used as literature has shown that green turtles shorten the duration of nest covering and camouflaging stages when exposed to camera flashes (Campbell 1994). Instead a small flashlight shone from above was used as the light source. For each nesting female we photographed, we also measured curved carapace length (CCL) and width (CCW) using a flexible measuring tape; we also attached tags (Inconel 625 Style 681c) on both front flippers. If the turtle was already tagged (either from the previous season(s) or previous nesting event) the tag numbers were read and recorded. After that, the sea turtle facial profile was photographed. We are uncertain if the use of flashlights would deter landing females from nesting or if the duration used to cover and camouflage the nests would be affected. This warrants further investigation.

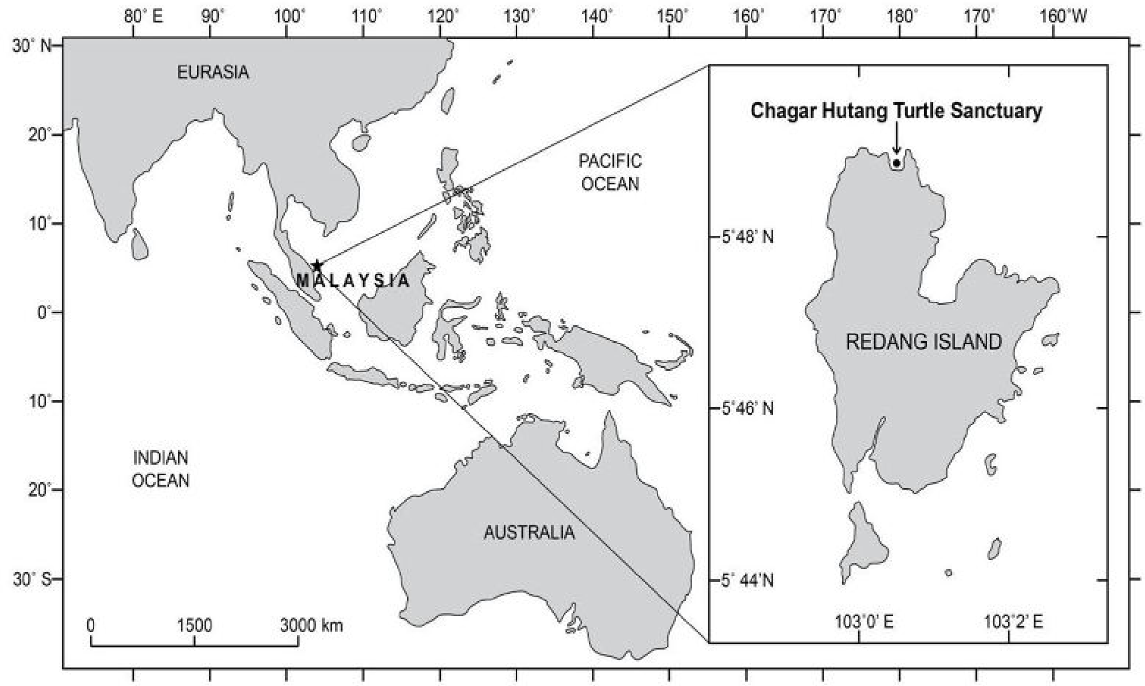

Figure 1. Map showing the location of Chagar Hutang, Redang Island.

To determine the stability of facial scute patterns, we collected 12 newly emerged green turtle hatchlings in June 2013 and maintained them in a fiberglass reinforced plastic tank (diameter = 1.35 m; height = 1.0 m) for one year (June 2013-June 2014). The tank was cleaned and seawater changed every four days. Hatchlings were fed a combination of turtle pellets, fish and squid. The left and right facial profiles of the hatchlings were photographed every 7-14 days. From January 2014 onwards, photographs were taken once a month.

Good quality images were selected and subjected to analysis using two different methods. The first method was based on Reisser *et al*. (2008) where the facial scute patterns were drawn and extracted using Adobe Photoshop CS4. In order to determine if the left and right profiles are symmetrical, the left facial profiles of all images catalogued were flipped horizontally using Microsoft Power Point 2007 and subsequently compared to the corresponding right facial profile. Images of the facial scute patterns were then compared manually and results were validated by checking the tag numbers. Whenever an individual turtle was photographed, the images were placed in their own folder. The folder was then labeled based on the date it was taken, e.g., 20.05.2014. If two or more individuals were photographed the same night, the folder was labeled as: 20.05.2014(1); 20.05.2014(2) and so on. The tag numbers of each individual sea turtle were noted in their respective folders.

When images were compared, only the folder name was written on the image. Thus, when two or more images were similar, the folders concerned were checked and tag numbers validated. Only two individual authors compared the images independently as the process was very time consuming. In the second method by Jean *et al*. (2010), each individual was assigned a unique identification code number. We assigned 3-digit codes for up to three rows of scutes. All results were verified by checking the corresponding tag numbers.

The left and right facial scute patterns of 155 adult female green turtles were photographed throughout the study. However, the images of only 140 green turtles could be analyzed and catalogued due to picture quality. As the inter-nesting interval for nesting females is approximately 10 days, some individual nesters were encountered for the second, third and even fourth time. The total number of different individuals analyzed in this study was 133 green turtles, with six turtles recaptured in subsequent years.

The visual comparison of the left and right profiles showed that nearly all turtles could be distinguished from each other through the shape and arrangement of facial scute patterns. The exception was the discovery of similar left and right profiles for two turtles: one that nested in 2010 and one that nested in 2013 (Fig. 2). In 2013, a newly tagged nester was observed to have similar left and right facial scute profiles to a 2010 nester. Unfortunately, we were unable to confirm if they were the same nester who lost both her tags or indeed two different nesters exhibiting similar facial scute profiles. Although it is suspected that these two nesters are in fact the same individual, we do not have concrete evidence to prove such a claim. The only way we could validate the photo-ID method would be to attach an additional tag to the sea turtle such as a passive integrated transponder (PIT) as done by McDonald & Dutton (1996).

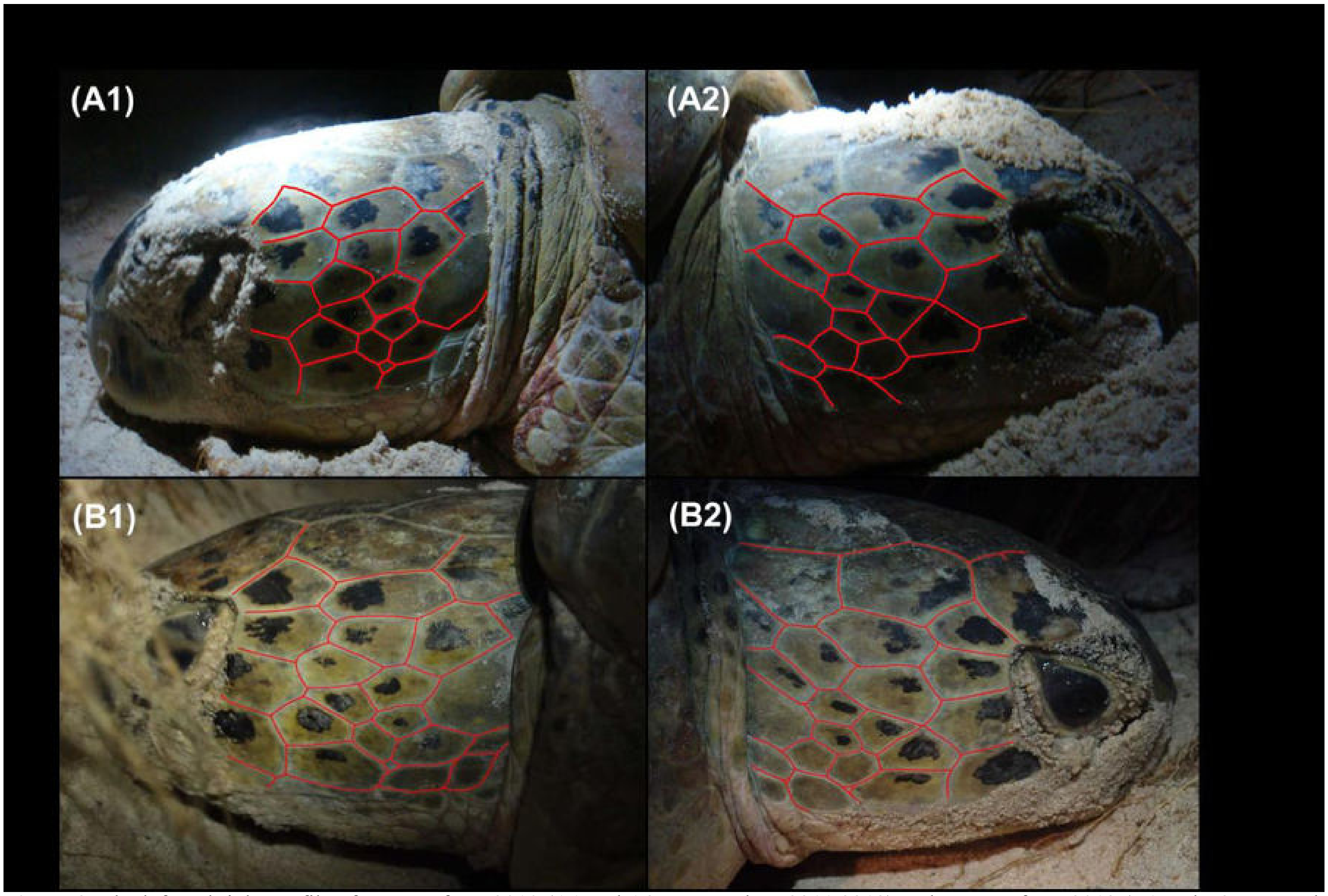

Figure 2. The left and right profile of a nester from 2010 (A1 and A2; tag number: 2311.2312) and a nester from 2013 (B1 and B2 tag number: 5466.5469). Both turtles display similar facial scute shapes and arrangement.

For all individual images catalogued, no symmetry between the left and right profiles was detected. No changes in the shape and arrangement of facial patterns were detected in remigrants that were re-sighted. Out of the 43 nesters catalogued in 2010, six returned to nest in 2013 and one in 2014. The images obtained were successfully matched with the first year images in 2010 of the respective individuals indicating the scute patterns had not changed and were stable over a period of at least four years.

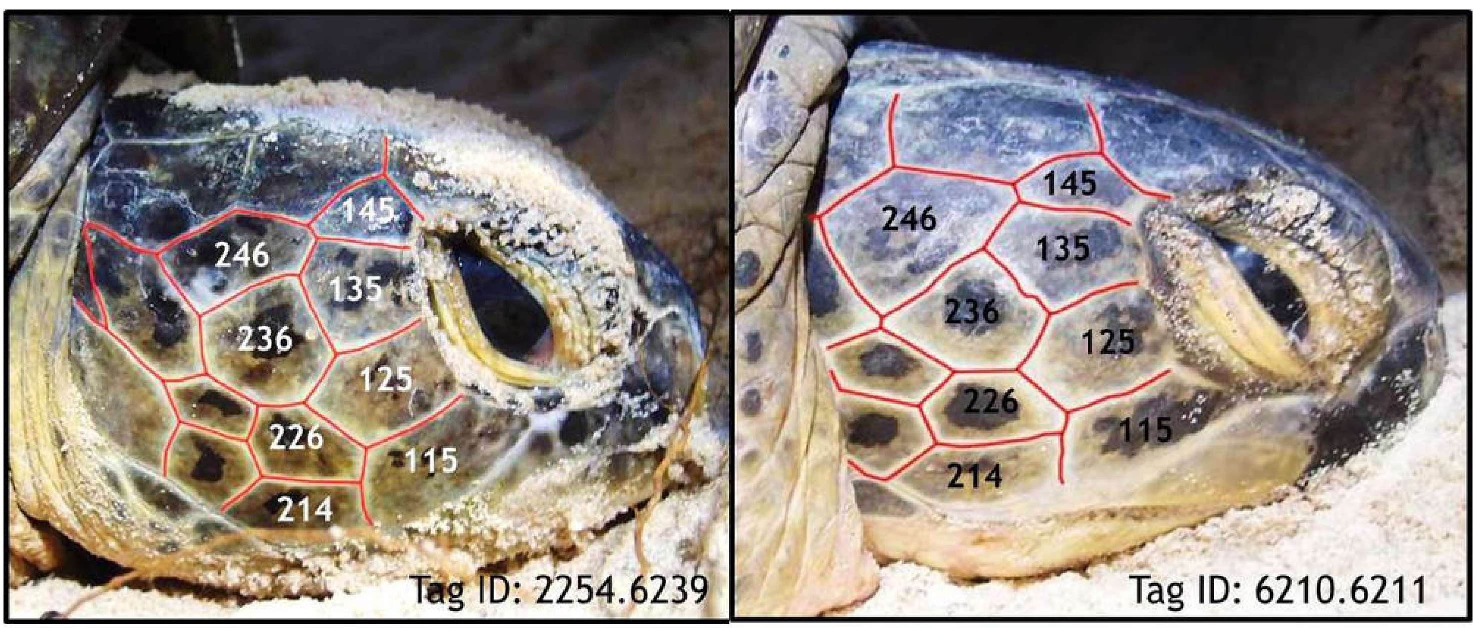

Figure 3. Right profile code of two different green turtles showing similar 3-digit series: 4-115-125-135-145-214-226- 236-246. The coding system is described in Jean *et al*. (2010). The first single digit of the code represents the number of post-ocular scutes. The first number of the 3-digit code corresponds to the row number; the second corresponds to the scute position in the row while the third corresponds to the number of sides on the scute. The code for the third row of Turtle 6210.6211 was not assigned as her neck was not fully extended. They were identified as different individuals after comparison with the codes of the left profile.

By implementing the method adapted from Jean *et al*. (2010), each image was assigned an individual photo-identification code, akin to a human's identification number. In this study, 3-digit codes were assigned up to the third row of scutes. For some turtles, codes could only be assigned until the second row as the nester did not fully extend her neck (Fig. 3). In addition, some individual turtles displayed similar codes up to the second row of scutes.

Positive results were obtained, as each individual could be distinguished from the others using the coding system, with the exception of the two nesters exhibiting similar left and right facial patterns. The maximum number of scutes observed in a row was six and the maximum number of sides to a scute recorded was 10.

The 12 hatchlings raised in captivity for one year showed a mean weight gain of 292.8 ± 63.3SD g while the mean increase in straight carapace length and straight carapace width was 8.8 ± 1.1SD cm and 7.9 ± 1.1SD cm respectively. Although the sample size was small, the experiment produced promising results in terms of utilizing photo-ID for tagging sea turtle hatchlings. Each individual hatchling was identified by comparing the images photographed on the first day with the most recently photographed images. It was apparent that the scutes increased in size during their growth but no changes were observed in the shape and arrangement of the scutes (Fig. 4).

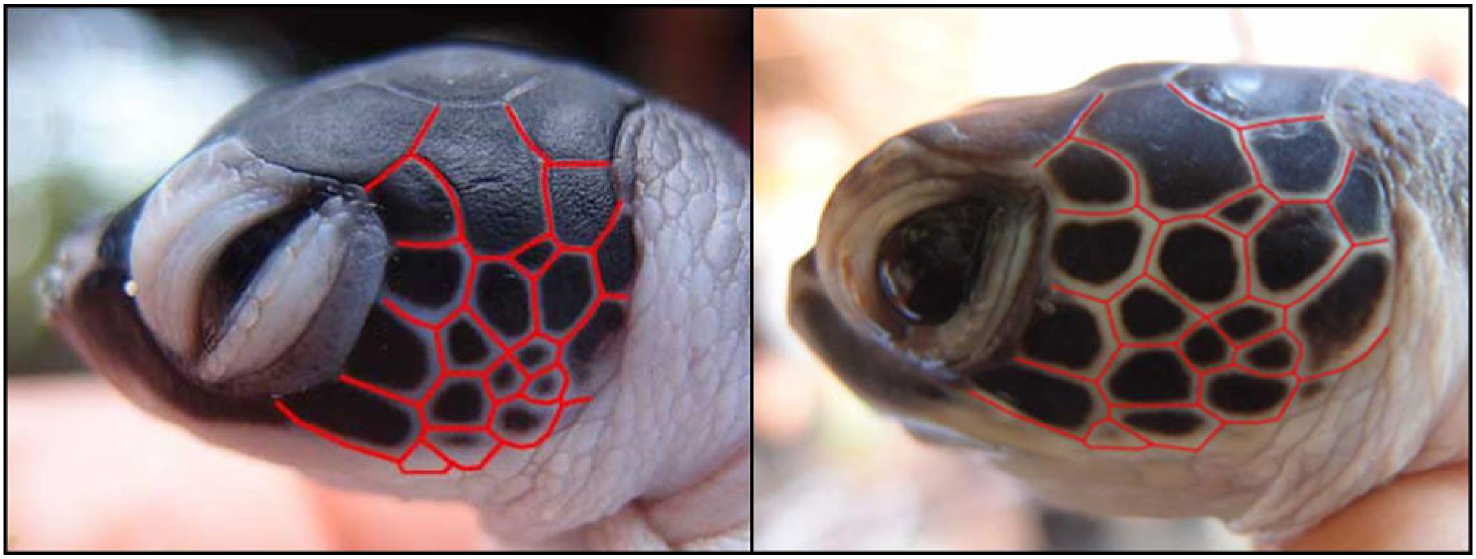

Figure 4. Left facial image of a green turtle hatchling captured on 10 June 2013 (A) and 26 June 2014 (B). Although the scute patterns remained stable for a year, there is the possibility that the pattern might change in the future. Thus photo-ID may be suitable for identifying hatchlings in the short term. More work has to be done for verification.

Results from this study demonstrate that the nesting turtles at Chagar Hutang displayed sufficient variability in facial scute patterns to identify individuals, a finding similar to other studies (Reisser *et al*. 2008; Schofield *et al*. 2008). Manual comparisons of the images were relatively easy as most individuals exhibited distinct pattern differences. An interesting finding was that some individuals displayed incomplete sides to a scute (Fig. 5). In many cases where natural markings are used for recognition purposes, the chances of patterns repeating are there, albeit very low. Such is the case for human fingerprinting identification where the chances of a person's fingerprint matching another individual in the population is one in a million (Cherry & Imwinkelried 2006). The incomplete side found in the scutes of sea turtles might therefore serve as nature's way to diversify the facial scute patterns that may be found on sea turtles. This variation in scute pattern would lower the probability of different sea turtle individuals having similar facial scute patterns.

Our study also showed that the facial scute patterns in green turtles are stable for at least four years. The stability of characteristics used as identifiers is fundamental for long-term monitoring purposes (Rocha & Rebelo 2014; Vincent *et al*. 2001; Waye *et al*. 2013). Evolution in the markings (patterns or pigmentations) have serious implications in population studies as misidentification of individuals may either underestimate or overestimate population size. In two separate studies, Kenyon *et al*. (2009) discovered that the dorsal spot patterns found on green-eyed tree frogs had a risk of changing significantly over the course of just two months while in Waye *et al*. (2013), spot patterns on tiger salamanders changed dramatically in less than a year. As far as sea turtles are concerned, however, photo-ID has thus far been successful for individual recognition of juvenile and adult turtles (Chassagneux *et al*. 2013; Feliz *et al*. 2013; Jean *et al*. 2010; Lloyd *et al*. 2012; Rees *et al*. 2013; Reisser *et al*. 2008; Schofield *et al*. 2008). In these studies, the longest time span between re-sightings of sea turtles using photo-ID was six years, recorded by Feliz *et al*. (2013) in their work on hawksbill turtles. Whether the facial scute patterns of sea turtles will remain as a unique identifier in the long run remains unknown. Continuous effort should be done to verify the feasibility of using photo-ID as an alternative method for tagging sea turtles.

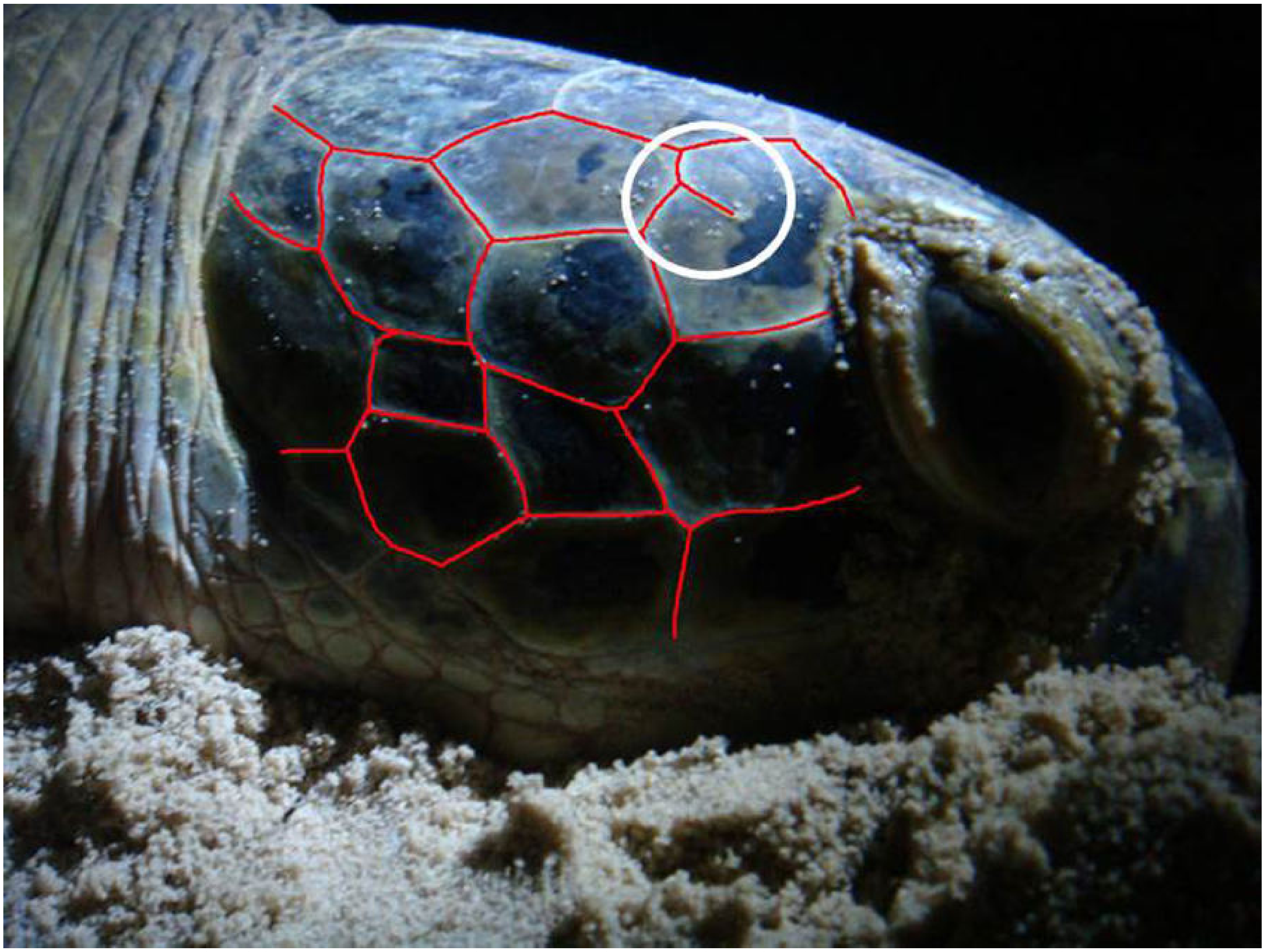

Figure 5. A green turtle exhibiting an incomplete side to a scute. This incomplete side refers to the line projecting midway through a scute from a point of intersection. In contrast to the other sides of a scute; this line has only one intersection point.

Various techniques have been employed to tag hatchlings such as carapace notching, internal placement of radio-active wires, plastral tattoos (Balazs 1978; Eckert & Eckert 1990), europium tagging (Shoop 1978) and autografting (Hendrickson & Hendrickson 1981; Wood & Wood 1993). However, as opposed to individual identification, tagging hatchlings using methods such as coded wire tags, notching and autografting is limited to identifying cohorts (Dutton & Stewart 2013). An emerging tool, still in its early stages, is genetic fingerprinting; this may allow hatchlings to be identified individually. To the authors' knowledge, ours is the first study to explore the feasibility of implementing photo-identification as a potential method for "tagging" green turtle hatchlings. The results obtained revealed that the facial scute shape and arrangement patterns of green turtle hatchlings are stable from hatch date to a time span of at least a year. Despite the small sample size and short study period, the findings signify the potential of using photo-ID as a method in tracking sea turtle hatchlings. Studies have shown that "living tags" created through autografting are retained from the hatchling phase until maturity (Bell & Parsons 2002; Rodríguez 2013). While photo-ID presents similar potential to autografting, this method should be subjected to continuous work and monitoring efforts before verification can be done.

Despite the many advantages photo-ID offers, there are limitations to this method. The main concern lies with the image quality of photographs taken. Poor quality images would decrease the matching success (Frish & Hobbs 2007; Speed *et al*. 2007). With large numbers of hatchling photos produced, the post-processing of images will be time consuming. Moreover, photo-ID is unsuitable at nesting sites with light ordinances and in areas where water visibility is limited.

Our results signify the importance of photographing two sides of a sea turtle profile to increase the success of identifying an individual. Moreover, as long as both sides of the facial profile have been documented, we would still be able to identify an individual even if only one side of the profile was photographed. Unlike sea turtles underwater, nesting turtles on occasion do not fully extend their necks and are sensitive to movement, causing them to contract their neck when disturbed. Thus, as the coding system is reliant on the degree of neck extension exhibited by the turtle, the ability to assign a complete set of codes to a certain individual will sometimes be hampered.

At present, we do not have an automated matching program developed for comparing and matching the images of the nesting sea turtle population at Redang Island. Although the images were compared manually in this study, it would be time consuming and laborious to process larger amounts of photographs. Having said this, in spite of the fact that the method adapted from Jean *et al*. (2010) resulted in certain nesters displaying similar codes, the process of counting the number of sides to scutes was no doubt more convenient. Given that the probability of two individuals having similar codes will decrease when the length of the code increases, it would therefore be interesting to determine this probability value. We did find, however, that photographic identification proved to be a feasible potential tagging method for nesting green turtles and green turtle hatchlings at Chagar Hutang, Redang Island.

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