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First Report of Physiological Color Change in a Crocodilian

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Physiological color change in animals is a rapid process involving the redistribution of pigments within chromatophores, and may have roles in camouflage, communication, or thermoregulation. Although observed in other ectotherms, it has not previously been documented in crocodilians. Using wild and captive Sunda gharials (*Tomistoma schlegelii*), we confirm rapid ventral color change in juveniles, as evidenced by significantly darker ventral scales in illuminated vs. dark environments. We also found that color varied significantly with body size, with larger individuals losing the ability to change color, and by testing environmental factors potentially triggering color change—temperature, ambient light, background color, or stress—we report that illumination was the only factor observed to explain the change. Our results reveal an unusual trait not corresponding to established countershading patterns, which provides a basis for further research not only into crocodilian behavioral ecology and physiology, but also into animal camouflage and convergent evolution. These results add further evidence to *Tomistoma* being an evolutionarily distinct lineage of crocodilian, confined to the threatened peat swamp habitats of Southeast Asia.

COLOR change in animals can be considered either morphological or physiological. Morphological color change is a slow process initiated by a change in the number or quality of pigment-containing chromatophores, usually happening over days or months (Stuart-Fox and Moussalli, 2009). Physiological color change is faster, usually taking from seconds to hours, and involves the movement of the pigment melanin within chromatophores (Greenberg, 2002).

Morphological color change can be induced by environmental stimuli and is associated with seasonal breeding, growth, or changes in habitat (Cooper and Greenberg, 1992). Physiological color change may be part of regular circadian rhythm (Boback and Siefferman, 2010), direct communication between individuals or species (Stuart-Fox and Moussalli, 2008), or may occur in response to environmental changes (Vroonen et al., 2012). In crocodilians, morphological color change of dorsal pigmentation in response to water color has been documented in *Crocodylus porosus* (Kirshner, 1985). Anecdotal reports (Neill, 1971) of short-term physiological color change of dorsal scales in *Caiman crocodilus* suggest a response according to temperature, but this has not been experimentally tested. These are the only accounts of color change in crocodilians documented to date, and only for the dorsal surfaces.

The Sunda gharial (*Tomistoma schlegelii*) is one of the largest and least studied of the crocodilians, inhabiting peat swamp forests and adjacent wetlands in Indonesia and Malaysia (Stuebing et al., 2006). During field surveys, we observed four juvenile *Tomistoma* change ventral color from being white when captured at night, to being dark gray the following morning. Juvenile *Crocodylus siamensis* inhabiting the same area were caught and handled in the same manner but did not exhibit any color change. All crocodiles are countershaded with dark dorsal scales and light ventral scales, including adults of *Tomistoma* (Rachmawan and Brend, 2009). Ventral color change is not widely encountered in reptiles (Norris and Lowe, 1964) and has not previously been described in any crocodilian.

The main objective of this study is to experimentally verify ventral color change in juvenile *Tomistoma* and identify the environmental factors inducing the change. We considered temperature, ambient light, background color, or stress as possible triggers for color change.

MATERIALS AND METHODS

Wild crocodile observations.—To document color change in wild animals, we photographed 23 wild-caught juvenile *Tomistoma schlegelii* (51–111 cm; mean 73.9 ± SD 18.4 cm) from Mesangat Lake, Indonesia (0.517, 116.7) in 2011 and 2012. We captured the crocodiles by hand at night, kept them in a white cloth bag until the next day, and then released them at the point of capture. We photographed each juvenile at night after capture (between 0000–0300 hours) and the following day (between 1200–1500 hours) against a standard 10-shade grayscale with a Nikon D40 digital single lens reflex camera.

Captive crocodile experimental design.—To test the factors influencing color change, we used five captive-bred juveniles (mean total body length 61.6 ± SD 3.5 cm) at Samutprakarn Crocodile Farm, Thailand. All animals were siblings, housed together in a concrete outdoor enclosure. We placed two 90 cm glass tanks separated by a partition in a room with no access to natural light. Tanks had either white or black walls and were filled with clear water to 7 cm; each had a separate standard 10W overhead reptile UV light and a temperature of 30–31°C. We made all observations between 1000 and 1700 hours. We caught all crocodiles by hand and photographed their ventral sides under natural light against the standard 10-shade grayscale.

We placed two *Tomistoma* in each tank and switched the overhead light on in one tank, and off in the other tank. After one hour we removed the animals and photographed them against the standard 10-shade grayscale. We then returned them to the tanks and reversed the light treatment. We repeated this process until all five crocodiles had been photographed following an hour of light and darkness in each tank. We also photographed each animal after an hour in natural daylight at 22°C and 32°C (corresponding to typical night and day temperatures, respectively).

Analysis.—All photographs were converted to 8-bit grayscale and opened in ImageJ 1.46r (Schneider et al., 2012) and

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calibrated following Hamilton et al. (2008). We measured the values of the throat and abdominal ventral scale regions on each photograph and used their mean as the ventral grayscale value for each crocodile. To calculate percentage reflectance of ventral scales on black and white backgrounds, we calibrated these values using barium sulfate standard images (Stevens et al., 2007). All statistical analyses were carried out in R 2.15.1 (R Core Team, 2012) using a significance level of 0.05.

RESULTS

In the 23 wild juvenile *Tomistoma*, the ventral scales were significantly darker during the day than during the night (ANCOVA $F_{40,3} = 35.84$, $P < 0.001$). The effects of body size (ANCOVA $F_{40,3} = 0.278$, $P = 0.601$) and the time-size interaction (ANCOVA $F_{40,3} = 0.848$, $P = 0.363$) were not significant. However, the degree of ventral color change decreased with body size ($R^2 = 0.166$, $F_{1,44} = 8.739$, $P = 0.005$, Fig. 1). The ventral color varied significantly with body size during the day ($R^2 = 0.307$, $F_{1,21} = 9.321$, $P < 0.01$), with smaller *Tomistoma* becoming darker than larger individuals in the same light conditions (Fig. 2). During the night, there was no significant variation in color with body size ($R^2 = 0.069$, $F_{1,21} = 1.565$, $P = 0.225$).

Ambient light was the only factor observed to trigger ventral color change in juvenile *Tomistoma*. Ventral scales of the five captive *Tomistoma* were significantly darker after one hour in the light and significantly lighter after one hour in the dark ($t_4 = 4.211$, $P = 0.014$). There was no significant difference in ventral color of the five crocodiles after being in the daylight in 22°C and 32°C ($t_4 = 0.631$, $P = 0.564$). The percentage reflectance values of the ventral scales of *Tomistoma* kept on the black and on the white background during the day were not significantly different between the two treatments ($t_4 = -1.286$, $P = 0.268$). Stress, caused by repeated capture, did not affect color change.

DISCUSSION

Our results show that ambient light triggers ventral physiological color change in juvenile *Tomistoma*. Both wild and captive *Tomistoma* had ventral scales significantly darker in light environments than in the dark. As the crocodiles grow larger, we show that this effect becomes less pronounced, and that not all individuals will turn darker in daylight. Several possible functions that may explain this physiological color change include thermoregulation, diel cycle, camouflage, or stress.

Darker animals can raise their body temperature faster than light-colored individuals, and thermoregulation has been suggested as the function of color change in *Caiman crocodilus* (Neill, 1971). This can be particularly important for hatchling and juvenile crocodilians, as it would reduce the time spent basking (Bustard, 1970), shortening the critical period during which the young are most vulnerable to predation. However, since we have only observed the skin darkening on the ventral side of *Tomistoma*, which is not exposed when basking, it is unlikely that thermoregulation alone is the reason for color response in this case. Similar diel activity and light-cycle patterns related to color change have been documented in nocturnal snakes (Boback and Siefferman, 2010) and geckos (Vroonen et al., 2012). Our experiments were carried out during the day, indicating that ambient light levels, rather than time of day alone, induce the change.

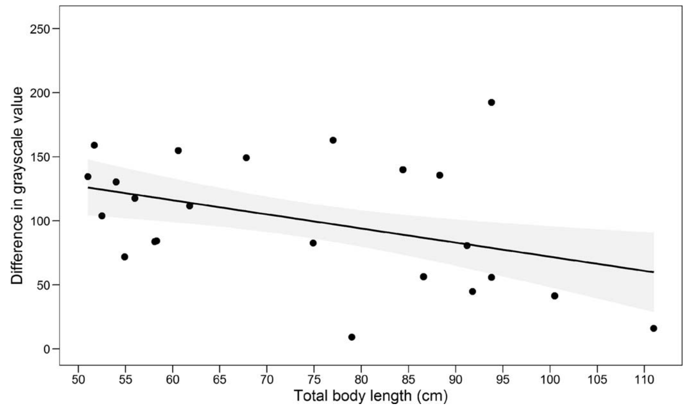

Fig. 1. Negative correlation between night vs. day difference in grayscale values of ventral scales and body size of 23 wild *Tomistoma schlegelii* (51–111 cm). Gray shaded area represents 0.95 confidence intervals of linear regression line.

As young crocodilians are vulnerable to predators (Somaweera et al., 2013), color change could aid camouflage. Countershaded coloration is thought to provide camouflage for aquatic animals in daylight by reducing the shadow that the animal casts on its underside when illuminated from above (Rowland, 2009). At night, when actively swimming in shallow water, countershading may help conceal crocodiles from both prey and predators when illuminated by moonlight. While most crocodilians retain their countershaded coloration during the day, ventral skin darkening in juvenile *T. schlegelii* indicates that they do not actively swim in daylight. *Tomistoma* inhabit peat swamp forests drained by tannin-stained blackwater rivers (Stuebing et al., 2006). Although our results show that the crocodiles do not actively match their daytime color in response to a background, dark ventral color in juveniles may still aid camouflage in these habitats.

Although our results show that stresses related to capture did not affect the observed color change, it may still play a role in body color control, particularly in larger individuals. In many reptiles, dominant animals are usually lighter (Greenberg, 2002), so dark coloration could thus serve as a recognition signal for adults and other juveniles in *Tomistoma*. Body color changes in response to catecholamines are common in reptiles, especially lizards (Greenberg, 2002). The differences in ventral color response to light observed in larger wild *Tomistoma* could be related to different stress hormone levels. The stress levels may also be different between wild and captive animals, which are more used to human presence and handling. While our data collected on the wild *Tomistoma* indicate that body color response to light reduces when the animals reach over 80 cm in body length, the sample size, associated with the rarity and difficulty of catching *Tomistoma*, prevents more definite conclusions.

The combined data, documenting color change of both captive and wild crocodiles, strongly support the hypothesis of ventral skin darkening in response to ambient light. Ventral color change is not common in vertebrates (Norris and Lowe, 1964), and our results reveal an unusual trait not corresponding with the established countershading patterns (Rowland, 2009), which provides the basis for further research not only into crocodilian behavioral ecology and physiology, but also animal camouflage and convergent

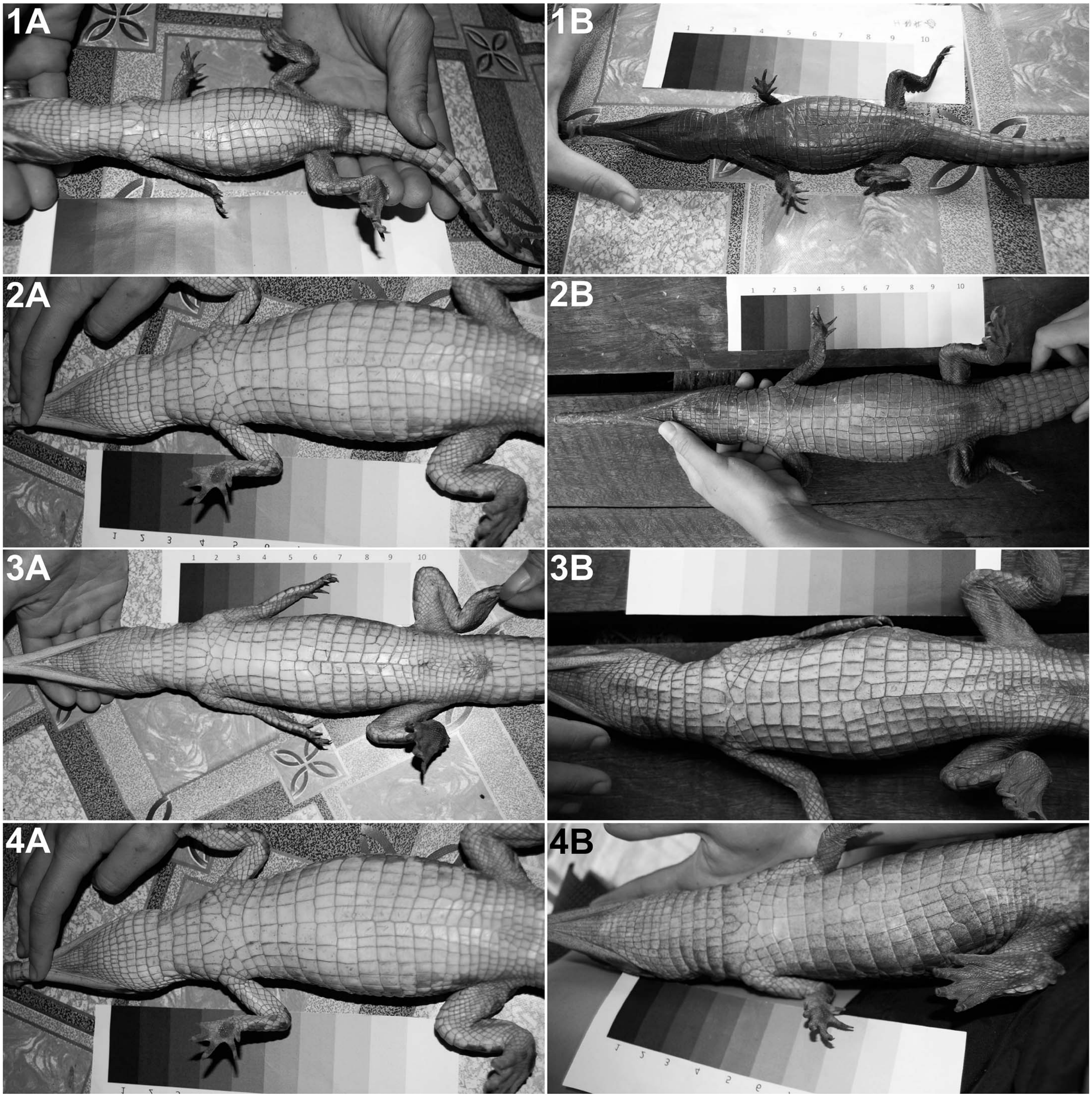

Fig. 2. Images of ventral scales of four wild juvenile *Tomistoma schlegelii* of different total body length: 51 cm (1A, 1B), 74.9 cm (2A, 2B), 86.6 cm (3A, 3B), and 92.1 cm (4A, 4B) photographed during the night after capture (A) and the following day (B).

evolution. The results of our study are also relevant to paleobiologists working on coloration of extinct archosaurs. As the only surviving species of the tomistomine crocodilian clade (Piras et al., 2007), *Tomistoma schlegelii* represents an important component of functional and phylogenetic diversity, emphasizing the importance in conserving its threatened peat swamp habitats.

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