The Common Mussurana, *Clelia clelia*, is a widespread colubrid found throughout much of Central and South America. One unusual feature of this snake is its ontogenetic color change, as juveniles (above; KU 181136) display markedly different coloration from adults, which are uniform bluish black. In the following study we quantitatively measured the coloration patterns of 105 juvenile museum specimens of this species from many localities throughout its range, and found strong geographic patterns in the shape and size of the head bands. To our knowledge, this information on color pattern variation has not been reported. 📷 © Luke Welton

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Geographic variation in head band shape in juveniles of *Clelia clelia* (Colubridae)

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Abstract: Color variability influences many aspects of organismal function, such as camouflage, mating displays, and thermoregulation. Coloration patterns frequently vary geographically and sometimes among life stages of the same species. One widely distributed snake species that shows ontogenetic color change is *Clelia clelia* (Colubridae). No quantitative studies, however, have assessed coloration patterns in this species. To fill this gap and to assess color pattern variation within this species, we measured the lengths of the head and neck bands of 105 specimens of *C. clelia* from across much of its geographic range. We found that the head band shape and length of specimens from Amazonia and the Atlantic Forest were significantly different compared to those from Central America and the Pacific or Caribbean coasts of South America, and that they stem from a difference in the shape of the first black collar on the snout and the anterior portion of the head. The Amazonian and Atlantic Forest groups contained significantly more specimens with straight head bands, whereas those from other regions contained more specimens with horseshoe-shaped head bands. These observations identify a previously unreported color pattern variation in this species, but the evolutionary or taxonomic importance of this variation remains unknown.

Key Words: Coloration, geographic patterns, mussurana, ontogenetic color change

Resumen: La variabilidad del color influye la función del organismo en muchos aspectos, como el camuflaje, las pantallas de apareamiento y la termorregulación. Los patrones de coloración con frecuencia varían geográficamente y, a veces, entre las etapas de la vida de la misma especie. Una especie de serpiente ampliamente distribuida que muestra un cambio de color ontogenético es *Clelia clelia* (Colubridae). Sin embargo, no hay estudios cuantitativos que hayan evaluado los patrones de coloración en esta especie. Para llenar este vacío y evaluar la variación del patrón de color dentro de esta especie, medimos las longitudes de las bandas de la cabeza y el cuello de 105 especímenes de *C. clelia* a través de gran parte de su rango geográfico. Encontramos que la forma y la longitud de la banda de la cabeza de los especímenes de la Amazonia y el Bosque Atlántico fueron significativamente diferentes en comparación con los de Centroamérica y el Pacífico o las costas del Caribe de América del Sur, y que provienen de una diferencia en la forma del primer collar negro en el hocico y la porción anterior de la cabeza. Los grupos de la Amazonia y el Bosque Atlántico contenían significativamente más especímenes con bandas de la cabeza recta, mientras que los de otras regiones contenían más especímenes con bandas de la cabeza en forma de herradura. Estas observaciones identifican una variación de patrón de color no reportada previamente en esta especie, pero la importancia evolutiva o taxonómica de esta variación permanece desconocida.

Palabras Claves: Cambio de color ontogenético, coloración, patrones geográficos, zopilota

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INTRODUCTION

Variation in animal coloration long has fascinated biologists. Color pattern variation is important for many organismal functions, including thermoregulation (Stuart-Fox and Moussalli, 2009), breeding success (Siefferman and Hill, 2003), and predator avoidance (Berenbaum and Miliczky, 1984). Many examples of color and color pattern variation are known in snakes (Bechtel, 1978; Allen et al., 2013). The sources of this variation have been attributed to, or are associated with, a diversity of factors, including aposematism (Pfennig et al., 2001), crypsis (King, 1987), geographic variation (Mooi et al., 2011), and sexual dimorphism (Forsman, 1995).

Ontogenetic color variation, a type of color pattern variation where individuals show conspicuously different color phenotypes as juveniles compared to adults, has received relatively little attention in snakes (Wilson et al., 2007). The Common Mussurana, *Clelia clelia*, a wide-ranging colubrid with a distribution that extends from northern Guatemala southward to northern Argentina, as well as in Trinidad and Antigua (Solórzano, 2004; Uetz et al., 2018), is a species known to exhibit conspicuous ontogenetic color change. As juveniles, the color pattern of the head and neck contains bold black and white colors arranged in discrete bands, whereas the remainder of the remainder of the dorsum is bright red, but the dorsum of adults is uniform glossy bluish black (Savage, 2002). Nonetheless, when this color transition occurs, and both what triggers it and its adaptive significance remain unknown. Given the broad distribution of *C. clelia*, one might expect geographic differences in juvenile coloration patterns among regions, but to our knowledge such differences have not been investigated. Using image analysis of photographs from museum specimens, herein we present the first data on geographic variation in the dorsal color patterns of juveniles of *C. clelia*.

MATERIALS AND METHODS

We quantified the dorsal color patterns of preserved museum specimens using ImageJ (Abramoff et al., 2004), by measuring the following characters: (1) the midline length of the first black collar on the anterior-most portion of the head; (2) the length of the white collar posterior to it; and (3) the length of the second black collar, posteriorly (Fig. 1). Because the second black collar often tapers gradually, we measured its length until the black pigmentation no longer covered at least five scales horizontally across the dorsum. We then measured the length of the remaining red coloration on the body. To avoid biases from body size differences among specimens, we used the proportions of the body represented by each color (rather than the raw measurements) in the analysis.

To analyze this data set, we separated the specimens into broad categories based on their biogeographic location, and used the following categories: Amazonia (*n* = 29); Atlantic Forest (*n* = 9); Pacific lowlands of South America (*n* = 6); Caribbean lowlands of South America (*n* = 12); highlands of Central America (*n* = 10); Pacific lowlands of Central America (*n* = 18); and Caribbean lowlands of Central America (*n* = 21). We placed the specimens in the Central American highlands category if their elevation was ≥ 1,200 m. For specimens that lacked detailed locality data, we categorized them geographically by using the center of their respective province, state department or region. Specimens that lacked locality data beyond country of origin were not used unless that entire country fell into one of the categories listed above.

Fig. 1. An example of a straight-banded specimen of *Clelia clelia* (AMNH 119924). (A) The first black collar; (B) the white collar; and (C) the second black collar. We took the measurements using the metric ruler as a reference. The inset shows an example of a horseshoe-banded specimen (MCZ R2712). © Lauren Vonnahme and Joseph Martinez (inset)

To test for significant differences in color proportion among the different biogeographic regions, we used a multivariate analysis of variance test (MANOVA) with subsequent univariate and post hoc tests, as appropriate. To test whether either of the documented black band shapes are found significantly more often in one geographic area over another, we used a likelihood ratio test. We performed all tests by using IBM SPSS Statistics version 24, after ensuring that test assumptions were met.

RESULTS

We measured 105 specimens from throughout much of the range of *Clelia clelia* (Appendix 1). We found significant differences in coloration proportions among the biogeographic regions (Wilk's Lambda = 0.503, $F = 3.018$, $P < 0.001$). Univariate tests revealed significant differences for the first black collar ($F = 7.417$, $df = 6$, $P < 0.001$), the second black collar ($F = 2.428$, $df = 6$, $P = 0.031$), and the white collar ($F = 8.394$, $df = 6$, $P < 0.001$), but not for the proportion of the body that was red ($F = 1.462$, $df = 6$, $P = 0.199$).

For both the first black collar and the white collar, post hoc tests revealed that specimens from the Atlantic Forest and Amazonia showed significant differences from specimens from all the other regions, except for the Central American highlands, which were intermediate (Fig. 2, 3). For the second black collar, the pattern was less clear. Among the regions we compared, the specimens from the Central American highlands showed significant differences from specimens from the Central American Pacific lowlands, but no other significant patterns were identified.

Our observations also revealed a distinct difference in the shape of the first black collar. In some specimens, the first black collar was distinctly horseshoe-shaped (Fig. 1, inset), whereas in others it was straight (Fig. 1, main photo). All of the specimens clearly fell into one of these two collar shape types, with no intermediates. A likelihood ratio test indicated a significant difference in the frequency of the straight and horseshoe-shaped black collars among the biogeographic regions ($D = 25.6$, $df = 6$, $P < 0.001$). The horseshoe-shaped black collars were significantly more common in specimens collected in Amazonia and the Atlantic Forest, but were rare or absent elsewhere (Fig. 4). Conversely, specimens with straight black collars were found with high frequency in Central America, as well as in Andean regions of South America. Specimens from the Central American highlands were intermediate, showing both collars types with near equal frequencies (Fig. 4).

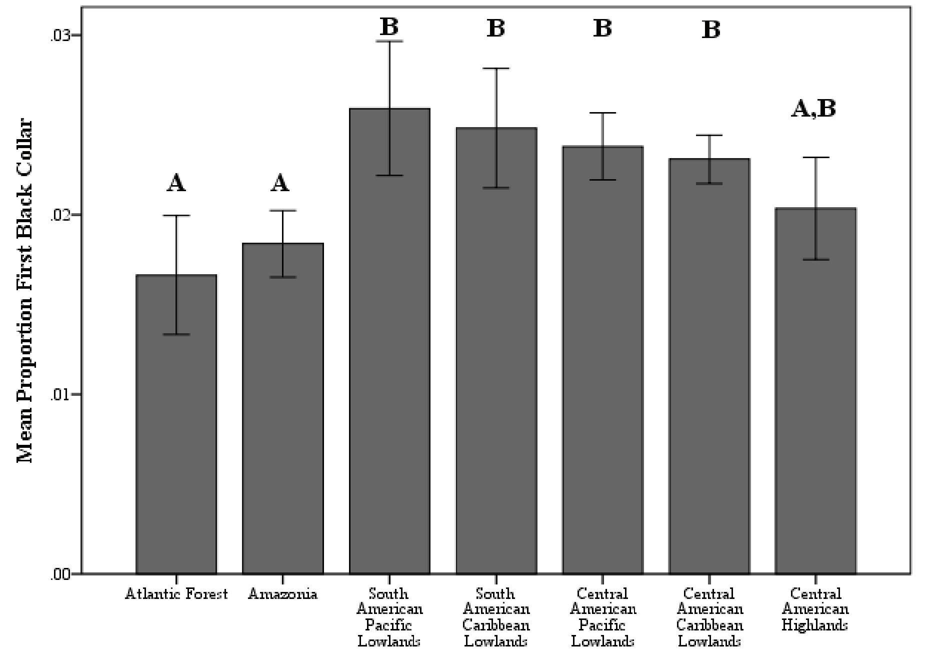

Fig. 2. Mean proportion of the total body length represented by the first black collar across the biogeographic regions. The mean proportion of the first black collar was more similar among the South Atlantic Forest and Amazonian groups (A), and all Caribbean and Pacific lowland groups were more similar to each other than to the other groups (B). The highland group was not statistically different from any other group. The error bars represent two standard errors, and the letters above the bars represent groups of specimens that are significantly different from the others.

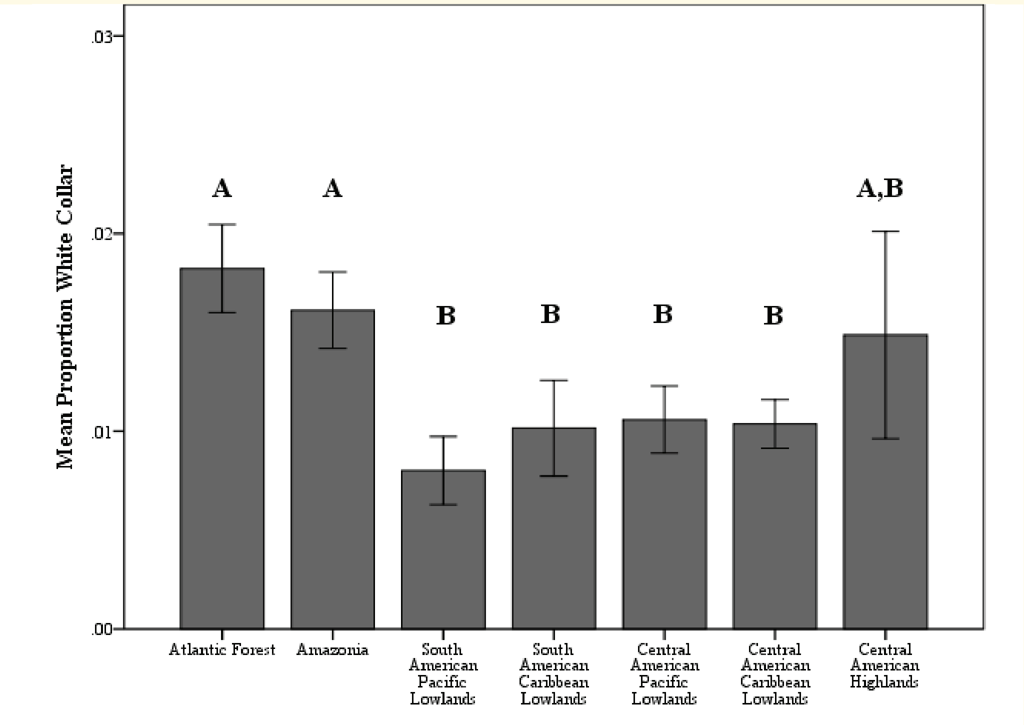

Fig. 3. Mean proportion of the total body length represented by the white collar across the biogeographic regions. The South Atlantic Forest and Amazonia groups (A) were statistically different in proportion than all Pacific and Caribbean lowland groups (B). Again, the highland category was not significantly different than any other group. The error bars represent two standard errors, and the letters above the bars represent groups of specimens that are significantly different from the others.

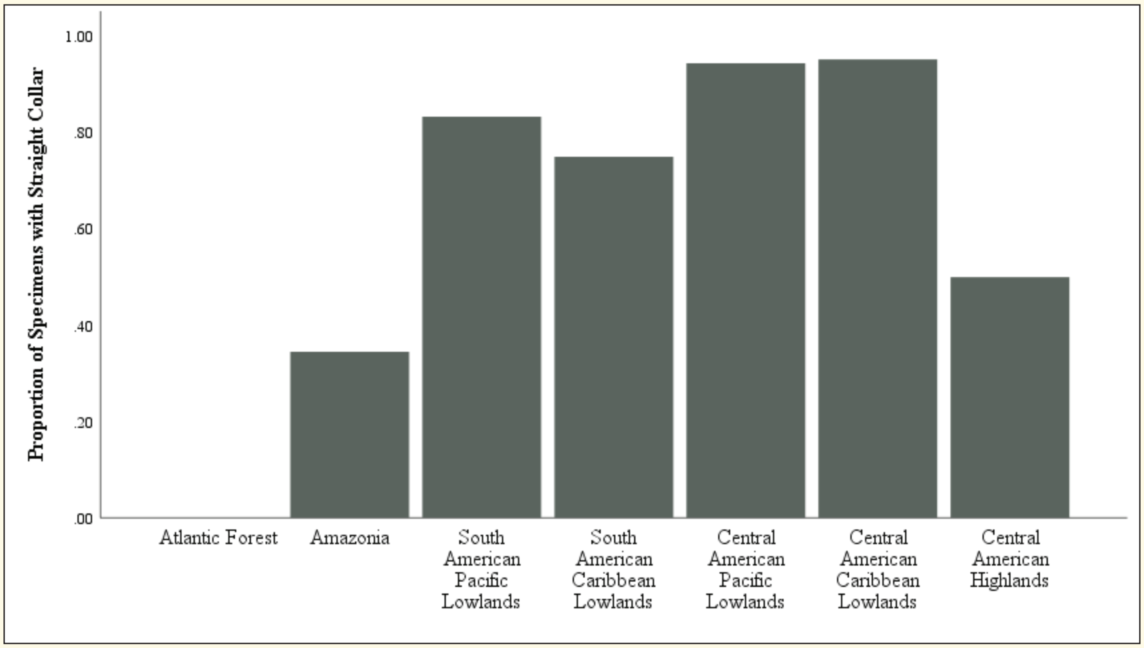

Fig. 4. Proportion of specimens with a straight first black collar from each biogeographic region.

Of the 105 specimens we examined, 12 were transitioning from the juvenile to adult coloration, i.e., the first and second black collars were extending posteriorly into the white and red regions, and beginning to obscure them. Nine of these specimens were the longest we analyzed, suggesting a positive relationship between body size and the beginning of the transition into the adult coloration. The average body size of a snake transitioning from the juvenile to the adult coloration was 718 mm in total length (SD = 174 mm; range = 429–980 mm).

DISCUSSION

To our knowledge, the presence of two distinct collar shapes in juveniles of *Clelia clelia* has not been reported. These different collar shapes underlie the differences in color proportions we found, as specimens with the horseshoe-shaped first black collar showed more black and less white overall coloration (see Fig. 1). We identified a clear geographic pattern, in which specimens from Amazonia and the Atlantic Forest differed from those in the other groups, except for the Central American highlands group, which was intermediate.

We provide several possible explanations for these patterns. The different collar shapes simply could be a previously unreported polymorphism that exists in different frequencies in different populations of this species. Since some evidence suggests that juveniles of *Clelia clelia* could be coralsnake mimics (Brodie, III, 1993; Hinman et al., 1997), the differences we identified here might result from co-evolutionary interactions with different coralsnake models in different areas of this species' range. Finally, the geographic trends in collar shape patterns we identified might indicate that more than one evolutionary lineage currently exists under the name *C. clelia* (e.g., see discussion in Sasa et al., 2010). Additional research is necessary to determine which of these explanations (or others) is the most likely.

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Appendix 1. Museum accession numbers and geographic locality information for the juvenile specimens of *Clelia clelia* measured. The numbers for the Biogeographic regions are as follows: 0 = Atlantic Forest; 1 = Amazonia; 2 = Pacific lowlands of South America; 3 = Caribbean lowlands of South America (including Trinidad); 4 = Pacific lowlands of Central America; 5 = Caribbean lowlands of Central America; and 6 = Central American highlands (elev. 1,200 m and greater).

Museum	Accession Number	Biogeographic Region	Country	Province, Department, Region, or State
AMNH	110582	1	Ecuador	Napo
AMNH	119924	1	Bolivia	Santa Cruz
AMNH	161967	5	Costa Rica	Limón
AMNH	35919	2	Ecuador	Chimborazo
AMNH	35994	1	Bolivia	Santa Cruz
AMNH	56147	1	Peru	Madre de Dios
AMNH	57305	1	Peru	Loreto
AMNH	64475	3	Trinidad	Trinidad
AMNH	71128	1	Peru	Loreto
AMNH	81446	3	Trinidad	Trinidad
AMNH	98232	3	Venezuela	Aragua
AMNH	119923	1	Bolivia	Santa Cruz
AMNH	59429	3	Venezuela	Miranda
MCZ	39779	4	Panama	Darién (Yavisa)
MCZ	37151	6	Panama	(Chagres River)
MCZ	3570	2	Ecuador	Guayas

MCZ	31778	6	Costa Rica	Heredia
MCZ	2712	4	Panama	Chiriquí
MCZ	26869	5	Honduras	Yoro
MCZ	22446	1	Brazil	Pará
MCZ	21725	5	Honduras	Yoro
MCZ	17886	0	Brazil	Santa Catarina
MCZ	173840	1	Ecuador	Napo
MCZ	15275	5	Costa Rica	Limón
MCZ	29608	5	Honduras	Atlántida
MCZ	189696	3	Suriname	Paramaribo
MCZ	156882	2	Ecuador	Pichincha
MCZ	156875	2	Ecuador	Los Ríos
UTA	22265	3	Trinidad	St. George
UTA	46167	5	Ecuador	Gracias a Dios
LSUMZ	47725	1	Ecuador	Napo
LSUMZ	28749	4	Costa Rica	Puntarenas
LSUMZ	33059	5	Costa Rica	Limón (Tortuguero)
LACM	150361	6	Costa Rica	Alajuela
LACM	150360	6	Costa Rica	Alajuela
LACM	150359	4	Costa Rica	Puntarenas
LACM	150358	5	Costa Rica	Heredia
LACM	150357	6	Costa Rica	Alajuela
LACM	150356	4	Costa Rica	Guanacaste
LACM	150355	4	Costa Rica	Puntarenas
LACM	103479	5	Mexico	Veracruz
KU	128253	1	Brazil	Pará
KU	182708	3	Venezuela	Aragua
KU	86578	5	Costa Rica	Heredia
KU	102499	4	Costa Rica	Puntarenas
KU	157550	5	Belize	Stann Creek
KU	31895	6	Costa Rica	Heredia (Isla Bonita)
KU	102498	4	Costa Rica	Puntarenas
KU	181136	3	Venezuela	Bolivar
KU	25746	6	Costa Rica	Alajuela
KU	31896	6	Costa Rica	Heredia (Cinchona)
KU	121862	1	Ecuador	Sucumbios
KU	35640	4	Costa Rica	Limón
KU	112961	5	Nicaragua	Zelaya (El Recreo)
KU	80222	5	Panama	Bocas del Toro
KU	75670	4	Panama	Canal Zone
KU	110279	4	Panama	Veraguas
KU	172911	4	Panama	Canal Zone (Balboa)
UMMZ	124288	3	Venezuela	Aragua
UMMZ	109048	0	Paraguay	Central
UMMZ	123271	4	Panama	Darién
UMMZ	142804	5	Panama	Canal Zone
UMMZ	152912	5	Panama	Canal Zone

UMMZ	155693	4	Panama	Canal Zone
UMMZ	147822	5	Panama	Canal Zone
UMMZ	67943	1	Bolivia	Santa Cruz
UMMZ	60753	1	Bolivia	Santa Cruz
UMMZ	60808	1	Bolivia	Santa Cruz
UMMZ	64156	1	Bolivia	Santa Cruz
UMMZ	67941	1	Bolivia	Santa Cruz
UMMZ	60806	1	Bolivia	Santa Cruz
UMMZ	60807	1	Bolivia	Santa Cruz
UMMZ	67940	1	Bolivia	Santa Cruz
UMMZ	62755	0	Brazil	Sâo Paulo
UMMZ	62754	0	Brazil	Sâo Paulo
UMMZ	62753	0	Brazil	Sâo Paulo
UMMZ	204147	0	Brazil	São Paulo
UMMZ	56894	1	Brazil	Rondônia
UMMZ	55871	3	Guyana	–
UMMZ	117727	6	Costa Rica	Alajuela
UMMZ	131304	6	Costa Rica	Alajuela
ANSP	25585	1	Colombia	Caquetá
ANSP	10164	4	Panama	Veraguas
ANSP	24700	4	Panama	Panamá
ANSP	22869	5	Nicaragua	Zelaya
ANSP	22866	5	Nicaragua	Zelaya
USNM	49543	1	Peru	Cuzco
USNM	348489	5	Panama	Bocas del Toro
USNM	32218	3	Venezuela	Distrito Federal
USNM	252673	3	Trinidad	St. George
USNM	24507	4	Panama	Darién
USNM	226393	4	Panama	Chiriquí
USNM	210849	1	Ecuador	Napo
USNM	19563	5	Nicaragua	Río San Juan
USNM	154019	5	Colombia	Antioquia
USNM	12349	2	Ecuador	Guayas
USNM	119015	1	Peru	Ucayali
FMNH	9381	0	Argentina	Misiones
FMNH	9337	0	Argentina	Misiones
FMNH	37440	1	Colombia	Putumayo
FMNH	35703	1	Bolivia	Santa Cruz
FMNH	78116	1	Colombia	Antioquia
FMNH	74375	1	Colombia	Cauca
FMNH	165444	2	Colombia	Valle
FMNH	42266	0	Paraguay	–

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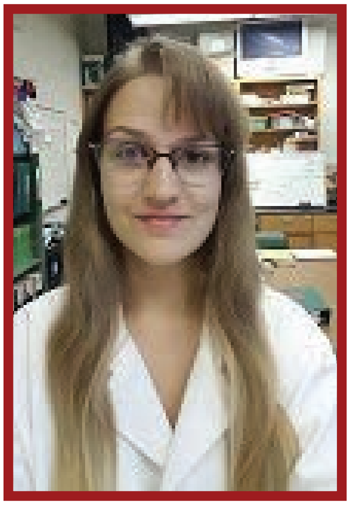

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