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Variation in the morphology of hatchlings of black iguanas *Ctenosaura pectinata* (Wiegmann, 1834) (Squamata: Iguanidae)

Variación en la morfología de crías de iguanas negras *Ctenosaura pectinata* (Wiegmann, 1834) (Squamata: Iguanidae)

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ABSTRACT. To contribute to the management of *Ctenosaura pectinata* under confinement conditions, this study presents statistical analyses of nine morphological variables of 127 hatchlings, which were obtained from production farms. The variables examined and processed with one-way analysis of variance, multivariate variance analysis, discriminant analysis, and analysis of covariance reveal that snout-vent length (males= 52.53 mm, females= 49.23 mm), tail length (males= 13.59 mm, females= 12.30 mm), forelimb (males= 25.99 mm, females= 23.08 mm) and hindlimb leg length (males= 44.43 mm, females= 38.08 mm) are the larger characters in males than in females hatchlings,

as occurs in adults. Sex morphologic differentiation of *C. pectinata* in an early stage of development is useful when working under farm conditions. To facilitate the recognition of the sex of the offspring, a dichotomous key with the most dimorphic characters is included.

Key words: Black iguanas; hatchlings; morphometry; sexual dimorphism

RESUMEN. Con el propósito de contribuir al manejo de *Ctenosaura pectinata* en condiciones de confinamiento, este estudio presenta análisis estadísticos de nueve variables morfológicas que fueron obtenidas de 127 crías, producidas bajo condiciones de criadero. Las variables examinadas y procesadas con análisis de varianza de una vía, análisis de varianza multivariada, análisis discriminante y análisis de covarianza revelan que la longitud-hocico cloaca (machos= 52.53 mm, hembras= 49.23 mm), la longitud de la cola (macho= 13.59 mm, hembras= 12.30 mm), la longitud de las patas delanteras (machos= 25.99 mm, hembras= 23.08 mm) y las patas traseras (machos= 44.43 mm, hembras= 38.08 mm) de las crías, son los caracteres más grandes en machos que en hembras, como ocurre en los adultos. La diferenciación morfológica del sexo de *C. pectinata* en estadio temprano de desarrollo es útil cuando se trabaja en condiciones de granja. Para facilitar el reconocimiento del sexo de las crías se incluye una clave dicotómica con los caracteres más dimórficos.

Palabras clave: iguanas negras; neonatos; morfometría, dimorfismo sexual

INTRODUCTION

Reptile hatchlings usually grow rapidly and in many cases during development, there is variation in body size between sexes. In several species of lizards, males develop larger and more robust bodies than females, thus sexual size dimorphism seems to be most common in several species (Braña, 1996; Olsson *et al.*, 2002; Bustos-Zagal *et al.*, 2011), while it is true that variation in body size is related to sexual selection, fecundity, and differential resource use between the sexes (Kratochvil & Frynta, 2002). It has also been suggested that this may occur due to differences in energy allocation during growth (Smith & Ballinger, 1994; Haenel & John-Alder, 2002), or even by the development of an increasingly aggressive behavior, which makes it possible to defend large territories with a lot of food and shelters available, as has been observed in herbivorous lizards (Carothers, 1984).

In *Ctenosaura pectinata*, as occurs in other species of Iguanidae (*e.g.*, *I. iguana*, *C. pectinata* Casas- Andreu & Valenzuela-López, 1984; *C. similis* Fitch & Henderson, 1977; *Dipsosaurus dorsalis* Dibble *et al.*, 2008; *Cyclura n. nubila* Gonzáles, 2018), morphological variation related to sex is also common and usually recognizable in adult individuals, which is possible due to the changes in the coloration pattern and the variation in the body size. Adult males have larger heads and are more robust than females (Fitch & Henderson, 1978). However, morphological variation in juvenile stages is a subject that has received little attention and should be addressed, due to changes that occur as a product of ontogenetic development (Durtsche, 2000). Sex recognition in *C. pectinata* hatchlings a few days after hatching is difficult, due to the leaf green color of most of the body and the apparent little variation in morphology. The first attempt to identify the sex in juveniles showed that only after 21 months of age (1.7 years), males and females can be recognized by the development of the dorsal crest, the femoral pores, the bulge of the hemipenes, the total weight and the length of the head (Arcos-

García *et al*., 2005), which undoubtedly indicates that they are subadult individuals. Generating information to facilitate sex recognition in juvenile stages is useful when it comes to managing *Ctenosaura pectinata* hatchlings in confinement conditions; above all, to estimate if there are differences in development rates related to sex. The divergence in growth rates between individuals in wild populations, and in those kept under captive conditions, has been recorded in *Crocodylus moreletti* (Serna-Lagunes *et al*., 2010), and it has been observed that confinement limits the development of individuals, as frequently occurs in *C. pectinata* farms (*personal observation*).

The objective of the study was to differentiate the sexes of *C. pectinata* offspring through morphological variation. This work also includes a key with the most divergent morphological characters with the purpose of making the identification of both sexes easier. Our test hypothesis that with the use of several morphological characters and their processing with traditional statistical techniques and multivariate analyses, it is possible to differentiate males and females in early stages of development.

MATERIALS AND METHODS

As part of a series of periodic visits to an iguanarium located in Morelos State, México (2 km south of Yautepec at 18° 49' 20" N, 99° 05' 37" W; 1,105 m elev.), we obtained morphometric data on 127 *C. pectinata* hatchlings (authorization Semarnat No. SGPA/DGVS/03490), produced by 4 females with an average size of 285.3 mm of snout-vent length (SVL) (± 21.5, range: 240.0 – 320.0), average clutch size = 31.1 eggs (±8.6), developed in an environment of tropical deciduous forest and semitropical climate (Fig. 1). To differentiate the sex of hatchlings, the following criteria according to Solorzano and Canales (2009) were considered: males with ovoid-shaped abdominal testicles connected to tubular spermatic cords; females with elongated abdominal ovaries and connected to wavy oviducts. The morphological measurements were obtained from dead individuals that only lived the first week after hatching, and were the following: SVL, tail length (TL), head length (HL, distance in a straight line between the anterior edge of the snout and the anterior edge of the eardrum), head depth (HD, vertical distance in a straight line between the lateral border of the throat and the parietal region), head width (HW, distance between the two edges of the head at the height of the postocular region), forelimb length (FLL, distance obtained by adding the length of the humerus, length of the radius, length of the metacarpus, and length of the phalanges of the longest toe), hindlimb length (HLL, linear distance obtained by adding the length of the femur, length of the tibia, length of the metatarsus, and length of the phalanges of the longest toe) (Hamilton & Sullivan, 2005; Herrel *et al*., 2007), the weight (g) and the physical robustness (Weight/SVL)*100. All measurements were obtained with a digital vernier (0.01 mm), and the weight was recorded with a scale (precision 0.01 g).

Our data were confirmed with the additional review of gonads of 25 preserved individuals of similar size and age than hatchlings analyzed, hosted in the herpetological collection of the Universidad Autónoma del Estado de Morelos (INE.MOR.REP.119.1101).

The normal distribution of all the data was verified with the Kolmogorov-Smirnov test and the homoscedasticity of the variables between the sexes was estimated with a Levene test (Table 1). The differences between characters that differentiate males from females were obtained with one-way analysis of variance (ANOVA). To estimate whether the variation in the data really could be attributable to sex, a multivariate analysis of variance was used (MANOVA) with the variables obtained. Subsequently, the reliability of the variables between the sexes due to use of individual morphological characters was estimated with the coefficient of variation CV = (standard

Deviation/mean) 100%, where CV ≤ 5.0% indicates that there is no variation and the use of the character is reliable, while CV ≥ 5.0% indicates variation (Castro-Franco *et al.*, 2011). To estimate whether all the variables contribute in the same magnitude to the separation of the sexes, a discriminant analysis was used (Johnson, 2000), which allows the identification of separation of groups with greater precision, considering the simultaneous effect of all analyzed variables. To increase the certainty in the separation of the sexes, an analysis of covariance was used (ANCOVA), using the variable HL as the dependent variable, SVL as the predictor variable, and sex as the categorical variable. These variables were used because it is known that the head and the body regularly have allometric relationships (Sánchez & Gutiérrez, 2020); males have large heads (Fitch & Henderson, 1978) and ANCOVA is a robust test that allows to identify if the variation in the structures can be attributable to sex. An alpha of P ≤ 0.05 was used in all statistical tests.

Figure 1. Artificial nesting, incubation and hatching of *Ctenosaura pectinata* in captivity. **A**) Nest for incubating eggs; **B**) Eclosion of hatchlings; **C**) and **D**) Newly hatched hatchlings. **SVL**: snout-vent length, **TL**: tail length, **HL**: head length, **HD**: head depth, **HW**: head width, **FLL**: forelimb length, **HLL**: hindlimb length. Photos by Anna Theresa Dietrich Frick.

Table 1. Normal distribution of nine morphological characters verified with the Kolmogorov-Smirnov test. The homoscedasticity of the variables between the sexes was estimated with a Levene test. Significance value P<0.05.

Morphological characters (mm)	Kolmogorov-Smirnov test	Homoscedasticity of variances Levene test
Snout-vent length (SVL)	D= 0.1277	F= 0.7222
Tail length (TL)	D= 0.0662	F= 0.0002
Head length (HL)	D= 0.1822	F= 0.0546
Head depth (HD)	D= 0.1838	F= 3.6602
Head width (HW)	D= 0.1633	F= 3.4111
Forelimb length (FLL)	D= 0.1233	F= 16.1100
Hindlimb length (HLL)	D= 0.0853	F= 22.3000
Weight (g)	D= 0.0947	F= 3.8280
Physical robustness (Weight/SVL)*100	D= 0.0760	F= 0.0001

RESULTS

The ANOVA (Table 2) showed that males were 3.3 mm longer than females in SVL character, 1.29 mm longer in the TL character 2.91 mm FLL and 6.35 mm in HLL. These characters were also those that showed a coefficient of variation below 10% which reveals that they are reliable characters to separate the sexes Although it is true that there were no significant differences in HL character ($F_{1,125}$= 0.11, p= 0.74560; Table 2), it also behaved as a character with little variation in both sexes (males CV= 8.77%, females CV= 7.08%). The characters with the greatest variation in the two sexes were the weight (CV= 13.91% males, CV= 12.58% females), and the physical robustness (CV= 14.36% males, CV= 12.66% females). The characters with the greatest variation only in females were head width (CV= 10.32% females, CV= 7.71% males), and forelimb length (CV= 14.64% females, CV= 7.83% males; Table 2). The MANOVA showed variation in the data because sex (λ *Wilks*= 0.3885, F_{98}= 17.1, p= 0.0000). Of the nine characters examined, snout-vent length, tail length, forelimb length, and hindlimb length presented statistically significant differences between the sexes (Table 2). Discriminant analysis also showed that SVL (λ *Wilks*= 0.621095), HL (λ *Wilks*= 0.595876) and HLL (λ *Wilks*= 0.7707) are the characters that best contribute to the separation of sexes due to the little variation they have (Table 3). Additionally, ANCOVA showed that head length varies depending on the SVL of hatchlings ($F_{1,121}$= 44.46, p= 0.0000) and the variation is attributable to sex ($F_{1,121}$= 5.20, p= 0.0242). The same happened with the hindlimb length, which varies depending on the SVL (ANCOVA $F_{1,121}$= 4.92, p= 0.0283) and the variation is also due to the sex ($F_{1,121}$= 97.96, p= 0.0000).

Table 2. Morphological variation in hatchlings of *Ctenosaura pectinata* from Morelos State. Measurements in mm, mean, below (minimum and maximum values), ± standard deviation, CV (coefficient of variation). The * shows significant differences with P≤ 0.05.

Morphological characters (mm)	Males $n= 68$	Females $n= 59$	Test value ANOVA
Snout-vent length (SVL)	52.53 (45.50-57.00) ±3.21 CV= 6.11%	49.23 (40.00-58.00) ±4.33 CV= 8.52%	$F_{1,125}= 24.05$ $p= 0.000003*$
Tail length (TL)	13.59 (10.20-15.50) ±1.24 CV= 9.16%	12.30 (10.10-14.90) ±1.21 CV= 9.47%	$F_{1,125}= 33.07$ $p= 0.00000*$
Head length (HL)	16.69 (10.30-19.20) ±1.46 CV= 8.77%	16.61 (14.00-19.00) ±1.17 CV= 7.08%	$F_{1,125}= 0.11$ $p= 0.74560$
Head depth (HD)	8.13 (7.00-9.00) ±0.59 CV= 7.27%	8.08 (5.00-10.00) ±0.90 CV= 9.96%	$F_{1,125}= 0.14$ $p= 0.71380$
Head width (HW)	10.31 (8.30-12.00) ±0.79 CV= 7.71%	10.08 (8.00-12.00) ±1.07 CV= 10.32%	$F_{1,125}= 1.79$ $p= 0.18330$
Forelimb length (FLL)	25.99 (20.50-29.00) ±2.03 CV= 7.83%	23.08 (16.00-33.00) ±3.58 CV= 14.64%	$F_{1,125}= 32.62$ $p= 0.0000*$
Hindlimb length (HLL)	44.43 (36.90-50.00) ±2.68 CV= 6.05%	38.08 (30.00-44.00) ±3.40 CV= 8.99%	$F_{1,125}= 131.08$ $p= 0.00*$
Weight (g)	4.83 (3.10-5.7) ±0.67 CV= 13.91%	4.55 (3.00-5.80) ±0.60 CV= 12.58%	$F_{1,125}= 5.98$ $p= 0.0158*$
Physical robustness (Weight/SVL)*100	9.04 (4.08-11.46) ±1.29 CV= 14.36%	9.34 (7.08-12.22) ±1.18 CV= 12.66%	$F_{1,125}= 1.71$ $p= 0.19299$

Table 3. Lambda values resulting from the discriminant analysis of five morphological variables among recently eclosioned hatchlings (males and females) of *Ctenosaura pectinata*. λ= 0.55238, $F_{5,120}$=19.449, p<0.0000. The variables that do not contribute to the separation of the sexes are the tail length, the head depth, and the length of the forelimb. The * shows significant differences with P≤0.05.

Morphological characters (mm)	Wilk's Lambda	Lambda partial	F-removal (1,118)	p level of significance
Snout-vent length (SVL)	0.621095	0.889357	14.92899	0.000182*
Tail length (TL)	0.558431	0.989155	1.31567	0.253653
Head length (HL)	0.595876	0.926997	9.45023	0.002614*
Head depth (HD)	0.568081	0.972352	3.41206	0.067185
Hindlimb length (HLL)	0.770777	0.716647	47.44642	0.000000*

DISCUSSION

The traditional way of evaluating sexual dimorphism in lizards has usually been through the variation in the body size of males and females (Fitch, 1978), and this has been observed in several families (*e.g*., Phrynosomatidae Fitch, 1978, Lacertidae-Braña, 1996, Liubisavliević *et al*., 2008; Scincidae Olsson *et al*., Anguidae Dashevsky *et al*., 2013; Chamaeleonidae Stuart Fox & Moussalli, 2007). In Iguanidae, species such as *C. similis* which is phylogenetically close to *C. pectinata* it has been observed that the SVL and the size of the head is larger in males than in females (Fitch & Henderson, 1977). The variation in the size of the hatchlings within the litters in *I. iguana* has been explained as an adaptative strategy to survive drought (Van Marker & Albers, 1993) or to facilitate the adaptation of various phenotypes in the face of unpredictable situations (Castro-Franco *et al*., 2011). All this observed variation has been explained by the effect of environmental factors (extrinsic) such as the differential allocation of energy during growth the development of aggressive behaviors in adults to protect large territories, sexual selection, fecundity, and the differential use of microhabitats (Carothers, 1984; Haenel & John-Alder, 2002; Kratochvil & Frynta. 2002). Only in recent years has it been observed that intrinsic factors such as the formation of chromosome races and genetic hybridization (*Sceloporus grammicus* Cruz-Elizalde *et al*., 2023; Lozano *et al*., 2023; *C. pectinata* Zarza *et al*., 2009; Zarza *et al*., 2019) also contribute to the variation in the body size of males and females. This reveals that the information generated on the subject has been focused on explaining the variation in body size of adults as a mechanism that offers evolutionary advantages of larger over smaller individuals. However, the difference between the sexes is not always due to variation in a single character such as SVL. We have evidence that morphological variation considering various characters can also occur in hatchlings which has been a virtually ignored topic. The data presented in this work show that dimorphism in *C. pectinata* can be identified in juvenile stages, considering the additive effect of various morphological characters, among which the snout vent length and the length of the forelimbs and hindlimbs stand out. This pattern of elongated bodies with long limbs in hatchlings is consistent with the same characters that define typical dimorphism in adult males of this species (Fitch & Henderson, 1977). This explains why in a previous study (Arcos-García *et al*., 2005) it was not possible to distinguish the sexes considering few characters, analyzed separately. Sex differences

in SVL, and adult limb length have also been recorded in *Sphenodon punctatus* tuatara (Herrel *et al.*, 2009). We suggest that the variation in body size between males and females of *C. pectinata* emerges as a character that is linked to sex, which produces a certain level of predisposition in hatchlings, so that the body and other structures such as the length of the head and legs, develop faster in males than in females, and behaves as an allometric function of the SVL (Sánchez & Gutiérrez, 2020). Variation in sex linked morphological characters (large heads in males vs large abdomen in females) has been observed in various groups of lizards (Braña, 1996; Scharf & Meiri, 2013), and it has been suggested that a specific sex is the mechanism that triggers the variation in morphology between the sexes. In parthenogenetic species where there is obviously only one sex body and head size should vary little between individuals, which has been observed in lizards of the genus *Aspidoscelis* where little variation in body size has been recorded (CV= 4.75% Hernández-Gallegos *et al.*, 2015). In conclusion, we suggest that more research is needed to verify if sexual dimorphism in body size and limb length also occurs in thermal sex dependent species, or if morphological variation does not exist in unisexual species. The use of multivariate analysis to document sex morphological differences of ranching specimens, is a useful tool considering the additive value of the variables involved.

KEY TO THE IDENTIFICATION OF SEXES IN NEONATES OF *Ctenosaura pectinata* USING MORPHOMETRIC CHARACTERS

1   A Body size (SVL) large (x= 52.53 mm ±3.21), three times longer than the head length.........2A
    B Body size (SVL) relatively small, no more than 50.00 mm (x= 49.23 mm ±4.33), and no more than three times the length of the head 2B
2   A Large hind legs (x= 44.43 mm ±2.68), almost twice the length ....Male
    B Relatively small hind legs (x= 38.08 mm, ±3.40), no more than 1.5 times the length of the front legs...Female

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