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Topographic anatomy and sexual dimorphism of *Bothrops erythromelas* AMARAL, 1923
(Squamata: Serpentes: Viperidae)

Topographische Anatomie und Geschlechtsdimorphismus von *Bothrops erythromelas* AMARAL, 1923 (Squamata: Serpentes: Viperidae)

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KURZFASSUNG

Informationen über die innere Anatomie brasilianischer Schlangen sind rar. Über *Bothrops erythromelas* AMARAL, 1923, eine endemische Art der Ökoregion Caatinga in Nordostbrasilien, ist auch biologisch wenig bekannt. Diese Studie untersucht die topographische Anatomie und den Geschlechtsdimorphismus bei dieser Lanzenotternart. Die Lage der inneren Organe wurde in Bezug auf die Bauchschuppen beschrieben, indem angegeben wurde, auf Höhe welcher Schuppen sie beginnen und enden. Folgende Parameter der Körpergestalt wurden zur Untersuchung des Sexualdimorphismus ausgewertet: Anzahl der Bauchschuppen, Kopf-Rumpf-Länge, Schwanzlänge, Kopflänge, Kopfbreite, Kopfhöhe und Kopfvolumen.

Die topographische Lage des Herzens folgt dem für Viperidae typischen Muster, bei denen dieses Organ vergleichsweise weit caudal positioniert ist. Das Vorhandensein einer Tracheallunge, ein für dieser Gruppe typisches Merkmal, wurde bestätigt. Die Geschlechtsunterschiede in der Kopf-Rumpf-Länge waren nahezu signifikant, mit höheren Werten für Weibchen, wahrscheinlich um Platz und Nahrungsressourcen für große Würfe zu gewährleisten. Die Werte für Höhe und Volumen des Kopfes waren ebenfalls bei den Weibchen größer.

ABSTRACT

Information on the internal anatomy of Brazilian serpents is scarce. *Bothrops erythromelas* AMARAL, 1923, native to the northeast Brazilian ecoregion called Caatinga, is also poorly known with regard to its biology. This study aimed to investigate the topographic anatomy and sexual dimorphism in *B. erythromelas*. The position of internal organs was characterized by the numbers of the ventral scales where they began and ended. To check for potential sexual dimorphism, the number of ventral scales, snout-vent length, tail length, head length, head width, head height and head volume was assessed.

The topographic situation of the heart follows the pattern found in the family Viperidae, in which this organ is in a comparatively caudal position. The presence of tracheal lungs, a distinctive feature of this group was verified. Differences in snout-vent length were near significant, with higher values for females, probably to provide space and nutritional resources for large litter. Also, the values in height and volume of the head were higher for females.

KEY WORDS

Squamata: Serpentes: Viperidae; *Bothrops erythromelas*, ecomorphology, morphometry; topographic anatomy, organ position, sexual dimorphism, Brazil

INTRODUCTION

As in other species, morphology and physiology of snakes is determined by natural selection as a result of their interaction with the habitat (VITT & VANGILDER 1983; GUYER & DONNELLY 1990; CADLE & GREENE 1993; LILLYWHITE & HENDERSON 1993). Anatomy is an essential tool for several lines of research, such as comparative morphology, veterinary medicine, ecology, systematics and evolution, and provides information for teaching, handling, and conserving of species. Thus, morphology and anatomy of snakes adds to the understanding of their phylogeny as well as functional anatomy, which establishes relationships between form, function, and adaptation (MASO 2002). Topographic anatomy describes the various anatomical structures in terms of their topography, i. e., extension, location and orientation relative to each other and the body surface. In snakes, in particular, it studies the relationship between the position and morphology of internal organs and external points of reference or structures such as the ventral scales, thus, helping locate these organs (Aveiro-Lins et al. 2006). The position of the organs in relation to the ventral scales was depicted by Collins & Carpenter (1970), Rossman et al. (1982) and Martinez et al. (1985). The heart is commonly positioned more anteriorly in arboreal snakes than in terrestrial species, reflecting adaptive strategies to gravitational variations in those habitats (Lillywhite 1988; Badeer 1998; Rocha-Barbosa et al. 1999, 2000). Besides facilitating descriptions and intra- and interspecific comparisons (Navega-Gonçalves 2009), knowledge of the topographic location of the organ of interest is useful for veterinary treatment as well as dissections (e.g., Bragdon 1953; Camazine et al. 1981). Topographic anatomy studies also led to ecomorphological studies of snakes (Aveiro-Lins et al. 2006; Gartner et al. 2010).

Sexual dimorphism in body size and shape is common in snakes (Shine 1993, 1994). In most species, females are larger than males, probably an adaptation to increase their reproductive output (Shine 1981, 1988, 1989, 1994; Madsen & Shine 1993). Several studies found intersexual differences in head shape, relative head size (Camilleri & Shine 1990; Luiselli et al. 2002; Mesquita & Brites 2003; Vincent et al. 2004; Matias et al. 2011) as well as tail length (Janeiro-Cinquini et al. 1992; Bonnet et. al 1998; Santos & Ribeiro 2005; Matias et al. 2011).

Brazil has a very diverse snake fauna, but anatomical studies of Brazilian species are scarce (e.g., Narchi 1973; Gomes et al. 1989; Gomes & Puorto 1993; Mesquita & Brites 2003; Aveiro-Lins et al. 2006). *Bothrops erythromelas* Amaral, 1923, commonly known as Caatinga Lancehead, is an endemic species of the Caatinga, a xeric shrubland and thorn forest. The snake is found in the semiarid north-eastern Brazil, from the northern state of Piauí, in a southerly direction, to the northeast of the state of Minas Gerais (Vanzolini et al. 1980; Campbell & Lamar 1989; Wallach et al. 2014). This small (about 50 cm), terrestrial, viviparous lancehead species feeds when young mainly on centipedes, later gradually substituting them for anurans, lizards and small mammals (Amaral 1977; Martins et al. 2002).

In this study, the authors aimed to investigate the gross topographic visceral anatomy of *B. erythromelas*, as well as assess whether or not there is sexual dimorphism in selected traits of size and proportions. It was expected to find that the topographic position of the internal organs of *B. erythromelas* in relation to the ventral scales is similar to that of other terrestrial species of the genus, specifically, that the heart is positioned more posteriorly than in arboreal members of Viperidae and that females would have a larger body size than males, following the general pattern for snake species without male combat.

MATERIALS AND METHODS

The topographic anatomy study comprised 27 specimens of *Bothrops erythromelas* (15 males, 12 females), the sexual dimorphism study, 36 specimens (18 males and 18 females). The samples originated from the Coleção de Herpetologia da Universidade Federal do Ceará (CHUFC), and the Instituto Butantan (IBSP) (see Appendix I).

The dissection procedures followed Gomes et al. (1989) and Gomes & Puorto (1993). An incision was made along the medial line of the ventral scales from the cloacal opening to the gular region. Sex determination was accomplished by hemipenis observation. Ventral scales were counted from the first (most cranial) that was wider than long to the scale before the cloacal plate (Dowling 1951). Organs were positioned in relation to the consecutively numbered ventral scales, i. e., at the level of which scale they began and ended (Gomes et al. 1989; Gomes & Puorto 1993). Also, cranial and caudal organ limits or positions were expressed in percent of the total number of ventral scales. The following visceral organs were considered: liver, heart, lung, trachea, stomach, esophagus, kidneys, testes, ovaries, reproductive ducts (oviducts and vasa deferentia), gall bladder and intestine. Pancreas, thyroid, spleen, thymus, adrenals and saccular portion of the right lung were not considered because they were difficult to locate in many specimens.

For the study of sexual dimorphism, the number of ventral scales (NVS), snout-vent length (SVL), tail length (TL), head length (HL), head width (HW) and head height (HH) were recorded. These measurements were taken with a string, then measured with a ruler (1 mm accuracy). In addition, head volume (HV) was calculated using the formula for the volume of a pyramid of rectangular base (Area of Base x Height/3), using head length as the height of the pyramid and head width and height as the measures of its base. In this note, terms such as anterior, before, or beginning refer to cranial, whereas posterior, behind and ending to caudal directions/positions.

All measured variables were log-transformed ($\log_e$) and showed normal distribution according to Shapiro-Wilk tests. The log-transformed SVLs of the males were compared to the females using the Welch two samples t-test (Zar 2009). Sexual dimorphism regarding the other measured variables (HL, HW, HH, HV, TL and NVS) was assessed using Generalised Linear Models (GLMs) with Gaussian errors and log-transformed SVL as a covariate; the models were selected by Analysis of Deviances (ANODEV) with F-tests.

Descriptive statistics are presented as mean ± 1 standard deviation. Analyses were performed using R ver. 3.0.1 software (R Development Core Team 2013).

Table 1: Topographic anatomy of *Bothrops erythromelas* Amaral, 1923. Position of visceral organs relative to the ventral scales consecutively numbered in cranio-caudal direction. NVS – total number of ventral scales; S_a – number of ventral scale corresponding with the anterior (cranial) end of organ; S_p – number of ventral scale corresponding with the posterior (caudal) end of organ; S_a (%) – S_a in percent of mean NVS; S_p (%) – S_p in percent of mean NVS; A – Number of ventral scales occupied. Mean NVS = 149, NVS_{min} = 139, NVS_{max} = 158; *n* = 27, except for right and left oviducts (*n* = 12) and right and left vasa deferentia (*n* = 15).

Tab. 1: Topographische Anatomie von *Bothrops erythromelas* Amaral, 1923. Lage visceraler Organe in Bezg zu den in cranio-caudaler Richtung fortlaufend numerierten Bauchschuppen. NVS – Gesamtzahl der Bauchschuppen; S_a – Nummer der Bauchschuppe auf Höhe des vorderen (cranialen) Organendes; S_p – Nummer der Bauchschuppe auf Höhe des hinteren (caudalen) Organendes; S_a (%) – S_a in Prozent der durchschnittlichen NVS; S_p (%) – S_p in Prozent der durchschnittlichen NVS; A – Anzahl der eingenommenen Bauchschuppen. Durchschnittliche NVS = 149, NVS_{min} = 139, NVS_{max} = 158; *n* = 27, mit Ausnahme der rechten und linken Eileiter (*n* = 12) bzw. der rechten und linken Samenleiter (*n* = 15).

Character	$S_a – S_p$	S_a (%) – S_p (%)	A
Trachea	0 – 53	0.0 –35.6	53
Lung	11 – 58	7.4 – 38.9	48
Esophagus	0 – 70	0.0 – 47.0	70
Heart	49 – 54	32.9 – 36.2	6
Liver	54 – 80	36.2 – 53.7	27
Stomach	70 – 89	47.0 – 59.7	20
Gall Bladder	92 – 96	61.7 – 64.4	05
Intestine	89 – 149	59.7 – 100.0	61
Right kidney	119 – 139	79.9 – 93.3	21
Left kidney	122 – 140	81.9 – 94.0	19
Right Ovary	100 – 112	67.1 – 75.2	13
Left Ovary	109 – 119	73.2 – 79.9	11
Right Testis	103 – 107	69.1 – 71.8	5
Left Testis	109 – 113	73.2 – 75.8	5
Right Oviduct	112 – 152	74.2 – 100	41
Left Oviduct	119 – 152	78.8 – 100	34
Right Vas Deferens	107 – 147	73.3 – 100	41
Left Vas Deferens	113 – 147	77.4 – 100	35

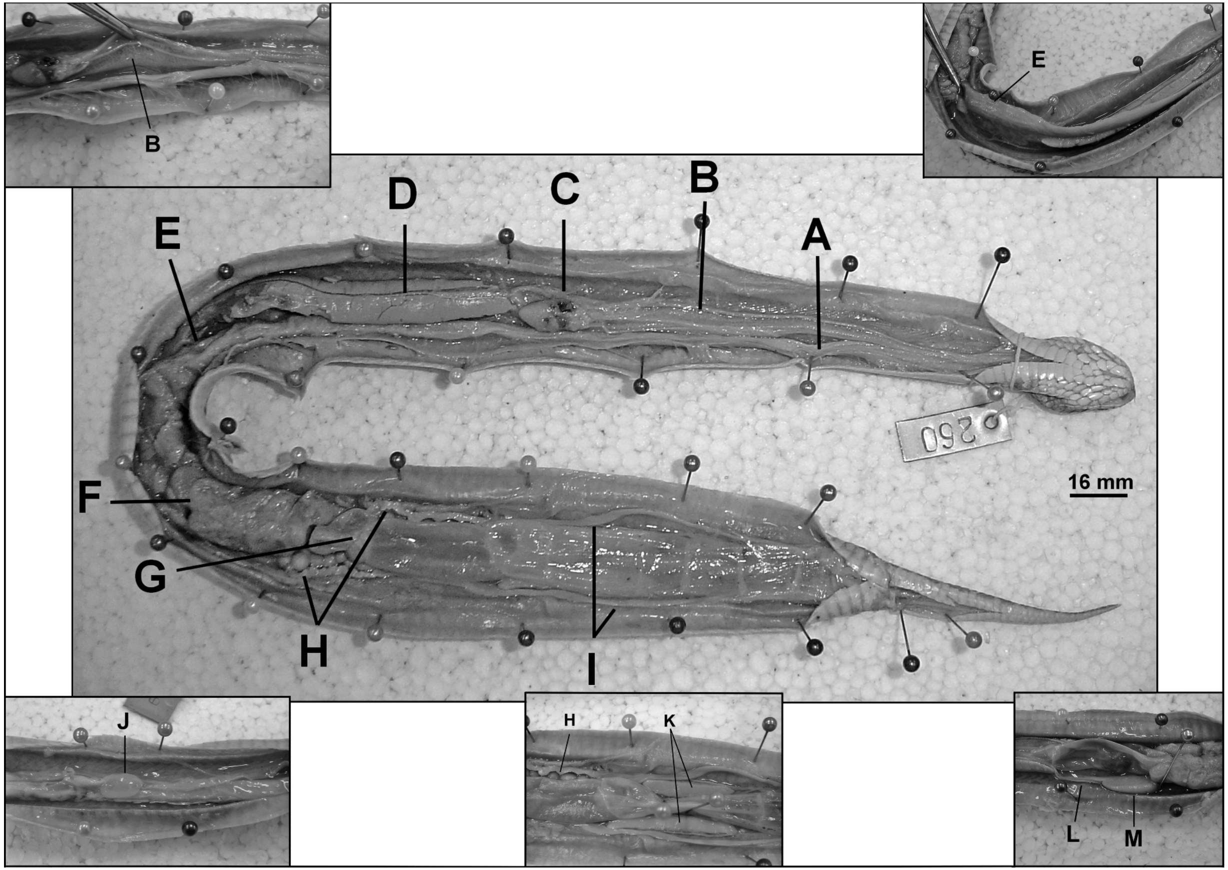

Fig. 1: Overview of the internal organs of *Bothrops erythromelas* AMARAL, 1923. A – Esophagus; B – Tracheal lung; C – Heart; D – Liver; E – Stomach; F – Small intestine; G – Colon; H – Ovaries; I – Oviducts; J – Gall bladder; K – Kidneys; L – Vas deferens; M – Testis. Scale bar represents 16 mm. Picture: Patrícia Gondim.

Abb. 1: Übersicht der inneren Organe der von *Bothrops erythromelas* AMARAL, 1923. A – Speiseröhre; B – Trachealllunge; C – Herz; D – Leber; E – Magen; F – Dünndarm; G – Dickdarm; H – Eierstöcke. I – Eileiter; J – Gallenblase; K – Nieren; L – Samenleiter; M – Hoden. Maßstab entspricht 16 mm. Bild: Patrícia Gondim.

Table 2: Variation in morphological traits among the studied male and female *Bothrops erythromelas* AMARAL, 1923. SVL – Snout Vent Length; HL – Head Length, HW – Head Width, HH – Head Height, HV – Head Volume, TL – Tail Length, and NVS – Number of Ventral Scales; $\bar{x}$ – Mean value; SD – Standard deviation; n = 18 males and 18 females, except for HW and HV (n = 17 for females).

Tab. 2: Variation der morphologischen Merkmale bei den untersuchten Männchen und Weibchen von *Bothrops erythromelas* AMARAL, 1923. SVL – Kopf-Rumpf-Länge; HL – Kopflänge; HW – Kopfbreite; HH – Kopfhöhe; HV – Kopfvolumen; TL – Schwanzlänge; NVS = Anzahl der Bauchschuppen; $\bar{x}$ – Mittelwert; SD – Standardabweichung; n = 18 Männchen und 18 Weibchen, ausgenommen HW und HV (n = 17 Weibchen).

Character	Females ($\bar{x}$ ± SD)	Males ($\bar{x}$ ± SD)	Test Result	*p*
SVL (cm)	46.65 ± 9.22	41.70 ±3.60	$t_{23.9} = 1.95$	0.063
HL (mm)	24.58 ± 3.77	22.03 ± 1.87	$F_{1,33} = 2.12$	0.154
HW (mm)	14.69 ± 2.41	13.71 ± 1.41	$F_{1,32} = 3.01$	0.092
HH (mm)	10.64 ± 1.80	8.86 ± 1.31	$F_{1,33} = 6.03$	0.019
HV (mm^3)	1448.91 ± 769.65	901.67 ± 216.90	$F_{1,32} = 7.97$	0.008
TL (cm)	6.24 ± 1.42	6.82 ± 1.18	$F_{1,33} = 16.73$	< 0.001
NVS	151.11 ± 4.61	146.44 ± 4.32	$F_{1,33} = 6.43$	0.016

RESULTS AND DISCUSSION

Topographic anatomy

The data of the topographic position of the internal organs of *Bothrops erythromelas* in relation to the ventral scales are shown in Table 1. Among the paired organs, the testes were smallest, occupying the least number of scales, whereas the ovaries were nearly two times longer than the testes. Similar results were obtained for *Boa constrictor* LINNAEUS, 1758 (GOMES et al. 1989), *Bothrops jararaca* (WIED, 1824) (GOMES & PUORTO 1993), *Bothrops moojeni* HOGE, 1966 (FARIA & BRITES 2003), and *Bothrops alternatus* DUMÉRIL, BIBRON & DUMÉRIL, 1854 (MESQUITA & BRITES 2003).

The topographic position of the cranial limit of the heart expressed as percent of the total number of ventral scales (32.9 %) was similar to that found by GOMES & PUORTO (1993) in *B. jararaca*. SEYMOUR (1987) and GARTNER et al. (2010), who, however, reported heart positions in percent of total length, found similar values (33.3 % and 31.9 %, respectively) for Viperidae. The heart is placed more posteriorly in terrestrial and more anteriorly in arboreal viperids (SEYMOUR 1987). A pronounced anterior location of the heart is often found in arboreal snakes. In contrast, it is centrally positioned in aquatic species, while in terrestrial snakes it generally takes an intermediary position between that of the aquatic and arboreal species (LILLYWHITE 1988; BADEER 1998; AVEIRO-LINS et al. 2006; GUIMARÃES et al. 2013). These differences reflect adaptive strategies to the particular effects of gravity in those habitats (LILLYWHITE 1988; ROCHA-BARBOSA et al. 1999, 2000).

From the topographic position of the organs, the authors observed that the liver was isolated from the gall bladder. MCDOWELL (1979) noted that having the gall bladder displaced far behind the liver is often cited as a distinctive feature of snakes. According to BRAZIL (1911), this distance is important so that the organ is not disturbed in its functions by the repletion of the stomach. Both trachea and lung occupied approximately the same position relative to the ventral scales (Table 1). This is due to the architecture of the tracheal lung (Fig. 1), which, according to KEOGH & WALLACH (1999), is a weakly vascularized membrane supported between the incomplete semicircular cartilaginous rings of the trachea. The respiratory system of viperid snakes is composed of the trachea, tracheal lung, right lung, and occasionally, a bronchus and vestigial left lung. The trachea typically extends into the right lung where it continues as an intrapulmonary bronchus.

Sexual dimorphism

Sexual dimorphism was found regarding the relative head height, relative head volume, and number of ventral scales, the values of which were significantly higher in females than males. Tails were significantly longer in males (Table 2).

The occurrence of sexual dimorphism in the number of ventral scales is found also in other species of the genus, such as *B. moojeni* (FARIA & BRITES 2003) and *B. alternatus* (MESQUITA & BRITES 2003). In this study, differences between male and female SVLs were only near significant (p = 0.063). This unexpected result may be due to insufficient sample size. A power analysis that considered the sample size of both sexes, the difference between the means of both groups and the standard deviation of the whole sample supports this assumption, as the power amounted to 44.5 % only. Usually, females have larger body size than males, probably to provide space and nutritional resources for large broods (SHINE 1981, 1988, 1989, 1994; MADSEN & SHINE 1993). Large females have bigger ovaries, allowing for a higher number of follicles and, consequently, larger litter size (SEMLITSCH & GIBBONS 1982). The longer tail in males shelters the hemipenes and retractor muscles (CLARK 1966; KING 1989; GREENE 2000).

The bigger size of the female head results most probably in larger venom glands and production of higher venom quantities (FARIA & BRITES 2003; MESQUITA & BRITES 2003). BIASI et al. (1976/1977) found that female *Bothrops leucurus* WAGLER, 1824 (*pradoi* in their paper) produced more venom than males, a result also found for *Bothrops atrox* (LINNAEUS, 1758) (BELUOMINI et al. 1991). Sexual differences in head size can also be related to food preferences for a particular type or size of prey (SHINE 1989, 1994; LUISELLI et al. 2002; SHETTY & SHINE 2002; SHINE et al. 2002; KRAUSE et al. 2003; VINCENT et al. 2004). Studies on the morphometry of venom glands and food habits of this species should be conducted to test these hypotheses.

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Appendix I: Studied specimens of *Bothrops erythromelas* Amaral, 1923, from Brazil.
Anhang I: Untersuchungsmaterial von *Bothrops erythromelas* Amaral, 1923 aus Brasilien.

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