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Trabajo

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Advertisement and release calls of *Rhinella scitula* (Caramaschi and Niemeyer, 2003) (Anura: Bufonidae)

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ABSTRACT
The anuran calls descriptions have given important taxonomic information in studies within problematic species group. Herein, we describe the advertisement and release calls of *R. scitula*. We analyzed calls recorded in three localities in Mato Grosso do Sul state, Brazil, including the type locality. Males were calling at the margins of permanent streams in forest fragments ca. 21:00 h. The advertisement call of *R. scitula* is multi-pulsed with interior amplitude modulation, resulting in pulse groups. Males emitted non-regular call series with duration of 0.27s ± 0.03 (0.23–0.36), note duration of 0.015s ± 0.004 (0.007–0.040), pulse duration of 0.008s ± 0.001 (0.005–0.015, n=180), pulse group per call of 6.6 ± 0.92 (5–8) and dominant frequency of 1439.7 Hz ± 46.1 (1378.1–1550.4). The release calls were characterized by a dominant frequency of 1115.8Hz ± 102.2 (947.5–1550.4), a frequency bandwidth of 2001.6Hz ± 527.4 (861.3–3876). They are formed by pulsed and/or pulsatile notes spaced by non-regular intervals or series of 2–19 calls. From all *R. margaritifera* species group with described advertisement calls, the most different to *R. scitula* and other species in the group was *R. magnussoni*, which has a structurally distinct call. The release calls in *R. granulosa* species group and *R. scitula* has the same pattern of pulsed and/or non-pulsed notes.

Key words: Bioacoustics; Vocalization; True toads; Animal behavior; Taxonomy.

Introduction

Amphibian communication occurs through different sensorial mechanisms, and vocalization is certainly the most important for Anura (frogs, toads, and treefrogs). Their diverse calls have major role in species-specific interactions, like mate attraction and reproduction, but can also be used during aggressive or defensive behaviors (Toledo *et al.*, 2014). The anuran calls descriptions have given important taxonomic information in studies within problematic species group, helping researchers to detect and diagnose cryptic species (Blair, 1972; Guerra *et al.*, 2011; Köhler *et al.*, 2017).

The *Rhinella margaritifera* species group is composed by 19 species of dead-leaf patterned toads, and evidence of cryptic diversity within taxa suggest that species richness in this group is probably higher than currently recognized (Fouquet *et al.*, 2007a; Moravec *et al.*, 2014; Vaz-Silva *et al.*, 2015). Such evidence stems mainly from molecular systematics approaches (Fouquet *et al.*, 2007a; Moravec *et al.*, 2014), and independent data sources that could complement them for taxonomic purposes, such as the description of bioacoustic parameters, are still scarcely published (Lima *et al.*, 2007; Ávila *et al.*, 2010; Roberto *et al.*, 2012; Vaz-Silva *et al.*, 2015). Among the recognized species in this group (including potential new ones), only *R. dapsilis*, *R. ocellata* and *R. yunga* have the release calls described (Zimmerman and Bogart, 1988; Caldwell and Shepard, 2007; Stynoski *et al.*, 2020) while only ten have their advertisement calls known: *R. dapsilis* (Zimmerman and Bogart, 1988), *R.* sp. from Bolivia (De La Riva *et al.*, 1996); *R.* sp. from French Guiana (clade A *sensu* Fouquet *et al.*, 2007b), *R. castaneotica* (Köhler and Lötters, 1999), *R. martyi* (Fouquet *et al.*, 2007b), *R. lescurei* (Fouquet *et al.*, 2007b), *R. magnussoni* (Lima *et al.*, 2007), *R. ocellata* (Caldwell and Shepard, 2007), *R. paraguayensis* (Ávila *et al.*, 2010), and *R. hoogmoedi* (Roberto *et al.*, 2012).

Rhinella scitula (Caramaschi and Niemeyer,

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2003) is a small toad occurs mainly on the margins of temporary or permanent streams near or within gallery forests (Caramaschi and Niemeyer, 2003). In Brazil, its geographic distribution ranges from southwestern to central Mato Grosso do Sul State. The species was also recorded in Paraguay, near the Brazilian border (Sugai *et al.*, 2014). Herein, we describe the advertisement and release calls of *R. scitula* from three localities in Brazil (including specimens from the type locality), and compare bioacoustic parameters of the advertisement calls with those from related species.

Materials and methods

We analyzed calls recorded in three localities in Mato Grosso do Sul state, Brazil: 1) Estância Mimosa Ecotourism, the type locality of the species, in the municipality of Bonito (-20.983160°S, -56.515635°W, 390 m a.s.l.); 2) Vale das Bruxas, Piraputanga district, Aquidauana municipality (-20.455140°S, -55.498411°W, 190 m a.s.l.); and 3) Morro do Paxixi, Camisão district, Aquidauana municipality (-20.451111°S, -55.621111°W, 377 m a.s.l.). We recorded the advertisement calls of one individual at Vale das Bruxas on 29 January 2016 using an Olympus LS 10 recorder. Five individuals to advertisement calls and one to release calls at Estância Mimosa Ecotourism on 14 May 2016 using a Tascam DR-40 recorder. Also, we recorded the release calls of two specimens at Morro do Paxixi on 15 August 2018 using a Tascam DR-40 recorder. The recorders' internal microphones were employed for recordings in all localities. We positioned the recorder about 1 m away from the calling toad and recorded the calls at a sample rate of 44 kHz and 16-bit resolution. Recorded males were collected and are housed in the Zoological Collection of Universidade Federal de Mato Grosso do Sul (ZUFMS-AMP 03740–45 and 11138–39). We analyzed ten bioacoustic parameters generally used for anuran (Köhler *et al.*, 2017), and specifically for species of the *R. margaritifera* group (see Roberto *et al.*, 2012) call description: call duration (s), note duration (s), pulse duration(s), pulse group per call, pulses per pulse group, number of pulses per call, pulse rate (pulse/s), inter-note interval (s), dominant frequency (Hz) and frequency bandwidth (Hz). Call parameters terminology follows the note-centered approach (Köhler *et al.*, 2017). For the advertisement calls, acoustic parameters of *R. scitula* were compared to those of other species in the *R. margaritifera* group. We analyzed all calls with Raven Pro v1.5 (Bioacoustics Research Program 2017) and built the oscillograms and spectrogram with R 3.4.3 (R Core Team, 2017). We also used the packages tuneR (Ligges *et al.*, 2013) and seewave (Sueur *et al.*, 2008), which analyze, manipulate, display and edit sound recordings. With these packages we process oscillograms and spectral contents (e.g. dominant frequency), and also build spectrograms.

Results

We observed males calling at the margins of permanent streams in forest fragments ca. 21:00 h. Snout vent length of collected males ranged from 38 to 42.5 mm (mean 40.3 mm). The advertisement call of *R. scitula* can be recognized as type II according to Martin (1972) classification, described as multipulsed calls with interior amplitude modulation, resulting in pulse groups (Fig. 1, Table 1). Males from both localities (Aquidauana and Bonito municipalities) emitted non-regular call series, each call with average duration of 0.27s ± 0.03 (0.23–0.36, n=28), mean note duration of 0.015s ± 0.004 (0.007–0.040, n=94), mean pulse duration(s) of 0.008s ± 0.001 (0.005–0.015, n=180), mean pulse group per call of 6.6 ± 0.92 (5–8, n=28) and mean dominant frequency of 1439.7 Hz ± 46.1 (1378.1–1550.4, n=28). In all calls analyzed, the inter-note interval (0.027s ± 0.007, 0.013–0.041; n=81) decreases from the first to the last (notes are emitted at a faster rate at the end of the call). When analyzing the number of pulses per pulse group (1.89 ± 0.53, 1–5; n=152), we found that most of the values are low (two or one), and only one specimen from Bonito emitted two distinct calls containing the last pulse group with five pulses. We found differences in call parameters between the two localities sampled in this study (Aquidauana and Bonito municipalities) when considering the number of pulses per call (12.7 ± 2.1, 9–17; n=28), pulse rate (46.4 pulses per second ± 8.1, 27.9–65.2; n=28) and frequency bandwidth (630.6 Hz ± 142.7, 430.7–947.5; n=28). The male from Morro do Paxixi, Aquidauana emitted calls with nine or ten pulses, lower pulse rate (27.9–36 pulses per second) and higher frequency bandwidth (861.3–947.5 Hz), while males from Bonito have calls with 11–17 pulses, higher pulse rate (40–65.2 pulses per second) and lower frequency bandwidth (430.7–602.9 Hz).

The release call of *Rhinella scitula* (Fig. 2) is

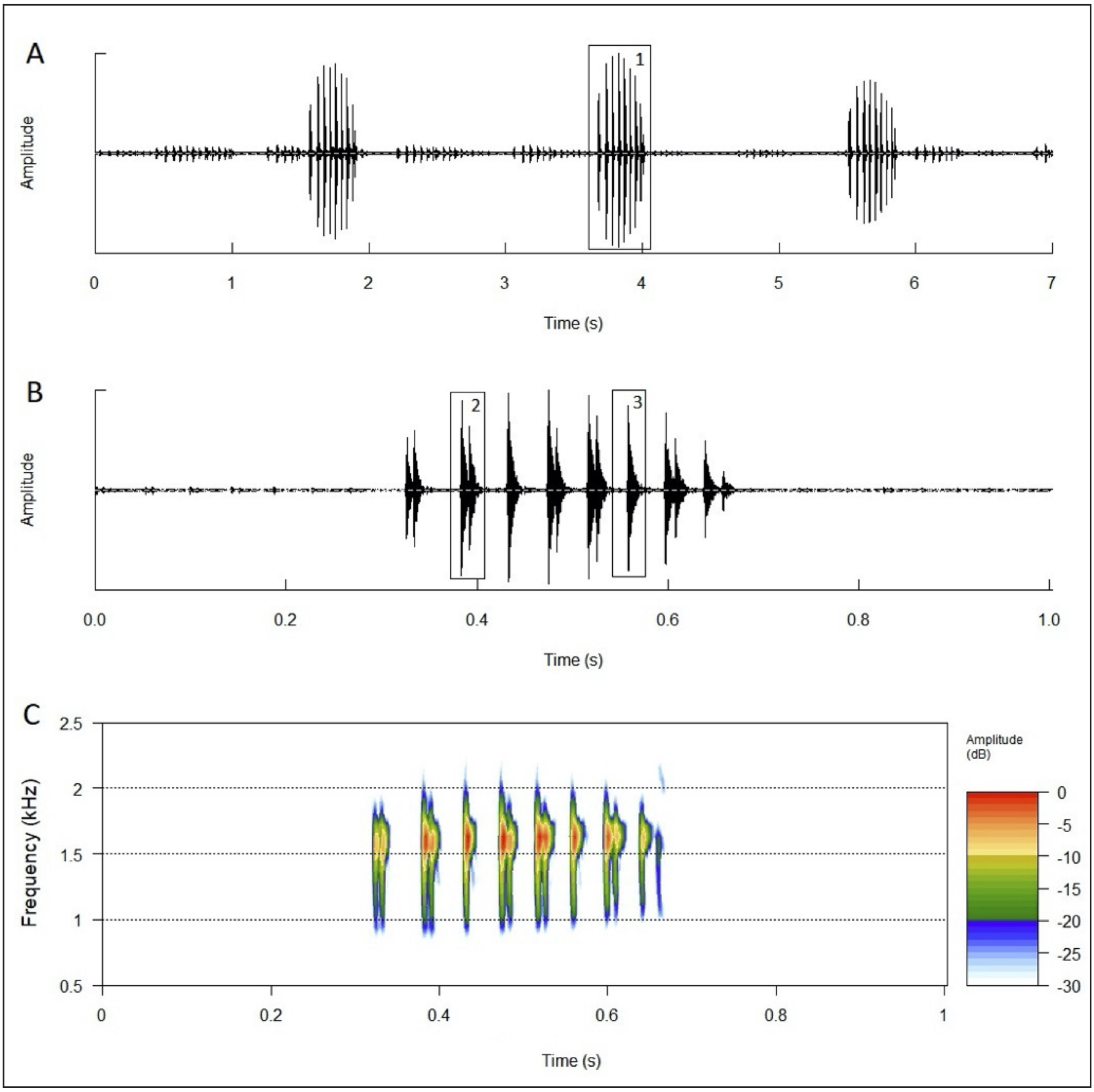

Figure 1. Advertisement call of *Rhinella scitula* (ZUFMS-AMP 03744). A: oscillogram of multiple calls (1 = delimitation of one call); B: oscillogram of single call highlighted at figure 1A (2 = pulse group; 3 = single pulse); C: spectrogram of single call.

composed by two types of notes. The first one, a more frequent emitted, is characterized by a pulsed structure with a dominant frequency of 1192.0 Hz ± 54.9 (1119.7–1292.0, n=32) and frequency bandwidth of 2097.8 Hz ± 485.5 (1205.9–3876.0, n=32). This note has a duration of 0.025s ± 0.014 (0.006–0.078, n=120) and has 2.9 ± 1.6 (1–8, n=120) pulses. The pulses present a duration of 0.006s ± 0.002 (0.002–0.021, n=120), emitted in a pulse-rate of 122.7 pulses/s ± 29.9 (41.67–230.77, n=120) separates by silent intervals. The second note type emitted in the release call present a non-pulsed structured (harmonics) and has a dominant frequency of 1033.6 Hz ± 258.4 (947.5–1722.7, n=9) and frequency bandwidth of 4038.7 Hz ± 4053.8 (1033.6–11369.5, n=9). Most of the dominant frequency are in the fundamental harmonic and this note is composed by 16.7 harmonics (±4.8, 5–22, n=9) with a duration of 0.017 ± 0.005 (0.009–0.032, n=120). When both notes are present in the same call, the second note is always the last one, and they can also be emitted alone (call formed by only one these notes types). The inter-note interval is 0.022s ± 0.012 (0.003–0.068, n=120).

Table 1. Acoustic parameters for *R.* gr. *margaritifera* species advertisement call. Values are presented as mean ± standard deviation (range).

Species	Call duration (s)	Note duration (s)	Pulse duration (s)	Pulse group per call	Pulses per pulse group	Number of pulses per call	Pulse rate (pulse/s)	Inter-note interval (s)	Dominant frequency (Hz)	Frequency bandwidth (Hz)	Reference
R. dapsilis	0.12±0.01 (0.08–0.14)	-	-	-	-	-	89.68±5.3 (79.88–97.02)	-	-	1560–3360	Zimmerman and Bogart, 1988
R. gr. *margaritifera* (Bolivia population)	0.238±0.02 (0.197–0.291)	-	0.005 (0.001–0.009)	6.3±0.6 (5–7)	2.0±0.5 (1–4)	-	102.1±17.4 (52.2–149.2)	-	1332.3±107 (1211.5–1544.7)	-	De La Riva *et al.*, 1996
R. castaneotica	0.2±0.03 (0.18–0.27)	0.01±0.004 (0.005–0.012)	-	-	1–4	9.1±1.1 (7–12)	-	-	1650	900–2600	Köhler and Lötters, 1999
R. martyi	0.3±0.01	-	0.01±0.001	6±0	4.75±0.96 2 (last pulse group)	-	-	0.026±0.004	1169±0.04	-	Fouquet *et al.*, 2007b
R. lescurei	0.03	-	0.004±0.0002	480/min	4.83±0.79	-	-	0.097±0.018	1161±0.015	-	Fouquet *et al.*, 2007b
R. gr. *margaritifera* (French Guiana population)	0.29±0.05	-	0.008±0.0007	6.75±0.957	3.25±0.5 2 (last pulse group)	-	-	0.026±0.007	1265±0.035	-	Fouquet *et al.*, 2007b
R. magnussoni	9.9±4.5 (3.3–23.1)	0.06±0.01 (0.02–0.09)	-	-	-	-	-	0.12±0.02 (0.07–0.26)	2260 (1890–2550)	-	Lima *et al.*, 2007
R. ocellata	0.28±0.01 (0.23–0.31)	-	0.017	-	-	11.4±1 (7–16)	27.9±0.9	-	1352.9±54.7 (1185–1501)	-	Caldwell and Shepard, 2007
R. paraguayensis	0.3±0.04 (0.20–0.42)	-	-	-	-	6.5±0.65 (5–8)	-	0.02±0.01 (0.01–0.04)	1438.7±70.5 (1113.7–1568.5)	-	Ávila *et al.*, 2010
R. hoogmoedi	0.23±0.02 (0.2–0.27)	0.028±0.01 (0.01–0.05)	-	5.44±0.54 (5–7)	2.4±1.15 (1–6)	13.12±1.154 (11–16)	26.47±3.52 (12.6–33.3)	0.015±0.008 (0.007–0.063)	1343.4±42.55 (1292–1378)	495.3–4474.2	Roberto *et al.*, 2012
R. scitula	0.27±0.03 (0.23–0.36)	0.015±0.004 (0.007–0.040)	0.008±0.001 (0.005–0.015)	6.6±0.92 (5–8)	1.89±0.53 (1–5)	12.7±2.1 (9–17)	46.4±8.1 (27.9–65.2)	0.027±0.007 (0.013–0.041)	1439.7±46.1 (1378.1–1550.4)	630.6±142.7 (430.7–947.5)	Present study
R. yunga	-	-	2–2.5	-	-	10±1.2 (6–13)	-	-	863 (689–947)	-	Stynoski *et al.*, 2020

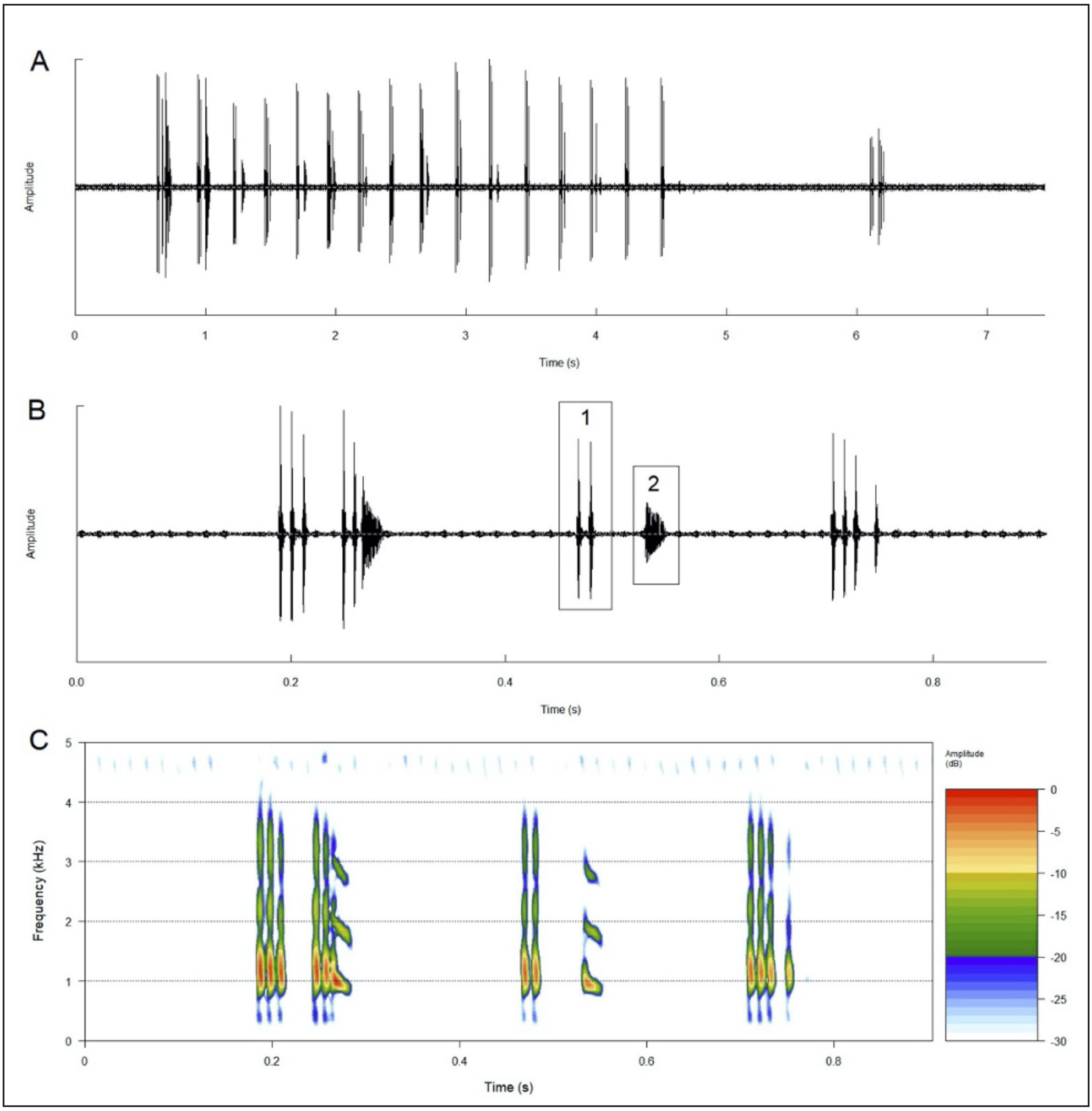

Figure 2. Release call of *Rhinella scitula* (ZUFMS-AMP 11139). A: oscillogram of a sequence of release calls; B: oscillogram of few release calls showing the different note types (1 = pulsed note; 2 = non-pulsed note); C: spectrogram of figure 2B.

Discussion

The advertisement call parameters and structure of *Rhinella scitula* follow the general pattern of *R. margaritifera* species group calls (except *R. magnussoni*), which composed of short pulsed notes, where pulses appear alone or in groups. *Rhinella scitula* values overlap with most of the species, except *R. magnussoni* (9.9 ± 4.5, 3.3–23.1), which has a longer call duration, and *R. dapsilis* (0.12 ± 0.01, 0.08–0.14), which is shorter. For *R. lescurei*, the duration of pulse group (mean = 0.003) was informed instead of call duration. Although there are differences in mean values, note duration overlaps between *R. scitula* and *R. castaneotica* (0.01 ± 0.004, 0.005–0.012), *R. magnussoni* (0.06 ± 0.01, 0.02–0.09), and *R. hoogmoedi* (0.028 ± 0.01, 0.01–0.05). Pulse duration is similar between *R. scitula* and *R. ocellata* (0.017 ± 0), *R.* sp. from French Guiana (0.008 ± 0.0007) and *R. martyi* (0.01 ± 0.001), and lower values are found in *R. lescurei* (0.004 ± 0.0002) and *R.* sp. from Bolivia (mean=0.005, 0.001–0.009), although the range values of the last species overlap with *R. scitula*. The pulse group per call values are similar or overlap between *R. scitula*, *R. hoogmoedi* (5.44 ± 0.54; 5–7,), *R.* sp. from French Guiana (6.75 ± 0.957) and *R. martyi* (6 ± 0) and *R.* sp. from Bolivia (6.3 ± 0.6, 5–7), while for *R. lescurei* only the pulse group per minutes (480) was informed due to the different call type. Pronounced overlap also occurs in pulses per pulse group between *R. scitula* and *R. hoogmoedi* (2.4 ± 1.5; 1–6,), *R.* sp. from French Guiana (2 ± 0), *R. martyi* (2 ± 0), *R. castaneotica* (1–4) and *R.* sp. from Bolivia (2 ± 0.5, 1–4), while for *R. lescurei* the values are higher (4.83 ± 0.79). The number of pulses did not overlap only between *R. scitula* and *R. paraguayensis* (6.5 ± 0.65, 5–8), which was lower. Overlap occurs between *R. scitula* and *R. hoogmoedi* (13.12 ± 1.154, 11–16), *R. ocellata* (11.4 ± 1, 7–16), and *R. castaneotica* (9.1 ± 1.1, 7–12). Pulse rate overlaps between *R. scitula*, *R. ocellata* (27.9 ± 0.9), and *R. hoogmoedi* (26.47 ± 3.52, 12.6–33.3), although the mean value was higher in *R. scitula*. Even higher values are found in *R.* sp. from Bolivia (102.1 ± 17.4, 52.2–149.2) and *R. dapsilis* (89.68 ± 5.3, 79.88–97.02). Inter-note interval overlaps between *R. scitula* and *R. hoogmoedi* (0.015 ± 0.008, 0.007–0.063), *R. paraguayensis* (0.02 ± 0.01, 0.01–0.04), *R.* sp. from French Guiana (0.026 ± 0.007) and *R. martyi* (0.026 ± 0.004), while it is higher in *R. lescurei* (0.097 ± 0.018) and *R. magnussoni* (0.12 ± 0.02, 0.07–0.26). Dominant frequency overlaps between *R. scitula* and *R. hoogmoedi* (1343.4 ± 42.55, 1292–1378), *R. paraguayensis* (1438.7 ± 70.5, 1113.7–1568.5), *R. ocellata* (1352.9 ± 54.7, 1185–1501), *R. castaneotica* (mean=1650, 900–2600) and *R.* sp. from Bolivia (1332.3 ± 107, 1211.5–1544.7). Lower dominant frequency is found in *R.* sp. from French Guiana (1265 ± 0.035), *R. lescurei* (1161 ± 0.015), and *R martyi* (1169 ± 0.04), while higher values are found in *R. magnussoni* (mean=2260, 1890–2550). Frequency bandwidth also overlaps between the species analyzed (table 1).

We found overlap between *R. scitula* and other species from the *R. margaritifera* species group in most of the acoustic parameters measured, indicating that they are (in general) not very useful as species diagnostic characteristic. Furthermore, most of the call description lack important parameters (e.g. pulse group per call and pulses per pulse group) for correctly describe *R. margaritifera* species group call. We recommend that future studies on bioacoustics in this species group consider greater number of parameters based on recent publications (Roberto *et al.*, 2012; Köhler *et al.*, 2017) and describe more release calls within the group in order to identify which ones can be used in species diagnosis (Grenat and Martino, 2013).

From all *R. margaritifera* species group with described advertisement calls, the most different to *R. scitula* and other species in the group was *R. magnussoni*, which has a structurally distinct call (simple pulse notes with no amplitude modulation; Lima *et al.*, 2007); it has much longer call duration, longer inter-note interval, and higher dominant frequency. The high similarity in advertisement call parameters and morphology between most of the *R. margaritifera* species group species reinforces the need of integrative taxonomy to understand the systematics of complex groups correctly. Descriptions of release calls from other species are needed, once they can also be used as diagnostic characters between cryptic species in anuran (Grenat and Martino, 2013).

There are only three release call described for species within the *R. margaritifera* species group (*R. dapsilis*, *R. ocellata* and *R. yunga*) (Zimmerman and Bogart, 1988; Caldwell and Shepard, 2007; Stynoski *et al.*, 2020). However, Roberto *et al.* (2011) informed that *R. hoogmoedi* emitted release calls when manipulated, and this seemed to trigger other males to start calling. We did not record such behavior for *R. scitula*. Both species within *R. margaritifera* group with release call described have pulsed call (Zimmerman and Bogart, 1988; Guerra *et al.*, 2020), however, *R. scitula* present both pulsed and non-pulsed structures, showing a more complex release call. Pulsed and non-pulsed notes or calls (depending on the approach used for description) also occur in release calls from *R. granulosa*, *R. marina* and *R. spinulosa* species groups (Guerra *et al.*, 2011; Vieira *et al.*, 2014). Comparing the call within the *R. margaritifera* group, the release call of *R. scitula* distinguished from the call of *R. dapsilis* by having a lower dominant frequency (4220–5250 Hz in *R. dapsilis*) and shorter duration (0.07–0.11 s in *R. dapsilis*). From the release call of *R. ocellata*, it distinguishes by the shorter duration (0.086–1.105s in *R. ocellata*). Lastly, from the release call of *R. yunga*, distinguishes by the shorter pulse duration (2–2.5 s in *R. yunga*) and higher dominant frequency (689–947 Hz in *R. yunga*). For a comparison among described release calls of the *Rhinella* species, Guerra *et al.* (2010) summarized data of all described release calls of genus.

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Trabajo

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¿Es importante el color de los machos de lagarto de Achala (*Pristidactylus achalensis*) para la elección de pareja?

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RESUMEN

La elección de pareja es un aspecto importante de la selección sexual, promoviendo el desarrollo de caracteres sexuales secundarios en los individuos del sexo opuesto, conocidos como ornamentos. El color puede ser entendido como una señal visual ya que puede transmitir información sobre diferentes aspectos de los individuos, como la condición reproductiva. Aun así, para ser considerado importante como señal para la elección de pareja requiere presentar además variabilidad entre los individuos. El objetivo de este trabajo fue evaluar la función de la coloración en la comunicación intersexual del lagarto de Achala, *Pristidactylus achalensis*. Para ello, se examinó la variabilidad de la coloración de machos en dos regiones del cuerpo consideradas dicromáticas, el flanco y el dorso; bajo el sistema de visión de lagartos, y se evaluó la elección de la hembra por la coloración de los machos, utilizando modelos de látex de distintas coloraciones. Los resultados mostraron una gran variabilidad en la coloración de los machos. Además, ambas regiones podrían ser distinguibles por los conespecíficos. Estos resultados están en concordancia con estudios que destacan a la región del flanco como importante para la comunicación entre conespecíficos, mientras que el dorso tendría una mayor importancia en relaciones interespecíficas como la depredación. Respecto a la elección de pareja, no se observó un patrón claro de elección de las hembras por la coloración de los machos, destacando una amplia variabilidad en la elección. La coloración es un rasgo complejo, que varía entre sexos e individuos de *Pristidactylus achalensis* y que puede participar en numerosas funciones además de la elección de pareja.

Palabras claves: Caracteres Sexuales; Comunicacion Intersexual; Elección Sexual; Variabilidad de Coloración.

Introducción

La elección de pareja es un aspecto importante de la selección sexual, promoviendo el desarrollo de caracteres sexuales secundarios en los individuos del sexo opuesto, conocidos como ornamentos (Andersson, 1994; Berglund *et al.*, 1996; Kokko *et al.*, 2003; Naretto *et al.*, 2014). La preferencia de la hembra por determinados ornamentos de los machos ha sido observada en numerosos taxones y en diversos caracteres fenotípicos (Tokarz, 1995; Basolo, 1996; Stuart-Fox y Ord, 2004; Bajer *et al.*, 2010; Calbacho-Rosa *et al.*, 2019). Al elegir una pareja, un individuo utiliza características físicas y/o comportamentales de las posibles parejas como predictores del desempeño del individuo o de sus recursos asociados (Halliday, 1983; Reynolds, 1990; Kokko *et al.*, 2003). Así, el individuo podría obtener diferentes tipos de beneficios como un mayor potencial reproductivo, mejor calidad de territorio, mayor cuidado parental, protección contra depredadores o calidad genética que proporcione variabilidad a la descendencia, lo que aumentaría su aptitud (Halliday, 1983; Kokko *et al.*, 2003; Siefferman y Hill, 2003).

Uno de los rasgos en lagartos que puede influir en la elección de pareja es la coloración de los individuos. Esto se debe a que el color es capaz de transmitir información sobre aspectos de los individuos, participando en la comunicación intraespecífica (Stuart-Fox *et al.*, 2006; Bradbury y Vehrencamp, 2011; Halloy *et al.*, 2013). Además, la coloración participa en otros procesos ecológicos

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y fisiológicos, como la termorregulación (Clusella-Trullas *et al.*, 2009) o la depredación (Stuart-Fox y Moussalli, 2008). Estos procesos pueden afectar directa o indirectamente la expresión de los caracteres sexuales, al establecer un compromiso entre reproducción y supervivencia. Por ejemplo, los costos y beneficios de la coloración pueden variar según el nivel de competencia o el riesgo de depredación. En poblaciones naturales de animales, la variación del color se encuentra ampliamente extendida (Sinervo y Lively, 1996; Paterson y Blouin-Demers, 2017), desde un polimorfismo discreto (incluso entre sexos) hasta una variación continua. Esta variabilidad plantea potencialidades para la elección de pareja, por ejemplo, machos con coloraciones más conspicuas podrían obtener ventajas de ser seleccionados como pareja y consecuentemente experimentar un mayor éxito reproductivo (Stuart-Fox y Ord, 2004; Bajer *et al.*, 2010; Lisboa *et al.*, 2017). La coloración también puede variar dentro de un mismo individuo. Distintas partes del cuerpo pueden estar sujetas a diferentes presiones selectivas (Abalos *et al.*, 2016). Por ejemplo, la coloración de regiones que principalmente se muestran a la vista de los conespecíficos estarían sujetas a mayores intensidades de selección sexual que aquellas regiones que están a la vista de depredadores como la región dorsal (Stuart-Fox *et al.*, 2003; Stuart-Fox y Ord, 2004). Por lo tanto, la distribución de las señales cromáticas en diferentes regiones del cuerpo es un factor importante a contemplar en el estudio de elección de pareja (Stuart-Fox *et al.,* 2006).

La percepción de la coloración depende del sistema de visión de los organismos. Por lo tanto, los estudios de elección de pareja deben ser abordados desde una perspectiva que tenga en cuenta la manera en que las especies perciben el color (Scholtyßek y Kelber, 2017; Maia *et al.*, 2019). Se ha observado en algunas especies de lagartos que poseen fotorreceptores sensibles al rango ultravioleta (UV) del espectro electromagnético (Fleishman *et al.*, 1993; Pérez i de Lanuza *et al.*, 2018); y siendo el sistema de visión filogenéticamente conservado entre lagartos diurnos (Fleishman *et al.*, 2011) se puede predecir que los lagartos pueden utilizar la coloración UV como señal. Por ejemplo, machos de *Lacerta agilis* presentan una coloración oscura, con parches verde brillante y reflectancia ultravioleta (UV) en áreas del vientre y flanco. Olsson *et al.* (2011) utilizando bloqueadores de UV durante la temporada de apareamiento sobre estas zonas, determinaron que aquellos machos sin los bloqueadores obtuvieron más parejas que los que presentaban bloqueadores. Actualmente se han desarrollado novedosas herramientas para mejorar la interpretación de las diferencias o discriminación entre colores considerando el sistema de visión de las especies (Troscianko y Stevens, 2015; Maia *et al.,* 2019). Por lo tanto, no solo la coloración sino la ubicación de los colores y la percepción de los mismos son factores que podrían influir en la elección de pareja.

Pristidactylus achalensis (Gallardo, 1964) es un lagarto de interés según el marco teórico abordado, ya que se caracteriza por un marcado dicromatismo sexual principalmente en el área dorsal y el flanco (Naretto y Chiaraviglio, 2020). Los machos presentan una coloración verde intensa con flancos celestes, mientras que las hembras presentan tonalidades de color marrón claro con flancos blancuzcos (Fig. 1). Además, los machos presentan mayor intensidad de coloración durante el período que poseen disponibilidad de esperma (octubre a diciembre) (Naretto y Chiaraviglio, 2020; Blengini *et al.,* en prensa). La época reproductiva de esta especie es un período relativamente acotado en los cuales la intensidad de competencia por apareamientos es alta (Naretto y Chiaraviglio, 2020). Considerando estas características de coloración en los machos, la hipótesis de nuestro trabajo postula que la variabilidad en la coloración de los machos de *Pristidactylus achalensis* promueve la elección de pareja por la hembra. Para ello, se examinó la variabilidad de la coloración de machos, considerando el sistema de visión de lagartos, y se evaluó la elección de la hembra por la coloración de los machos, utilizando modelos experimentales de látex de diferentes coloraciones.

Materiales y métodos

Especie y área de estudio

El lagarto de Achala (*Pristidactylus achalensis*) es un lagarto diurno, que habita en roquedales aislados por encima de los 1800 msnm y hasta el punto más alto (3000 msnm), en las Sierras Grandes de Córdoba (Etheridge y Williams, 1985; Minoli y Avila, 2017), siendo endémico de esta región. Los individuos son territoriales y manifiestan comportamientos agresivos (Torres *et al.*, 2019; Naretto y Chiaraviglio, 2020). Relacionado a esto se ha observado cambio de coloración hacia coloraciones más oscuras frente a encuentros agonísticos (S. Naretto, datos no publicados). Esto fue tenido en cuenta a la hora de

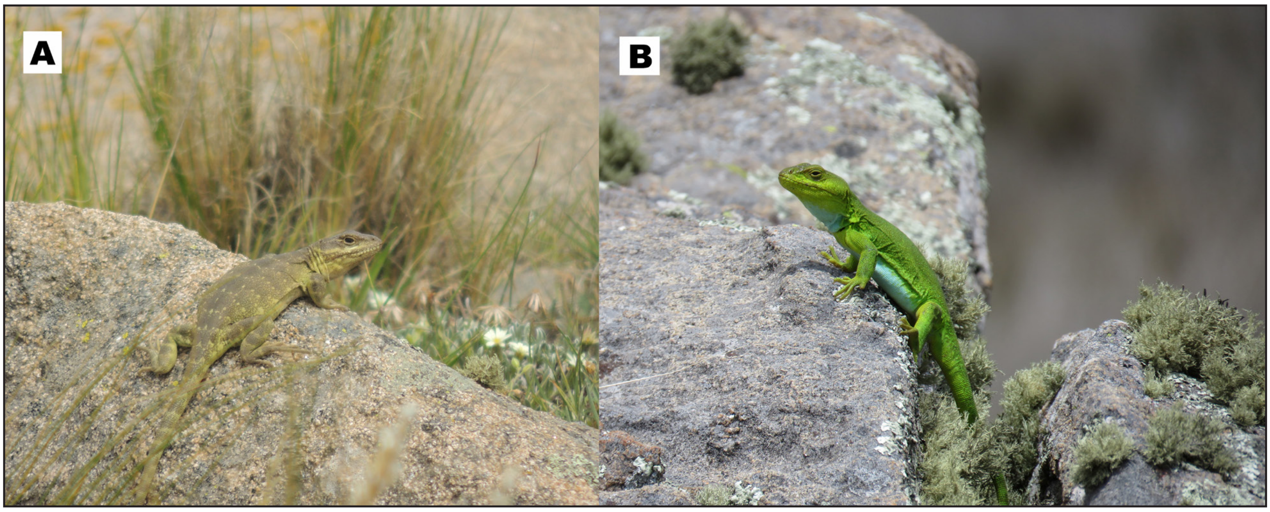

Figura 1. Ejemplares del lagarto de Achala *Pristidactylus achalensis*. A. Hembra de *P. achalensis.* B. Macho de *P. achalensis*.

plantear el rango de coloración con el que se pintaron los modelos utilizados. Los lagartos tienen actividad estacional, están activos durante los meses cálidos y húmedos (octubre-abril) e hibernan durante la época fría y seca (mayo- septiembre).

Se capturaron con lazo individuos adultos (25 machos de tamaños mayores a 92.5 mm LHC y 17 hembras de tamaños mayores a 90 mm LHC, el sexo es reconocido por su coloración) de poblaciones de los alrededores del paraje La Posta (31°36' S, 64°52' O) en Pampa de Achala, provincia de Córdoba, durante cinco semanas (10 de octubre al 11 de noviembre) del año 2018, dentro del período donde los individuos se encuentran reproductivamente activos (Blengini *et al., en prensa*). Una vez capturados, los individuos fueron colocados individualmente en bolsas de tela para su traslado al laboratorio de campo, en inmediaciones del sitio de captura (menos de 5 km) y fueron alojados en cajas plásticas individuales (30cm X 40cm X 40cm). Estos habitáculos contaban con piedras para refugio y agua *ad libitum* y se alimentaron a los lagartos con larvas de tenebrio (*Tenebrio* sp.) y flores de *Taraxacum officinale* (L. Weber, 1780). Los ejemplares se mantuvieron hasta 5 días bajo condiciones semi controladas en su ambiente natural, cerca de la zona de captura, para reducir el estrés por el traslado y mantener las mismas condiciones climáticas y de luz que las poblaciones naturales. Al finalizar las experiencias, los lagartos fueron fotografiados para su identificación (basada en la mancha humeral) y evitar recapturas, y posteriormente fueron liberados en el sitio donde habían sido capturados (registrado con GPS Garmin e-trex).

Variabilidad de la coloración de machos

La coloración fue evaluada siguiendo la metodología propuesta por Troscianko y Stevens (2015), en el rango del espectro visible y el UV. Se tomaron fotografías digitales a campo sin flash durante días despejados inmediatamente después de la captura de los individuos, desde una perspectiva ventral (ya que se puede apreciar el área del flanco) y dorsal de los machos. Se utilizó una cámara fotográfica Nikon D3400 con un lente Nikon de 50 mm con sensor modificado montada sobre un trípode a 64 cm de altura. Se tomaron fotografías con dos filtros distintos: un filtro que bloquea UV (B+W; transmite entre 400 y 700nm) y otro filtro que solo permite el paso de UV (UVROptics, transmite entre 300 y 400nm). Usamos un dispositivo personalizado a fin de intercambiar rápidamente los filtros y mantener la iluminación y posición del ejemplar en idénticas condiciones. Las fotografías fueron tomadas en formato RAW para prevenir modificación de la información por ajustes automáticos de la cámara (Stevens *et al.*, 2007). En todas las fotografías fueron incluidos 3 estándares, uno de 24 colores (Colorchecker Passport, mini X-Rite Inc., Gran Rapids, MI), un estándar de reflectancia blanco (Ocean Optics, Dunedin, Estados Unidos) y un estándar negro, que reflejan la luz al 87% y 8%, respectivamente, entre 300nm y 700nm. Los parámetros de sensibilidad y apertura de diafragma fueron constantes (ISO=200 y F=22) y se modificó la velocidad del obturador de acuerdo a la iluminación con el objetivo de obtener una correcta exposición, evitando imágenes sobresaturadas. Utilizamos el plugin "Image Calibration and Analysis toolbox" del software ImageJ (1.52a) para combinar las fotografías visibles y UV en una única imagen multiespectral. Además, obtuvimos las curvas de reflectancia de cada color del estándar mediante espectrofotometría, para conocer la sensibilidad espectral de nuestra cámara a través del mismo plugin. Con los datos obtenidos, aplicamos un modelo de visión de los lagartos a la imagen multiespectral. Mediante la función *cone mapping process* del mismo plugin se combinaron las sensibilidades de la cámara con las del modelo de visión de lagarto. Este proceso arroja valores de captura de conos para cada uno de los canales del modelo de visión: ultravioleta (UV), ondas cortas (SW), ondas medias (MW) y ondas largas (LW). Los valores de captura de conos representan la sensibilidad de los conos del ojo frente a las diferentes longitudes de onda que emite un estímulo, representando cómo ve el modelo de estudio y permitiéndonos hacer una correcta interpretación de los resultados. En lagartos, la visión tetracromática es filogenéticamente conservada (Fleishman *et al.*, 2011) por esto, y ante la ausencia de las sensibilidades espectrales de *P. achalensis*, utilizamos los datos espectrales de *Anolis sagrei* (Loew *et al.,* 2002). Otro de los parámetros requeridos para el modelado es el tipo de iluminación, por lo que se utilizó la de un cielo despejado (“bluesky”) provista por el paquete para R PAVO (2.0) (Maia *et al.,* 2019). Para obtener los valores de captura de conos de las dos regiones del cuerpo (el dorso y el flanco), se seleccionaron regiones de interés (ROI) con ImageJ excluyendo zonas con sombras de la imagen. Para explorar la variabilidad de color en los machos se graficaron los valores de captura de conos en un espacio de color tetraédrico para cada región, utilizando la función *colspace* del paquete *PAVO* del software R v.3.6.2 (R Core Team, 2018).

A partir de los valores de captura de conos se calcularon las distancias cromáticas y acromáticas en unidades de distancias perceptibles (*JND*- del inglés “Just Noticiable Differences”, Vorobyev y Osorio, 1998) usando la función *coldist* del paquete *PAVO* para R v.3.6.2 (Maia *et al.*, 2019). Las distancias “JND” son valores umbrales que determinan dos estímulos como diferentes. Las distancias cromáticas permiten medir cuan similares son dos colores teniendo en cuenta el tono y la saturación de los mismos, mientras que las distancias acromáticas analizan similitudes en la luminosidad o brillo de dos colores. Se establece que distancias perceptibles menores a 1 indican que dos colores no son discriminables para el sistema de visión en particular, valores entre 1 y 3 son considerados difíciles de discriminar excepto bajo condiciones óptimas de luz (Siddiqi *et al.*, 2004; Marshall y Stevens, 2014) y valores mayores a 3 son discriminables. Uno de los parámetros requeridos para calcular los JND es la fracción de Weber y se utilizó un valor de 0,1 según Fleishman (2011). Para evaluar si la coloración es variable en cada una de las áreas se compararon los JND entre los machos a fin de interpretar si un lagarto puede percibir diferencias de colores entre dorsos y diferencias de color entre flancos de distintos machos. Se graficaron histogramas de frecuencia relativa de los valores de JND cromáticos y acromáticos para cada una de las regiones y se calculó la proporción de valores de JND mayores a 1 y la proporción de valores de JND mayores a 3.

Para evaluar si el flanco de los animales es percibido como un color distinto a la coloración dorsal, se obtuvo para cada individuo el valor de JND entre dichas regiones. Se graficaron histogramas de frecuencia relativa de los valores de JND cromáticos y acromáticos entre las regiones.

Elección de pareja de la hembra

Se realizaron experiencias de elección dicotómica de pareja utilizando modelos de machos realizados con látex. La arena de experimentación consistió en un espacio (75cm x 61,5cm x 35cm) dividido en tres compartimientos según se muestra en la Fig. 2-A. En los dos compartimentos más pequeños (Fig. 2-A, Estímulo 1 y Estímulo 2) se colocaron modelos de lagartos de látex con distintas coloraciones dentro del rango de color de los machos. Los compartimentos estaban separados de la hembra mediante un acrílico transparente, impidiendo que la hembra entre en contacto con los modelos. El compartimento central se diferenció en 3 áreas para el análisis de los datos: un área de Elección del Estímulo 1, un área de Elección del Estímulo 2 y otra de No Elección. Las experiencias se realizaron al aire libre permitiendo la iluminación del sol durante los horarios de 10:00 am hs a 16:00 pm hs maximizando la temperatura e iluminación natural. Las experiencias se realizaron durante días soleados y la orientación de la arena de experimentación fue rotada durante el transcurso del día de modo de permitir la incidencia de la luz solar desde el mismo ángulo. De esta manera se priorizó la iluminación lo más homogénea posible entre las experiencias. Se tomaron las temperaturas de las hembras al final de la experiencia, las cuales se encontraron en un rango de 25.4ºC a 29.4ºC

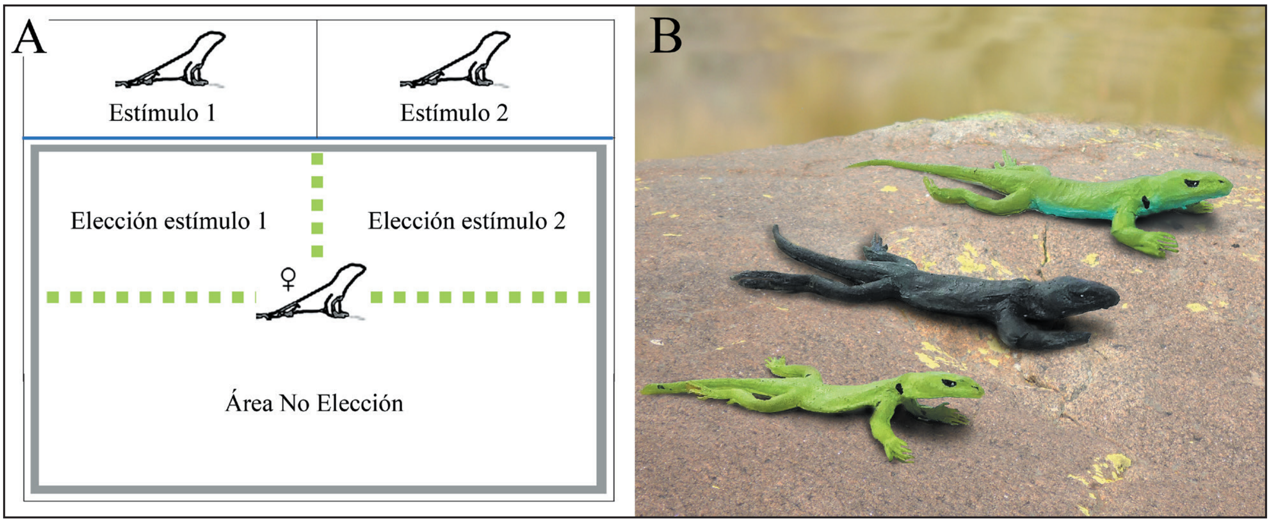

Figura 2. A. Habitáculo empleado para la elección de pareja: se identifican dos compartimentos donde se colocaron los modelos de látex (Estímulo 1 y Estímulo 2) y las tres áreas determinadas en el análisis (dos áreas de elección y un área de no elección). B. Fotografía de los tres modelos de látex utilizados.

concordante con las temperaturas corporales de los ejemplares cuando son capturados.

Se utilizaron modelos de látex con el objetivo de controlar variables morfológicas siendo todos del mismo tamaño y forma. Se manipuló el color de los modelos de manera artificial mediante pintura acrílica. Se pintaron asegurando que la coloración se encuentre dentro del rango observado en machos, consiguiéndose 3 modelos diferentes: uno verde oscuro, uno verde claro y otro, verde claro con flancos azules (Fig. 2-B). Los modelos fueron presentados sobre piedras similares, para descartar el efecto del tamaño de la piedra para la elección.

Se establecieron cuatro tratamientos experimentales:

Elección. Se presentaron al azar un modelo de color verde claro y otro verde oscuro como estímulos a la hembra.

Azul. Se presentaron un modelo de color verde claro con flancos azules y un modelo verde claro sin los flancos azules como estímulos a la hembra.

Vivo. Se presentó un lagarto macho vivo en uno de los compartimentos como estímulo a la hembra.

Control. No se presentó ningún estímulo a la hembra.

Dado que por cuestiones logísticas no se pudo realizar en cada hembra la totalidad de los tratamientos, en aquellos casos en los que se realizó el Control-, siempre fue el primero de los tratamientos en realizarse. Por otra parte, a cada hembra se asignó 2 de los tratamientos restantes, y el orden de los mismos al azar. De esta forma se evaluó la preferencia de cada hembra en 3 ocasiones durante los días de experimentación.

Cada experiencia tuvo una duración de 40 minutos. Las hembras alojadas en sus habitáculos individuales fueron colocadas directamente en la arena de experimentación y durante los primeros 10 minutos se les otorgó un periodo de aclimatación mientras se impidió la visualización de los compartimentos mediante una barrera opaca. Al minuto 10 se removió la barrera y durante los 30 minutos siguientes, las hembras se encontraron frente a los diferentes estímulos de elección. Las experiencias fueron registradas utilizando una videocámara Sony HDR CX230 colocada en un soporte a 2 metros de altura. Se cuantificó el tiempo de permanencia en cada área (áreas de elección y área de no elección) y se registró el área de elección donde la hembra ingresó primero, utilizando el software Solomon Coder (17.03.22) (Péter, 2011). La manipulación de los ejemplares fue realizada por una sola persona y minimizando ruidos, la filmación de la experiencia se realizó de manera remota, precauciones para disminuir los factores de estrés. Las hembras tuvieron un tiempo entre experiencias de al menos 20 horas, a fin de minimizar el tiempo total de cautividad.

Se tomaron en cuenta dos criterios para determinar elección de pareja por la hembra. Para el primer criterio se contempló el tiempo de permanencia de la hembra en cada área de elección. Se determinó que la hembra selecciona el estímulo cuando el tiempo de permanencia frente a él, supera el 50% del tiempo total en la arena. Para el segundo criterio se tuvo en cuenta el estímulo del área de elección donde la hembra ingresó por primera vez. Para este último criterio, se descartaron para el análisis, dos hembras que no se encontraban en el área de no elección luego del período de aclimatación, una perteneciente al tratamiento Control - y otra perteneciente al tratamiento Azul.

Por motivos logísticos no todas las hembras realizaron la totalidad de los tratamientos. De las 17 hembras capturadas se obtuvieron datos de los siguientes tratamientos: 11 control-, 8 de elección, 7 azul y 5 vivo. Se eliminaron 2 experiencias del tratamiento control- porque las hembras no realizaron ningún comportamiento ni desplazamiento.

Para visualizar la elección de las áreas por parte de la hembra, según el primer criterio se graficó un histograma de frecuencias de tiempo de permanencia en cada área por individuo. También se calculó la proporción de hembras que eligieron cada estímulo. Se realizó un test de chi-cuadrado para ver la existencia de algún patrón a nivel poblacional para los tratamientos elección y azul. También se realizó un test de chi-cuadrado para comparar los tiempos de permanencia en el área de no elección y las áreas de elección tomando a ambas áreas como una única área, a fin de poder comparar entre los tratamientos. En relación al segundo criterio se realizó un gráfico de barras con el porcentaje de individuos que ingresaron primero a cada una de las áreas de elección de los diferentes tratamientos. Se incluye también la proporción de hembras que se desplazaron solo en el área de no elección.

Resultados

Variabilidad de la coloración de machos

En los espacios de color tetraédricos para cada una de las regiones del cuerpo analizadas (flanco y dorso), se destaca la baja expresión de coloración UV (los puntos se encuentran por debajo del centro del tetraedro) y existe una tendencia hacia una mayor expresión en los canales de longitudes de ondas más largas (MW y LW) (Fig. 3).

La variabilidad de colores entre machos para cada región se observa en el histograma de frecuencias de los JND (Fig. 4). Los datos de la media y la mediana para ambas áreas se observan en la Tabla 1. Tanto en el dorso como en el flanco existe una gran proporción de valores mayores a 1, manifestando que son regiones variables (Tabla 1). A su vez, se destaca que la región del flanco a nivel acromático presenta mayor porcentaje de valores mayores a 3 (Tabla 1) lo cual indica mayores diferencias entre individuos en dicha característica.

Además, se destaca que la coloración dorsal y la del flanco son discriminables entre sí, lo que se manifiesta en valores de JND con un sesgo hacia JND más altos que el valor 3 (Fig. 5). La media para el nivel cromático es de 6,44 ± 2,39 con una mediana de 6,42 y con el 92,50% de JND mayores a 3. Para el aspecto acromático la media es de 9,27 ± 3,25 con una mediana de 9,45 y con 96,52% de JND mayores a 3.

Elección de la hembra por la coloración del macho

La Fig. 6 muestra el tiempo de permanencia de cada hembra experimental en las diferentes áreas, dependiendo del tratamiento. En el tratamiento elección, se observa una gran variabilidad en la respuesta de elección, destacándose que el 75% de las hembras eligieron (ya que estuvieron más del 50% en una de las áreas de elección) y el 25 % restante se movió principalmente solo en el área de no elección. Esta variabilidad en la elección se encuentra también soportada por el test de chi cuadrado donde no se observan diferencias en las frecuencias relativas de permanencia entre áreas ($FR_{Claro} = 0{,}29 \pm 0{,}34$, $FR_{Oscuro} = 0{,}31 \pm 0{,}29$, $FR_{NoElección} = 0{,}4 \pm 0{,}29$; $X^2=2{,}06$, gl=2 p=0,3570). En el tratamiento azul, si bien el 42% de las hembras eligieron, no se observó un patrón de

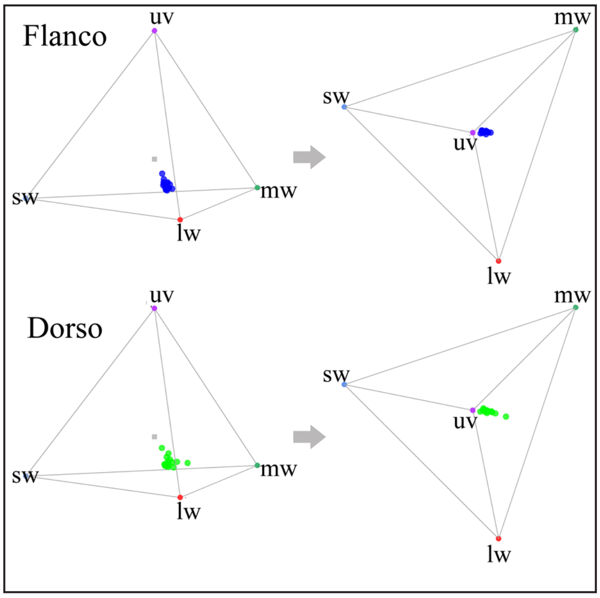

Figura 3. Espacio de color de las diferentes regiones del cuerpo analizadas (Flanco y Dorso). Cada punto del tetraedro está determinado por la estimulación relativa de los cuatro canales de color: uv (ultravioleta), sw (ondas cortas), mw (ondas medias) y lw (ondas largas).

Tabla 1. Valor Medio y Mediana para cada tipo de JND para cada una de las áreas analizadas. Además, se presenta la proporción de valores de JND mayores a 1 y 3.

Área	JND Tipo	Media	Mediana	Porcentaje >1	Porcentaje >3
Dorso	Cromático	2,65 ± 1,61	2,31	86,15%	37,23%
	Acromático	2,13 ± 1,43	1,82	74,03%	25,97%
Flanco	Cromático	2,84 ± 1,81	2,38	87,50%	38,97%
	Acromático	3,22 ± 2,33	2,74	82,35%	44,85%

elección por los flancos azules. La frecuencia del tiempo de permanencia fue mayor en el área de no elección ($FR_{VerdeconFlancosAzules} = 0{,}23 \pm 0{,}31$, $FR_{VerdesinFlancosAzules} = 0{,}19 \pm 0{,}37$, $FR_{NoEleccion} = 0{,}58 \pm 0{,}40$; $X^2 = 27{,}62$, gl=2, p <0,0001). En el tratamiento vivo, ninguna de las hembras eligió ya que las hembras permanecieron mayor tiempo en el área de no elección.

Con respecto al área de elección a la cual las hembras ingresaron primero (Fig. 7), se observa que para el control –, la proporción para ambas áreas es similar. En los restantes tratamientos se observa: una proporción más alta relacionada al modelo claro sobre el oscuro en el tratamiento elección; el modelo con flancos azules obtuvo una proporción mayor que el modelo sin los flancos azules (tratamiento azul); y finalmente, en el tratamiento vivo la proporción para el individuo vivo fue mayor con respecto al área sin estimulo, aunque también observamos una proporción similar de individuos que se desplazaron solo por el área de no elección.

Discusión y conclusiones

En el presente trabajo se busca comprender la función del color en la comunicación intersexual de *Pristidactylus achalensis*, es decir, cómo la coloración de machos puede influir en la elección de las hembras bajo el marco de la selección sexual.

Variabilidad de la coloración de machos

Se observó variabilidad de los colores en cada una de las regiones analizadas. Además, al comparar entre la región dorsal y la región del flanco, el 95% de los valores JND obtenidos fueron mayores a 3 y siguiendo criterios conservadores (Marshall y Stevens, 2014; Pérez i de Lanuza *et al.,* 2018) que manifiestan que con esas diferencias se podrían distinguir fácilmente dos colores, podemos determinar que los lagartos son capaces de discriminar los colores de ambas regiones. Estos resultados, sumado a que ambas regiones poseen una coloración diferente entre sexos, con distintos tonos e intensidades (Naretto y Chiaraviglio, 2020), destacan que el rol de la coloración podría tener diferentes funciones de señalización. Se observó variabilidad de los colores en cada una de las regiones analizadas, es decir los machos son diversos y diferentes entre sí lo que permitiría que las hembras los discriminen. Por un lado, el flanco es ampliamente reconocido como una región de importancia en la comunicación intraespecífica en lagartos, por ejemplo, para la señalización territorial y sexual (Stuart-Fox y Ord, 2004; Paterson y Blouin-Demers, 2017; Paterson y Blouin-Demers, 2018; Rossi *et al.,* 2019). Es interesante destacar que no encontramos una expresión de la coloración ultravioleta inclusive en el parche de coloración azul del flanco. Numerosos estudios en lagartos muestran que parches de color azul usualmente están acom-

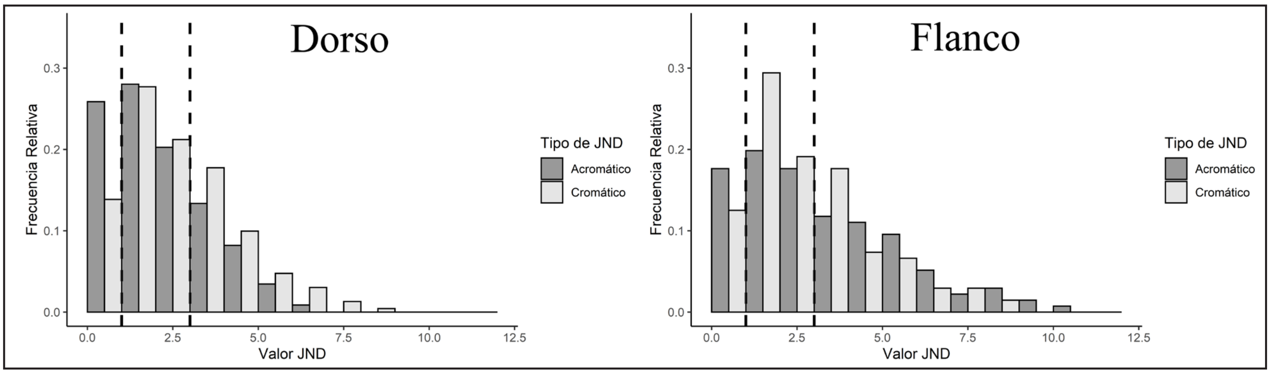

Figura 4. Histogramas de frecuencia de los JND cromáticos y acromáticos que comparan el dorso entre machos y el flanco entre machos. Las líneas punteadas indican los valores de criterio de discriminación visual (1 y 3 JND).

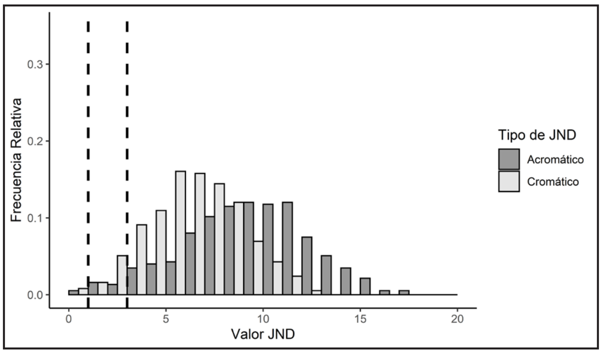

Figura 5. Histograma de frecuencia de los JND cromáticos y acromáticos comparando la región del dorso con la del flanco. Las líneas punteadas indican los valores de criterio de discriminación visual (1 y 3 JND).

pañados por un componente UV (Pérez i de Lanuza y Font, 2010, Badiane *et al.*, 2018) y que recientes estudios indican la importancia en la selección sexual (Badiane *et al*., 2020). Para que una coloración pueda ser interpretada como señal es requisito que exista variabilidad entre los individuos y que pueda ser identificada por el sistema visual del observador (Kemp *et al.*, 2015). Estos estudios nos permiten interpretar nuestros resultados de variabilidad en la coloración, es decir, la existencia de diversas coloraciones podría relacionarse con diferencias en el mensaje a transmitir. Por otra parte, el color del dorso podría estar relacionado principalmente con aspectos de comunicación interespecífica, ya que varios estudios plantean una relación de esta región con la coloración del fondo para incrementar el camuflaje ante depredadores, moduladas por presiones de selección natural (Stuart-Fox y Ord, 2004; Marshall y Stevens, 2014). Entre la diversidad de colores del dorso de los machos se podría esperar que algunos posean un mayor nivel de similitud con el fondo lo que disminuiría los riesgos de depredación y podría otorgarle beneficios relativos (Torres, 2019). Se plantea en este estudio que la variabilidad en la coloración podría estar mediada por diversas fuerzas selectivas, que no necesariamente actúen de manera direccional.

Elección de pareja

Teniendo en cuenta que el tratamiento vivo fue ideado para funcionar como control positivo, esperando que la hembra seleccione al ejemplar de lagarto, el criterio de permanencia en el área de influencia del macho, no mostró un claro patrón de elección, ya que todas las hembras permanecieron más tiempo en el área no elección. Para el segundo criterio, si bien la proporción de individuos que se desplazaron solo por el área de no elección fue similar a la observada en el estímulo vivo, de manera preliminar podríamos

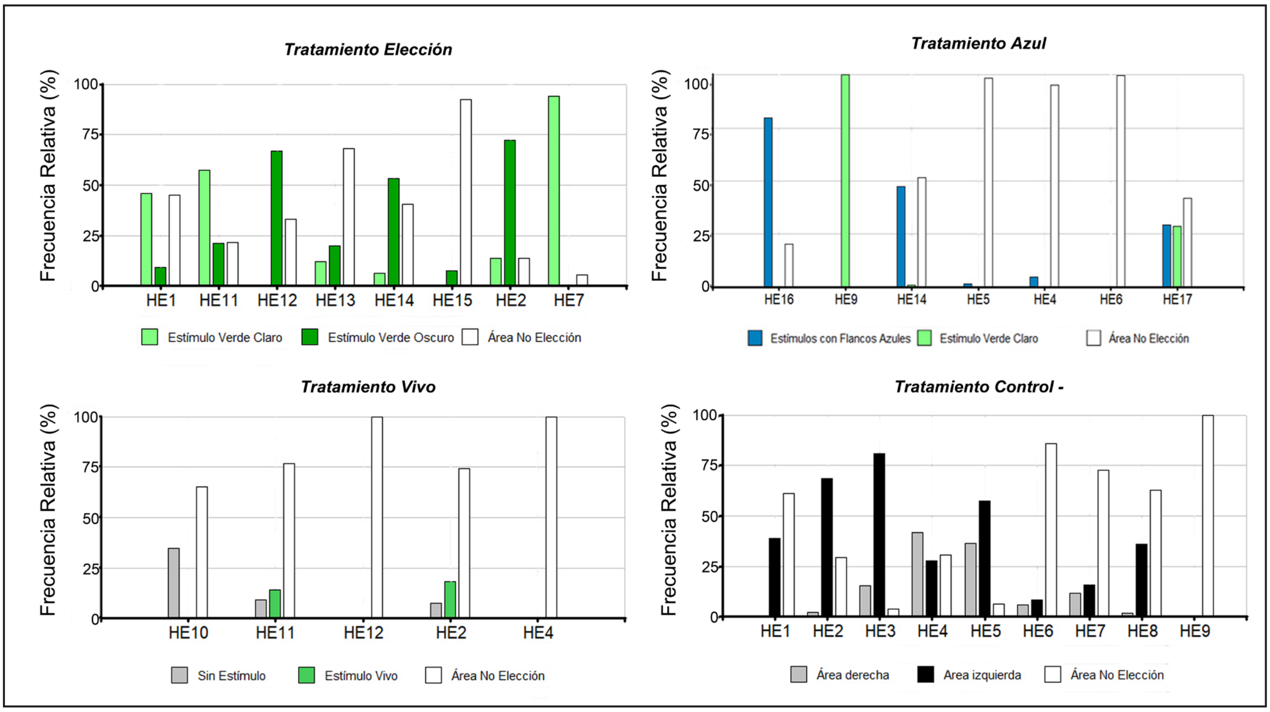

Figura 6. Frecuencia relativa del tiempo de permanencia de cada hembra (HE) en las áreas experimentales, durante los distintos tratamientos (Elección, Azul, Vivo y Control -). Se considera que la hembra elige un área en particular cuando supera el 50% del tiempo en alguna de las áreas de elección.

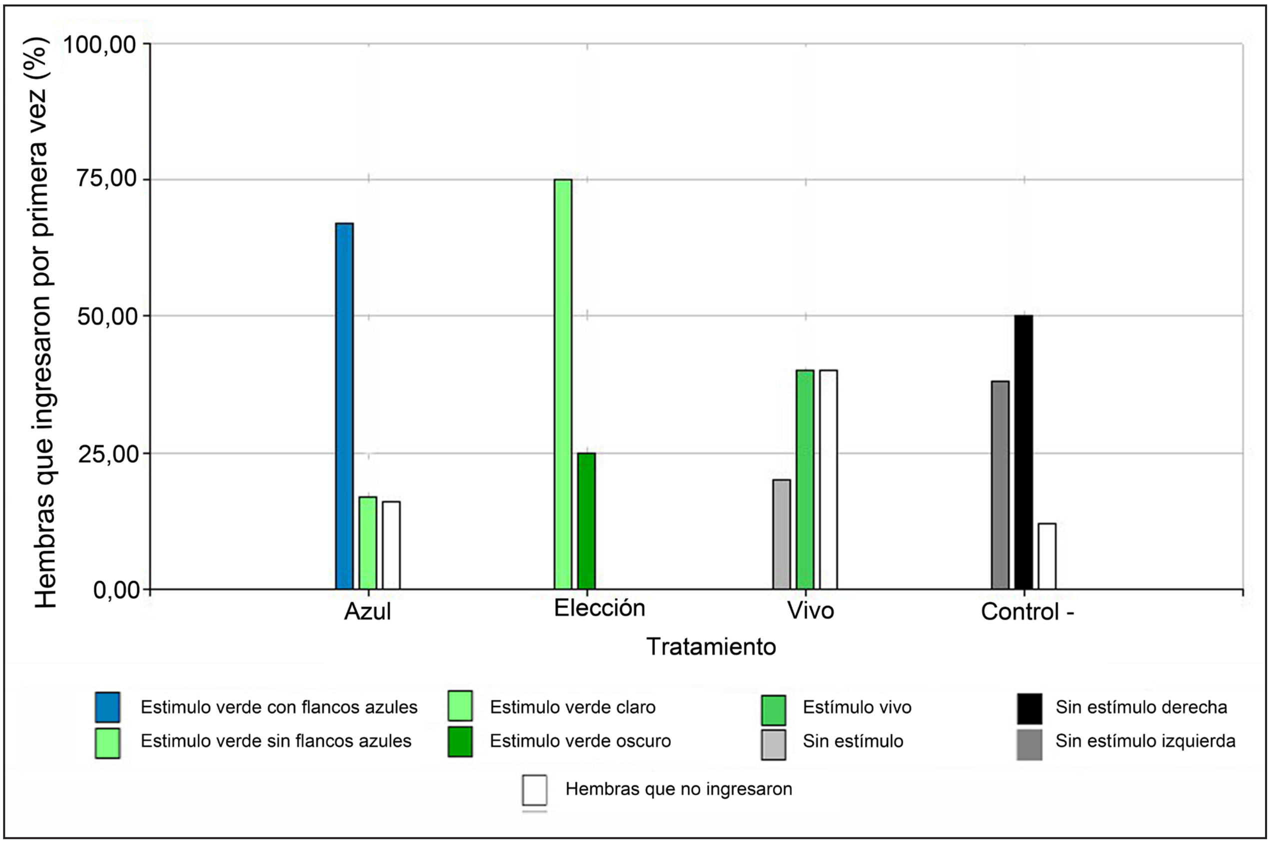

Figura 7. Porcentaje de hembras que ingresaron por primera vez a las distintas áreas de elección dependiendo de cada tratamiento. Además, se encuentra el porcentaje de hembras que se movieron solo por el área de no elección.

observar un patrón de elección seleccionando al estímulo vivo. Los resultados muestran una mayor proporción a elegir al ejemplar vivo, también elección del modelo de color verde con flancos azules frente al modelo verde sin flancos azules y elección del color verde claro frente al oscuro, coincidiendo con la elección esperada en base al contexto de selección sexual. Si bien nuestros resultados resultan preliminares, sugieren que es difícil de integrar el patrón de elección observado en los diferentes criterios. Los criterios no refuerzan la misma direccionalidad de la elección motivando a preguntarse sobre los motivadores de la elección de las hembras, sobre cuáles son los indicadores que seleccionan las hembras, y qué comportamientos o parámetros nos ayudan a comprender esa elección para mejorar los diseños experimentales.

Si bien existen algunos estudios que mostraron un patrón de elección (Pérez i de Lanuza *et al.*, 2012; Lattanzio *et al.*, 2014; Lisboa *et al.*, 2017), otros estudios (Alonzo y Sinervo, 2001; Healey *et al.*, 2008; Sacchi *et al.*, 2015; López Juri, 2019) encontraron que las hembras no mostraron elección por machos de diferentes fenotipos, es decir, no se encontró una correlación directa entre la elección activa por parte de la hembra con el fenotipo de los machos. Cabe destacar que la elección de pareja podría estar influida por diversas características, por ejemplo, comportamiento, personalidad animal e incluso indicadores ambientales como el territorio asociado (Sacchi *et al.*, 2017). Además, diferentes hembras pueden variar su elección; o hembras presentando un desinterés por caracteres fenotípicos particulares basando su elección en la calidad integral del macho (Lattanzio *et al.*, 2014; Sacchi *et al.*, 2017). Un punto para tener en cuenta, es lo propuesto por Swierk *et al.* (2012) donde la hembra evaluaría de manera integral el fenotipo y no solo la dicotomía cromática aquí planteada. En su caso, Swierk *et al.* (2012) distingue que las hembras prefieren a los machos que tienen mejor condición corporal, cabezas relativamente más grandes, gargantas más anchas y machos que desplegaban determinados comportamientos. Estudios en los que exista la interacción entre los individuos podrían ayudar a clarificar cuáles son los atributos que seleccionan las hembras para la elección de un macho en particular. Si bien son trabajos más integrales, también requieren mayor número de muestras y diseños experimentales complejos. En este trabajo se pretendió evaluar la coloración como rasgo determinante y podemos destacar que las hembras (sólo según los resultados del área a la que ingresó primero) eligieron la coloración azul del flanco de los machos, una región que es ampliamente variable bajo el modelo de visión del lagarto. Por otra parte, otro concepto a tener en cuenta es el planteado por Roughgarden y Akçay (2010), el concepto de diversidad en la preferencia, donde manifiestan la existencia de múltiples óptimos relativos a las hembras, lo que podría explicar los resultados del criterio de área de elección para el tratamiento Elección, donde el 50% de las hembras seleccionan un color y el otro 50% otro, incluso la elección de las hembras puede estar influida por experiencias previas (Yang *et al.*, 2013).

La coloración tiene un rol importante en la comunicación intersexual y los requisitos de variabilidad de coloración en los machos, y que las regiones analizadas son fácilmente distinguibles por los conespecíficos, nos permiten interpretar la coloración de los machos como señales visuales potencialmente funcionales en la elección de pareja por parte de la hembra. Cabe destacar que una de las experiencias controladas nos permite interpretar que el flanco de coloración azul podría ser un indicador a ser seleccionado por las hembras, pero nuevos diseños experimentales serían necesarios para una mejor comprensión. Diseños que logren combinar escenarios en las poblaciones naturales con experiencias de laboratorio pueden ayudarnos a conseguir una mirada completa de la importancia de la coloración como señal. Considerando que la coloración es un rasgo complejo que varía entre sexos e individuos de *Pristidactylus achalensis* y que puede participar en numerosas funciones, este trabajo aporta nuevas evidencias para comprender cómo la coloración actúa en el marco de la comunicación intraespecífica. Este trabajo también genera nuevos interrogantes, por ejemplo, qué caracteres morfológicos eligen las hembras e incluso si existe una relación entre caracteres sexuales morfológicos y la coloración.

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Trabajo

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Natural history notes of *Rhinella proboscidea* (Anura, Bufonidae) from a non-flooded forest in central Amazon

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ABSTRACT
Rhinella proboscidea, a toad of the *Rhinella margaritifera* species group, is found in non-flooded forests in central Amazon. This species is an explosive breeder that reproduces in streamside ponds and uses small seedling plants to rest during the night. In this study, we present data on the variation in abundance, body size and body mass of adults and juveniles, on the relationship between body mass and size, operational sex ratio (OSR), and we characterize the resting sites and some aspects of *R. proboscidea* reproductive events throughout two rainy seasons. We captured 366 individuals (59 males, 84 females, 204 juveniles and19 undetermined). Highest abundance of females and juveniles was found in the fifth sampling period; males were more abundant than females in three sampling periods. We did not detect sexual dimorphism in body size and mass between males and females. The OSR varied from 0.12:1 to 1.50:1 (males:females). There was a positive relationship between body size and mass considering all individuals. Reproductive events were observed from the middle to the end of the rainy season on few occasions. We found ontogenetic variation in the height of resting sites in which juveniles were found in lowest height when compared to adults. In conclusion, juvenile recruitment events occurred in the end of the rainy seasons, and the abundance of individuals are probably related to variation in water body availability for reproduction along the studied periods. Moreover, the absence of sexual dimorphism in body size and mass for this species disagrees with what is known for other species in the *R. margaritifera* group.

Key words: Abundance; Recruitment; Sexual dimorphism; Snout-vent length; Body mass.

RESUMO
Rhinella proboscidea, pertencente ao grupo de espécies de *Rhinella margaritifera*, é encontrada em florestas de terra firme na Amazônia central. Esta espécie possui reprodução explosiva em pequenas poças nas margens de riachos, e utiliza plântulas como sítio de repouso durante a noite. No presente estudo, apresentamos dados sobre a variação na abundância, tamanho e massa de adultos e juvenis, a relação entre massa corporal e comprimento rostro-cloacal, a razão sexual operacional (OSR) e caracterizamos os sítios de repouso e alguns aspectos reprodutivos de *R. proboscidea* ao longo de duas estações chuvosas. Capturamos 366 indivíduos (59 machos, 84 fêmeas, 204 juvenis e 19 indivíduos indeterminados). A maior abundância de fêmeas e juvenis ocorreu no quinto período de amostragem; machos were more abundantes do que as fêmeas em três períodos de amostragem. Não detectamos dimorfismo sexual no tamanho ou massa corporal entre machos e fêmeas. A OSR variou de 0.12:1 a 1.50:1 (machos:fêmeas). Detectamos uma relação positiva entre o tamanho corporal e massa considerando todos os indivíduos. Observamos eventos reprodutivos entre o meio e o fim das estações chuvosas, e em poucas ocasiões. Nós encontramos variação ontogenética na altura dos sítios de repouso onde os juvenis foram encontrados utilizando sítios mais baixos quando comparados aos adultos. Concluindo, os eventos de recrutamento de juvenis ocorreram no final das estações chuvosas, e a abundância de indivíduos está provavelmente relacionada com a variação na disponibilidade de corpos d'água para reprodução ao longo dos períodos estudados. A ausência de dimorfismo sexual no tamanho corporal e massa desta espécie é contrário ao registrado para outras espécies do grupo de *R. margaritifera*.

Palavras-chave: Abundância; Recrutamento; Dimorfismo sexual; Comprimento rostro-cloacal; Massa.

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Introduction

Despite the great Neotropical Anura diversity (Vasconcelos *et al.*, 2019) species population studies are not common and information on the variation in abundance and size of adults and juveniles and on seasonal recruitment events is available only for a few species of Aromobatidae, Bufonidae, Craugastoridae, Leptodactylidae and Microhylidae (e.g. Moreira and Lima, 1991; Galatti, 1992; Donnelly, 1999; Watling and Donnelly, 2002; Waldez *et al.*, 2011). Still, such basic information on population structure and associated variation factors are essential to the understanding of population dynamics and life history aspects of anurans (Schmidt *et al.*, 2002). In this context, information on the population structure are also important to future evaluation on anuran populations due the increasing of "amphibian declines crisis" around the world (Stuart *et al.*, 2004).

Bufonids are widely distributed in temperate and tropical regions, and the Neotropical genus *Rhinella* has the highest species richness, with 92 described species (Frost, 2020). This genus comprises seven species groups, including the *Rhinella margaritifera* group, currently composed of 19 species (Vaz-Silva *et al.*, 2015). Species within this group are found mainly in the leaf litter of primary forests, and reproduce in lentic water bodies (e.g. pools, swamps, Brazil nut empty fruit capsules, artificial ponds) during the rainy season (e.g. Wells, 1979; Aichinger, 1987; Caldwell, 1991; Fouquet *et al.*, 2007; Lima *et al.*, 2007; Roberto *et al.*, 2011), or during the dry season (Bernarde, 2007). These frogs can have explosive or prolonged reproduction patterns (Wells, 1979; Caramaschi and Niemeyer, 2003; Duellman, 2005; Menin *et al.*, 2006).

The leaf toad *Rhinella proboscidea* (Spix, 1824) is distributed along the Amazon River from Peru to Manaus in Brazil (Frost, 2020). It has diurnal habits and can be found during the night, resting perched on the vegetation above the ground (Zimmerman and Bogart, 1988; Lima *et al.*, 2012). However, nocturnal activity was found in males of this species which call during day and night for two or three days after intense rainfall, forming choruses of 50-100 males (Menin *et al.*, 2006). Clutches and tadpoles can be found in temporary ponds near streams or in stream headwaters (Menin *et al.*, 2006).

In this study, developed throughout two rainy seasons in a non-flooded forest in the Brazilian Amazon, we present data on the variation in abundance, size and mass of adults and juveniles, the relationship between body mass and size, and we characterize the resting sites and some reproductive aspects of *R. proboscidea*, a species of the *R. margaritifera* group.

Materials and methods

Study area

Our study was carried out at Reserva Florestal Adolpho Ducke (02º55' and 03º01'S, 59º53' and 59º59'W), in the city of Manaus, State of Amazonas, Brazil. Reserva Ducke covers about 10 000 ha of non-flooded forest, with a closed canopy of 30-37 m height, emergent trees growing up to 40-45 m height, and an understory of abundant sessile palm trees (*Astrocaryum* spp. and *Attalea* spp.; Ribeiro *et al.*, 1999). The climate is Am (Köppen-Geiger system; tropical monsoon with no dry season) (Peel *et al.*, 2007). Rainy season is usually from November to May and average annual rainfall was 2 362 mm between 1985 and 2006 (Rodrigues *et al.*, 2010). The rainiest months are March and April, generally with rainfall > 300 mm per month (Marques-Filho *et al.*, 1981). Air temperature varies between 24.6°C and 26.9°C (Araújo *et al.*, 2002). During the period of our study, annual rainfall was 2 702 mm in 2002, 2 044 mm in 2003 and 2 618 mm in 2004. The months of January (50.4 mm), February (178.1 mm) and March (213.9 mm) 2003 were below the 21-year average for these months (268, 285 and 318 mm, respectively; Rodrigues *et al.*, 2010) (Fig. 1).

Data collection

Individuals of *R. proboscidea* were sampled in five nocturnal surveys (November–December 2002, March–May 2003, November–December 2003, January–March 2004 and April–May 2004) during two rainy seasons. At each sampling period, we collected data for 72 plots (each plot 250 m long and positioned along altitudinal contour lines) systematically distributed over a 64-km^2 grid, formed by 8-km long trails inside Reserva Ducke (Menin *et al.*, 2008). We conducted nocturnal visual encounter surveys (Crump and Scott, 1994) between 18:30 h and 22:00 h. Despite diurnal habits of this species, individuals were easily found during nocturnal samplings than diurnal samplings at the same study area (Menin *et al.*, 2008). At every 5 min, in each

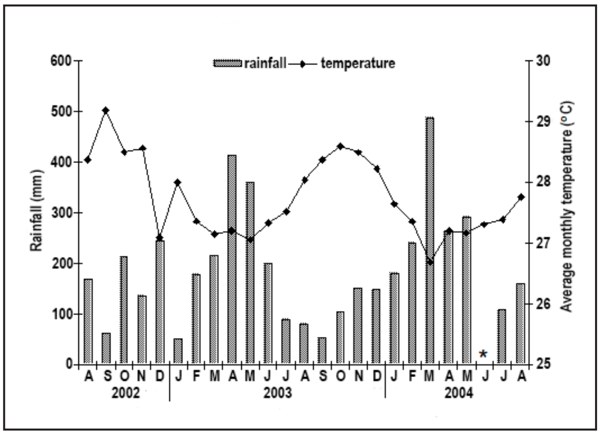

Figure 1. Monthly precipitation levels and average monthly temperatures from August 2002 to August 2004 at the Reserva Florestal Adolpho Ducke, Manaus, Amazonas, Brazil. Data obtained from the meteorological station at the Reserva Ducke. *= missing data.

plot, two observers stopped and searched the litter and the vegetation for specimens. All individuals located within 20 m of the center line of the plot were recorded. Occasional observations were made in areas outside of the plots. We (1) measured the size of each individual as the length from the tip of the snout to the vent (SVL) with a vernier calliper (0.05 mm precision), (2) measured body mass using a Pesola® spring balance (0.1 g precision), (3) classified the individual as male, female or juvenile, (4) recorded its activity (resting, moving, calling, breeding), and (5) characterized the resting site by registering perch height above the ground and type of substrate (leaves, stem, fallen trunks, leaf litter). Males and females were identified by dorsal surface skin or calling activity (dorsal surface is smooth in males and granular in females; Lima *et al.*, 2012). We considered juveniles the individuals with SVL < 39.9 mm (the size of the smallest male found in the samplings). Juveniles that were smaller than 21 mm in SVL and adult individuals that escaped did not have their body mass determined. We measured abundance as the number of individual detections in each sampling period. Vouchers are housed in the Amphibians and Reptiles Collection of the Instituto Nacional de Pesquisas da Amazônia (INPA-H 11815 to 11831, 11855, 11922, 11923).

Statistical analysis

Since our data on body size and body mass showed non-normal distribution, we used non-parametric Mann-Whitney U-Test to compared male and female body size and body mass (Zar, 1999). The differences in perching height between males, females and juveniles were compared using a Kruskal-Wallis test (Zar, 1999). Operational sex ratio was estimated by dividing the number of males by the number of females present in each sampling period and considering total number of individuals in all sampling periods. We also checked the adult sex ratio by using the Chi-square test with Yates' Correction (Fowler *et al.*, 1998). Relationship between body size and mass was determined through Linear Regression model (Zar, 1999). Data was log10 transformed prior to the regression analysis in order to meet the assumptions of the model. The results are expressed as mean ± standard deviation. All statistical analyses were conducted with a significance level of 0.05.

Results

We captured 366 individuals of *R. proboscidea* in the five sampling periods (59 males, 84 females and 204 juveniles; Fig. 2). We could not determine the sex of 19 individuals between 39.1 mm and 42.4 mm of SVL. Among sampled individuals, juveniles were more abundant in four of the five sampling periods (Fig. 2). Males were not detected in the first sampling period. Considering all sampling periods, the operational sex ratio was 0.51:1 males/females (Table 1). However, considering each sampling period individually, the OSR varied from 0.12:1 (males/females) in the fifth sampling period, to 1.50:1 in the fourth sampling period (Table 1). Females were

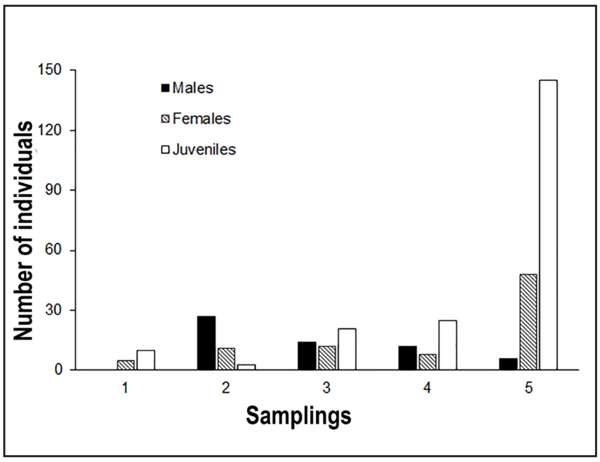

Figure 2. Number of males, females and juveniles of *Rhinella proboscidea* recorded in five nocturnal visual samplings at Reserva Florestal Adolpho Ducke, Manaus, Amazonas, Brazil. The numbers represent sampling periods: 1) November-December 2002, 2) March-May 2003, 3) November-December 2003, 4) February-March 2004, and 5) April-May 2004.

Table 1. Body size (snout-vent length; in mm) and body mass (in grams) of *Rhinella proboscidea*, measured in five nocturnal visual samplings at the Reserva Florestal Adolpho Ducke, Manaus, Amazonas, Brazil. Values are presented as mean ± standard deviation, range (in parenthesis) and sampling size (N), with the exception of periods with only one or two individuals, for which values were given. (*) Juveniles smaller than 21 mm in SVL did not have their body mass determined. A Mann-Whitney U-test was applied for comparisons between body size and body mass of males and females. OSR: operational sex ratio (number of males divided by the number of females). A Chi-squared test with Yates' Correction was used to evaluate adult sex ratio.

Sampling/Sex	Nov-Dec/2002	Mar-May/2003	Nov-Dec/2003	Feb-Mar/2004	Apr-May/2004	Total
Body Size						
Males	-	49.41 ± 3.87 (41.1-54.5) N= 11	45.89 ± 3.42 (38.3-50.5) N= 14	46.64 ± 4.29 (40.0-52.5) N= 12	50.35 ± 3.28 (45.1-54.9) N= 6	47.62 ± 4.06 (38.3-54.9) N= 43
Females	47.24 ± 4.79 (41.7-51.5) N= 5	48.14 ± 4.52 (43.3-55.5) N= 11	48.21 ± 4.21 (42.5-56.6) N= 12	48.39 ± 2.18 (45.0-51.6) N= 8	46.10 ± 3.32 (40.7-56.7) N= 48	46.90 ± 3.70 (40.7-56.7) N= 84
Juveniles	23.07 ± 7.23 (10.4-35.5) N= 10	36.87 ± 1.70 (34.9-37.9) N= 3	25.32 ± 4.02 (17.9-33.9) N= 21	34.55 ± 3.30 (29.6-39.9) N= 24	11.54 ± 8.50 (5.4-38.7) N= 145	16.60 ± 11.40 (5.4-39.3) N= 203
Mann-Whitney test (males *versus* females)	-	U= 50.00 P= 0.490	U= 107.50 P= 0.227	U= 58.00 P= 0.440	U= 49.50 P= 0.009	U= 1,567.00 P= 0.223
OSR (males:females)	-	1:1	1.17:1	1.50:1	0.12:1	0.51:1
Chi-squared test	-	χ^2= 5.92, gl=1, p> 0.05	χ^2= 0.16, gl=1, p> 0.80	χ^2= 0.45, gl=1, p> 0.70	χ^2= 31.13, gl=1, p< 0.01	χ^2= 12.60, gl=1, p< 0.01
Body Mass						
Males	-	9.0-11.0 N= 2	10.36 ± 1.98 (6.0-14.0) N= 14	9.67 ± 2.61 (5.5-14.5) N= 12	11.00 ± 2.02 (9.0-14.0) N= 6	10.20± 2.20 (5.5-14.5) N= 34
Females	9.80 ± 2.59 (6.0-13.0) N= 5	10.50 ± 3.64 (6.0-16.5) N= 8	11.63 ± 3.22 (7.0-18.0) N= 12	10.94 ± 3.91 (7.0-17.0) N= 8	9.33 ± 1.81 (6.0-16.0) N= 48	10.00 ± 2.60 (6.0-18.0) N = 81
Juveniles*	3.50 N= 1	5.17 ± 1.44 (3.5-6.0) N= 3	2.79 ± 0.77 (2.0-4.5) N= 17	3.88 ± 1.11 (2.0-6.0) N= 24	4.8 ± 0.90 (3.0-6.5) N= 15	3.80 ± 1.20 (2.0-6.5) N= 60
Mann-Whitney test (males *versus* females)	-	U= 6.50 P= 0.691	U= 94.50 P= 0.586	U= 53.50 P= 0.668	U= 68.00 P= 0.035	U= 1,181.00 P= 0.227

more abundant than males only in the fifth sampling period (Fig. 2). Highest abundance of juveniles was also found in the fifth sampling period (Fig. 2).

We did not detect significant differences in body size and mass between males and females in most of the sampling periods, and when considering all sampling periods together (Table 1). However, significant differences were found in both body size and body mass in the fifth sampling period, in which the males were greater and heavier than the females (Table 1). Additionally, adult males found at the end of the two rainy seasons (second and fifth sampling periods) were larger than those found in other sampling periods, while the size of females was similar along the sampling periods (Table 1, Fig. 3A and 3B). Juveniles found at the end of the second rainy season were smaller than those found in the other sampling periods (Table 1, Fig. 3C). SVL of smallest juvenile was 5.4 mm. We also detected a positive relationship (Fig. 4) between body size (SVL) and mass (weight) when considering all individuals together (males, females, juveniles and undetermined) ($F_{1,192}$ = 933.690, $P < 0.001$, n = 194).

Reproductive activity was observed in six occasions but the number of males was estimated in four of these occasions between April and May 2003 (5 males calling in a pool of about 1 m^2, 10 in a pool of about 1 m^2, and 20 in a pool of about 0.5 m^2) and

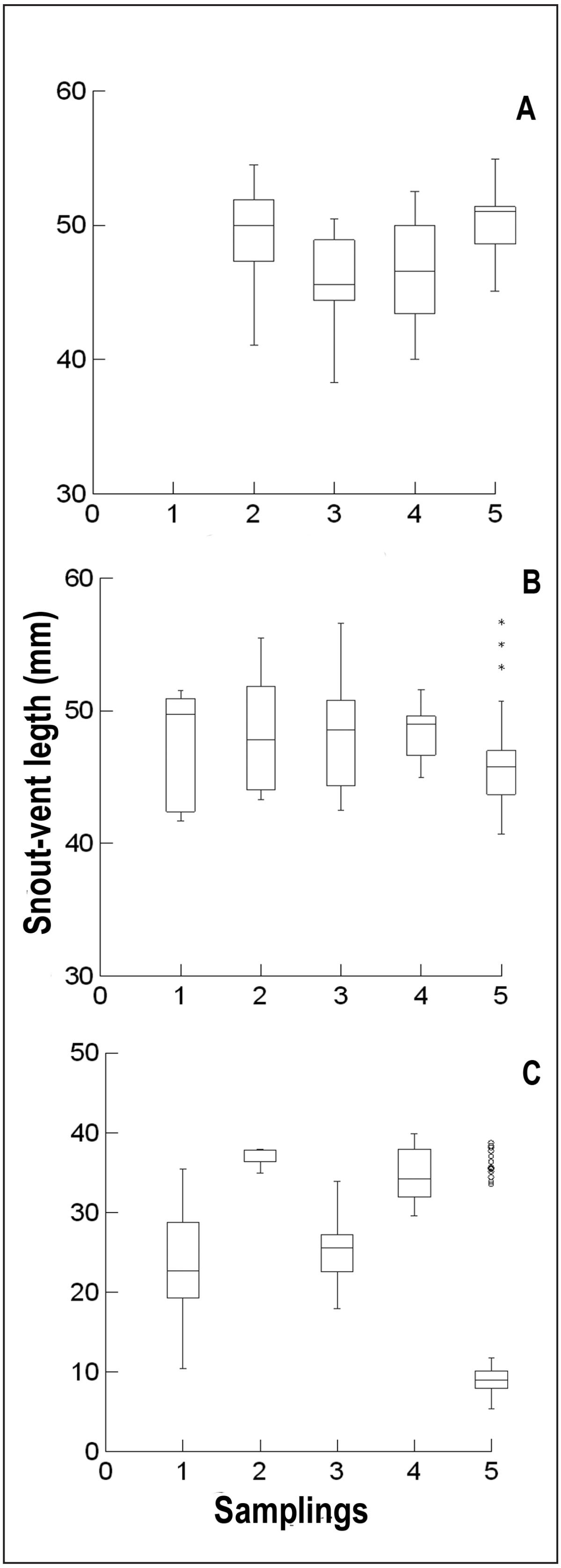

Figure 3. Variation in snout-vent length of 43 males (A), 84 females (B) and 203 juveniles (C) of *Rhinella proboscidea* measured in five nocturnal visual samplings at Reserva Florestal Adolpho Ducke, Manaus, Amazonas, Brazil. Sampling periods as Figure 2.

March and April 2004 (40 males calling in a pool of about 2 m^2). All ponds were shallow, temporary and in edges of streams. SVL of three amplectant pairs varied from 45.8 to 54.5 mm for males and from 50.3 to 55.5 for females.

Most adults and all juveniles were found resting on leaves of small shrubs (319 individuals), on stems or fallen tree trunks (13 individuals) or leaf litter (three individuals); 25 males were found in calling activity and only three individuals were found moving on the leaf litter during the night, far from water bodies. The height of resting sites was significantly highest for males (mean perching height= 25.69 cm ± 18.80, range= 5–100 cm, n= 55) and for females (mean perching height= 23.54 cm ± 17.12, range= 3–100 cm, n= 81) than for juveniles (mean perching height= 15.79 cm ± 13.10, range= 0–70 cm, n= 201) (Kruskall-Wallis= 32.450, df= 2, $P< 0.001$).

Discussion

Adults and juveniles of *R. proboscidea* were detected throughout the studied rainy seasons, with an abundance peak at the end of the rainy season. The highest abundance of juveniles at the end of the rainy season indicate events of recruitment. This pattern was also observed for *R. hoogmoedi*, another member of the *R. margaritifera* species group (Brito *et al.*, 2013), and for other Neotropical anuran species as *Pristimantis* aff. *fenestratus*, *Adenomera andreae* and *Anomaloglosus stepheni* (Moreira and Lima, 1991; Waldez *et al.*, 2011). Likewise, other anurans have recruitment events that occur mainly in the dry season (Moreira and Lima, 1991; Watling and Donnelly, 2002) or continuously (Chinchilla-Lemus *et al.*, 2020). The high juvenile body size values detected at the beginning of the two studied rainy seasons probably represent individuals that were born in the previous rainy season, a pattern also detected for *Physalameus marmoratus* from the Brazilian Cerrado (Giaretta and Menin, 2004). The same reasoning could be applied to the variation in body size of males: males with smaller sizes were detected in the beginning of the rainy season, indicating the entrance of these individuals in the reproductive stage; whereas those with greater sizes were found in the end of the rainy season - a fact also observed for *R. hoogmoedi* (Brito *et al.*, 2013).

The small number of reproductive events observed in *R. proboscidea* throughout the studied periods indicates seasonal reproduction, with a peak

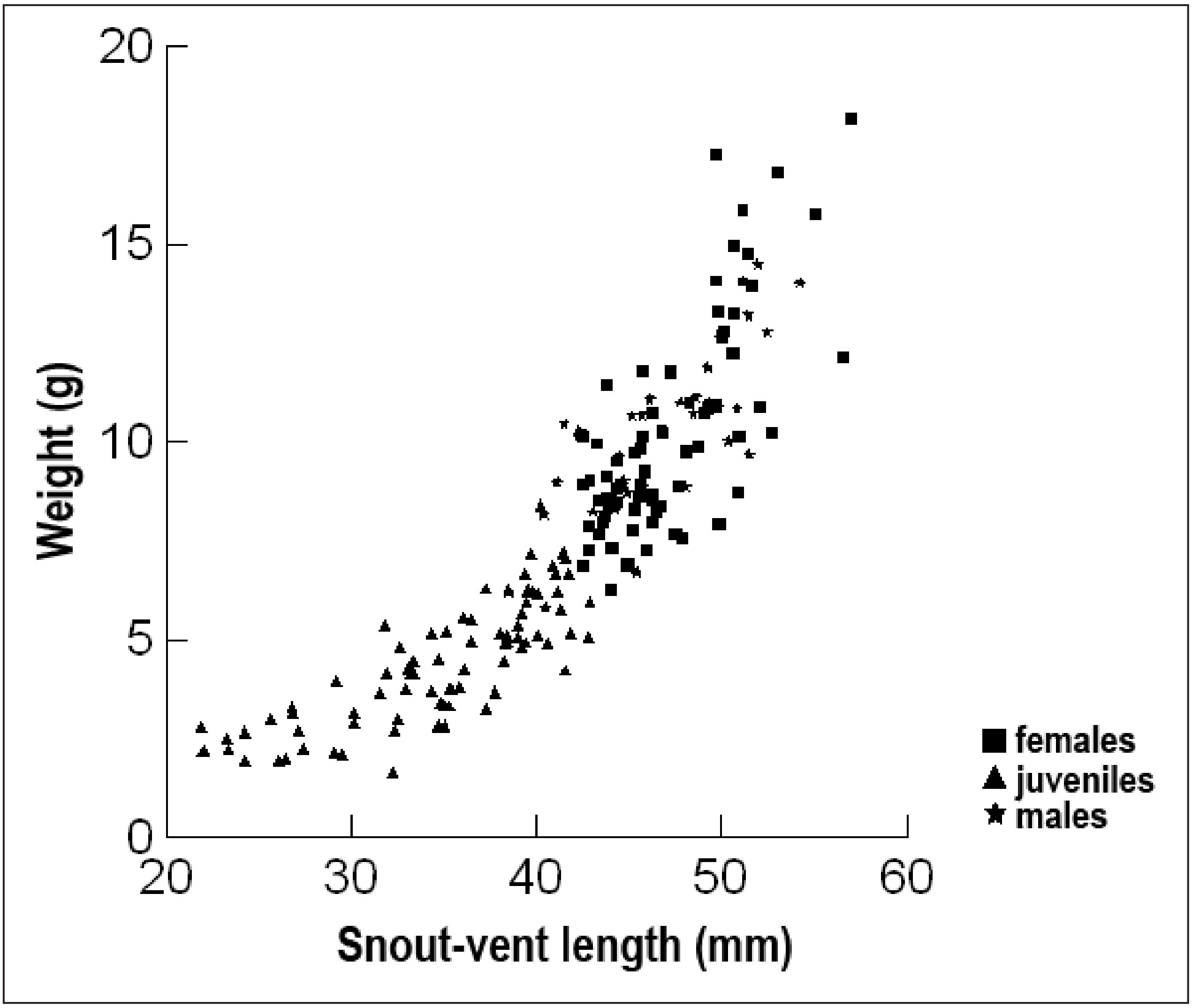

Figure 4. Relationship between body size (snout-vent length, in mm) and mass (in grams) for 191 individuals of *Rhinella proboscidea* measured in five nocturnal visual samplings at Reserva Florestal Adolpho Ducke, Manaus, Amazonas, Brazil. Data not transformed.

from the middle to the end of the rainy season. This fact is probably related to the necessity of temporary ponds for the reproduction of this species, since the availability of temporary water bodies vary in time (Rodrigues *et al.*, 2010). Moreover, the variation in abundance of adults and juveniles throughout the studied rainy seasons should be related to the variation in number of water bodies at the Reserva Ducke. Rodrigues *et al.* (2010) detected precipitation levels below the annual averages for the months of January to March 2003, but observed above average rainfall in March 2004. The authors also stressed that the number of ponds strongly varied, and estimated that there were two times more ponds in 2004 than when compared to 2003 at the Reserva Ducke. We suggest that this variation in water body availability throughout the years should be related to the small number of individuals, mainly juveniles and females of *R. proboscidea,* found in our study at the end of the first rainy season. Anurans with aquatic reproduction in Central Amazonia depend on streamside pond availability (Menin *et al.*, 2011), and should be especially vulnerable to changes in weather patterns. Thus, changes in pond availability due alterations in the precipitation regime should affect juvenile recruitment events and consequently affect the species reproductive success (Greenberg *et al.*, 2017).

The operational sex ratio varied among sampling periods, and it was near to 1:1 (males:females) in three of the sampling periods. However, considering only the fifth sampling period (where we detected the highest number of small-sized juveniles, indicating greater reproductive activity) and all sampling periods together, we found a female-biased sex ratio. This fact can be a result of the higher motility of females, which makes them conspicuous and easily detected. We suggest that males probably stand more near to the reproduction areas, while females may be more mobile, searching for reproductive male aggregations in explosive reproduction. On the other hand, Izzo *et al.* (2012) detected a male-biased sex ratio for *R. proboscidea* in the same studied area, but the authors collected these data only in water bodies with male aggregations in calling activity. In contrast, we collected our data systematically along standardized sampling units in the two rainy seasons.

We did not detect significant sexual dimorphism in body size and mass between males and females of *R. proboscidea* when considering the whole dataset, although we found significative differences in both measurements in the fifth sampling period. Our results contrast to what was found for all other species in the *R. margaritifera* group, where females are larger (Caramaschi and Niemeyer, 2003; Lima *et al.*, 2007; Ávila *et al.*, 2010) and heavier than males (Hoogmoed and Avila-Pires, 1991; Caldwell and Araújo, 2005; Duellman, 2005). This type of sexual size dimorphism is a common characteristic of most anuran species (Shine, 1979), including many bufonid species (e.g., Monnet and Cherry, 2002; Arantes *et al.*, 2015). The absence of sexual dimorphism in size detected by us may be related to territorial defense and fighting (Shine, 1979). As previously mentioned, *R. proboscidea* is an explosive breeder (Menin *et al.*, 2006), and males use a lot of energy in the search for females in large mating aggregations in small water bodies, and also when fighting for females (Wells, 1977, 2007). The fighting is relatively common, and results in mating balls that are often fatal to some females (Wells, 2007; Izzo *et al.*, 2012).

In our study, adults and juveniles of *R. proboscidea* were found resting during the night, perched on leaves of small shrubs and on stems or fallen tree trunks above the ground, as observed by Zimmerman and Bogart (1988) for both this species and other species of the *R. margaritifera* species group, such as *R. castaneotica*, *R. magnussoni* and *R. hoogmoedi* (Caldwell and Araújo, 2005; Lima *et al.*, 2007; Brito *et al.*, 2013). The use of perching sites during the night should be related to the avoidance of ground dwelling predators, as described for snakes, by Martins (1993). We also detected ontogenetic variations in the use of resting sites: juveniles were found in lowest sites when compared to adults (males and females), which probably relates to the ability of adults in accessing higher resting places.

In conclusion, *R. proboscidea* varies in adult and juvenile body size and body mass throughout the rainy seasons. Juvenile recruitment events occurred mainly at the end of the second rainy season, and the abundance of individuals are probably related to variation in water body availability for reproduction along the studied periods. Moreover, the absence of sexual dimorphism in body size and mass for this species disagrees with what is known for other anuran species and species of the *R. margaritifera* group.

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Trabajo

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Contributions to the knowledge of parasitic nematodes of amphibians from the Dry Chaco ecoregion in Argentina

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ABSTRACT

In the Argentinean Gran Chaco, numerous investigations have dealt with the diversity of vertebrates, including amphibians and reptiles. The parasitofauna of these organisms, has been more studied in some groups such as amphibians; however, from a biogeographic perspective, most of these studies were carried out in the Humid Chaco. The goal of this work is to present the nematofauna found in six species of amphibians, *Rhinella diptycha*, *Chacophrys pierottii*, *Ceratophrys cranwelli*, *Scinax nasicus*, *Leptodactylus bufonius*, and *Odontophrynus lavillai*, collected in different locations within the Dry Chaco, in the department of Matacos, Formosa, Argentina. In a total of 34 amphibians analyzed, we found 1,526 parasitic nematodes (total prevalence: 61.7%; total mean intensity: 72.6±90.3; total mean abundance: 44.9±78.9), belonging to 9 taxa: adults of *Rhabdias* sp., *Oswaldocruzia* sp., *O. subauricularis*, *Schulzia travassosi*, *Aplectana hylambatis*, *Cosmocerca podicipinus*, *Oxyascaris caudacutus*, *Schrankiana formosula*, and larvae of *Physaloptera* sp. These nematodes were collected from the lungs, small intestine, large intestine, gastric mucosa, and gallbladder of hosts. This work is the first record of helminths from *C. pierottii* and *O. lavillai* and the first record of nematodes from *Ce. cranwelli* throughout its geographical range. The species *Schrankiana formosula* (Atractidae) is recorded for the first time in Argentinean amphibians. Thus, we expand the knowledge of the biological diversity of helminth parasites of amphibians from the Dry Chaco ecoregion. This base-line information, would allow to understand the factors that influence the patterns of host and geographic distribution of vertebrate parasites in an area with extreme characteristics such as the Dry Chaco.

Key Words: Anurans; Chaco; Nematode Parasites.

RESUMEN

Contribuciones al conocimiento de nematodos parásitos de anfibios de la ecorregión Chaco Seco de Argentina. En el Gran Chaco Argentino, numerosas investigaciones han tratado la diversidad de los vertebrados, incluida su fauna herpetológica. En cuanto a la parasitofauna de estos organismos, algunos grupos como el de los anfibios han sido más estudiados; sin embargo, biogeográficamente, la mayor parte de estos estudios fueron realizados en el Chaco Húmedo. El objetivo de este trabajo es presentar la nematofauna hallada en seis especies de anfibios, *Rhinella diptycha*, *Chacophrys pierottii*, *Ceratophrys cranwelli*, *Scinax nasicus*, *Leptodactylus bufonius* y *Odontophrynus lavillai*, colectadas en distintos puntos del Chaco Seco en el departamento de Matacos, Formosa, Argentina. En un total de 34 anfibios analizados encontramos 1.526 nematodes parásitos (prevalencia total: 61,7%; intensidad media total: 72,6±90,3; abundancia media total: 44,9±78,9), pertenecientes a 9 taxones, adultos de *Rhabdias* sp., *Oswaldocruzia* sp., *O. subauricularis*, *Schulzia travassosi*, *Aplectana hylambatis*, *Cosmocerca podicipinus*, *Oxyascaris caudacutus*, *Schrankiana formosula* y larvas de *Physaloptera* sp. Estos nematodos se colectaron de

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los pulmones, el intestino delgado, el intestino grueso, la mucosa gástrica y la vesícula biliar de los hospedadores. Este trabajo constituye el primer registro de helmintos para *C. pierottii* y *O. lavillai* y el primero de nematodes para *Ce. cranwelli* en toda su área de distribución geográfica. La especie *Schrankiana formosula* (Atractidae) es registrada por primera vez en anfibios argentinos. De este modo, ampliamos el conocimiento de la diversidad biológica de los helmintos parásitos de anfibios de la ecorregión Chaco Seco. Esta información permitirá conocer cuáles son los factores que influyen en los patrones de ocurrencia en hospedadores y distribución geográfica de helmintos parásitos de vertebrados, en un área con características tan extremas como el Chaco Seco.

Palabras claves: Anuros; Chaco; Nematodos Parásitos.

Introduction

Studies on amphibian parasites are relevant because they can provide information regarding the structure and relationships of trophic networks, as many parasites may pass from one host to another through predator-prey relationships. Besides, parasitological data may provide indirect information about the host diet, as the presence of certain parasites with known indirect cycles would indicate the consumption of certain type of prey. The study of parasites may indicate the state of ecosystems, as their presence or absence makes possible to infer the richness of their intermediate and definitive hosts, and provide also information related to host stress because some agrochemicals are known to depress the immune system and turn amphibians more susceptible to certain parasites (see for instance Marcogliese and Cone, 1997; Lafferty *et al.*, 2008; Koprivnikar and Redfern, 2012; Koprivnikar *et al.*, 2012), among other aspects. However, despite the fact that their study may give us a wealth of information, parasites are not generally considered in biodiversity studies (Gómez and Nichols, 2013; Rocha *et al.*, 2016).

In recent investigations, Campião *et al.* (2014; 2015) compiled data on helminth parasites of amphibians from South America; one of the results of their investigation was that the most studied hosts were anurans of the families Bufonidae, Hylidae and Leptodactylidae, mainly *Leptodactylus latrans* (Steffen, 1815) and *Rhinella marina* (Linnaeus, 1758). In addition, they found that Brazil was the origin of most studies with over 50% of the total output. This evidence large information gaps regarding several host species and large geographical areas that remain unsurveyed.

Particularly for the Argentinean Chaco, parasitological studies have focused on diverse vertebrate hosts, including mammals such as monkeys (Perea-Rodriguez *et al.*, 2010), wolves (Orozco *et al.*, 2014), and armadillos (Ríos *et al.*, 2020), and sauropsids such as birds (Dueñas Díaz *et al.*, 2018; Lunaschi *et al.*, 2018), snakes (Lunaschi and Drago, 2007; 2010), lizards (Lamas and Zaracho, 2006; Zaracho and Lamas, 2006), caimans and turtles (Lunaschi and Drago, 2007). Regarding amphibians, the majority of helminth studies were carried out in the Humid Chaco, mainly in the province of Corrientes (Hamann *et al.*, 2013a; 2020; Draghi *et al.*, 2015a, b; González and Hamann, 2015a; González *et al.*, 2019), while such studies are only incipient for the Dry Chaco region (Hamann and González, 2015; González and Hamann, 2015b; González *et al.*, 2020).

The aim of the present work is to provide records of nematode parasites for six amphibian species collected in a locality within the Dry Chaco ecoregion, Argentina.

Materials and methods

Thirty-four adult amphibians belonging to six species of five families, namely *Rhinella diptycha* (Cope, 1862), *Scinax nasicus* (Cope, 1862), *Chacophrys pierottii* (Vellard, 1948), *Ceratophrys cranwelli* Barrio, 1980, *Leptodactylus bufonius* Boulenger, 1894, and *Odontophrynus lavillai* Cei, 1985, were collected for this study. Table 1 presents the number of specimens for each amphibian species analyzed, with the respective collection date, locality, and collection accession number.

Amphibians were collected within the Dry Chaco ecoregion (Morello *et al.*, 2012), approximately 32 km south from the city Ingeniero Juárez, Matacos Department, in Formosa, Argentina. In this area the vegetation cover is adapted to dry conditions (xerophytic deciduous forest), with predominance of small leaves deciduous and thorny species tolerant to large fluctuations in water availability, as well as to seasonal thermal variations. Woody vegetation is sparse, and the herbaceous species are predominantly grasses. The landscape is flat, gently sloping toward the east. The climate is characterized by low rainfall (mean annual precipitation <700 mm), and high temperatures, at times exceeding 47°C since this area comprises part of the South American Heat Pole (Maldonado *et al*., 2006).

Amphibians were hand-captured between 18 and 21 hours using the sampling technique defined as “visual encounters survey” (Crump and Scott Jr., 1994) and "Audio Strip Transect" method (Zimmerman, 1994) in the most favorable environments such as shallow lakes, temporary puddles and ditches. Frogs were subsequently transported alive to a field-mounted laboratory, euthanized topically using 10% lidocaine cream and necropsied. Their snout-vent length (SVL) and body weight (g) were recorded. We examined under a stereoscopic microscope the digestive tract, lungs, liver, gallbladder, kidneys, urinary bladder, coelomic cavity, and musculature. Parasites were observed *in vivo*, counted, and then fixed in hot 70% ethyl alcohol. Posteriorly, they were clarified in Amman’s lactophenol, mounted on temporary slides and examined under a light microscope. For morphological examination and measurements, a Leica DM2500 microscope equipped with a drawing tube was used. Amphibian taxonomy followed Frost (2020). Specimens were preserved in 70% ethyl alcohol and deposited in the Helminthological Collection of Centro de Ecología Aplicada del Litoral (CECOAL) Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Corrientes city, Corrientes, Argentina. Amphibian specimens were deposited in the Colección Herpetológica of the Universidad Nacional del Nordeste (UNNEC); accession numbers are shown in Table 1.

Table 1. Amphibian species from the Dry Chaco Ecoregion analyzed for nematode parasites: collection date, locality and accession numbers.

Family	Species and number of specimens analyzed	Collection date	Locality - Geographical coordinates	Accession number
Bufonidae	*Rhinella diptycha* (n= 3)	Dec. 2016; May 2017; Feb. 2018	-24.215401, -61.981015	UNNEC 13601
Ceratophryidae	*Chacophrys pierottii* (n= 8)	Dec. 2016; Feb. 2018; Dec. 2019	-24.216001, -61.979434	UNNEC 13602
	Ceratophrys cranwelli (n= 1)	Dec. 2016	-24.216001, -61.979434	UNNEC 13603
Hylidae	*Scinax nasicus* (n= 8)	Dec. 2016; Feb. 2017; Jul. 2017	-24.223394, -61.958458	UNNEC 13604
Leptodactylidae	*Leptodactylus bufonius* (n= 12)	Dec. 2016; April 2018; Jul. 2018	-24.223394, -61.958458	UNNEC 13605
Odontophrynidae	*Odontophrynus lavillai* (n= 2)	Dec. 2016; Feb. 2017; May 2017; Jul. 2017	-24.191161, -62.04896	UNNEC 13606

Prevalence, mean intensity (MI) and mean abundance (MA) of infection for parasites were calculated following Bush *et al.* (1997).

Results

We found 21 amphibians parasitized by nematodes (total prevalence: 61.7%), from which we recovered 1,526 nematode specimens (total MI: 72.6±90.3; total MA: 44.9±78.9) of the following species and families: Rhabdiasidae: *Rhabdias* sp. (CECOAL 18021944, 1 hermaphrodite female); Molineidae: *Oswaldocruzia* sp. (CECOAL 16122115; 3 FF), *O. subauricularis* (Rudolphi, 1819) (CECOAL 17050809; 5 FF, 5 MM), *Schulzia travassosi* Durette-Desset, Baker and Vaucher, 1985 (CECOAL 18071603; 2 FF, 2 MM);

Table 2. Nematode parasites found in six amphibian species from the Dry Chaco ecoregion, Argentina. n: number of nematode parasites collected; %: prevalence of infection; MI±SD: mean intensity (± 1 standard deviation); MA±SD: mean abundance (± 1 standard deviation).

Amphibian species	Nematode parasites	Stage	n	%	MI±SD	MA±SD	Site of infection
	Rhabdias sp.	A	2	33.3 (1/3)	-	0.66±1.1	LU
R. dyptycha	*O. subauricularis*	A	62	33.3 (1/3)	-	20.6±35.7	SI
	A. hylambatis	A	112	66.6 (2/3)	56±49.4	37.3±47.6	LI
	A. hylambatis	A	55	37.5 (3/8)	20.6±29.8	7.75±19.2	LI
C. pierottii	*C. podicipinus*	A	4	25.0 (2/8)	2.0±1.4	0.5±1.1	LI
	Physaloptera sp.	L	3	12.5 (1/8)	-	0.37±1.1	GM
	Oswaldocruzia sp.	A	3	100.0 (1/1)	-	-	SI
Ce. cranwelli	*A. hylambatis*	A	202	100.0 (1/1)	-	-	LI
	C. podicipinus	A	3	100.0 (1/1)	-	-	SI
S. nasicus	*O. caudacutus*	A	25	25.0 (2/8)	12.5±12.0	3.12±7.35	SI
L. bufonius	*Rhabdias sp.*	A	1	8.3 (1/12)	-	0.08±0.2	LU
	S. travassosi	A	6	33.3 (4/12)	1.5±0.5	0.5±0.7	SI-GB
	A. hylambatis	A	835	83.3 (10/12)	83.5±80.1	69.5±79.4	LI
	C. podicipinus	A	6	25.0 (3/12)	2±1	0.5±1	SI-LI
	S. formosula	A	150	16.6 (2/12)	75±59.3	12.5±34.2	LI
	Physaloptera sp.	L	13	8.3(1/12)	-	1.08±3.7	GM
O. lavillai	*A. hylambatis*	A	44	100.0 (2/2)	22±15.5	11±15.5	LI

Stage: A: adult; L: larval; Site of infection: LU: lung; SI: small intestine; LI: large intestine; GM: gastric mucosa; GB: gallbladder.

Cosmocercidae: *Aplectana hylambatis* (Baylis, 1927) Travassos, 1931 (CECOAL 18042419; 10 FF, 10 MM), *Cosmocerca podicipinus* Baker and Vaucher, 1984 (CECOAL 18042402; 1 F, 1 M), *Oxyascaris caudacutus* (Freitas, 1958) Baker and Vaucher, 1984 (CECOAL 17072407; 5 FF, 1 M); Atractidae: *Schrankiana formosula* Freitas, 1959 (CECOAL 16122124; 10 FF; 10 MM); Physalopteridae: *Physaloptera* sp. (CECOAL 16122136; 5 larvae).

A more precise identification of the specimens of genus *Rhabdias* Stiles and Hassall, 1905 obtained from *R. diptycha* and *L. bufonius*, and those of *Oswaldocruzia* Travassos, 1917 identified in *Ce. cranwelli* was not possible because the number of worms was very low.

Table 2 presents the number of nematode parasites found in each host species, as well as quantitative parasitological descriptors, stage (larval or adult), and site of infection.

Discussion

To our knowledge, no parasites have been reported for *C. pierottii* or *O. lavillai* throughout their geographical ranges. For *Ce. cranwelli*, there is one report about the finding of *Haematoloechus longiplexus* (Trematoda, Plagiorchiidae) from Santa Fe province, Argentina (Hamann and Pérez, 1999). In contrast, *Rhinella diptycha*, *S. nasicus* and *L. bufonius* have been previously analyzed for their parasitic helminths several times and from different localities of their geographical ranges (see Lunaschi and Drago, 2007; Hamann *et al.*, 2013a; Campião *et al.*, 2014; González and Hamann, 2015a).

All the nematode taxa found in this study correspond to generalist species, i.e. they can parasitize a broad range of amphibians, even from different families (Table 3). This was not surprising as generalist parasites are the major components of helminth parasitic communities in amphibians (Aho, 1990).

One of these nematode species, *S. formosula*, has not been reported in amphibians from Argentina until the present work.

Schrankiana formosula is an atractid nematode that has only been found in leptodactylid hosts; it was found in *Leptodactylus fuscus* (Schneider, 1799) and *L. syphax* Bokermann, 1969 from Brazil (Campião *et al*., 2014; Souza Linz *et al*., 2017), and in *L. fuscus* and *L. elenae* Heyer, 1978 from Paraguay (Baker and Vaucher, 1988). Our record is the first for *L. bufonius* throughout the distribution area of this species.

Table 3. Amphibian nematode parasites found in this study and previous reports from Argentina, after the checklist of González and Hamann (2015a). Adult nematodes not identified at species level were not included in this table.

Nematode species	Amphibian species	Locality	References
O. subauricularis	*R. diptycha*£	Matacos Department, Formosa	Present study
	Rhinella major (Müller and Hellmich, 1936)	Corrientes, Corrientes	Hamann et al., 2013b
S. travassosi	*R. major*	Las Lomitas, Formosa; Concepción del Bermejo, Chaco	Hamann and González, 2015
A. hylambatis	*R. major*	Las Lomitas and Ingeniero Juárez, Formosa; Concepción del Bermejo and Taco Pozo, Chaco	Hamann and González, 2015; González et al., 2019
	Trachycephalus typhonius (Linnaeus, 1758)	Pirané, Formosa	Draghi et al., 2015b
	L. bufonius	Las Lomitas, Formosa; Taco Pozo, Chaco	González et al., 2019
		Matacos Department, Formosa	Present study
	Dermatonotus muelleri (Boettger, 1885)	Las Lomitas, Formosa	González et al., 2019
	D. muelleri	Ingeniero Juárez, Formosa	González et al., 2020
	R. diptycha	Matacos Department, Formosa	Present study
	*C. pierottii**	Matacos Department, Formosa	Present study
	Ce. cranwelli *	Matacos Department, Formosa	Present study
	*O. lavillai**	Matacos Department, Formosa	Present study
C. podicipinus	*Leptodactylus elenae; L. podicipinus* (Cope, 1862)	Corrientes, Corrientes	González and Hamann, 2016
	R. major	Las Lomitas, Formosa	Hamann and González, 2015
	P. albonotatus	Corrientes, Corrientes	González et al., 2019
	D. muelleri	Ing. Juárez, Formosa	González et al., 2020
	C. pierottii *	Matacos Department, Formosa	Present study
	Ce. cranwelli *	Matacos Department, Formosa	Present study
	L. bufonius	Matacos Department, Formosa	Present study
O. caudacutus	*S. nasicus*£	Matacos Department, Formosa	Present study
S. formosula	*L. bufonius**£	Matacos Department , Formosa	Present study
Physaloptera sp.	*R. major*	Las Lomitas and Ing. Juárez, Formosa; Concepción del Bermejo, Chaco	Hamann and González, 2015
	Melanophryniscus klappenbachi Prigioni and Langone, 2000	Resistencia, Chaco	Hamann et al., 2014
	*C. pierottii**	Matacos Department, Formosa	Present study
	L. bufonius	Matacos Department, Formosa	Present study

* new host record; £ new geographical record

Campião *et. al.* (2015) established that gastrointestinal roundworms of the family Cosmocercidae are the most reported helminth parasites in South American amphibians. Concurrently, González and Hamann (2015a) found that *Cosmocerca parva* Travassos, 1925 and *C. podicipinus* (Cosmocercidae), collected as adults, are the most commonly occurring nematodes in Argentinean amphibians; each of them was found parasitizing 14 amphibian species. In the present study, cosmocercids were also the most common nematodes; they were represented by the greatest number of species (*A. hylambatis*, *C. podicipinus*, and *O. caudacutus*), and in general, the highest quantitative descriptors. In addition, they parasitized all the amphibian species analyzed, except for the hylid *S. nasicus*. However, it was not a species of the genus *Cosmocerca* Diesing, 1861 the one most frequently occurring, but *A. hylambatis* which presented the highest values of abundance and infection prevalence in the amphibians analyzed (see Table 2). This is in agreement with recent studies on the helminth fauna of anurans from the Dry Chaco ecoregion (Hamann and González, 2015; González *et al.*, 2019; 2020). We herein expand the hosts list of *A. hylambatis* in Argentina to thirteen species (see González and Hamann, 2015a and Table 3).

Nematodes of the families Rhabdiasidae, Cosmocercidae, and Molineidae have direct life cycles (Anderson, 2000); infection may occur in some cases by active penetration of the infective larvae (L3) that occur in the soil and penetrates through the skin, or through the mucosa or eye cavity (e.g., *Rhabdias*, *Oswaldocruzia*, *Cosmocerca*); in others, amphibians ingest the larvae present in the environment and acquire the infection (e.g., *Aplectana*). For *Oxyascaris* Travassos, 1920, no information is available about their life cycle yet.

Nematodes of the family Atractidae have been found in amphibians, reptiles, mammals and fishes. Eggs develop to third-stage larvae in utero and autoinfect the host; these autoinfections are generally accompanied by high intensities, low pathogenesis and large larvae. Their transmission from host to host is not understood for most species (Anderson, 2000).

Of all the nematodes found in this study, only those of the genus *Physaloptera* Rudolphi, 1819 present an indirect life cycle. Amphibians become infected when they consume intermediate hosts (e.g. orthopterans and coleopterans) that contain L3. In amphibians, these nematodes do not develop or grow, and, only complete their life cycle when consumed by the definitive hosts, which include all types of terrestrial vertebrates (Anderson, 2000; de Quadros *et al.*, 2014). Accordingly, the amphibians analyzed in this study are the definitive hosts in the life cycle of eight nematode taxa, while only in the case of genus *Physaloptera* found as larvae, the frogs *L. bufonius* and *C. pierottii* represent paratenic hosts.

In recent years, the Gran Chaco ecoregion has been the focus of relevant studies spanning related topics such as the biodiversity of amphibians, the loss of that diversity as a consequence of human activities, the regional distribution of biodiversity according to land-use and land-cover, and the determination of priority conservation areas (see Medina *et al.*, 2016; Nori *et al.*, 2016; Torres *et al.*, 2014). At this point, we would like to highlight that the loss of amphibian species also represents the loss of a whole other dimension of diversity that is usually not taken into account, i.e. the diversity of organisms that parasitize them. Future studies in new host species, will provide further knowledge of the helminth fauna of these vertebrates in one of the most threatened subtropical woodland savannas in the world.

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Trabajo

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Diet of the Cuban giant frog, *Eleutherodactylus zeus* (Anura: Eleutherodactylidae) in Viñales National Park, Cuba

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ABSTRACT

Eleutherodactylus zeus is an endemic frog of the family Eleutherodactylidae from Western mountains of Cuba, that inhabits in rocky karstic caves and tropical forest adjacent to caves. Here, we report the first approach to the diet of a Cuban cave-rock dwelling amphibian. We surveyed seven cave-karstic habitats and tropical forest habitats adjacent to those caves from May to July. The samples (N = 70) of stomach content were obtained using the stomach-flushing method. Preys were present in 68.6% of stomachs: 76.9% in the forest and 44.4% in the cave samples. The most represented preys in *E. zeus* stomach were Aranae, Orthoptera, Lepidoptera larvae, Blattodea, and Gasteropoda. In addition, we report the presence of an anuran (*Eleuthorodactylus*), a lizard (*Anolis*) and a plathyhelminth in the stomach content of this species. In this species, larger frogs consumed larger and fewer prey, but difference in prey length and number of prey consumed depends on the habitat they temporary exploited.

Key Words: Feeding Ecology, Cuba, *Eleutherodactylus zeus*, Conservation, Caves.

RESUMEN

Eleutherodactylus zeus es una rana endémica de las montañas de la parte más occidental de Cuba, que habita en áreas cársicas, dentro de cuevas y en el bosque adyacente a estas cavidades. Aquí reportamos el primer acercamiento a la dieta de un anfibio petrícola-cavernícola Cubano. Muestreamos siete cuevas cársicas y cuatro áreas de bosque tropical adyacentes a estas cavidades, desde mayo hasta julio. Las muestras (N = 70) del contenido estomacal fueron obtenidas mediante el método de lavado estomacal. Se observaron presas en el 68.6% de los estómagos analizados: el 76.9% en el bosque, y el 44.4% en las cuevas. Esta especie depredó principalmente arañas, grillos, larvas de lepidópteros, cucarachas y moluscos terrestres. Se reporta la presencia de un anuro, un lagarto y un platelminto en el contenido estomacal de esta especie. En *E. zeus*, los individuos más grandes consumieron por lo general presas más grandes, aunque esto varió entre los sexos dependiendo del hábitat donde se encuentren forrajeando.

Palabras claves: Ecología Alimentaria, Cuba, *Eleutherodactylus zeus*, Conservación, Cuevas.

Introduction

Trophic interactions are important to understand the population dynamics of a species because the acquisition of prey resources could affect some factors, such as population densities and individual fecundity (Mahan and Johnson, 2007). In general, anuran´s adults feed on invertebrate community (Duellman and Trueb, 1986), being the rest of species specialist predators (Pertel *et al.*, 2010).

Eleutherodactylus zeus is an endemic species of the family Eleutherodactylidae in Cuba, with a notorious longitude variation between females (127.4 mm, García, 2012) and males (63.7 mm, Schwartz, 1958). This species has a local distribution in rocky karstic areas of Cordillera de Guaniguanico (Henderson and Powell, 2009) where it is reproduce in caves (Alonso *et al.*, 2015). In addition, it also fre-

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quent in the tropical forest habitat adjacent to caves-kartic habitats (Henderson and Powell, 2009). This species is included in the IUCN red list as Endanger B1ab (iii), threatened by habitat loss or fragmentation (Hedges and Díaz, 2004). The foraging behavior and diet of *E. zeus* is unknown as it also for other Cuban *Eleutherodactylus* species. Anecdotal data reported a large roach (*Eurycotis* spp.) disgorged by an adult female (Schwartz, 1958), and a juvenile mollusk in a male (Alonso and Rodríguez, 2003) from Moncada, Viñales. We studied the diet composition of *E. zeus* inside caves-karstic habitats and in the adjacent tropical forest habitat. We expected that *E. zeus* feeds mostly in the forest adjacent to the cave, were there are more prey available that in the cave, as we observed in previous studies (García-Padron, *unpubl. data*).

Materials and methods

Sampling and study sites

We carried out field samples on the cave-karstic habitats on Santo Tomás Great Cavern (STGC), and in the adjacent tropical forest habitat, El Moncada, Viñales, Pinar del Río, Cuba (83º50937.0240 W; 22º32942.8630 N; WGS 84; 130-300 m a.s.l.). Two consecutive nights (21:30-01:00 h) were monitored during each of three months on the rainy season (May, June and July) in 2018. The seven sites in the cave-karstic habitat (CKH hereafter) were Puñales, Incógnita, Increíble, Rivero, Lechuza, Dos Dolinas, and Magalognus. The tropical forest habitat (TFH hereafter) were three karst depressions (*Hoyos*) (Hoyo de Fanía, Hoyo del Yagrumón, and Hoyo de la Palma) and the northeastern slope of Sierra de Quemados. In each TFH and in the CKH, we surveyed one 100 m transect with 3 m each side from the middle of the track; each site (forest or gallery) was at least 150 m apart from any other. The sampled area then was calculated, in both CKH and TFH, as 100 x 6 m in each site surveyed.

We analyzed N = 70 *Eleutherodactylus zeus* individuals (Figure 1A and B), 52 (20 males and 32 females) in TFH and 18 in CKH. Each frog was measured snout–vent length (SVL) with a caliper (to the nearest 0.05 mm), and sexed (following Massip, 2016) *in situ*. All individuals were stomach-flushed following Mahan and Johnson (2007); two different catheter tubes were used (with 4 mm for males and 5.5 mm for females) of outer diameter because of the size difference between sexes. The stomach content was preserved in vials with 75% ethanol for further analysis. After stomach-flushed, each individual was released at the site of capture. In the laboratory we measured the body length of each item (only unbroken items) using a digital caliper (to the nearest 0.01 mm), and classified them to order level when possible using a stereoscope; also data of some samples identified at genus and/or species level are given (Espinosa and Ortea, 2009; Mancina and Cruz, 2017).

We calculate the volume of each item using the ellipsoid formula (Magnusson *et al*., 2003): $V = 4/3\pi$ (lenght/2) (breadth/2)2. In addition, we calculated the number of consumed items (*N*) and its percent ($N_{\%}$); the frequency of occurrence (*F*, number of stomachs in which a given prey category was found), and it's percent ($F_{\%}$), and the volume percent ($V_{\%}$) for each prey category. The Index of Relative Importance (IRI) was calculated for every prey category by IRI=$N_{\%} + V_{\%} + F_{\%} / 3$. We used the Levins´ index (*B´*) (Krebs 1989) to calculate the trophic niche breadth:

were Pi = fraction of items in the food category i; for standardization of niche breadth (B_A), we use

$$ß = \frac{1}{\sum_{i=1}^{n} P_i^2}$$

the Hurlbert (1978) proposal: dividing *B´* by the total number of resource states after correcting for a finite number of resources; range = 0 (no diversity, exclusive use of a single prey type, specialist) to 1 (highest diversity, prey items of all categories, generalist). In addition, we calculated niche overlap between juveniles and adults, and males and females, using Pianka's overlap index (*POI* hereafter) (Pianka 1973), which varies from 0 (no overlap) to 1 (complete overlap).

Karst and forest characteristic

More than 65% of the soil in Cuba is karst (Molerio, 2004), and the typical morphology of the karstic hills of Sierra de los Órganos is conic karst (*mogotes*) with internal karstic valleys (poljes) locally known as *hoyos* (Acevedo-González, 1967). The forest in these karstic hills is named "*complejo de vegetación*" (vegetation complex) because it has a mesophyll semi-deciduous forest distributed in strip-shape at the base of the *mogotes*; a mesophyll evergreen forest in their internal valleys (*hoyos*), and a xerophytic thicket in the upper part in the karstic hill (Luis,

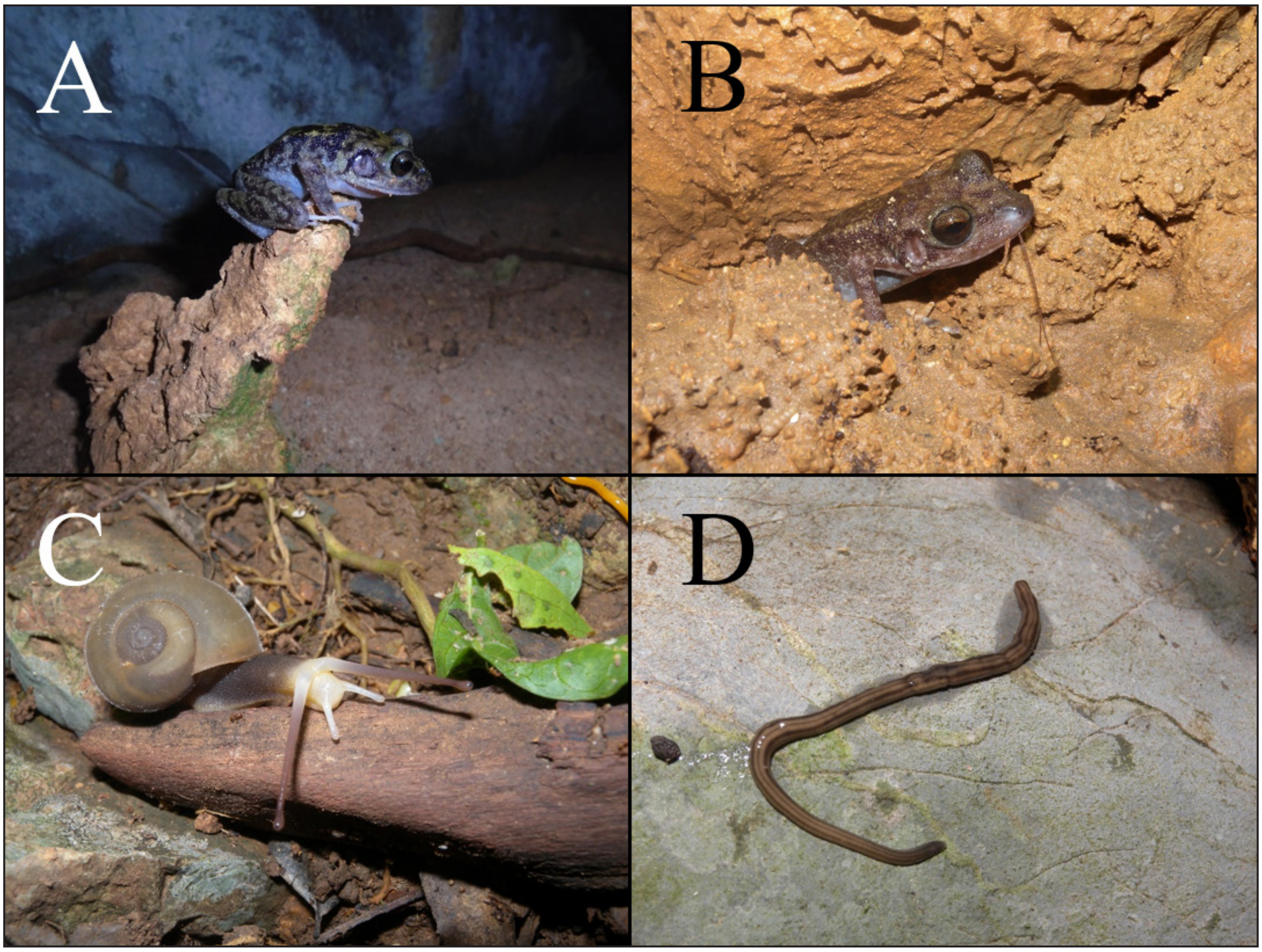

Figure 1. Female of *E. zeus* in hunting attitude inside a gallery (A), a male consuming an *Otteius thoracicus* (B), an adult of the land snail *Satipellis stigmatica* (Stylommatophora) (C), a plathyhelminth in the study area (D). (Photographed by LYGP).

2000). The typical vegetation is composed of *Oxandra lanceolata, Pachira emarginata*, *Spathelia brittonii*, large trees, as *Ficus* spp., had many bromeliads (genus *Tillandsia, Hohenbergia, Guzmania*, etc.).

Statistical analysis
We applied the Kolmogorof-Smirnof normality test and the Levene variance homogeneity test, and for both tests *a*>0.05. We analyzed the ratio of maximum prey size (MPS)/SVL of all individuals with prey in the stomach. We used t-test comparing both cave and forest samples for number of prey/sex, and prey length/sex for independent samples of stomach content. In addition, one factor ANOVA was calculated for the observed differences for prey per stomach.

Results

Were captured 37 females of *E. zeus* (SVL = 90.5±13.54; mean ± 1 SD; range 73.25-127.7 mm), and 33 males (SVL = 68.15±4.08; mean ± 1 SD; range 63.1 to 75.1 mm). Difference were found in abundance in TFH with respect to CKH (*a*=0.008; *df*=9). The tropical forest habitat had with 0.03 frog/m^2, meanwhile, the cave-karstic habitat had 0.004 frog/m^2. The male/female ratio was 0.63/1 in the forest, and 2.6/1 in the galleries.

Forty-eight *E. zeus* (68.6%) had stomach content, and only 22 had empty stomach (31.4%). In the forest, most of frogs (76.9%) had prey in the stomach, only three males and nine females had an empty stomach; on the contrary, in the galleries, most frogs had an empty stomach (seven males and three females), six males and two females were found with prey consumed (44.4%). We observed 131 prey items (2.93 mean prey items per stomach, range= 1-10), representing 26 prey categories, plus plant material and dirt/rock (Table 1). Males consumed more and smaller prey items than females in forested areas; in the galleries, females consumed more prey items at smaller size than males (Table 2).

We observed that *E. zeus* consumed more

Table 1. Diet composition of *Eleutherodactylus zeus* from El Moncada, Viñales, given by Classes and Orders. F, frecuency of occurrence (percent), N, number of consumed items (percent), V, volume of each item (percent), given in mm3, and IRI, is the index of relative importance.

Class	Order	Females (n=25)				Males (n=23)				Total (n=48)			
		F	N	V	IRI	F	N	V	IRI	F	N	V	IRI
Diplopoda	Spirobolida	2 (4.26)	2 (3.77)	32.83 (0.37)	2.8	1 (2.33)	1 (1.89)	39.86 (0.74)	1.65	3 (3.37)	3 (2.83)	36.35 (0.42)	2.21
	Polydesmida	1 (2.13)	1 (1.89)	172.69 (1.96)	1.99	0	0	0	0	1 (1.12)	1 (0.94)	172.69 (1.99)	1.35
Arachnida	Amblypygi	1 (2.13)	1 (1.89)	420.15 (4.77)	2.93	2 (4.65)	2 (3.77)	90.51 (1.67)	3.36	3 (3.37)	3 (2.83)	255.33 (2.94)	3.05
	Uropygi	2 (4.26)	2 (3.77)	**2533.81** **(28.78)**	12.27	1 (2.33)	1 (1.89)	**2253.05** **(41.69)**	15.3	3 (3.37)	3 (2.83)	2393.43 (27.56)	**11.25**
	Aranae	4 (8.51)	5 (9.43)	18.09 (0.21)	6.05	7 (16.28)	7 (13.21)	5.64 (0.1)	9.86	**11** **(12.36)**	**12** **(11.32)**	11.87 (0.14)	7.94
	Opiliones	0	0	0	0	1 (2.33)	1 (1.89)	6.09 (0.11)	1.44	1 (1.12)	1 (0.94)	6.09 (0.07)	0.71
	Scorpiones	2 (4.26)	2 (3.77)	**2546.01** **(28.92)**	12.32	1 (2.33)	1 (1.89)	378.22 (7.0)	3.74	3 (3.37)	3 (2.83)	1462.12 (16.84)	7.68
Hexapoda	Orthoptera	2 (4.26)	2 (3.77)	149.41 (1.7)	3.24	7 **(16.28)**	7 **(13.21)**	**856.44** **(15.85)**	**15.11**	**9** **(10.11)**	**9** **(8.49)**	502.93 (5.79)	8.13
	Hymenoptera	2 (4.26)	4 (7.55)	0.06 (0.0007)	3.94	1 (2.33)	6 (11.32)	0.38 (0.01)	4.55	3 (3.37)	10 (9.43)	0.22 (0.003)	4.27
	Diptera	0	0	0	0	4 (9.3)	7 (13.21)	2.48 (0.05)	7.52	4 (4.49)	7 (6.6)	2.48 (0.03)	3.71
	Lepidoptera (larvae)	5 (10.64)	5 (9.43)	37.89 (0.43)	6.83	4 (9.3)	5 (9.43)	59.33 (1.1)	6.61	**9** **(10.11)**	**10** **(9.43)**	48.61 (0.56)	6.7
	Blattodea	5 (10.64)	5 (9.43)	262.76 (2.98)	7.68	4 (9.3)	4 (7.55)	42.16 (0.78)	5.88	**9** **(10.11)**	**9** **(8.49)**	152.46 (1.76)	6.79
	Coleoptera	3 (6.38)	3 (5.66)	95.02 (1.08)	4.37	3 (6.98)	4 (7.55)	82.89 (1.53)	5.35	6 (6.74)	7 (6.6)	88.96 (1.02)	4.79
	Zygentoma	0	0	0	0	1 (2.33)	1 (1.89)	106.94 (1.98)	2.07	1 (1.12)	1 (0.94)	53.47 (0.62)	0.89
	Hemiptera	1 (2.13)	1 (1.89)	554.52 (6.3)	3.44	0	0	0	0	1 (1.12)	1 (0.94)	554.52 (6.39)	2.82

	Not-identified insect	1 (2.13)	1 (1.89)	0.46 (0.01)	1.34	1 (2.33)	1 (1.89)	0.02 (0.0004)	1.41	2 (2.25)	2 (1.89)	0.24 (0.03)	1.39
Chilopoda	Scolopendromorpha	1 (2.13)	1 (1.89)	26.4 (0.3)	1.44	1 (2.33)	1 (1.89)	13.54 (0.25)	1.49	2 (2.25)	2 (1.89)	19.97 (0.23)	1.46
Clitellata	Haplotaxida	0	0	0	0	1 (2.33)	1 (1.89)	488.28 (9.03)	4.42	1 (1.12)	1 (0.94)	488.28 (5.62)	2.56
Turbellaria	Tricladida	2 (4.26)	2 (3.77)	57.18 (0.65)	2.89	0	0	0	0	2 (2.25)	2 (1.89)	57.18 (0.66)	1.6
Gastropoda	Stylommatophora	**6** **(12.77)**	7 (13.21)	**1445.91** **(16.42)**	**14.13**	0	0	0	0	**6** **(6.74)**	**7** **(6.6)**	1445.91 (16.65)	**10.0**
	Littorinimorpha	2 (4.26)	2 (3.77)	93.2 (1.06)	3.03	0	0	0	0	2 (2.25)	2 (1.89)	93.2 (0.11)	1.42
	Cycloneritimorpha	2 (4.26)	4 (7.55)	22.81 (0.26)	4.02	1 (2.33)	1 (1.89)	11.39 (0.21)	1.48	2 (2.25)	5 (4.72)	17.1 (0.02)	2.33
	Not-identified gasteropod	1 (2.13)	1 (1.89)	0.41 (0.005)	1.34	0	0	0	0	1 (1.12)	1 (0.94)	0.41 (0.005)	0.69
Crustacea	Isopoda	1 (2.13)	1 (1.89)	267.87 (3.04)	2.35	0	0	0	0	1 (1.12)	1 (0.94)	267.87 (3.09)	1.72
VERTEBRATA													
Reptilia	Squamata	0	0	0	0	2 (4.65)	2 (3.77)	967.43 (17.9)	8.77	2 (2.25)	2 (1.89)	483.72 (5.57)	3.24
Amphibia	Anura	1 (2.13)	1 (1.89)	67.48 (0.77)	1.6	0	0	0	0	1 (1.12)	1 (0.94)	67.48 (0.78)	0.95
Plant material		13	0	0	0	6	0	0	0	19	0	0	0
dirt/rocks		2	0	0	0	3	0	0	0	5	0	0	0

Table 2. Number of stomachs with prey and prey length between sexes of *E. zeus*.

	Males		Females	
	Galleries	Forest	Galleries	Forest
Prey/stomach	1,5 (1-2) (N=6)	2,59 (1-10) (N=17)	2,5 (1-4) (N=2)	2,13 (1-4) (N=23)
N items	9 (N=6)	44 (N=17)	5 (N=2)	49 (N=23)
Prey length (mm)	20,97 (7,53-54,0) (N=7)	11,62 (0,78- 64,58) (N=28)	5,92 (1,17- 6,42) (N=4)	14,30 (2,35- 40,6) (N=35)

amounts of prey, and types of prey ($\alpha \leq 0.05$) in the forest than in galleries (Table 3). No difference were found in number of prey per stomach ($\alpha=0.928$), and prey length ($\alpha=0.998$), in males and females. Aranae was the most abundant prey in the stomachs ($F_{\%}=12.36$; $IRI=7.94$), detected in 27.5% of frogs in the forest, followed by Lepidoptera larvae ($F_{\%}=10.11$; $IRI=6.7$), Orthoptera ($F_{\%}=10.11$; $IRI=8.13$), Blattodea ($F_{\%}=10.11$; $IRI=6.79$), and Gasteropoda ($F_{\%}=6.74$; $IRI=10.0$) (Table 1). Females consumed more type of preys ($B_A=0.55$) than males ($B_A=0.39$) ($\alpha<0.05$). In the forest, individuals consumed more type of preys (females, $B_A=0.53$; males, $B_A=0.39$) than in the galleries (females, $B_A=0.12$; males, $B_A=0.17$) ($\alpha<0.05$). Difference was observed in prey preference was observed between males and females: Females consumed mainly Gasteropoda ($F_{\%}=12.77$; $IRI=14.13$), meanwhile males preferred Orthoptera ($F_{\%}=16.28$; $IRI=15.11$) and Aranae ($F_{\%}=16.28$; $IRI=9.86$). We observed high overlap between males and females ($POI=0.65$): in Aranae, Lepidoptera (larvae), Blattodea, and Coleoptera (Table 1). The higher overlap occur in the forest ($POI=0.64$), but no overlap was detected in the gallery ($POI=0.00$).

Some taxa were able to be identified to genus or species: amblypygid (*Phrynus* sp.), uropygid (*Mastigoproctus* sp.), cockroaches (*Eurycotis* sp., *Euthlatoblatta diaphana*, and *Nauphoeta cinerea*), scorpion (*Centruroides* cf. *guanensis*), crickets and grasshoppers (*Otteius thoracicus* and *Gryllodes* sp.), land snails (*Setipellis stigmatica* [Fig. 1C], *Alcadia* sp., *Zachrysia* cf. *guanensis*, and *Chondropometes* sp.), a partially digested individual of *Eleutherodactylus*, an egg and a juvenile of *Anolis* sp.

Discussion

In the present study the diet of *E. zeus* was first described from two different habitats. A higher number of empty stomachs (55.5%) and a lower dietary richness of frogs from the CKH of frogs from the CKH when compared with TFH indicate that this species forage mostly in the later habitat. Limitation in prey abundance carry to a high number of individuals without prey in their stomachs, whereas a lower dietary diversity is indicative of a greater resource availability (Toft, 1980; Whitfield and Donnelly, 2006; Luría-Manzano and Ramírez-Bautista, 2017). However, some studies pointed out that the abundance and diversity of arthropods are lower inside caves than in surroundings (Prous *et al.*, 2004; Tobin *et al.*, 2013). The higher richness of prey consumed by *E. zeus* in the forest, seems to reveal that total prey availability, independent of prey taxa, is lower in the caves of STGC. However, an adequate abundance or density research of invertebrate fauna in this area is required to corroborate this hypothesis.

In this study, most *E. zeus* were observed in the TFH adjacent to CKH of STGC during the study (May to July). We observed that females leave the galleries after hatching, but males apparently stay much longer and can feed upon the cave resources (García-Padrón, *unpubl. data*). This could explain why males were more abundant than females in galleries (2.6/1, male/female rate), in spite of the small sample size, whereas in the forest the opposite occurred (0.63/1) during the study time. High density of frogs, less empty stomachs, and more dietary taxa consumed in the TFH may indicate that *E. zeus* leave the CKH after reproduction, as observed Alonso *et al.* (2015). The migration to adjacent forest to feed may be explained in terms of high diversity of arthropods (Prous *et al.*, 2004; Tobin *et al.*, 2013).

Despite high overlap between males and females of *E. zeus* ($POI=0.65$), females preferred gastropods ($F_{\%}=23.4$; $IRI_{\%}=22.52$), and males, Orthoptera and Aranae ($F_{\%}=32.56$; $IRI_{\%}=24.96$, combined). Large preys are also consumed (females: Uropygi and scorpions; Males: Uropygi), but no preference for these type of prey seems to occur because of its

Table 3. Percent of prey categories in the stomach content of *E. zeus* in forest and galleries. The "% total" represents the percent from the total of prey category in both ecosystems.

Class	Order	Galleries (N=8)		Forest (N=40)	
		N	% total	N	% total
Diplopoda	Spirobolida	0	0	3	7.5
	Polydesmida	0	0	1	2.5
Arachnida	Amblypygi	1	12.5	2	5
	Uropygi	0	0	3	7.5
	Aranae	1	12.5	11	27.5
	Opiliones	0	0	1	2.5
	Scorpiones	1	12.5	2	5
Hexapoda	Orthoptera	2	25	8	20
	Hymenoptera	2	25	8	20
	Diptera	0	0	7	17.5
	Lepidoptera	2	25	8	20
	Blattodea	2	25	7	17.5
	Coleoptera	1	12.5	6	15
	Zygentoma	0	0	1	2.5
	Hemiptera	0	0	1	2.5
	NI 1	0	0	2	5
Chilopoda	Scolopendromorpha	0	0	2	5
Annelida, Class Clitellata	Haplotaxida	0	0	1	2.5
Phyllum Nemertina	NI 2	0	0	2	5
Gastropoda	Stylommatophora	1	12.5	6	15
	Littorinimorpha	1	12.5	1	2.5
	Cycloneritimorpha	0	0	5	3.47
	NI 3	0	0	1	2.5
Crustacea	Isopoda	0	0	1	2.5
VERTEBRATA					
Reptilia	Squamata	0	0	2	5
Amphibia	Anura	0	0	1	2.5
Plant material		2	25	17	42.5
dirt/rocks		1	12.5	4	10
TOTAL	17		144		

low frequency (Table 1). In the five cases (see Table 1), no more than a single (large) prey per stomach was observed. Seems that large prey, besides occupy most of its stomach capacity, can fulfill its nutritious needs in this species. No overlap in the galleries were observed, maybe because most of *E. zeus* had empty stomach, and the number of frogs with prey in the stomach were too low.

With these results, we reinforced previous unpublished data regarding to ethology of *E. zeus*. This species remain into forest for feeding and gaining energy after reproduction, whereas males spend more time in the caves exploiting the food resources available. The cockroaches were an important component in the diet of *E. zeus* (see Tables 1 and 2). Schwartz (1958) observed a large roach (*Eurycotis*) disgorged by an adult female; we observed and identified three species of roaches: *Eurycotis* sp., *Euthlatoblatta diaphana* and *Nauphoeta cinerea*. Alonso and Rodríguez (2003) report a mollusk in a juvenile of the Cuban Giant Frog. In this study, four species of land snails (*Setipellis stigmatica* [Figure 1C], *Alcadia* sp., *Zachrysia* cf. *guanensis*, and *Chondropometes* sp.), plus one not identified specimen were observed in *E. zeus* stomach content. From all organisms identified in the cave samples, the cave cricket (*Otteius thoracicus*) (Figure 1B) was the most common prey observed in the stomach of this species. Rodríguez-Cabrera and Torres (2019) reported the presence of the exotic land platyhelminth *Bipalium kewense* for central and western Cuba. We detected the presence of a Platyhelminthes (order Tricladida, family Geoplanidae), probably *B. kewense* (see Rodríguez-Cabrera and Torres, 2019), consumed by two females (91.6mm and 94.4mm SVL) in the forest; this is the first record of a non-parasitic land Plathyhelminthes consumed by an anuran (Figure 1D). Here, we report the presence of an *Eleutherodactylus* frog in the stomach of a female (74.35mm) in the forest; this item was partially digested, but according to the size of it (8.26mm of maximum length) could be a neonate of *E. zeus* or an adult of *E. klinikowskii*, both very common on the forest floor. Also in the forest we found a male (67.9mm SVL) with a partially digested individual (probably female) and an egg of *Anolis*; could be *A. homolechis* or *A. mestrei,* because both species seek refuge on the forest floor in that area during nighttime.

Difference exploitation of dietary resources in each habitat was detected. Females consumed more type of prey than males in the TFH; on the contrary, in the CKH males had broaded diet than females. In *E. zeus* no significant difference was observed in prey length between sexes, but larger individual consumed larger preys; although, this role apparently can change between sexes, and depends on the habitat they temporarily exploited. We observed that males of *E. zeus* consumed more and smaller prey than females in the forest; meanwhile females, on the contrary, consumed more prey items at smaller size than males in the caves (Table 2).

We can classify properly *Eleutherodactylus zeus* as a "non-ant specialist" and a "sit-and-wait" predator according to Toft (1981): because of the type of prey consumed (large and solitary prey, such as spiders, mollusks, crickets, roaches, and caterpillars), its crypticity, its wide mouth, and the lower number of prey consumed (2.93 prey/stomach). We observed that this frog forages from the ground to 2 m above ground level, on rocks, walls of galleries, or trunks of bushes, ambushing its prey. This ambushed attitude is largely known for anurans (Pertel *et al*., 2010).

The generalized diet of *Eleutherodactylus zeus* seems to have contributed to its success in both caves and forest, but the growing modification and/or destruction of the habitat, and consequently the disturbance in the food web, may determinate the future, survival and emergence of morphological abnormalities (García-Padrón and Alonso, 2019; García-Padrón *et al*., 2020) of this local endemic and ecologically restricted frog.

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Trabajo

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Variation in feeding ecology of five cnemidophorine lizard species along Brazilian eastern coast

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ABSTRACT
Feeding ecology of a particular species is associated to population dynamics and contributes for the understanding of natural history and trophic interactions in ecosystems. We investigated changes in the feeding habits of 16 populations belonging to five cnemidophorine lizard species (being four bisexual and one unisexual) along approximately 4000 km extension of the Brazilian eastern coast. Fieldwork was carried out in 15 areas of restinga habitats and for each cnemidophorine population, the composition of the diet was analyzed based on the number, volume (mm^3) and frequency of each prey category or plant material.The arthropods were categorized in the taxonomic level of Order or Family (e.g.Formicidae). Cnemidophorine populations/species studied were mainly carnivorous and had, in general, a diet consisting predominantly of larvae and/or termites, with few instances of plant material consumption. The availability of termites locally at each restinga habitat was not a determinant factor in the increase of termite consumption by the local cnemodophorine population. However, differences in diet composition among populations partially resulted from differential consumption of termites, leading to the formation of two distinct groups depending on higher or lesser consumption of termites. Some populations had onthonegetic variation in diet, but males and females of different populations/species tended to have similar diet composition. The head width of lizards affected significantly the volume and the length of the largest prey ingested in 60% (3/5) of the species studied, indicating that adults tended to consume larger food items compared to coespecifics juveniles. The high level of importance of termites and larvae in almost all populations/species probably contributed to the low intra-and interspecific differences in food habit. The diet of cnemidophorine species studied in restinga habitats in general, tended to be similar to that found to other cnemidophorines, regardless its geographic distribution. Invertebrates were the dominant prey on cnemidophorines diet, but predation on vertebrates was also registered.

Key Words: Diet; Restinga; Teiidae; Whiptail Lizard; Sand Dune.

RESUMO
A ecologia alimentar de uma determinada espécie está associada à dinâmica da população e contribui para a compreensão da história natural e das interações tróficas nos ecossistemas. No presente estudo, investigamos mudanças nos hábitos alimentares de 16 populações pertencentes a cinco espécies de lagartos cnemidophorines (quatro bissexuais e uma unissexual) ao longo de aproximadamente 4000 km da costa leste brasileira. O trabalho de campo foi realizado em 15 áreas de restinga. A composição da dieta de cada população foi baseada no número, volume (mm3) e frequência de cada categoria de presa e de material vegetal. Os artrópodes foram categorizados no nível taxonômico de Ordem ou Família (por exemplo, Formicidae). As populações/espécies de cnemidophorines estudadas foram principalmente carnívoras com uma dieta constituída predominantemente por larvas e/ou cupins, com pouco consumo de material vegetal. A disponibilidade de cupins localmente em cada restinga não foi um fator determinante no aumento do consumo de cupins pela população do lagarto cnemidophorino local. No entanto, as diferenças na composição da dieta entre as populações resultaram do consumo diferencial de cupins, levando à formação de dois grupos distintos, dependendo do consumo maior ou menor de cupins. Algumas populações apresentaram variação ontogenética na dieta, mas machos e fêmeas tenderam a ter uma composição alimentar semelhante em cada população/espécie. A largura da cabeça afetou significativamente o volume e o comprimento da maior presa ingerida

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em 60% (3/5) das espécies estudadas, indicando que os adultos tendem a consumir itens alimentares maiores que os jovens coespecíficos. O elevado índice importância de cupins e larvas em quase todas as populações/espécies provavelmente contribuiu para as baixas diferenças intra e interespecíficas no hábito alimentar. A dieta das espécies estudadas, em geral, tendeu a ser semelhante à encontrada para outros cnemidophorinos, independentemente de sua distribuição geográfica. Os invertebrados foram as presas predominantes na dieta, mas a predação em vertebrados foi também registrada.

Palavras-chave: Dieta; Restinga; Teiidae; Whiptail lizard; Sand Dune.

Introduction

Feeding ecology of a particular species is associated to population dynamics and contributes for the understanding of natural history and trophic interactions in ecosystems, being essential to the comprehension of the amounts of energy allocated to growth (body size and mass), maintenance, reproduction and storage (e.g. Doughty and Shine, 1997; Huey *et al.,* 2001). Parameters of prey consumption of a species or related group of lizard species may vary along space, including the energy balance of each population (Flynn et al., 2020). To keep an appropriated energy balance, active foraging lizards tend to consume prey with low mobility (e.g. insect larvae), with an aggregated distribution in the environment (like termites) and usually in large numbers per unit of time spent foraging compared to sit-and-wait lizards (e.g. Huey and Pianka, 1981; Pianka, 1986; Bergallo and Rocha, 1994).

Cnemidophorine lizards are active foragers and occur only in America (e.g. Wright, 1993; Reeder *et al.,* 2002; Harvey *et al.,* 2012). They are usually found in open habitats with sandy soil (e.g. Schall and Ressel, 1991; Dias and Rocha, 2007), high temperatures (e.g. Menezes and Rocha, 2011) and relative low humidity (e.g. Pianka, 1970; Vitt *et al.,* 1993). Although cnemidophorines species apparently have a diverse diet, prey like insect larvae and/or termites generally predominate in their diets, which seems to result from active foraging strategy (e.g. Pianka, 1977, 1986; Magnusson *et al.,* 1985; Bergallo and Rocha, 1994; Menezes *et al.,* 2006; 2008). Only two insular species (*Cnemidophorus arubensis* - Schall and Ressell, 1991 and *C. murinus* - Dearing and Schall, 1992) are known to have preference for ingesting plant material (flowers, fruits and leaves).

The geographical variation in environmental conditions is an important factor that can influence some ecological patterns of lizards' populations/species like food habit (e.g. Vitt *et al.,* 1998; Siqueira *et al.,* 2013). The diet of brazilian cnemidophorine lizards is known primarily for isolated populations of bisexual species (e.g. Vitt, 1991; Magnusson and Silva, 1993; Mesquita and Colli, 2003; Teixeira-Filho *et al.,* 2003; Menezes *et al.,* 2006; 2011; Dias and Rocha, 2007) with few studies addressing geographical variation in diet composition for populations/species, especially parthenogenetic ones (e.g. Bergallo and Rocha, 1994; Mesquita and Colli, 2003; Menezes *et al.,* 2008). It is expected that different populations of a particular species , despite having a same foraging strategy, might differ in their diet composition especially for those with broad geographic distribution as a result of local differences in prey availability (Cooper and Vitt, 2002).

In Brazil, some fragments of restinga habitats (plain sand-dunes) are found along approximately 4000 km of the coast. Five cnemidophorines species (see Pyron *et al.,* 2013 and Goicoechea *et al.* 2016 for a nomenclature review) (*Ameivula ocellifera, Glaucomastix abaetensis, Ameivula nativo, Glaucomastix littoralis* and *Contomastix lacertoides*) are distributed along these remnants (Menezes and Rocha, 2013), being good models to study geographical variation in diet among populations and species. In this study, we investigated changes in feeding strategies and diversity of diet of 16 populations belonging to these five cnemidophorine species (being four bisexual and one unisexual - *A. nativo*) along the coast of Brazil. Specifically, we aimed to (1) evaluate the diet composition of the different bisexual and unisexual populations/species throughthout its geographic distribution along Brazilian coast, (2) determine to what extent the consumption of termites and larvae would be similar among species/populations, (3) to evaluate if the preference by termites of a local cnemidophorine population is related to the frequency of termite nests locally at each restinga, (4) to evaluate if the diversity of prey consumed follows a latitudinal pattern.

Materials and methods

Study area

Fieldwork was carried out in 15 areas of restinga habitats along approximately 4000 km of the Brazilian eastern coast (Fig. 1). Restingas are coastal sandy dune habitats located between the sea and the mountains of the Brazilian eastern coast and are part of the Atlantic Forest biome. This habitat originated in the Quaternary as a result of successive marine regressions which occurred throughout the Holocene and Pleistocene periods (Suguio and Tessler, 1984).

Figure 1. Distribution of cnemidophorine species occurring in the restinga habitats along the eastern coast of Brazil.

Sampling Methods and Analysis

All samples were carried out during the rainy season (October-May) along three years (2004-2006, depending on the area), excepting for Guaratiba, where we also included data from the dry season to expand the sample size, since we did not find differences in diet between seasons.

We followed the procedures of the Society for the Study of Amphibians and Reptiles outlined in the *Guidelines for Use of Life of Amphibians and Reptiles in Field Research,* which recommend the use of anesthetics prior to euthanizing the animals. Lizards were collected during their activity period (09:00-16:00 h) with rubber bands or pellet rifles, euthanized with ether and immediately fixed in 10% formalin.

In the laboratory, we measured the snout–vent length (SVL) and head width of each individual collected, with a Vernier caliper (accuracy of 0.1 mm) and tested the morphological differences between sexes by ANOVA (Zar, 1999). The stomach contents of lizards were counted and identified. The arthropods were categorized in the taxonomic level of Order or Family (in the case of Formicidae). Unidentified arthropod remains were grouped in a separate category ("unidentified parts of arthropods") and were considered only for volumetric analyses. For each cnemidophorine population, the composition of the diet was based on the number, volume (mm^3) and frequency of each prey category. Each food item was measured in its length and width with a Vernier caliper (to the nearest 0.1 mm), and its volume was estimated by the ellipsoid formula: $4/3\pi$ (length/2) (width/2)^2 (Dunham, 1983). The number of food items was counted and the mean length and the mean volume of the five largest prey were estimated for each lizard. We used the largest prey length and higher prey volume for regression statistics and analysis of variance with the morphological variables of lizards. To perform statistical analysis, data were tested for normality (Kolmogorov-Smirnov test, Lilliefors´ correction) and homoscedasticity (Zar, 1999). Due to the great variation in length, volume, and number of items found in stomachs, these variables were converted to their decimal logarithm.

The differences in prey consumption based on the number, volume and length of the largest prey (log transformed) among different populations/species and, within the same population, between males and females and between juveniles and adults, were tested by analysis of variance for one factor (One-Way ANOVA) (Zar, 1999). For each lizard population, the value of the relative importance index (I_x) for each category of prey in the diet of the lizards was estimated by the sum of the proportional values of volume, number and frequency of occurrence of prey in the diet divided by three (see Howard *et al.,* 1999).

To estimate an index of density of termites (one of the most consumed prey type by cnemidophorine species) in each area sampled, we established straight-line transects of 500m. We recorded termite nests within 5 m to each side of the observer, totaling 0.5 ha sampled area (500 m length x 10 m width of transect). For each area, the relative availability of termite nests was expressed as the number of termite nests recorded per hectare. The association between the relative availability of termite nests in the environment and number (percentage) and frequency of termites found in the diet of each population studied (arcsine transformed) was tested using Spearman rank correlation analysis (Zar, 1999). The relationship between the number/volume of termites and larvae consumed in each population was tested by Spearman rank correlation (Zar, 1999). Latitudinal variation in the comsumption of termites was tested by linear regression analysis after removing the effect of SVL.

To assess differences in the feeding patterns among the populations and species studied, similarity analysis was performed by Non-Metric Multidimensional Scaling (NMDS) (McCune and Grace, 2002), applying the Bray-Curtis distance. The lines (objects) of the NMDS matrix were the populations, the columns (variables) were food categories, and cells contained numeric percentage of prey values. These data were reduced to one dimension and related with the latitude by simple regression analysis (Zar, 1999). Descriptive statistics are presented throughout the text as mean ± standard deviation. Statistical analyses were performed using Systat 11.0 (Wilkinson, 1990) and R program.

Results

A total of 566 individuals of cnemidophorines were analyzed in this study, ranging from 23 specimens of *Glaucomastix abaetensis* to 241 specimens of *A. nativo* (Table 1 and 2). Lizard species varied significantly in body size (SVL of adults) (ANOVA, $F_{4,342} = 55.581$, $R^2 = 0.394$, $P < 0.001$), being *A. ocellifera* the smallest and *G. littoralis* the largest one (Table 1). Intraspecifically, the populations of *Ameivula nativo* differed in SVL (ANOVA, $F_{5,122} = 7.256$, $R^2 = 0.229$, $P < 0.001$), with individuals from Setiba (ES) differing from other populations (Table 1). *Ameivula ocellifera* populations also varied in SVL (ANOVA, $F_{4,138} = 5.077$, $R^2 = 0.128$, $P = 0.001$), with Genipabu individuals being, in average, larger than individuals from Piaçabuçu (AL) and Praia do Porto (SE) (Post hoc Scheffe $P < 0.05$) (Table 1). *Glaucomastix littoralis* populations did not vary in SVL (ANOVA, $F_{2,61} = 2.544$, $R^2 = 0.077$, $P = 0.087$). Males, in general, had larger head width than females (pooled data for each species, Table 1).

Only 4.2% (24/567) of the lizards analysed had empty stomachs, being the population of *G. littoralis* in Grussaí (BA) the one with the highest proportion of empty stomachs (Table 2). In general, prey types consumed varied from eight to 16 (Table 2) among populations/species. The diet of cnemidophorines was composed mainly of arthropods, especially insect larvae and termites (Table 2, see attached material). The population of *C. lacertoides* in the restinga of Joaquina (SC) was the only one in which larvae and/or termites did not constitute one of the most important items in the diet of coastal cnemidophorine lizards (Table 2). Lizards from Comboios, ES (*A. nativo*) and from Barra dos Coqueiros, SE (*A. ocellifer*) consumed few termites, but larvae were the most important prey in the diet (Table 2, see attached material).

The number of prey consumed by individuals varied among the populations/species (ANCOVA, $R^2 = 0.17$, $F_{14,1,400} = 5.690$, $P < 0.001$), however only the population of Joaquina, SC (*C. lacertoides*) differed from others (*A. ocellifera* in Guarajuba, BA; *A. ocellifera* in Piaçabuçu, AL; *A. nativo* in Guriri, ES and *G. littoralis* in Maricá, RJ - Post hoc Scheffe $P < 0.05$) (Table 2). For most populations, there were no sex or ontogenetic variation in the mean number of prey consumed by individuals, except for *A. nativo* from Maraú, where juveniles consumed a smaller number of prey, and for *A. ocellifera* from Barra dos Coqueiros, where females consumed a greater number of prey (Table 3).

The volume of the largest prey consumed varied among populations/species (ANCOVA, $R^2 = 0.31$, $F_{14,1,400} = 4.625$, $P < 0.001$), but the post hoc test was significant only between populations of Comboios, ES (*A. nativo*) and of Joaquina, SC (*C. lacertoides*) (Post hoc Scheffe, $P < 0.05$). Males and females did not vary regarding prey volume consumed (Table 3) in each population. For some populations, juveniles ingested a smaller volume of prey than adults did, but it is not a rule for most populations/species (Table 3).

The mean length of the largest prey consumed by lizards varied among populations/species (ANCOVA, $R^2 = 0.25$, $F_{15,1,380} = 5.610$, $P < 0.001$), but the Post Hoc Scheffe showed no differences between populations/species (Table 2). Males and females

Table 1. Morphological measurements (snout-vent length and head width, in mm) of cnemidophorines in the coast of Brazil. Data are presented as mean + SD (in bold), range in parenthesis and N is the sample size. The columns ANOVA have the results of the variation in snout-vent length and in head width between sexes (adults).

Species	Localities	Snout-vent length (mm)					Head width (mm)			
		Juveniles	Adults	Males	Females	ANOVA between sexes	Juveniles	Males	Females	ANOVA between sexes
Contomastix lacertoides	Joaquina, SC	50.0 N =1	**58.7 ± 4.4** (44.8 - 65.8) N = 39	**56.0 ± 4.4** (44.8 - 62.6) N = 25	**57.9 ± 4.2** (51.1 - 65.8) N = 14	$F_{1,37} = 1.827$ $R^2 = 0.047$ P = 0.185	8.3 N = 1	**8.8 ± 0.9** (7.4 -10.5) N = 25	**8,1 ± 0,5** (7,4 a 8,9) N = 14	$F_{1,36} = 22.827$ $R^2 = 0.544$ $P < 0.001$
Glaucomastix littoralis	Maricá, RJ	**39.1 ± 3.0** (34,9 - 43,1) N = 9	**66.4 ± 5.2** (56.7 - 74.8) N = 16	**69.0 + 4.1** (64.4 - 74.8) N = 8	**63.9 + 5.2** (56.7 - 74.3) N = 8	$F_{1,14} = 4.782$ $R^2 = 0.255$ $P < 0.05$	**6.5 ± 0.4** (5.9 - 6.9) N = 9	**12.6 ± 1.5** (9.9 -14.2) N = 8	**9.5 ± 0.9** (8.6 -10.6) N = 8	$F_{1,14} = 23.708$ $R^2 = 0.629$ $P < 0.001$
	Jurubatiba, RJ	**43.6 ± 7.5** (37,4 - 52,0) N = 3	**62.7 ± 6.5** (48.0 - 76.1) N = 29	**63.4 + 7.7** (48.0 -76.1) N = 19	**61.1 + 2.7** (57.7 - 66.0) N = 10	$F_{1,27} = 0.809$ $R^2 = 0.029$ P = 0.377	**6.2 ± 0.4** (5.9 - 6.7) N = 3	**9.1 ± 1.2** (7.0 - 11.0) N = 16	**8.5 ± 0.7** (8.0 - 9.9) N = 8	$F_{1,22} = 1.348$ $R^2 = 0.058$ P = 0.258
	Grussaí, RJ	**37.9 ± 6.5** (31,5 - 52,4) N = 8	**62.0 ± 6.9** (48.6 - 71.6) N = 19	**60.9 + 7.8** (48.6 -71.6) N = 9	**62.9 + 6.1** (54.1 - 69.2) N = 10	$F_{1,17} = 0.396$ $R^2 = 0.023$ P = 0.537	**6.1 ± 0.6** (5.5 - 7.6) N = 8	**9.2 ± 1.4** (7.6 - 11.6) N = 9	**8.9 ± 0.9** (7.4 - 10.6) N = 10	$F_{1,17} = 0.342$ $R^2 = 0.020$ P = 0.566
	Pooled data	**39.3 ± 5.4** (31.5 - 52.4) N = 20	**63.7 ± 6.3** (48.0 - 76.1) N = 64	**39.3 ± 5.4** (31.5 - 52.4) N = 20	**62.6 ± 4.8** (54.1 - 74.3) N = 28	$F_{1,62} = 0.814$ $R^2 = 0.013$ P = 0.370	**6.3 ± 0.5** (5.5 - 7.6) N = 20	**10.0 ± 2.0** (7.0 - 14.2) N = 33	**9.0 ± 0.9** (7.4 - 10.6) N = 26	$F_{1,56} = 5.146$ $R^2 = 0.533$ $P < 0.05$
Ameivula nativo	Setiba, ES	**39.2 ± 49.7** (39.2 - 49.7) N = 7	**61.7 ± 8.0** (50.6 - 79.5) N = 35		**61.7 ± 8.0** (50.6 - 79.5) N = 35		**7.2 ± 0.5** (6.2 - 7.6) N = 7		**8.6 ± 0.9** (7.1 - 10.4) N = 35	
	Comboios, ES	**43.3 ± 4.1** (33.6 - 49.7) N = 27	**57.3 ± 5.8** (47.2 - 63.7) N = 16		**57.3 ± 5.8** (47.2 - 63.7) N = 16		**6.3 ± 0.6** (5.0 - 7.3) N = 30		**8.3 ± 0.7** (7.4 - 9.5) N = 13	
	Guriri, ES	**42.2 ± 3.6** (36.3 - 46.6) N = 14	**54.1 ± 5.8** (46.9 - 65.1) N = 20		**54.1 ± 5.8** (46.9 - 65.1) N = 20		**6.1 ± 0.4** (5.5 - 6.9) N = 14		**7.5 ± 0.6** (6.8 - 8.6) N = 21	
	Guaratiba, BA	**40.0 + 5.4** (30.9 - 48.3) N= 53	**56.1 ± 4.5** (48.8 - 67.4) N = 48		**56.1 ± 4.5** (48.8 - 67.4)		**6.3 ± 0.7** (4.6 - 7.4) N = 53		**8.1 ± 0.6** (6.6 - 9.4) N = 48	
	Prado, BA	**38.3 ± 3.1** (33.7 - 43.0) N = 8	**64.7 ± 1.0** (63.6 - 65.4) N = 3		**64.7 ± 1.0** (63.6 - 65.4) N = 3		**6.2 ± 1.1** (4.9 - 8.0) N = 8		**9.7 ± 0.2** (9.5 - 9.8) N = 3	

	Maraú, BA	**33.5 ± 1.4** (32.6 - 35.9) N = 5	**60.1 ± 1.7** (57.7 - 62.2) N = 8		**60.1 ± 1.7** (57.7 - 62.2) N = 8		**5.5 ± 0.2** (5.2 - 5.7) N = 5		**8.6 ± 0.4** (7.9 - 8.9) N = 8	
	Pooled data	**40.9 ± 5.1** (30.9 - 49.7) N = 117	**58.1 ± 10.7** (46.9 - 79.5) N = 130		**58.1 ± 6.4** (46.9 - 79.5) N = 128		**6.3 ± 0.7** (4.6 - 8.0) N = 117		**8.2 ± 0.8** (6.6 - 10.4) N = 128	
Glaucomastix abaetensis	Guarajuba, BA	**39.7 ± 4.3** (34.4 - 45.5) N = 9	**64.1 ± 5.3** (54.0 - 73.3) N = 14	**65.4 ± 5.2** (57.8 - 73.3) N = 9	**61.6 ± 5.0** (54.0 - 65.1) N = 5	$F_{1,12} = 1.779$ $R^2 = 0.129$ $P = 0.207$	**6.2 + 0.5** (5.7 - 6.9) N = 9	**9.5 + 0.7** (8.5 - 10.6) N = 8	**8.7 + 0.7** (7.7 - 9.4) N = 5	$F_{1,10} = 7.353$ $R^2 = 0.918$ $P < 0.05$
Ameivula ocellifera	Guarajuba, BA	**39.0 ± 3.4** (32.9 - 45.7) N = 14	**50.2 ± 4.4** (41.3 - 61.8) N = 41	**50.1 ± 5.6** (41.3 - 61.8) N = 21	**50.3 ± 2.7** (45.9 - 56.4) N = 20	$F_{1,39} = 0.026$ $R^2 = 0.001$ $P = 0.872$	**6.2 ± 0.3** (5.5 - 6.7) N = 14	**7.8 ± 0.9** (6.6 - 9.4) N = 21	**7.3 ± 0.3** (6.6 - 8.0) N = 20	$F_{1,39} = 5.468$ $R^2 = 0.123$ $P < 0.05$
	Praia do Porto, SE	**34.3 ± 4.0** (28.8 - 42.1)	**52.7 ± 4.1** (42.4 - 67.9) N = 44	**53.6 ± 5.7** (42.4 - 67.9) N = 18	**52.0 ± 2.4** (48.2 - 58.1) N = 26	$F_{1,42} = 1.720$ $R^2 = 0.039$ $P = 0.197$	**5.9 ± 0.5** (5.3 - 6.9) N = 8	**8.9 ± 1.0** (6.6 - 10.7) N = 18	**7.8 ± 0.3** (7.4 - 8.7) N = 26	$F_{1,42} = 26.451$ $R^2 = 0.622$ $P < 0.001$
	Barra dos Coqueiros, SE	**36.4 ± 3.1** (33.3 - 39.4) N = 3	**50.3 ± 4.2** (41.8 - 57.7) N = 15	**48.7 ± 3.9** (41.8 - 53.5) N = 8	**52.1 ± 4.1** (44.6 - 57.7) N = 7	$F_{1,13} = 2.754$ $R^2 = 0.175$ $P = 0.121$	**5.8 ± 0.5** (5.3 - 6.1) N = 3	**7.8 ± 0.8** (6.6 - 8.9) N = 8	**7.7 ± 0.5** (7.0 - 8.5) N = 7	$F_{1,13} = 0.142$ $R^2 = 0.011$ $P = 0.712$
	Piaçabuçu, AL	**41.0 ± 2.2** (39.2 - 44.1) N = 4	**50.9 ± 4.2** (41.9 - 60.9) N = 36	**51.0 ± 5.5** (41.9 - 60.9) N = 19	**50.7 ± 2.1** (46.2 - 53.4) N = 17	$F_{1,34} = 0.050$ $R^2 = 0.001$ $P = 0.824$	**7.0 ± 1.0** (6.4 - 8.6) N = 4	**8.1 ± 0.9** (6.5 - 9.9) N = 19	**7.7 ± 0.4** (6.8 - 8.2) N = 17	$F_{1,34} = 3.427$ $R^2 = 0.092$ $P = 0.073$
	Genipabu, RN	**34.7 ± 2.3** (31.9 - 39.7) N = 11	**57.6 ± 8.9** (42.3 - 65.5) N = 7	**57.2 ± 10.3** (42.3 - 65.5) N = 4	**58.1 ± 8.9** (47.9 - 64.3) N = 3	$F_{1,5} = 0.015$ $R^2 = 0.003$ $P = 0.906$	**6.2 ± 1.3** (5.4 - 10.0) N = 11	**8.0 ± 2.3** (5.4 - 10.5) N = 4	**8.5 ± 0.9** (7.5 - 9.1) N = 3	$F_{1,5} = 0.123$ $R^2 = 0.024$ $P = 0.740$
	Pooled data	**36.9 ± 3.9** (28.8 - 45.7) N = 40	**51.9 ± 10.8** (41.3 - 67.9) N = 136	**51.5 ± 6.0** (41.3 - 67.9) N = 70	**51.5 ± 3.3** (44.6 - 64.3) N = 73	$F_{1,141} = 0.000$ $R^2 = 0.000$ $P = 0.995$	**6.2 ± 0.8** (5.3 - 10.0) N = 40	**8.2 ± 1.1** (5.4 - 10.7) N = 70	**7.6 ± 0.5** (6.6 - 9.1) N = 73	$F_{1,141} = 14.255$ $R^2 = 0.266$ $P < 0.001$

Table 2. Main prey consumed (based on the highest importance index), percentage of empty stomachs and number of prey types found in the stomachs of each population/species of cnemidophorine studied. Number, volume (mm3) and length (mm) of the largest prey consumed in each population are represented by mean ± standard deviation with amplitude in parenthesis and sample size (N). *Vertebrate prey found in the stomach (one gekkonid lizard at Maricá and one unidentified frog at Jurubatiba, Rio de Janeiro state).

Species	Locality	Main prey	Empty stomachs (%)	Prey types	Nº of prey	Volume of prey	Prey length
Contomastix lacertoides	Joaquina, SC	Ants, spiders and cricket	7.3 (3/41)	11	4.4 + 3.7 (1 – 18) N = 37	189.5 + 185.1 (10.8 - 820.5) N = 37	12.4 + 4.4 3.7-20.5 N = 37
Glaucomastix littoralis	Grussaí, RJ	Insect larvae, cockroaches and spiders	12.5 (3/24)	8	7.0 + 5.1 (1 - 23) N = 21	136.5 + 125.3 (0.1 - 435.5) N = 21	13.4 + 6.7 (3.0 - 34.6) N = 21
	Jurubatiba, RJ	Insect larvae, termites and cockroaches*	6.9 (2/29)	13	19.2 + 25.1 (1 - 96) N = 28	113.9 + 124.1 (2.0 - 509.1) N = 27	23.3 + 52.6 (4.0 - 289.7) N = 28
	Maricá, RJ	Insect larvae, termites and cockroaches*	0 (0/30)	11	37.5 + 52.4 (1 - 181) N = 26	120.6 + 237.3 (0.4 - 1001.7) N = 26	10.6 + 6.4 (1.7 - 22.2) N = 26
Ameivula nativo	Setiba, ES	Insect larvae, termites and spiders	11.1 (4/36)	16	8.5 + 7.5 (1 - 33) N = 34	106.8 + 184.6 (10.0 - 851.4) N = 34	13.1 + 6.2 (1.5 - 24.9) N = 34
	Comboios, ES	Insect larvae, ants and spiders	7.7 (3/39)	16	13.2 + 15.1 (1 - 77) N = 37	15.5 + 16.1 (0.3 -73.9) N = 37	10.7 + 5.3 (2.9 - 21.9) N = 37
	Guriri, ES	Insect larvae, termites and spiders	0 (0/35)	14	30.0 + 35.4 (1 - 134) N = 31	74.5 + 133.7 (0.7 - 516.9) N = 31	9.5 + 5.0 (2.2 - 18.6) N = 31
	Prado, BA	Insect larvae, termites and spiders	0 (0/11)	11	22.6 + 34.7 (1 - 117) N = 10	18.0 + 25.5 (1.1 - 87.1) N = 10	9.5 + 6.6 (1.7 - 18.2) N = 10
	Guaratiba, BA	Insect larvae, termites and spiders	2.7 (3/101)	16	23.9 + 27.7 (1 - 202) N = 98	44.7 + 79.0 (0.8 - 676.9) N = 98	12.0 + 5.7 (1.9 - 32.8) N = 98
	Maraú, BA	Insect larvae, termites and spiders	9.11/11	8	20.7 + 15.3 (2 - 46) N = 11	54.3 + 81.4 (0.7 - 249.4) N = 11	15.9 + 6.2 (0.9 - 23.3) N = 11
Glaucomastix abaetensis	Guarajuba, BA	Termites, spiders and insect larvae	0 (0/23)	9	11.0 + 9.7 (1-32) N = 22	38.3 + 43.2 (1.6 - 131.1) N = 22	7.3 + 3.6 (1.8 - 14.2) N = 22

Ameivula ocellifera	Guarajuba, BA	Insect larvae, termites and crickets	2.2 (1/45)	11	24.3 + 19.6 (1 - 75) N = 44	49.3 + 91.5 (0.9 - 414.7) N = 44	7.5 + 5.7 (1.5 - 25.1) N = 44
	Piaçabuçu, AL	Insect larvae, beatles and termites	5.3 (2/38)	14	20.3 + 21.6 (1 - 125) N = 37	69.9 + 97.7 (0.1 - 423.3) N = 37	13.0 + 5.8 (1.4 - 24.9) N = 37
	Praia do Porto, SE	Insect larvae, spiders and ants	2.0 (1/51)	11	16.2 + 12.4 (1 - 50) N = 46	40.6 + 77.3 (1.0 - 514.0) N = 46	11.1 + 6.0 (2.1 - 26.4) N = 46
	Barra dos Coqueiros, SE	Insect larvae, homoptera and beetles	0 (0/18)	13	12.7 + 8.0 (2 - 31) N = 18	27.5 + 35.5 (1.4 - 143.2) N = 18	10.3 + 5.4 (2.6 - 19.4) N = 18
	Genipabu, RN	Termites, cockroaches and insect larvae	5.9 (1/17)	12	18.1 + 23.4 (1 - 76) N = 16	192.2 + 295.8 (1.4 - 899.4) N = 16	11.3 + 8.8 (2.6 - 35.5) N = 16

Table 3. Differences in number of prey, volume and length ingested by juveniles (J) and adults (A) and by males (M) and females (F) (one-way analysis of variance test - ANOVA) in 15 populations of five cnemidophorine species at restinga habitats throughout Brazilian east coast. Effect of snout-vent length on log volume (V), number (N) and length (L) of prey consumed in each population studied (regression analysis test). Significant results are in bold. *Small sample size.

SPECIES/POPULATIONS	**NUMBER OF PREY**			**VOLUME** (largest prey)			**LENGTH** (largest prey)		
Contomastix lacertoides	ANOVA **J x A**	ANOVA **M x F**	Regression **SVL x N**	ANOVA **J x A**	ANOVA **M x F**	Regression **SVL x V**	ANOVA **J x A**	ANOVA **M x F**	Regression **SVL x L**
Joaquina, SC	*	$F_{1,34} = 2.434$ P = 0.128	$F_{1,35} = 3.879$ P = 0.057	*	$F_{1,34} = 0.079$ P = 0.781	$F_{1,35} = 0.063$ P = 0.803	*	$F_{1,34} = 0.197$ P = 0.660	$F_{1,35} = 2.100$ P = 0.156
Glaucomastix littoralis									
Maricá, RJ	$F_{1,24} = 2.744$ P= 0.111	$F_{1,15} = 0.003$ P = 0.959	$F_{1,24} = 1.698$ P = 0.205	$F_{1,24} = 9.306$ $R^2 = 0.279$ **P < 0.05**	$F_{1,15} = 0.648$ P = 0.433	$F_{1,34} = 12.329$ $R^2 = 0.339$ **P < 0.05**	$F_{1,24} = 6.513$ $R^2 = 0.213$ P < 0.05	$F_{1,15} = 4.109$ R2 = 0.215 P = 0.061	$F_{1,24} = 5.441$ $R^2 = 0.185$ **P < 0.05**
Jurubatiba, RJ	*	$F_{1,25} = 0.735$ P= 0.400	$F_{1,2} = 106.638$ $R^2 = 0.810$ **P < 0.001**	*	$F_{1,25} = 3.662$ $R^2 = 0.128$ P = 0.067	$F_{1,26} = 1.049$ P = 0.315	*	$F_{1,25} = 0.009$ P = 0.926	$F_{1,26} = 0.045$ P = 0.834
Grussaí, RJ	$F_{1,19} = 0.007$ P = 0.933	$F_{1,13} = 0.012$ P = 0.916	$F_{1,19} = 0.017$ P = 0.897	$F_{1,19} = 8.803$ $R^2 = 0.317$ **P < 0.05**	$F_{1,13} = 2.012$ P = 0.180	$F_{1,34} = 2.434$ R2 = 0.433 **P < 0.05**	F1,19 = 3.129 P = 0.093	$F_{1,13} = 1.447$ P = 0.251	$F_{1,19} = 3.234$ P = 0.088
Ameivula nativo									
Setiba, ES	F1,32 = 0.528 P = 0.473	-	F1,32 = 0.217 P = 0.644	F1,32 = 1.720 P = 0.199	-	F1,20 = 1.215 P = 0.283	F1,32 = 7.323 R2 = 0.186 **P < 0.05**	-	F1,20 = 3.677 P = 0.070
Comboios	$F_{1,34} = 1.015$ P = 0.321	-	$F_{1,34} = 0.660$ P = 0.422	$F_{1,34} = 0.125$ P = 0.725	-	F1,34 = 1.132 R2 = 0.032 P = 0.295	$F_{1,34} = 1.777$ P= 0.191	-	$F_{1,34} = 1.132$ P = 0.336
Guriri	$F_{1,29} = 2.828$ P = 0.103	-	$F_{1,29} = 0.271$ P = 0.607	$F_{1,29} = 1.098$ P = 0.303	-	$F_{1,29} = 9.245$ R2 = 0.242 **P < 0.05**	$F_{1,29} = 0.332$ P= 0.569	-	$F_{1,29} = 2.859$ P = 0.102
Prado	*	-	*	*	-	*	*	-	*
Guaratiba	$F_{1,96} = 2.735$ P = 0.101	-	$F_{1,96} = 8.137$ $R^2 = 0.078$ **P < 0.05**	$F_{1,96} = 8.680$ $R^2 = 0.083$ **P < 0.05**	-	$F^{1,96} = 17.611$ $R^2 = 0.155$ **P < 0.001**	$F_{1,96} = 2.505$ P= 0.117	-	$F_{1,96} = 5.123$ $R^2 = 0.051$ **P < 0.05**
Maraú	F1,9 = 14.732 R2 = 0.621 **P < 0.05**	-	F1,9 = 18.246 R2 = 0.670 **P < 0.05**	F1,9 = 12.934 R2 = 0.590 **P < 0.05**	-	F1,9 = 13.837 R2 = 0.606 **P < 0.05**	F1,9 = 7.587 R2 = 0.457 **P < 0.05**	-	F1,9 = 7.634 R2 = 0.459 **P < 0.05**
Ameivula abaetensis	$F_{1,20} = 0.001$ P = 0.975	$F_{1,11} = 0.471$ P = 0.507		$F_{1,20} = 0.223$ P = 0.642	$F_{1,11} = 0.633$ P = 0.443	$F_{1,20} = 0.732$ P = 0.402	$F_{1,20} = 1.415$ P = 0.248	$F_{1,11} = 0.864$ P = 0.372	$F_{1,20} = 1.816$ P = 0.193

Ameivula ocellifera									
Guarajuba, BA	$F_{1,42}$ =0.224 P = 0.638	$F_{1,36}$ = 1.728 P= 0.197	$F_{1,42}$ = 1.825 P = 0.184	$F_{1,42}$ = 0.366 P= 0.548	$F_{1,36}$ = 2.423 P= 0.128	$F_{1,42}$ = 9.187 R^2 = 0.179 **P < 0.05**	$F_{1,42}$ = 0.280 P = 0.599	$F_{1,36}$ = 0.368 P = 0.368	$F_{1,42}$ = 5.323 R^2 = 0.112 **P < 0.05**
Barra dos Coqueiros, SE	*	$F_{1,12}$ = 5.065 R^2 = 0.297 **P < 0.05**	$F_{1,16}$ = 0.765 P = 0.395	*	$F_{1,12}$ = 0.344 P = 0.568 $F_{1,13}$ = 0.318 P = 0.582	$F_{1,16}$ = 1.197 P = 0.290	*	$F_{1,12}$ = 2.101 P = 0.173	$F_{1,42}$ = 0.333 P = 0.572
Praia do Porto, SE	*	$F_{1,38}$ = 0.271 P = 0.606	$F_{1,44}$ = 3.284 P = 0.08	*	$F_{1,38}$ = 1.915 P= 0.175	$F_{1,44}$ = 11.752 R^2 = 0.211 **P < 0.05**	*	F1,38 = 1.395 P = 0.245	$F_{1,44}$ = 5.883 R^2 = 0.118 **P < 0.05**
Piaçabuçu, AL	$F_{1,35}$ = 0.898 P = 0.350	$F_{1,27}$ = 0.952 P = 0.338	$F_{1,35}$ = 0.517 P = 0.477	$F_{1,35}$ = 0.038 P = 0.847	$F_{1,27}$ = 0.117 P = 0.070	$F_{1,35}$ = 1.342 P = 0.255	$F_{1,35}$ = 0.814 P = 0.373	$F_{1,27}$ = 3.271 P= 0.082	$F_{1,35}$ = 0.138 P = 0.712
Genipabu, RN	*	*	$F_{1,14}$ = 0.594 P = 0.454	*	*	$F_{1,14}$ = 11.248 R^2 = 0.445 **P < 0.05**	*	*	$F_{1,14}$= 11.321 R^2 = 0.447 P < 0.05

did not differ in prey length ingested (Table 3). Some *Ameivula nativo* (Setiba, Maraú and Guaratiba) and *Glaucomastix littoralis* populations (Maricá and Grussaí) presented ontogenetic differences in the size of prey consumed (Table 3).

The number, volume and length of the largest prey consumed also varied in terms of the mean SVL for some species/populations (Table 3). The similarity in diet composition varied among different populations/species and formed two distinct groups based on isoptera consumption (Fig. 2). The NMDS-ordination of prey frequency (based on number of prey ingested) showed low values of stress (0.0099) for 2D NMDS plot. The diet of *G. littoralis* from Grussaí differed from the two other populations studied, mainly due to the consumption of few termites and of many gastropoda. *Ameivula ocellifera* diet from Genipabu differed from others population of the same species by the higher consumption of Blattodea (Fig. 2, see attached material). *Ameivula nativo* populations had a geographic difference in the diet by state. *Ameivula nativo* populations from Bahia state were more similar in diet than those populations from Espirito Santo state, being Guriri the exception (the northern population from Espirito Santo state) that was more similar to Bahia state populations (Fig. 1 and 2). The configuration in one dimension represented a great part of original distance between objects for all populations.

Latitude had no influence on the consumption of termites ($F_{1,13} = 0.361$, $P = 0.558$) or on prey diversity consumed by lizards (Regression Analysis: $F_{1,13} = 0.614$, $P = 0.477$). There was no association between availability of termites in the environment (number of termite nests/ha) and the numerical percentage ($r = 0.134$, $P > 0.05$, $N = 10$) or the frequency of occurrence of termites ($r = 0.091$, $P > 0.05$, $N = 10$) in the diet of the lizards.

The number of termites and insect larvae consumed in each population were not related to each other (Spearman rank correlation, $r_s = 0.135$, $P > 0.05$) as well as the volume of termites and larvae ingested (Spearman rank correlation, $r_s = 0.109$, $P > 0.05$).

Discussion

Cnemidophorine lizards from restinga areas along the eastern coast of Brazil have food habits consisting predominantly of larvae and/or termites, usually followed by spiders, cricket, ants and beetles. A diet composition characteristic of cnemidophorine species in different seasons and locations (e.g. Vitt, 1991; Vitt *et al.,* 1997; Eifler and Eifler, 1998; Mesquita and Colli, 2003; Menezes *et al.,* 2006; Dias and Rocha, 2007). As phylogenetically close species tend to be similar in foraging mode, the niche conservatism in diet is common in different strains of Squamata that occur in distinct geographic areas (e.g. VanSluys, 1993; Vitt *et al.,* 2003).

Differences in diet composition among the studied cnemidophorine populations/species, partially resulted from the differential consumption of termites among them. The populations with higher consumption of termites in the diet formed a separated group on NMDS graph. *Glaucomastix littoralis,* in the restinga of Grussaí (RJ), consumed few food items, being larvae the only prey category with a high importance index (79.3), which may explain this species, be further from other populations. The population of *C. lacertoides* from restinga da Joaquina (SC), also differed consistently from some populations/species. *Contomastix lacertoides* was the only species that did not consume termites, even though there were termites in the area, having preferences (at least during the study period) for preys such as ants and spiders. However, there are records of frequent consumption of termites and ants by other *C. lacertoides* populations (e.g. Milstead, 1961; Aún and Martori, 1996). In the literature available, only few populations/species of cnemidophorines did not consume termites (e.g. *Cnemidophorus lemniscatus* in Curuá-Una, Amazon – Vitt *et al.,* 1997; *C. nigricolor* in Venezuela – Paulissen and Walker, 1994).

In the present study, the populations/species in which the importance index of termites was not the highest one, larvae formed the most important prey, excepting *C. lacertoides* (Joaquina, SC) which had ants as the most important prey in diet. The high frequency of termites and larvae found in the stomach of most populations, indicate that these prey are the basis of the diet of populations/species of cnemidophorines in the eastern coast of Brazil. Despite the small size of termites, they, together with larvae, seemed to be an important source of energy, being the food items preferred by many populations of cnemidophorine species (including the genus *Aspidoscelis* from North America) (e.g. Pianka, 1970; Paulissen *et al.,* 1988; Vitt, 1991; Mesquita and Colli, 2003; Teixeira-Filho *et al.,* 2003; Dias and Rocha, 2007). The low mobility of larvae and the clustering occurrence of termites tend to compensate the cost

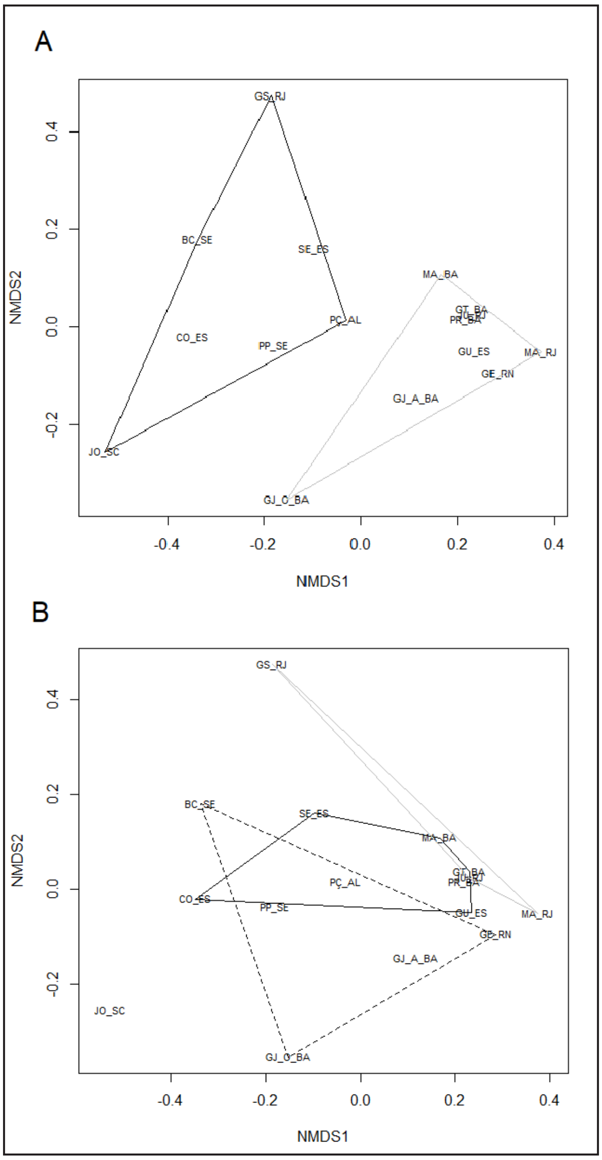

Figure 2. Two-dimensional non-metric multidimensional scaling ordination diagram to show the consumption of prey by cnemidophorines populations/species: A) grouped by isoptera consumption, B) grouped by species. BC_SE (Barra dos Coqueiros, SE), CO_ES (Comboios, ES), GE_RN (Genipabu, RN), GJ_O_BA (Guarajuba - *Ameivula ocellifera*, BA), GJ_A_BA (Guarajuba - *Glaucomastix abaetensis*, BA), GS_RJ (Grussaí, RJ), GT_BA (Guaratiba, BA), GU_ES (Guriri, ES), JO_SC (Joaquina, SC), JU_RJ (Jurubatiba, RJ), MA_BA (Maraú, BA), MA_RJ (Maricá, RJ), PÇ_AL (Piaçabuçu, AL), PP_SE (Praia do Porto, SE), PR_BA (Prado, BA), SE_ES (Setiba, ES).cnemidophorine studied. Number, volume (mm3) and length (mm) of the largest prey consumed in each population are represented by mean ± standard deviation with amplitude in parenthesis and sample size (N). *Vertebrate prey found in the stomach (one gekkonid lizard at Maricá and one unidentified frog at Jurubatiba, Rio de Janeiro state).

of active foraging and prey capture by increasing the efficiency of resource exploitation by these lizards (Pianka, 1986; Schoner, 1971; Nagy *et al.*, 1984; Etheridge and Wit, 1993). As active foragers, cnemidophorine species have ecophysiological and behavioral characteristics (as to extend and retire their tongs fastly during foraging search) that provide them the ability to detect and recognise chemically prey before attacking, favoring the finding of prey with low mobility that are hidden in leaf litter or under the topsoil (Cooper, 1990).

The availability of termites in the restinga area was not a determinant factor in the increase of termite consumption by these lizards, indicating that other factors results in differences of food preferences (especially termites) in the diet. For example, in the restinga of Joaquina (SC) termite nests occurred in a frequency comparable to other areas, but the local population of *C. lacertoides* did not consumed termites. Other studies also showed a lack of relationship between the availability of prey in the environment and the prey consumed by cnemidophorine lizards, indicating a degree of food preference by some populations (e.g. Dearing and Schall, 1992).

According to Pianka (1970), there is a latitudinal variation in the consumption of termites by lizards of the species *Aspidoscelis tigris*, which occurs from the United States to northern Mexico. In this species, populations with a further south distribution consumed higher amounts of Isoptera than northern populations. Parker and Pianka (1975) found the same trend for the species *Uta stansburiana*, which occurs in the same geographic area. In this study, we did not find a latitudinal trend in the diversity of prey consumed or in the consumption of termites by populations.

The intraspecific variations in the SVL of adults among areas were also found in other studies (e.g. Vitt, 1983, 1991; Feltrim, 2002; Mesquita and Colli, 2003; Dias and Rocha, 2007) and probably are due to the interactions of local factors that determine the size of individuals of each species (e.g. Meiri, 2007). There was a biological tendency to increase the mean number of items consumed with the increase of lizard SVL in each population studied. For *G. littoralis* pooled data, there was an increase in the number of items consumed with increasing SVL of lizards, mainly due to the number of termites in the diet. In other species, *A. ocellifera* and *A. nativo*, the relation was reversed, the number of items tended to decrease with the increase in SVL (pooled data), suggesting that larger lizards tend to consume fewer number of prey than smaller lizards, indicating an energy advantage of larger lizards to consume fewer but larger prey (e.g. Díaz and Carrascal, 1993; Brooks *et al.,* 1996) which is supposed made by the lizard to keep a positive energy balance.

The head width size of lizards affected significantly the volume and the length of the larger prey ingested in 60% (3/5) of the species studied. Adults tend to consume larger food items than juveniles, as demonstrated for other species of lizards (e.g. Van Sluys, 1993; Rocha *et al.,* 2004). However, in spite of the significant relationship (for population pooled data), the morphological variables of lizards (SVL and HW) explained less than 20% of the length and of the volume of prey ingested, probably due to the high consumption of termites (that have low size variation). This low or lack of relationship is commonly found for cnemidophorine species (e.g. Mesquita and Colli, 2003; Teixeira-Filho *et al.,* 2003; Menezes *et al.,* 2006).

In general, males and females from different species/populations of cnemidophorines studied consumed basically similar types of prey. However, females had a tendency to consume a greater number of prey in relation to males. In compensation, males had a tendency to consume a greater volume of prey in relation to females, probably due to the tendency of an increased size of the mandible shown by males. There were no differences in SVL between males and females, excepting for *G. littoralis* whose males were comparatively larger than females. Males of all species tended to have a greater head width (HW). This sexual dimorphism on HW between males and females of cnemidophorines, was also found for other species in Brazil (e.g. Vitt, 1983; Vitt *et al.,* 1997; Rocha *et al.,* 2000; Feltrim, 2002; Mesquita and Colli, 2003; Teixeira-Filho *et al.,* 2003; Dias and Rocha, 2007) and probably stems from the fact that males with a greater mandible width can get more advantages during the agonistic interactions with other males of the same species (i.e. result of the intrasexual component of sexual selection) (e.g. Vitt, 1983; Rocha, 1996).

The frequency of lizards with empty stomachs (0% to 7.3%) is consistent which is generally found for cnemidophorine species from different locations (e.g. Teixeira-Filho *et al.,* 2003; Mesquita *et al.,* 2006; Dias dados não publicados). This result suggests a positive energy balance, a constant energy gain (Huey *et al.,* 2001).

We concluded that the eastern populations of cnemidophorine lizards in restinga habitats of Brazil are omnivorous, but consum mainly arthropods, with few consumption of plant material. The high level of importance of termites and larvae in almost all populations/species probably contributed to the low intra-and interspecific differences in food habit. The diet of cnemidophorine species studied in restinga habitats, in general, tend to be similar to that found to other cnemidophorines, regardless its geographic distribution. Males and females of different populations/species tend to have similar diet composition.

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Appendix

Number (N), volume (V) (in mm3) and frequency (F) of each prey category consumed by each cnemidophorine species studied at restinga habitats along the Brazilian east coast. Ix = importance index of each prey category in diet (most important prey are in bold). (In portuguese).

Contomastix lacertoides		**Joaquina, SC (N = 38)**		
ITEM	**N (%)**	**V (%)**	**F (%)**	**IX**
Gastropoda	1 (0.6)	37.9 (0.2)	1 (2.6)	1.1
Arachnida				
Araneae	**56 (34.6)**	**3116.0 (16.1)**	**20 (52.6)**	**34.4**
Acari	9 (5.6)	12.0 (>0.1)	7 (18.4)	7.9
Hexapoda				
Orthoptera	7 (4.3)	1449.4 (7.5)	7 (18.4)	10.1
Blattodea	1 (0.6)	43.8 (0.2)	1 (2.6)	1.1

Hemiptera	2 (1.2)	12.5 (>0.1)	1 (2.6)	1.2
Coleoptera				
Adultos	6 (3.7)	182.4 (1.0)	5 (13.2)	5.9
Larvas	2 (1.2)	78.5 (0.4)	2 (5.3)	2.2
Hymenoptera				
Formicidae	**71 (43.8)**	**8417.4 (43.7)**	**22 (57.9)**	**48.5**
Outros	1 (0.6)	12.1 (>0.1)	1 (2.6)	1.0
Larvas de Lepidoptera	4 (2.5)	151.7 (0.8)	4 (10.5)	4.5
Diptera				
Adultos	1 (0.6)	66.8 (0.3)	1 (2.6)	1.1
Larvas	1 (0.6)	9.4 (>0.1)	1 (2.6)	1.0
Total de larvas	7 (4.3)	239.6 (1.2)	6 (15.8)	7.3
Artropodes não Identificados	-	5705.2 (29.6)	-	-
TOTAL	162	19295.8		

Glaucomastix littoralis	**Maricá, RJ (N = 30)**				**Jurubatiba, RJ (N = 29)**				**Grussaí, RJ (N = 24)**			
ITEM	**N (%)**	**V (%)**	**F (%)**	**I_x**	**N (%)**	**V (%)**	**F (%)**	**I_x**	**N (%)**	**V (%)**	**F (%)**	**I_x**
Gastropoda									10 (6.8)	87.0 (1.3)	7 (29.2)	12.4
Arachnida												
Araneae	6 (0.6)	123.1 (1.6)	5.0 (16.7)	6.3	17 (3.0)	41.7 (0.5)	5 (17.2)	6.9	8 (5.4)	122.2 (1.8)	7 (29.2)	12.1
Pseudoscorpiones					1 (0.2)	0.6 (0.0)	1.0 (3.4)	1.2				
Malacostraca												
Isopoda	2 (0.2)	11.5 (0.2)	2.0 (6.7)	2.3	8 (1.4)	67.8 (0.9)	4.0 (13.8)	5.4				
Chilopoda									1 (0.7)	30.6 (0.5)	1 (4.2)	1.8
Hexapoda												
Thysanura	1 (0.1)	3.1 (0.04)	1.0 (3.3)	1.2								
Orthoptera	2 (0.2)	3.9 (0.1)	2.0 (6.7)	2.3	6 (1.1)	88.7 (1.2)	5.0 (17.2)	6.5				
Isoptera	**879 (90.8)**	**2089.4 (27.5)**	**22.0 (73.3)**	**63.9**	**378 (67.3)**	**815.7 (10.8)**	**21.0 (72.4)**	**50.2**	6 (4.1)	7.5 (0.1)	1 (4.2)	2.8
Operário	870 (89.9)	2048.1 (27.0)	22.0 (73.3)	63.4	378 (67.3)	815.7 (10.8)	21.0 (72.4)	50.2	6 (4.1)	7.5 (0.1)	1 (4.2)	2.8
Soldado	9 (0.9)	41.3 (0.5)	4.0 (13.3)	4.9								
Blattodea	**11 (1.1)**	**3649.3 (48.1)**	**6.0 (20.0)**	**23.1**	**14 (2.5)**	**840.4 (11.2)**	**7.0 (24.1)**	**12.6**	**5 (3.4)**	**1649.2 (24.7)**	**3 (12.5)**	**13.5**
Hemiptera					7 (1.2)	225.5 (3.0)	3.0 (10.3)	4.8				
Homoptera	2 (0.2)	2.9 (0.04)	2.0 (6.7)	2.3	2 (0.4)	29.9 (0.4)	2.0 (6.9)	2.6				
Coleoptera												
Adultos	4 (0.4)	314.1 (4.1)	4.0 (13.3)	6.0	7 (1.2)	154.8 (2.1)	4.0 (13.8)	5.7	2 (1.4)	29.8 (0.4)	2 (8.3)	3.4

Larvas	40 (4.1)	424.6 (5.6)	11.0 (36.7)	15.5	35 (6.2)	186.1 (2.5)	6.0 (20.7)	9.8	41 (27.7)	257.8 (3.6)	16 (66.7)	32.7
Neuroptera												
Adultos					2 (0.4)	289.8 (3.8)	1.0 (3.4)	2.5				
Larvas	4 (0.4)	99.5 (1.3)	2.0 (6.7)	2.8	8 (1.4)	293.2 (3.9)	4.0 (13.8)	6.4	72 (48.6)	4442.4 (66.4)	18 (75.0)	63.4
Hymenoptera Formicidae	6 (0.6)	62.7 (0.8)	3.0 (10.0)	3.8	4 (0.7)	2.5 (0.0)	3.0 (10.3)	3.7	3 (2.0)	7.2 (0.1)	2 (8.3)	3.5
Hexapoda												
Larvas de Lepidoptera	6 (0.6)	47.2 (0.6)	5.0 (16.7)	6.0	29 (5.2)	1923.2 (25.6)	15.0 (51.7)	27.5				
Diptera					2 (0.4)	0.5 (0.0)	2.0 (6.9)	2.4				
Larvas não identificadas					3 (0.5)	13.6 (0.2)	3.0 (10.3)	3.7				
Total de larvas	**50 (5.2)**	**571.3 (7.5)**	**17 (56.7)**	**23.1**	**75 (13.4)**	**2416.1 (32.2)**	**19 (65.5)**	**37.0**	**113 (76.3)**	**4700.2 (70.0)**	**22 (91.7)**	**79.3**
Ooteca					1 (0.2)	4.7 (0.1)	1.0 (3.4)	1.2				
Pupa					7 (1.2)	495.7 (6.6)	4.0 (13.8)	7.2				
Casulo					1 (0.2)	7.1 (0.1)	1.0 (3.4)	1.2				
Artropodes não Identificados		401.4 (5.3)				2850.1 (24.6)				53.1 (0.8)		
Material Vegetal												
Frutos	1 (0.1)	80.5 (1.1)	1.0 (3.3)	1.5								
Folhas	3 (0.3)	11.0 (0.1)	3.0 (10.0)	3.5								
Outros		6.0				10.4 (0.1)	3.0 (10.3)					
Lacertilia					1 (0.2)	177.0 (2.3)	1.0 (3.4)	2.0				
Amphibia	1 (0.1)	255.0 (3.4)	1.0 (3.3)	2.3								
TOTAL	968	7585.1			559	7519.0			148	6686.9		

Ameivula nativo	**Setiba, ES (N =36)**				**Comboios, ES (N = 39)**				**Guriri, ES (N = 35)**			
ITEM	**N (%)**	**V (%)**	**F (%)**	**I_x**	**N (%)**	**V (%)**	**F (%)**	**I_x**	**N (%)**	**V (%)**	**F (%)**	**I_x**
Gastropoda	1 (0.4)	9.2 (0.1)	1 (2.8)	1.1	7 (1.4)	26.5 (0.7)	2 (5.1)	2.4	8 (0.9)	46.0 (0.7)	6 (17.1)	6.2
Arachnida												
Araneae	38 (13.4)	287.4 (4.1)	18 (50.0)	22.5	76 (15.5)	505.4 (13.0)	28 (71.8)	33.4	17 (1.8)	87.7 (1.4)	10 (28.6)	10.6
Opiliones					3 (0.6)	1.2 (0.03)	2 (5.1)	1.9				
Acari	1 (0.4)	0.008 (0.0)	1 (2.8)	1.0					1 (0.1)	0.1 (0.0)	1 (2.9)	1.0

Pseudoscorpiones					1 (0.2)	0.4 (0.01)	1 (2.6)	0.9	2 (0.2)	1.2 (0.02)	2 (5.7)	2.0
Malacostraca												
Isopoda	2 (0.7)	11.0 (0.1)	1 (2.8)	1.2	1 (0.2)	7.2 (0.2)	1 (2.6)	1.0				
Diplopoda	1 (0.4)	2.6 (0.04)	1 (2.8)	1.1								
Hexapoda												
Thysanura					3 (0.6)	17.0 (0.4)	3 (7.7)	2.9				
Orthoptera												
Adultos	5 (1.8)	152.4 (2.1)	4 (11.1)	5.0	8 (1.6)	65.6 (1.7)	7 (17.9)	7.0	2 0.2)	6.5 (0.1)	2 (5.7)	2.0
Ninfa	1 (0.4)	8.0 (0.1)	1 (2.8)	1.1								
Isoptera	74 (26.1)	140.4 (2.0)	11 (30.5)	19.6	34 (6.9)	63.6 (1.7)	5 (12.8)	7.1	672 (72.9)	1158.9 (18.3)	20 (57.1)	49.4
Operário	74 (26.1)	140.4 (2.0)	11 (30.5)	19.6	32 (6.5)	61.2 (1.6)	5 (12.8)	7.0	669 (72.6)	1152.2 (18.2)	20 (57.1)	49.3
Soldado					2 (0.4)	2.4 (0.1)	1 (2.6)	1.0	3 (0.3)	6.7 (0.1)	2 (5.7)	2.0
Mantodea									1 (0.1)	12.1 (0.2)	1 (2.9)	1.1
Blattodea	21 (7.4)	3096.5 (43.8)			2 (0.4)	155.7 (4.0)	2 (5.1)	3.2	15 (1.6)	3226.2 (51.0)	5 (14.3)	22.3
Hemiptera	1 (0.4)	13.7 (0.2)	1 (2.8)	1.1	4 (0.8)	41.1 (1.0)	4 (10.2)	4.0	3 (0.3)	29.4 (0.5)	3 (8.6)	3.1
Homoptera	2 (0.7)	11.2 (0.2)	2 (5.5)	2.1	6 (1.2)	8.5 (0.2)	5 (12.8)	4.7	13 (1.4)	139.5 (2.2)	8 (22.9)	8.8
Adultos					5 (1.0)	8.5 (0.2)	4 (10.2)	3.8				
Ninfa					1 (0.2)	1.2 (0.03)	1 (2.6)	0.9				
Coleoptera												
Adultos	8 (2.8)	284.8 (4.0)	7 (19.4)	8.8	50 (10.2)	510.7 (13.1)	15 (38.5)	20.6	7 (0.8)	76.6 (1.2)	7 (20.0)	7.3
Larvas	66 (23.3)	1235.2 (17.5)	17 (47.2)	29.3	73 (14.9)	678.5 (17.5)	21 (53.8)	28.7	39 (4.2)	345.1 (5.5)	15 (42.9)	17.5
Neuroptera												
Larvas	4 (1.4)	118.0 (1.7)	2 (5.5)	2.9	4 (0.6)	50.3 (1.0)	4 (10.2)	3.9	11 (1.2)	132.3 (2.1)	5 (14.3)	5.9
Hymenoptera												
Formicidae	17 (6.0)	19.3 (0.3)	9 (25.0)	10.4	180 (36.8)	217.2 (5.6)	23 (59.0)	33.8	115 (12.5)	88.7 (1.4)	11 (31.4)	15.1
Outros	1 (0.4)	2.9 (0.04)	1 (2.8)	1.1								
Lepidoptera												
Adultos					3 (0.6)	76.4 (2.0)	2 (5.1)	2.5	1 (0.1)	43.9 (0.7)	1 (2.9)	1.2
Larvas	20 (7.1)	820.6 (11.6)	14 (38.9)	19.2	13 (2.6)	322.4 (8.3)	10 (25.6)	12.2	12 (1.3)	175.2 (2.8)	10 (28.6)	10.9
Diptera												
Adultos	3 (1.1)	1.2 (0.02)	2 (5.5)	2.2	3 (0.6)	39.8 (1.0)	3 (7.7)	3.1				

Larvas não identificadas	11 (3.9)	79.8 (1.1)	8 (22.2)	9.1	5 (1.0)	34.2 (0.9)	2 (5.1)	2.3				
Total de larvas	**101 (35.7)**	**2253.6 (31.9)**	**26 (72.2)**	**46.6**	**95 (19.1)**	**1085.4 (27.7)**	**28 (71.8)**	**39.5**	**62 (6.7)**	**652.6 (10.4)**	**20 (57.1)**	**24.7**
Ovo	3 (1.1)	2.7 (0.04)	1 (2.8)	1.3								
Pupa									1 (0.1)	146.9 (2.3)	1 (2.9)	1.8
Artropodes não Identificados		688.6 (9.7)				1050.7				561.7 (8.9)		
Material Vegetal												
Flores												
Frutos												
Sementes	3 (1.1)	28.6 (0.4)	2 (5.5)	2.3	13 (2.6)	11.3 (0.3)	4 (10.2)	4.4	2 (0.2)	19.6 (0.3)	2 (5.7)	2.1
Folhas												
Outros		56.1 (0.8)	3 (8.3)	3.4						25.5 (0.4)	7 (20.0)	
TOTAL	283	7070.2			489	3884.9			922	6322.9		

Ameivula nativo	**Prado, BA (N = 11)**				**Guaratiba, BA (N = 101)**				**Maraú, BA (N = 11)**			
ITEM	**N (%)**	**V (%)**	**F (%)**	**IX**	**N (%)**	**V (%)**	**F (%)**	**IX**	**N (%)**	**V (%)**	**F (%)**	**IX**
Gastropoda									1 (0.4)	0.6 (0.02)	1 (9.1)	3.2
Arachnida												
Araneae	**12 (5.3)**	**43.5 (4.2)**	**8 (72.7)**	**27.4**	**59 (2.6)**	**486.3 (3.1)**	**37 (36.6)**	**14.1**	**9 (4.0)**	**15.6 (0.4)**	**6 (54.5)**	**19.7**
Opiliones	1 (0.4)	12.0 (1.2)	1 (9.1)	3.6								
Pseudoscorpiones					3 (0.1)	0.9 (0.01)	3 (3.0)	1.0	1 (0.4)	0.2 (0.01)	1 (9.1)	3.2
Diplopoda					1 (0.04)	8.2 (0.05)	1 (1.0)	0.4				
Hexapoda												
Orthoptera												
Adultos					4 (0.2)	26 (0.2)	4 (4.0)	1.5				
Ninfa	2 (0.9)	21.0 (2.0)	2 (18.2)	7.0	1 (0.04)	5.0 (0.03)	1 (1.0)	0.3				
Isoptera	**161 (70.9)**	**512.5 (49.5)**	**5 (45.4)**	**55.3**	**1690 (75.0)**	**5925.0 (38.4)**	**72 (71.3)**	**61.6**	**142 (62.8)**	**870.8 (23.9)**	**10 (90.9)**	**59.2**
Operário	160 (70.8)	511.3 (49.4)	5 (45.4)	55.2	1463 (64.9)	3737.2 (24.2)	62 (61.4)	50.2	142 (62.8)	870.8 (23.9)	10 (90.9)	59.2
Soldado	1 (0.4)	1.2 (0.1)	1 (9.1)	3.2	16 (0.7)	28.0 (0.2)	8 (7.9)	2.9				
Alado					211 (9.4)	2159.8 (14.0)	16 (15.8)	13.1				
Blattodea	1 (0.4)	1.6 (0.1)	1 (9.1)	3.2	11 (0.5)	1098.4 (7.1)	5 (4.9)	4.2				

Hemiptera					9 (0.4)	315.2 (2.0)	7 (6.9)	3.1	1 (0.4)	18.4 (0.5)	1 (9.1)	3.3
Homoptera	3 (1.3)	3.8 (0.4)	2 (18.2)	6.6	11 (0.5)	26.2 (0.2)	10 (9.9)	3.5				
Psocoptera					1 (0.04)	2.5 (0.02)	1 (1.0)	1.1				
Mallophaga					1 (0.04)	31.9 (0.2)	1 (1.0)	0.4				
Coleoptera												
Adultos	3 (1.3)	29.4 (2.8)	2 (18.2)	7.5	22 (1.0)	272.3 (1.8)	19 (18.8)	7.2	5 (2.2)	196.6 (5.4)	4 (36.4)	14.7
Larvas	16 (7.1)	106.8 (10.3)	6 (54.5)	24.0	207 (9.2)	2381.5 (15.4)	64 (63.4)	29.3	52 (23.0)	951.3 (26.1)	8 (72.7)	40.6
Neuroptera												
Larvas	3 (1.3)	142.0 (13.7)	2 (18.2)	11.1	86 (3.8)	1946.3 (12.6)	38 (37.6)	18.0	3 (1.3)	280.9 (7.7)	3 (27.3)	12.1
Hymenoptera												
Formicidae	8 (3.5)	14.6 (1.4)	2 (18.2)	7.7	33 (1.5)	66.1 (0.4)	11 (10.9)	4.3				
Outros	4 (1.8)	0.4 (0.04)	1 (9.1)	3.6	2 (0.1)	173.1 (1.1)	2 (2.0)	1.1				
Lepidoptera												
Adultos									1 (0.4)	4.0 (0.1)	1 (9.1)	3.2
Larvas	7 (3.1)	82.1 (7.9)	4 (36.4)	15.8	80 (3.5)	1777.1 (11.5)	34 (33.7)	16.2	9 (4.0)	253.5 (7.0)	4 (36.4)	15.8
Díptera					2 (0.1)	91.3 (0.6)	1 (1.0)	0.6				
Larvas não identificadas	1 (0.4)	1.1 (0.1)	1 (9.1)	3.2	3 (0.1)	13.4 (0.1)	2 (2.0)	0.7	2 (0.9)	2.5 (0.1)	2 (18.2)	6.4
Total de larvas	**27** **(11.9)**	**252.0** **(24.4)**	**7** **(63.6)**	**33.3**	**378** **(16.8)**	**6209.6** **(40.2)**	**84** **(83.2)**	**46.7**	**66** **(29.2)**	**1488.2** **(40.9)**	**11** **(100.0)**	**56.7**
Ovo	4 (1.8)	1.0 (0.1)	1 (9.1)	3.7	10 (0.4)	46.6 (0.3)	2 (2.0)	0.9				
Pupa					19 (0.8)	436.3 (2.8)	12 (11.9)	5.2				
Artrópodes não Identificados		59.9 (5.8)				305.6 (2.0)				1048.9 (28.8)		
Restos de material vegetal		2.3 (0.2)	1 (9.1)							0.01 (0.0)	1 (9.1)	
TOTAL	226	1034.2			2255	15435.15			226	3643.4		

Glaucomastix abaetensis	**Guarajuba, BA (N = 23)**			
ITEM	**N (%)**	**V (%)**	**F (%)**	**I_x**
Arachnida				
Araneae	**39 (16.3)**	**244.9 (9.2)**	**15 (65.2)**	**30.2**
Hexapoda				
Orthoptera				
Adulto	14 (5.8)	369.9 (13.8)	8 (34.8)	18.2
Ninfa	3 (1.3)	8.8 (0.3)	2 (8.7)	3.4
Isoptera	**144 (60.7)**	**796.9 (29.8)**	**18 (78.3)**	**56.3**
Operário	138 (57.5)	746.0 (27.9)	18 (78.3)	54.6
Soldado	6 (2.5)	50.9 (1.9)	4 (17.4)	7.3
Blattodea				
Adulto	2 (0.8)	196.2 (7.3)	2 (8.7)	5.6
Ninfa	1 (0.4)	5.1 (0.2)	1 (4.3)	1.7
Hemiptera	4 (1.7)	4.6 (0.2)	1 (4.3)	2.1
Coleoptera				
Adultos	20 (8.3)	203.5 (7.6)	5 (21.7)	12.6
Larvas	2 (0.8)	17.8 (0.7)	2 (8.7)	3.4
Hymenoptera Formicidae	1 (0.4)	0.1 (0.004)	1 (4.3)	1.6
Lepidoptera (larva)	7 (2.9)	100.9 (3.8)	6 (26.1)	10.9
Diptera (adulto)	2 (0.8)	2.1 (0.1)	2 (8.7)	3.2
Artropodes não Identificados		622.3 (23.3)		
Total de larvas	**10 (4.1)**	**212.5 (8.0)**	**7 (30.4)**	**14.2**
Larvas não identificadas	1 (0.4)	93.8 (3.5)	1 (4.3)	2.8
Material Vegetal		6.4 (0.2)	4 (17.4)	
TOTAL	240	2673.5		

Ameivula ocellifera	**Guarajuba, BA (N = 45)**				**Barra dos Coqueiros, SE (N = 18)**				**Praia do Porto, SE (N = 51)**			
ITEM	**N (%)**	**V (%)**	**F (%)**	**I_x**	**N (%)**	**V (%)**	**F (%)**	**I_x**	**N (%)**	**V (%)**	**F (%)**	**I_x**
Gastropoda					6 (2.6)	45.5 (2.5)	5 (27.8)	11.0				
Arachnida												
Araneae	57 (5.3)	683.0 (8.1)	24 (53.3)	22.3	20 (8.8)	38.8 (2.2)	11 (61.1)	24.0	**111 (14.9)**	**210.8 (3.8)**	**35 (68.6)**	**29.1**
Acari					3 (1.3)	5.6 (0.3)	2 (11.1)	4.2				
Pseudoscorpiones					1 (0.4)	0.4 (0.02)	1 (5.5)	2.0				
Hexapoda												
Thysanura					3 (1.3)	9.2 (0.5)	3 (16.7)	6.2				
Odonata									1 (0.1)	44.2 (0.8)	1 (2.0)	1.0

Orthoptera												
Adultos	**69 (6.5)**	**1818.4 (21.6)**	**21 (46.7)**	**24.9**					13 (1.7)	108.1 (2.0)	9 (17.6)	7.1
Ninfa	14 (1.3)	122.0 (1.5)	3 (6.7)	3.1					1 (0.1)	1.4 (0.03)	1 (2.0)	0.7
Isoptera operário	**782 (73.2)**	**3732.5 (44.4)**	**35 (77.8)**	**65.1**	9 (3.9)	12.1 (0.7)	4 (22.2)	8.9	163 (21.8)	221.0 (4.0)	24 (47.1)	24.3
Mantodea									1 (0.1)	8.3 (0.2)	1 (2.0)	0.7
Blattodea	3 (0.3)	64.3 (0.8)	3 (6.7)	2.6								
Hemiptera												
Adultos	13 (1.2)	46.0 (0.5)	8 (17.8)	6.5	1 (0.4)	2.8 (0.1)	1 (5.5)	2.0	13 (1.7)	180.1 (3.3)	10 (19.6)	8.2
Ninfa	4 (0.4)	66.3 (0.8)	3 (6.7)	2.6					2 (0.3)	7.7 (0.1)	2 (3.9)	1.4
Homoptera												
Adultos	6 (0.6)	30.7 (0.4)	4 (8.9)	3.3	**65 (28.5)**	**110.6 (6.2)**	**13 (72.2)**	**35.6**	21 (2.8)	442.8 (8.0)	15 (29.4)	13.4
Ninfa	5 (0.5)	35.7 (0.4)	3 (6.7)	2.5					2 (0.3)	14.7 (0.3)	1 (2.0)	0.8
Coleoptera												
Adultos	40 (3.7)	164.6 (2.0)	13 (28.9)	11.5	**30 (13.1)**	**141.2 (7.9)**	**16 (88.9)**	**36.6**	78 (10.4)	1046.5 (19.0)	29 (56.9)	28.8
Larvas	20 (1.9)	516.2 (6.1)	15 (33.3)	13.8					6 (0.8)	20.2 (0.4)	6 (11.8)	4.3
Neuroptera												
Larvas	1 (0.1)	18.7 (0.2)	1 (2.2)	0.8								
Hymenoptera												
Formicidae	15 (1.4)	13.9 (0.2)	8 (17.8)	6.4	11 (4.8)	10.2 (0.6)	7 (38.9)	14.8	**148 (19.8)**	**129.5 (2.4)**	**29 (56.9)**	**26.3**
Outros					1 (0.4)	1.2 (0.1)	1 (5.5)	2.0				
Lepidoptera												
Adultos	1 (0.1)	0.05 (0.0)	1 (2.2)	0.8	2 (0.9)	7.8 (0.4)	2 (11.1)	4.1	11 (1.5)	373.1 (6.8)	8 (15.7)	8.0
Larvas	30 (2.8)	385.1 (4.6)	18 (40.0)	15.8	59 (25.9)	732.3 (40.8)	15 (83.3)	50.0	99 (13.3)	1352.1 (24.5)	32 (62.7)	33.5
Diptera												
Adultos	3 (0.3)	50.7 (0.6)	2 (4.4)	1.8	5 (2.2)	7.7 (0.4)	2 (11.1)	4.6	2 (0.3)	518.4 (9.4)	2 (3.9)	4.5
Larva					1 (0.4)	2.4 (0.1)	1 (5.5)	2.0				
Ninfa									1 (0.1)	8.5 (0.2)	1 (2.0)	0.8
Larvas não identificadas	5 (0.5)	100.3 (1.2)	5 (11.1)	4.3					5 (0.7)	29.9 (0.5)	4 (7.8)	3.0
Total de larvas	**56 (5.3)**	**1020.3 (12.1)**	**27 (60.0)**	**25.8**	**60 (26.3)**	**734.7 (40.9)**	**15 (83.3)**	**50.2**	**110 (14.8)**	**1402.2 (25.4)**	**35 (68.6)**	**36.3**
Ovo									5 (0.7)	0.8 (0.02)	3 (5.9)	2.2
Pupa					1 (0.4)	5.2 (0.3)	1 (5.5)	2.1	2 (0.3)	45.6 (0.8)	2 (3.9)	1.7

Artropodes não Identificados		543.6 (6.5)				654.0 (36.4)						
Material Vegetal										603.3 (10.9)		
Flores	1 (0.1)	7.9 (0.1)	1 (2.2)	0.8					3 (0.4)	16.3 (0.3)	3 (5.9)	2.2
Frutos									1 (0.1)	30.5 (0.6)	1 (2.0)	0.9
Sementes					10 (4.4)	8.1 (0.4)	2 (11.1)	5.3	54 (7.2)	53.3 (1.0)	15 (29.4)	12.5
Folhas									4 (0.5)	15.0 (0.3)	3 (5.9)	2.2
Outros		13.8 (0.2)	2 (4.4)							27.5 (0.5)	5 (9.8)	
TOTAL	1069	8413.8			228	1795.1			747	5509.9		

Ameivula ocellifera	**Piaçabuçu, AL (N = 38)**				**Genipabu, RN (N = 17)**			
ITEM	**N (%)**	**V (%)**	**F (%)**	**I_x**	**N (%)**	**V (%)**	**F (%)**	**I_x**
Gastropoda	6 (0.7)	12.4 (0.2)	6 (15.8)	5.6	2 (0.7)	8.7 (0.2)	2 (11.8)	4.2
Arachnida								
Araneae	31 (4.0)	116.0 (1.6)	20 (52.6)	19.4	3 (1.0)	51.7 (1.0)	2 (11.8)	4.6
Diplopoda	8 (1.0)	15.9 (0.2)	4 (10.5)	3.9				
Hexapoda								
Orthoptera								
Adultos	10 (1.3)	742.8 (10.1)	9 (23.7)	11.7	5 (1.7)	1778.7 (33.8)	5 (29.4)	21.7
Isoptera	**280 (36.5)**	**722.4 (9.8)**	**16 (42.1)**	**29.5**	**237 (81.7)**	**405.4 (7.7)**	**8 (47.1)**	**45.5**
Operário	277 (36.1)	719.5 (9.8)	15 (39.5)	28.5	237 (81.7)	405.4 (7.7)	8 (47.1)	45.5
Soldado	3 (0.4)	2.9 (0.04)	1 (2.6)	1.0				
Blattodea	3 (0.4)	490.5 (6.7)	3 (7.9)	5.0	**9 (3.1)**	**1854.1 (35.3)**	**3 (17.6)**	**18.7**
Hemiptera	21 (2.7)	178.3 (2.4)	14 (36.8)	14.0	4 (1.4)	249.5 (4.7)	4 (23.5)	9.9
Homoptera								
Adultos	38 (4.9)	345.2 (4.7)	17 (44.7)	18.1	7 (2.4)	76.5 (1.5)	4 (23.5)	9.1
Ninfa					1 (0.3)	3.4 (0.1)	1 (5.9)	2.1
Coleoptera								
Adultos	**95 (12.4)**	**373.7 (5.1)**	**29 (76.3)**	**31.3**	7 (2.4)	47.9 (0.9)	5 (29.4)	10.9
Larvas	13 (1.6)	19.1 (0.3)	5 (13.1)	5.0	5 (1.7)	153.4 (2.9)	5 (29.4)	11.4

Larva de Neuroptera	1 (0.1)	4.8 (0.1)	1 (2.6)	0.9	2 (0.7)	53.2 (1.0)	2 (11.8)	4.5
Hymenoptera								
Formicidae	41 (5.3)	124.9 (1.7)	24 (63.1)	23.4	2 (0.7)	1.0 (0.02)	2 (11.8)	4.2
Outros	7 (0.9)	62.0 (0.8)	6 (15.8)	5.8				
Lepidoptera								
Adultos	10 (1.3)	653.6 (8.9)	8 (21.0)	10.4	1 (0.3)	1.6 (0.03)	1 (5.9)	2.1
Larvas	139 (18.1)	2370.3 (32.3)	33 (86.8)	45.8	2 (0.7)	25.4 (0.5)	2 (11.8)	4.3
Diptera								
Adultos	3 (0.4)	33.5 (0.4)	2 (5.3)	2.0	3 (1.0)	11.3 (0.2)	1 (5.9)	2.4
Larva	2 (0.3)	14.5 (0.2)	2 (5.3)	1.9				
Total de larvas	**155 (20.1)**	**2408.7 (32.9)**	**34 (89.5)**	**47.5**	**9 (3.1)**	**232.0 (4.4)**	**7 (41.2)**	**16.2**
Pupa	1 (0.1)	4.8 (0.1)	1 (2.6)	0.9				
Artropodes não Identificados		929.1				537.5 (10.2)		
Material Vegetal								
Sementes	50 (6.5)	69.5 (0.9)	11 (28.9)	12.1				
Folhas	1 (0.1)	5.6 (0.1)	1 (2.6)	0.9				
Outros		9.5 (0.5)						
	767	7325.9			290	5259.5		

Trabajo

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Riqueza e composição, lacunas de conhecimento e conservação dos crocodilianos do estado do Tocantins, ecótono Amazônia-Cerrado, Brasil

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ABSTRACT
Six species of alligatorids occur in Brazil, but only three have been recorded from the state of Tocantins: *Melanosuchus niger, Caiman crocodilus and Paleosuchus palpebrosus*. The objectives of this study are to present a synthesis of the knowledge of crocodilians of Tocantins, describing geographical distribution of species, and some aspects of conservation. The exhaustive compilation of records in the literature, herpetological collections and surveys carried out in the field by the authors confirmed the occurrence also of *Paleosuchus trigonatus*. Although a specimen of *C. latirostris* was reported from Tocantins, it was possibly erroneously labeled. To investigate knowledge gaps, we divided the state into 95 grid cells of 300,000 hectares. Only 55% of Tocantins State has received some sampling for crocodilians, regardless of the method of registration. Only 12% of the state has been sampled considering any level of documentation and only 1.6% of the state had collection and deposit of specimens in a scientific collection. Only *P. trigonatus* does not have records within full-protection conservation areas and/or indigenous lands in Tocantins State. Hunting pressure and dams on the Tocantins River may lead to a decrease in the density of *M. niger*. A systematic program for monitoring and surveying for caimans is suggested to consolidate the knowledge of this animal group in Tocantins State.

Key Words: Caimans of Tocantins; *Caiman crocodilos; Melanosuschus niger*; *Paleosuchus palpebrosus*; *Paleosuchus trigonatus*; Tocantins-Araguaia Basin.

RESUMO
Das seis espécies de jacarés do Brasil apenas três são reconhecidas para o estado do Tocantins: *Melanosuchus niger, Caiman crocodilus e Paleosuchus palpebrosus*. São objetivos deste estudo apresentar uma síntese do conhecimento de crocodilianos do Tocantins abordando riqueza e composição de espécies, distribuição geográfica e aspectos sobre a conservação. A compilação exaustiva de registros na literatura, coleções herpetológicas e dados primários efetuados em campo pelos autores confirmou a presença de *Paleosuchus trigonatus,* além das três espécies conhecidas. Embora um espécime de *C. latirostris* foi reportado em território tocantinense, isto é possivelmente um equívoco na etiquetagem. Para verificação de lacunas de conhecimento nós dividimos o estado em 95 quadrículas de 300.000 hectares. Um total de 55% da área territorial do estado do Tocantins foi alvo de algum esforço amostral de crocodilianos, independente do método de registro. Apenas 12% foram amostrados considerando algum nível de documentação e somente 1,6% do território tocantinense teve coletas e depósito de espécimes em coleção científica. Apenas *P. trigonatus* não possui registros no interior de unidades de conservação de proteção integral e/ou terras indígenas no estado do Tocantins. A pressão de caça e os sucessivos barramentos no rio Tocantins sugerem uma diminuição da população de *M. niger*. Um programa de buscas e monitoramento é sugerido para consolidar o conhecimento deste grupo animal em todo território do estado do Tocantins.

Palavras-chave: Bacia Tocantins-Araguaia; Jacarés do Tocantins; *Caiman crocodilus*; *Melanosuschus niger*; *Paleosuchus palpebrosus*; *Paleosuchus trigonatus*.

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Introdução

Existem no mundo 23 espécies de crocodilianos, divididos nas famílias Crocodylidae, Gavialidae e Alligatoridae. Na América do Sul são encontradas oito espécies de crocodilianos, sendo dois crocodilos: *Crocodylus acutus* e *C. intermedius*; e seis alligatorideos: *Paleosuchus palpebrosus*, *Paleosuchus trigonatus*, *Melanosuchus niger*, *Caiman crocodilus*, *C. yacare* e o *C. latirostris* (Medem, 1983). No Brasil, ocorrem as seis espécies de alligatorideos listadas, enquanto no estado do Tocantins, apenas três são registradas: *M. niger, C. crocodilus e P. palpebrosus* (Costa e Bérnils, 2018).

No estado do Tocantins, estudos sobre jacarés focaram predominantemente em aspectos da sua biologia reprodutiva e ecologia de populações na região do rio Javaés, entre o Centro de Pesquisa Canguçu, norte da Ilha do Bananal no entorno do Parque Nacional do Araguaia e a porção sul do Parque Estadual do Cantão (Malvasio e Salera-Junior, 2006; Soares, 2007; Portelinha *et al.* 2019). No rio Tocantins, os estudos conduzidos na região do reservatório da Usina Hidrelétrica Luís Eduardo Magalhães (UHE LEM), avaliaram dados populacionais e a caracterização dos habitats frequentados pelas espécies no período de enchimento do reservatório (Villaça, 2004). Aspectos históricos e impactos da caça sobre jacarés no Tocantins também foram abordados nestes últimos anos (Pereira e Malvasio, 2014; Pereira *et al.* 2019).

Portanto, o conhecimento sobre jacarés no estado do Tocantins são de uma área bastante restrita. Os estudos no rio Javaés e no reservatório da UHE LEM, parecem ser os únicos estudos sistematizados publicados abordando crocodilianos ao longo das bacias dos rios Tocantins-Araguaia em território tocantinense. Outra fonte de dados são os registros de jacarés dispersos em relatórios de Estudos de Impacto Ambiental (EIAs), coleções herpetológicas em museus de zoologia, planos de manejo de Unidades de Conservação (UCs) e outras publicações.

Isto indica que o conhecimento sobre crocodilianos no estado do Tocantins, com a maior bacia hidrográfica exclusivamente brasileira, da Tocantins-Araguaia, é limitado. Portanto, são objetivos deste estudo apresentar uma síntese do conhecimento de crocodilianos no estado do Tocantins, destacando registros baseados em evidências documentais. A riqueza e composição de espécies, distribuição geográfica, lacunas de amostragem e aspectos sobre a conservação dos jacarés em território tocantinense serão principais pontos considerados.

Materiais e Métodos

Área de Estudo

O estado do Tocantins, com uma área de 277.620 km², localizado na porção central do Brasil, representa 5,4% do território da Amazônia Legal e está na região geopolitica Norte do país (Seplan, 2012), sendo a população estimada para 2019 de 1.572.866 habitantes (Sefaz, 2019a). A temperatura média anual é de 29°C com máximas de 40ºC e mínimas de 14°C enquanto a pluviosidade média anual variou de 1.300 mm na região sudeste, a 2.100 mm para a região centro-oeste do estado, (Seplan, 2012). O clima presente é transição entre o equatorial e tropical, com gradativa diminuição da umidade entre o eixo noroeste-sudeste (Seplan, 2012).

O estado do Tocantins está inserido integralmente na bacia hidrográfica dos rios Tocantins-Araguaia, cujas bacias possuem 173.430 km² (62,3% do território estadual) e 104.991 km² (37,7% do território estadual), respectivamente (Seplan, 2012). O bioma Cerrado compõe 63% do território do Tocantins, a Amazônia aproximadamente 6% enquanto o percentual restante de 31% são regiões de tensão ecológica entre savanas, florestas ombrófilas, florestas estacionais (Seplan, 2012). As formas aluviais e solos hidromórficos predominam na planície da Ilha do Bananal (Seplan, 2012).

A Ilha do Bananal, maior ilha fluvial do mundo com área de 1.916.225 hectares, delimitada pelo rio Araguaia a oeste, e pelo rio Javaés a leste; é uma região ecotonal entre Amazônia e Cerrado e importante Sitio Ramsar (MMA *et al.*, 2001). Atualmente, a Ilha do Bananal é reconhecida como Terra Indígena e Parque Nacional (Zanatto, 2014). Neste estudo devido a suas particularidades hidrológicas e geomorfológicas, seria tratada como uma unidade distinta.

Procedimentos

Para a compilação das espécies de jacarés do estado do Tocantins, foram utilizados os seguintes métodos: i) busca exaustiva de registros na literatura, ii) visita a museus e coleções zoológicas, iii) solicitação dos registros às coleções e museus de zoologia quando inviável a visita, como no casos das coleções estrangeiras e algumas coleções nacionais e iv) registros primários efetuados em campo.

Os registros da literatura foram obtidos em artigos científicos, relatórios de Estudos de Impacto Ambiental (EIA), planos de manejo e publicações oficiais sobre determinação de áreas prioritárias de conservação do Tocantins. Foram visitadas as coleções herpetológicas do Museu de Zoologia da Universidade de São Paulo (MZUSP), o Museu Paraense Emilio Goeldi (MPEG) e o Museu Nacional do Rio de Janeiro (MNRJ). Registros das coleções e museus não visitados foram obtidos através de consulta ao portal Vertnet (http://portal.vertnet.org/search), sobretudo nos acervos de museus internacionais. Os registros da Coleção Herpetológica da Universidade de Brasília (CHUNB) e do Museu Nacional de Historia Natural e Antropologia de Montevidéu no Uruguai (MNHNA) foram requisitados diretamente aos seus curadores. Devido à recente criação do estado do Tocantins, ocorrida em 1988, vários registros de crocodilianos atribuídos ao Tocantins, têm como procedência o estado de Goiás. Desta forma, todos os registros anteriores a 1988 foram comparados ao mapa político do estado do Tocantins, para a confirmação da localidade de registro (Seplan, 2012).

Localidades de registros sem coordenadas geográficas correspondentes foram consultadas no gazetteer (Paynter e Traylor, 1999) ou no software Google Earth PRO (GEPRO). Nas situações quando o uso do gazetteer e/ou GEPRO não se mostrou efetivo, as coordenadas geográficas da sede do município associado àquela localidade foram usadas.

A listagem das espécies de crocodilianos para o Tocantins distingue os registros providos de evidência documental ou somente avistamentos. São evidências documentais os itens disponíveis, para consulta independente, na forma exclusiva de espécime integral ou parcial, fotografia ou vídeo, que permitam a determinação segura do táxon.

Para identificar lacunas de conhecimento na distribuição de crocodilianos, foram elaborados mapas, com auxílio de software DIVA-GIS 7.5 (www.diva-gis.org/). O estado do Tocantins foi divido em uma malha de quadrículas de aproximadamente 300.000 ha (54,7 km de lado), cada uma correspondendo a 1,1% do território tocantinense. Quadrículas limítrofes as fronteiras políticas do estado tiveram suas áreas totais subtraídas da porção extrapolada. Descontadas as áreas extrapoladas, território tocantinense ficou dividido em 95 quadrículas (277.650 km^2 divididos por 300.000 ha). A riqueza de espécies de jacarés no interior de cada quadrícula foi determinada a partir do número de espécies registrado.

Para avaliar o status de conservação dos jacarés estado de Tocantins foram sobrepostas as localidades compiladas com registros sobre os limites das Unidades de Conservação de Proteção Integral (UCPIs) e Terra Indígenas (TI) (Sefaz, 2019b). As Unidades de Conservação de Uso Sustentável (UCUS) não foram consideradas nas análises em razão de presumivelmente ter menor efetividade na proteção à biodiversidade.

Resultados e Discussão

Foram encontrados registros de cinco espécies (Tabela 1), das quais, quatros foram devidamente confirmadas para Tocantins: *C. crocodilus*, *M. niger*, *P. palpebrosus*, *P. trigonatus*.

Materiais testemunhos que atestam a ocorrência dessas espécies em território tocantinense estão depositados nas seguintes coleções herpetológicas: CHUNB, MPEG, MNHNA, MZUSP, MNRJ (Apêndice). Nossas observações confirmam a ocorrência de *P. trigonatus* no extremo norte do estado. Esta espécie amazônica pode ocorrer em áreas de transição entre os biomas Amazônia e Cerrado (Campos *et al.,* 2013; Campos *et al.*, 2017; Campos *et al.*, 2019).

Os registros da espécie no baixo Araguaia, nas proximidades das Serras das Andorinhas, e no médio rio Tocantins, na região de Serra Quebrada, eram somente conhecidos de registros visuais (Atizengen e Silva, 1999; THEMAG, 2000). Entretanto, espécimes coletados no rio Itacaiúnas, em Marabá, estado do Pará (MPEG 21, 22) e nos rios Capim (MZUSP 2187) e Gurupi (MZUSP 2188), no Pará e Maranhão, respectivamente, indicavam a potencial ocorrência da espécie no extremo leste amazônico, alcançando os trechos tocantinenses do rio Araguaia e Tocantins e seus afluentes. Três indivíduos foram capturados no baixo rio Araguaia, entre Esperantina e Araguatins, por AM e MC, no período entre setembro e outubro de 2010, (Fig. 1A), um deles (macho com 100 cm de comprimento total, cabeça-cloaca 54,6 cm e cabeça 15,2 cm), encontra-se depositado no MPEG (No. 135 – Fig. 1B). Estes registros confirmam a ocorrência da espécie ao longo região ecotonal entre Amazônia e Cerrado (Campos *et al.,* 2017), alcançando o extremo leste amazônico.

Paleosuchus trigonatus tem relação com canais e corpos d`água mais turbulentos no interior das florestas, sendo os canais com densa vegetação os ambientes selecionados para construção de seus ninhos (Magnussom e Lima, 1991; Rueda-Almonacid *et al.,*

Tabela 1. Espécies de jacarés registradas para o estado do Tocantins após compilação de registros. COL: espécimes depositados em coleção herpetológica; FOT: registros fotográficos; VIS: registros visuais e/ou capturas sem fotografia e/ou coleta.

Espécies	COL	FOT	VIS	Total
Caiman crocodilus (Linnaeus, 1758)	20	14	91	125
Caiman latirostris (Daudin, 1802)	2	-	-	2
Melanosuchus niger (Spix, 1825)	2	13	17	32
Paleosuchus palpebrosus (Cuvier, 1807)	6	11	49	66
Paleosuchus trigonatus (Schneider, 1801)	1	2	5	8

2007; Campos *et al.,* 2019). No estado do Tocantins estas condições ambientais apontadas podem ser observadas nos rios Barreiro, Corda, Lontra e São Martinho, na região norte, com forte influência do bioma Amazônico (Seplan, 2012) onde novas expedições são necessárias.

Caiman latirostris, por sua vez, não foi inserida na lista de jacarés do Tocantins embora haja dois registros disponíveis por causa de possíveis equívocos na etiquetagem dos espécimes testemunhos. O estado do Tocantins está longe dos limites de distribuição geográfica conhecida para espécie (Coutinho *et al.,* 2013) e sua ocorrência nos limites tocantinenses é relatada por meio de dois espécimes. O primeiro, MZUSP 2136, visto em coleção, tem origem de coleta atribuída a Ilha do Bananal (Fig. 1C). Nos últimos 15 anos, estudos sistematizados efetuaram a captura de inúmeros jacarés na região da Ilha do Bananal, exceto *C. latirostris* (Malvasio e Salera-junior, 2006; Soares, 2007; Pereira e Malvasio, 2014; Portelinha *et al.,* 2019). Várias incursões para inventários herpetológicos foram conduzidas nos limites municipais de Lagoa da Confusão e Pium, por WSP entre os anos de 2013 e 2019, sem encontrar a espécie.

Faltam informações sobre o ano de coleta e coletor do espécime MZUSP 2136, no entanto, pressupõe ter sido coligido pelo herpetólogo Alphonse R. Hoge durante expedição à serviço do Instituto Butatan, na Ilha do Bananal, em 1948/1949 (Hoge, 1952). Uma série de espécimes provenientes da Ilha do Bananal (*C. crocodilus* - MZUSP 2106, 2107, 2108, 2109; *Paleosuchus palpebrosus* - MZUSP 2173, Figura 1D) coletados por A. Hoge sugere que o exemplar MZUSP 2136 tenha sido coletado nessa mesma expedição.

Entretanto Hoge (1952) menciona coletas herpetológicas em Goiânia, entreposto durante o deslocamento entre São Paulo e a Ilha do Bananal, podendo ser esta a origem do referido exemplar. Goiânia está inserida na distribuição geográfica de *C. latirostris* (Coutinho *et al.,* 2013), porém a falta de informações sobre coletor e data de coleta não permite confirmar sua origem.

Por outro lado, vale ressaltar os relatos do jornalista Willy Aureli de *C. latirostris* em seus manuscritos sobre suas expedições à região, entre as décadas de 1930 e 1940 (Aureli, 1960). Dotado de espírito explorador e não necessariamente científico, ele descreve o abate de alguns indivíduos de *C. latirotris*, mas não fornece fotos ou figuras. Afirma ainda que a região, já naquele período, era alvo de intensas caçadas por moradores locais e cidadãos do Brasil meridional, com abate de centenas a milhares de indivíduos de jacarés. Portanto, assumindo de fato que a região da Ilha do Bananal possa ter tido populações pequenas de *C. latirostris* (Verdade *et al.,* 2002), as evidências de caça intensa na região descrita por W. Aureli, que persistem desde aquela época até dias atuais (Pereira *et al.*, 2019), pode refletir em uma eventual extinção local dessa população.

O segundo espécime de *C. latirostris*, atribuído ao Instituto Butantan, NS 547 (Pessoa *et al.,* 1972) não foi visto ou manuseado em coleção, mas teria sido coligido na região do munícipio de Almas, sudeste do Tocantins. No entanto, é possível que o mesmo tenha se perdido em incêndio que consumou os acervos zoológicos da instituição no ano de 2010.

Apesar de toda incerteza sobre a ocorrência da espécie na região da Ilha do Bananal, a presença de *C. latirostris* no estado do Tocantins pode ser conjecturada. Existem espécimes coletados em Flores de Goiás (MZUSP 2311, 2314, 2315, 2320, 2321, 2322) e Alvorada do Norte (CHUNB 32705), ambos os municípios da região nordeste de Goiás e inseridos na bacia do rio Paranã, cuja foz desemboca no rio Tocantins, em território tocantinense. Estes espécimes amparam uma suposta ocorrência de *C. latirostris* em regiões à jusante aos pontos das coletas mencionadas, inferido na possível presença da es-

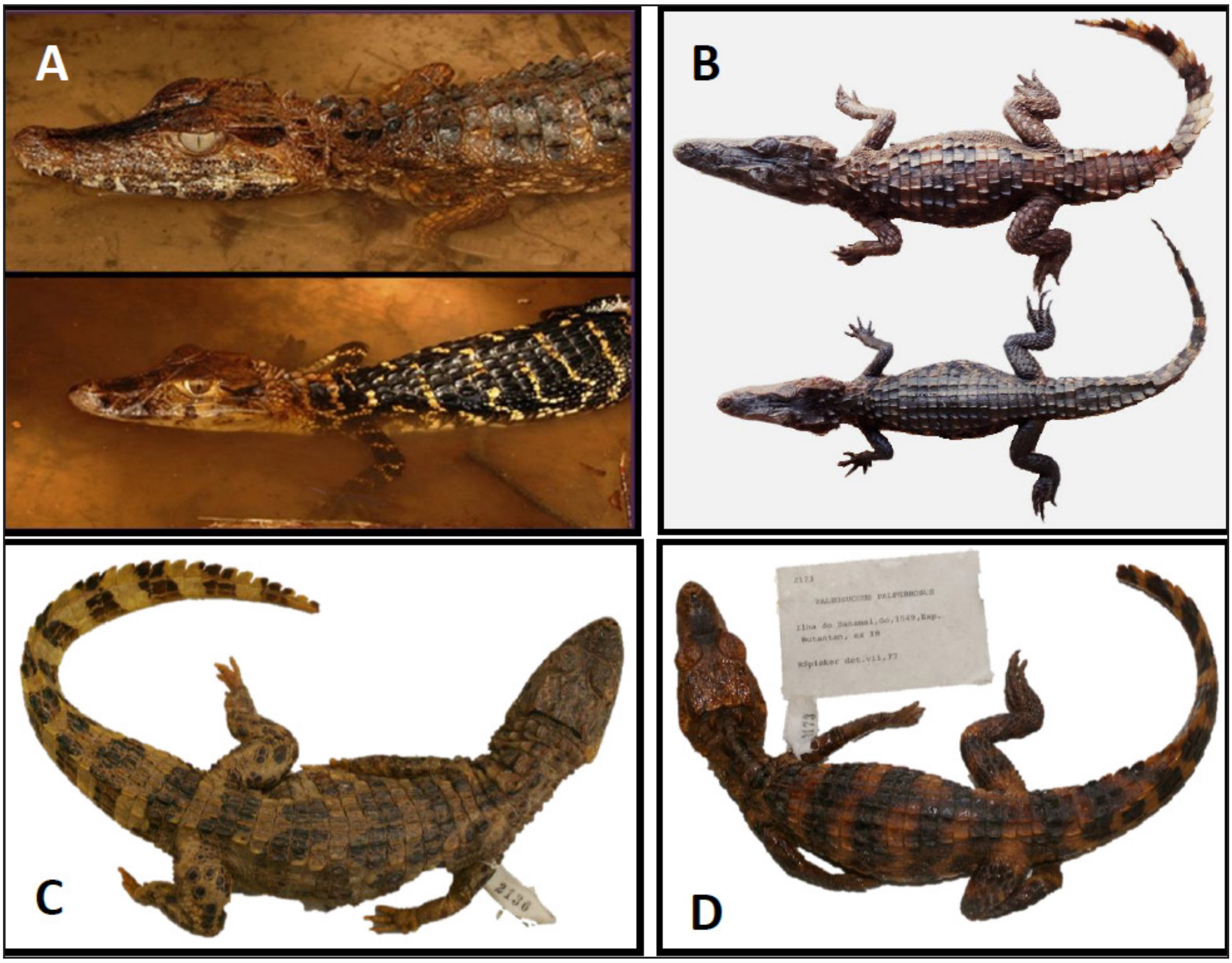

Figura 1. Fotografias de *Paleosuchus trigonatus* (acima) e *P. palpebrosus* (abaixo), no rio Araguaia, Araguatins, em A e B. Em B, espécimes MPEG 135 de *P. trigonatus* e MPEG 134 de *P. palpebrosus* distintos dos indivíduos em A (Fotos: Mauro Celso). Em C, espécime MZUSP 2136 de *Caiman latirostris* proveniente da Ilha do Bananal. D, espécime MZUSP 2173 de *P. palpebrosus*, coletado pelo herpetólogo Alphonse Hoge, na Ilha do Bananal em 1949. Fotos: Tulio Dornas.

pécie na porção sul-sudeste do estado do Tocantins. A condução de expedições de busca por jacarés, em especial *C. latirostris*, em toda porção sul do estado, necessita ser priorizada.

Com relação aos dados compilatórios, 233 registros de jacarés foram compilados, em 159 diferentes localidades de amostragem, distribuídas em 56 municípios mais a Ilha do Bananal. Destes 233 registros, 71 possuem evidência documental, ou por espécime depositado em coleção herpetológica, ou fotografia, enquanto 162 compreendem registros sem documentação (Tabela 1).

Independente do método de registro, dentre as 159 diferentes localidades com amostragem de crocodilianos (Apêndice), 110 tiveram uma única espécie registrada, 40 tiveram duas, sete tiveram três e apenas duas localidades tiveram quatro espécies registradas. Os registros de *C. latirostris* não foram considerados.

As lacunas de conhecimento de distribuição através da verificação de quadrículas de amostragem aponta que dos 277.650 km² correspondente ao território tocantinense, 149.018 km², cerca de 54%, possuem registro de pelo menos uma espécie de jacaré, independentemente do método. Entretanto, somente 27.737 km², cerca de 10% do território do Tocantins, tiveram registro de pelo menos 3 espécies de jacarés independente de uma evidência documental dos registros. Além disso, apenas 33.187 km², aproximadamente de 12% do território estadual, possuem pelo menos uma espécie depositada em coleção herpetológica, enquanto apenas 4.500 km², 1,6 % do território do Tocantins tiveram a coleta e depósito em coleção de no mínimo duas e no máximo três espécies de crocodilianos.

Das quatro espécies de jacarés confirmadas para o estado do Tocantins, três possuem registros no interior dos limites das Unidades de Conservação de Proteção Integral (UCPIs) e/ou Terras Indígenas (TI) tocantinenses. Apenas *P. trigonatus* está ausente de registros dentro de uma dessas duas categorias de áreas protegidas (Fig. 2). Nenhuma das espécies de jacarés do Brasil está ameaçada de extinção em nível global (IUCN, 2020) assim como em nível nacional (MMA, 2014).

No Tocantins, *Caiman crocodilus* ocorre no Parque Estadual do Cantão (PEC), Parque Nacional (PARNA) do Araguaia, Monumento Natural das Árvores Fossilizadas do Tocantins (MONAFTO), regiões fronteiriças entre o Parque Estadual do Jalapão (PEJ) e Estação Ecológica Serra Geral do Tocantins (EESGT), na TI do Araguaia, TI Khraô e TI Xerente (Fig. 2). É a espécie mais comum nos corpos hídricos do estado, ocorrendo em toda extensão da bacia Tocantins-Araguaia, quase sempre em abundância expressiva.

Na região da RPPN Centro de Pesquisa Canguçu, no rio Javaés, oeste do Tocantins, a densidade média de indivíduos da espécie vistos variou entre 1,5 a 4,2 ind/km com máximas de ate 12 ind/km (Portelinha *et al.*, 2019). Nas proximidades de Palmas, ao longo do reservatório da UHE LEM, a espécie já apresentou densidades de até 60 ind/km^2 dependendo do ambiente amostrado (Villaça, 2004). Levantamentos noturnos de aproximadamente seis horas não registrou jacarés nas proximidades da sede municipal de Palmas, conforme tentativa de contagem de jacarés feita por AM e MC em 20 junho de 2019. Segundo relatos de pescadores locais, carcaças abatidas nas margens dos reservatórios são constantes, de modo que a dificuldade de encontrar jacarés possivelmente é reflexo da forte pressão de caça. Pereira *et al.* (2019) demonstraram que a caça de jacarés na região é constante desde os anos 2000.

Existem registros de *M. niger* no PARNA do Araguaia, PEC e TI do Araguaia (Fig. 2). As ocorrências da espécie ao longo do rio Araguaia, desde a formação do rio Javaés até sua confluência com rio Tocantins, indica que a região abriga uma população mais numerosa do que ao longo do rio Tocantins. Na RPPN Centro de Pesquisa Canguçu, as densidades médias observadas variaram entre 0,5 e 1,5 ind/km com máximas de até 5 ind/km de rio (Portelinha *et al.*, 2019).

Entre outubro de 2002 e julho de 2003, nenhum indivíduo de *M. niger* foi registrado durante monitoramento no reservatório da UHE LEM, na região de Palmas (Villaça, 2004). O relato do Brigadeiro Lysias Rodrigues, em 1931, do furto de um pescado por um exemplar de quase 6 metros, em uma ilha fluvial do rio Tocantins, nas redondezas de São Salvador durante expedição à Porto Nacional (Rodrigues, 2001), mostra-se o registro mais a montante compilado da espécie no rio Tocantins. O abate de pelo menos dois indivíduos foi testemunhado para região da foz do ribeirão Taquaruçu Grande, na primeira metade da década de 1990, contudo não se sabe do paradeiro dos crânios, que foram fotografados na época (Leandro Ramos, com. pessoal).

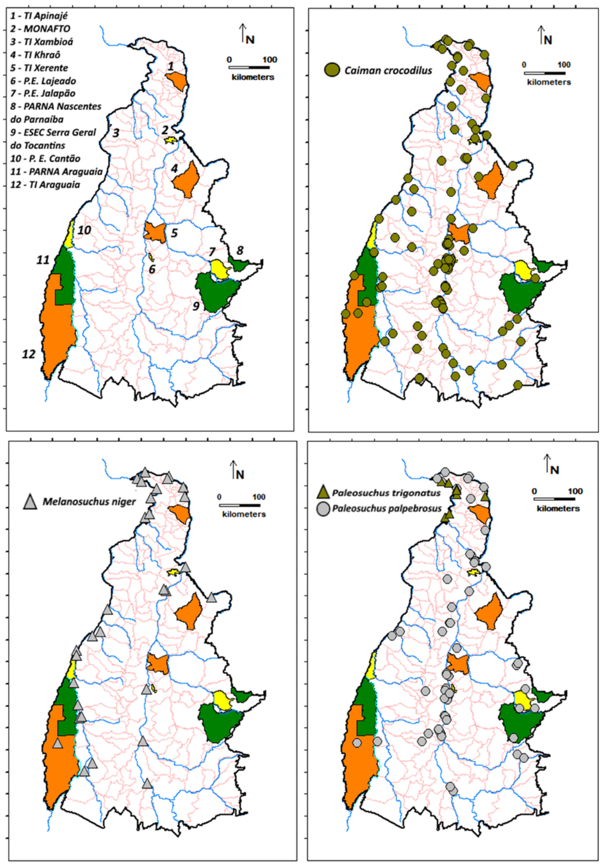

Figura 2. No sentido horário, Unidades de Conservação de Proteção Integral e Terras Indígenas no estado do Tocantins. Em seguida, mapas representando as localidades onde ocorreram registros de *Caiman crocodilus*, *Paleosuchus palpebrosus*, *P. trigonatus* e *Melanosuchus niger*, respectivamente, no estado do Tocantins em 1949.

Registro de um crânio (MZUSP 2374) na confluência do rio Manuel Alves e rio Tocantins, por volta do ano 2000, próximo à sede municipal de Ipueiras aparentava ser o mais recente registro documentado no rio Tocantins. Entretanto vídeos de julho de 2017 (g1.globo.com/to/tocantins1) e julho de 2019 (g1.globo.com/to/tocantins2) mostram indivíduos da espécie de tamanho considerável no reservatório da UHE LEM e próximo à praia da Tartaruga, no rio Tocantins, no município de Peixe, sul do estado.

Embora não haja ainda evidências contundentes (Marioni *et al.,* 2013), suspeita-se que as sucessivas barragens construídas ao longo do rio Tocantins funcionem como barreiras, fragmentando as populações a montante e jusante do eixo de barramento, e portanto, comprometendo o fluxo gênico da espécie. Além disso, o intenso tráfego de embarcações por razões de recreação ou mesmo trânsito de pessoas aumentou o contanto entre as mesmas e as populações remanescentes da espécie. No reservatório da UHE de Santo Antônio, no rio Madeira, em Rondônia, mais de 70% dos jacarés encontrados mortos no período pós-enchimento foram *M. niger* (42 indivíduos), sendo 52% com mais de 4 metros de comprimento total e a caça a principal razão da morte de *M. niger* neste monitoramento (Campos, 2015).

O mesmo cenário pode ser aplicado para a população de *M. niger* do rio Tocantins. Ainda que a espécie não esteja ameaçada de extinção em níveis nacional e global (MMA, 2014; IUCN, 2020), no âmbito estadual é corroborada uma significativa redução de sua população. Pereira *et al.* (2019) apontam que somente no rio Araguaia, entre as décadas de 1950 e 1980, um único grupo de caçadores podiam caçar até 4 mil indivíduos no período de um ano, com estimativas portanto de até 120 mil jacarés ao longo dos 30 anos amostrados. Certamente, devido a maior oferta de carne e couro, *M. niger* era preferido diante de *C. crocodilus.*

Entre 1992 e 2014, foram reportados 80 ocorrências de caça de jacarés com total de 134 animais abatidos no Tocantins (Pereira *et al.*, 2019). Embora drasticamente menor que os valores apresentados entre 1950 e 1980, a média de 5,83 indivíduos de jacarés caçados por ano, notificados em autuações ambientais demostram que a caça se mantém até os dias atuais. Essa atividade ilegal combinada com os sucessivos barramentos no rio Tocantins, que catalisam a caça de jacarés (Campos *et al.*, 2006), demonstram os riscos e a forte depleção que atravessa as populações de *M. niger* no rio Tocantins. Uma recomendação importante em detrimento desta situação são estudos populacionais sistematizados da espécie no Tocantins (Portelinha *et al.*, 2019).

A ausência de registros de *M. niger* em monitoramento entre outubro de 2002 e julho de 2003 (Villaça, 2004), avistamentos e vídeos pontuais de banhistas nos últimos 5 anos, do que pode ser um único indivíduo, associados à caça, deterioração e perda de ambientes pelos enchimentos de sucessivos reservatórios são indicativos de diminuição da população de *M. niger* nos limites do estado. As atividades de caça no estado do Tocantins desde a década de 1950 até os dias atuais mostram que a espécie teve significativas reduções populacionais (Pereira *et al.*, 2019). O estado de conservação da espécie deve ser encarado como moderadamente preocupante, carecendo a espécie de maior atenção e ações em favor de sua conservação.

Paleosuchus palpebrosus é uma espécie de jacaré típica de corpos d'agua estreitos no interior de florestas de várzea e matas de galeria. No Tocantins foi encontrada no PEJ, na EESGT e nas regiões fronteiriças entre ambos. Também foi registrada na TI do Araguaia e possui registros no entorno imediatos do PEL, MONAFTO, TI Xerente e TI Apinajé (Fig. 2). Em Palmas o reservatório da UHE LEM apresentou densidades de até 110 ind/km^2 dependendo do ambiente amostrado (Villaça, 2004). Contagem sazonal realizada no Parque Municipal Cesamar, também em Palmas, mostrou uma densidade aparente de quase cinco indivíduos por quilometro de margem percorrida com 29 indivíduos detectados de *P. palpebrosus* (25 deles capturados) (AM e MCHS em preparação). A ampla distribuição da espécie no Tocantins, ocupando praticamente qualquer ribeirão e córrego nos limites do estado demonstra pouca probabilidade de ameaça de extinção em nível estadual.

Em contrapartida, *P. trigonatus* é a única espécie de jacaré que não tem confirmação de registros em Unidades de Conservação e/ou Terras Indígenas no estado do Tocantins. Até o presente momento, a ocorrência da espécie foi registrada somente ao extremo norte do Tocantins. Registros não documentados na região da Serra dos Martírios e pedral de Santa Isabel, no rio Araguaia, indicam a presença da espécie no interior do Parque Estadual Serra das Andorinhas, no lado paraense do rio Araguaia (Atizengen e Silva, 1999). O relato de registro, também não documentado na Ilha de Serra Quebrada, no rio Tocantins, indica potencial ocorrência na TI Apinajé.

Somente com a coleta de exemplar testemunho (MPEG 135), em 2012, a presença da espécie no baixo Araguaia e médio rio Tocantins foi confirmada. Nessa região, não há unidade de conservação de proteção integral federal ou estadual. Na década de 1990, foi proposto o Parque Estadual Encontros das Águas (PEEA), com mais de 6 mil hectares, na confluência dos rios Tocantins e Araguaia, município de Esperantina (Seplan, 2012).. A região ainda é apontada como área potencial para a conservação da biodiversidade do Tocantins (Seplan, 2012; Sefaz, 2019) com dezenas de espécies de peixes reofílicos em vulnerabilidade (Akama, 2017; Hrbek *et al.*, 2018) e o ameaçado boto-do-Araguaia (Hrbek *et al.*, 2014).

Conclusões

Evidências documentais confirmam a presença de quatro espécies de jacarés nos limites do Tocantins: *Caiman crocodilus, Melanosuchus níger, Paleosuchus palpebrosus e Paleosuchus trigonatus.* A confirmação de *Paleosuchus trigonatus* no baixo Araguaia expande a ocorrência da espécie para o extremo leste da Amazônia e reforça a presença da espécie na zona ecotonal entre Amazônia e Cerrado.

Embora haja evidências documentais de *C. latirostris* em território tocantinense, possíveis equívocos na etiquetagem da espécie aliado a um padrão de distribuição geográfica incongruente, justificam sua não confirmação na listagem estadual atual. Expedições de buscas pela espécie, sobretudo na porção sul do estado do Tocantins, entre a Ilha do Bananal, Rio Tocantins e Serra Geral são altamente desejáveis, uma vez que essas regiões podem ser consideradas aquelas com maiores chances de detecções.

As análises de lacunas de conhecimento de distribuição geográfica demostraram que praticamente 55% da área territorial do estado do Tocantins foi alvo de algum esforço amostral de crocodilianos, sem qualquer restrição quanto aos métodos de registros, seja eles documentados (coleta e/ou fotografias) ou avistamento. Quando considerado regiões do Tocantins amostradas com algum nível de documentação, o percentual de conhecimento amostral cai para 12% do território do estado.

O estado de conservação das espécies de crocodilianos no Tocantins mostra-se relativamente preocupante. Três espécies apresentam ocorrências confirmadas no interior de Unidades de Conservação de Proteção Integral ou Terras Indígenas tocantinenses, áreas protegidas com efetividade de proteção mais elevada que as Unidades de Conservação de Uso Sustentável. Contudo, a pressão de caça e os sucessivos barramentos para estabelecimentos de UHEs no rio Tocantins, e até mesmo no rio Araguaia, podem estar causando uma diminuição da população de *Melanosuchus niger*. Nos últimos dez anos, registros da espécie no rio Tocantins são escassos.

Por sua vez, *P. trigonatus* aparenta ocorrer somente no extremo norte do Tocantins, onde não coincide com os limites de nenhuma UCPI ou TI do estado. Há suspeitas da ocorrência da espécie na TI Apinajé. Portanto, a criação de UCPIs no extremo norte do Tocantins pode contribuir com a proteção legal da espécie em âmbito estadual. O escasso conhecimento sobre a ocorrência da espécie em território estadual, atrelado a possibilidade de inundação dos rios Tocantins e Araguaia pelos sucessivos projetos de usinas hidrelétricas refletem um estado de conservação bastante preocupante para *P. trigonatus*, sugerindo categorias de ameaça mais severas em eventuais avaliações estaduais da fauna ameaçada de extinção.

Por fim, monitoramentos contínuos das populações das espécies, em especial *M. niger e P. trigonatus*, associado às pesquisas com abordagens voltadas à definição da distribuição geográfica nos limites tocantinenses da bacia Tocantins-Araguaia são substancialmente desejáveis. Estudos desta natureza poderão definir os reais riscos de extinção regional e auxiliar na proposta de ações a favor da conservação destas espécies.

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Apêndice

Espécies de crocodilianos registradas para o estado do Tocantins e suas respectivas fontes de consulta, localidades e coordenadas. Em fonte, C = captura; V = visual/avistamento; F = fotografia. CHUNB: Coleção Herpetológica da Universidade Brasília; MNHN: Museo Nacional de Historia Natural (Montevideo, Uruguai); MPEG: Museu Paraense Emílio Goeldi; MZUSP: Museu de Zoologia da Universidade de São Paulo.

ESPÉCIE	FONTE	LOCALIDADE	MUNICÍPIO	ANO	LATITUDE	LONGITUDE
Caiman crocodilus (Linnaeus, 1758)	AM, MCHS e equipe - V, F	Rio Tocantins 1	São Sebastiao	2010	-5,187125	-48,428166
Caiman crocodilus (Linnaeus, 1758)	AM, MCHS e equipe - V, F	Rio Tocantins 2	Sampaio	2010	-5,28575	-47,87807
Caiman crocodilus (Linnaeus, 1758)	AM, MCHS e equipe - V, F	Rio Tocantins 3	Esperantina	2010	-5,336767	-48,594754
Caiman crocodilus (Linnaeus, 1758)	AM, MCHS e equipe - C, V, F	Parque Cesamar	Palmas	2019	-10,20833333	-48,31944444
Caiman crocodilus (Linnaeus, 1758)	Bioterra, 2005 - C, V	Faz. Dois Rios	Lagoa da Confusão	2005	-10,75722222	-49,89833333
Caiman crocodilus (Linnaeus, 1758)	Brandao e Perez-Jr, 2001 - V	Estrada Graciosa	Palmas	2000	-10,16361111	-48,3825
Caiman crocodilus (Linnaeus, 1758)	Brandao e Perez-Jr 2001 - V	Desconhecido	Palmas	2000	-10,16361111	-48,3921
Caiman crocodilus (Linnaeus, 1758)	CHUNB 24751-52	Centro de Pesquisa Canguçu	Pium	2001	-9,978055556	-50,03638889
Caiman crocodilus (Linnaeus, 1758)	CHUNB 15231	Desconhecido	Palmas	2000	-10,16361111	-48,3825
Caiman crocodilus (Linnaeus, 1758)	CHUNB 49928	Estrada Graciosa	Palmas	2000	-10,16361111	-48,3825
Caiman crocodilus (Linnaeus, 1758)	CHUNB 10742	Ilha do Bananal - Santa Terezinha	Ilha do Bananal	1995	-10,48166667	-50,47138889
Caiman crocodilus (Linnaeus, 1758)	CHUNB 45441-42-43	Parque Estadual do Cantao - Rio do Coco	Pium	2005	-7,205833333	-47,75527778

Caiman crocodilus (Linnaeus, 1758)	CMT, 2006 - C, V	Projeto Irrigação Manuel Alves	Dianópolis	2006	-11,61	-47,00611111
Caiman crocodilus (Linnaeus, 1758)	CMT, 2008 - C, V	Trecho BR 010 - 1	Aparecida do Rio Negro	2007	-9,933333333	-48,01666667
Caiman crocodilus (Linnaeus, 1758)	CMT, 2008 - C, V	Trecho BR 010 - 2	Rio Sono	2007	-9,433333333	-47,81666667
Caiman crocodilus (Linnaeus, 1758)	CMT, 2008 - C, V	Trecho BR 010 - 4	Goiatins	2007	-8,266666667	-47,68333333
Caiman crocodilus (Linnaeus, 1758)	CNEC, 2003 - V	Estrada Palmeirante - Bielândia	Palmeirante do TO	2003	-7,885555556	-47,95138889
Caiman crocodilus (Linnaeus, 1758)	Consplan, 2004 - C, V	Sete Barracas	São Miguel do TO	2004	-5,541944444	-47,56027778
Caiman crocodilus (Linnaeus, 1758)	FADESP, 1999 - V	Ilha Santa Maria Velha	Couto Magalhaes	1997	-8,303611111	-49,26527778
Caiman crocodilus (Linnaeus, 1758)	FADESP, 1999 - C, V	Lajeado Grande	Lajeado	1997	-9,666666667	-48,35
Caiman crocodilus (Linnaeus, 1758)	FAET, 1999 - V	Projeto Agricola Campos Lindos	Campos Lindos	1999	-8,0301	-46,921
Caiman crocodilus (Linnaeus, 1758)	Farol, 2002 - V	Sitio 2 - Cerrado Sensu Strictu	Arraias	2001	-12,4877	-47,1609
Caiman crocodilus (Linnaeus, 1758)	Farol, 2002 - V	Sitio 3 - Cerrado Sensu Strictu	Paranã	2001	-12,46222222	-48,26472222
Caiman crocodilus (Linnaeus, 1758)	Magna 2002 - V	Projeto Sampaio	Sampaio	2002	-5,345833333	-47,92
Caiman crocodilus (Linnaeus, 1758)	Malvasio e Salera-Jr., 2006 C, V, F	Rio Javaés	Ilha do Bananal	2005	-9,966666667	-50,03333333
Caiman crocodilus (Linnaeus, 1758)	AM, MCHS e equipe - V, F	Rio Araguaia	Esperantina	2010	-5,412195	-48,498413
Caiman crocodilus (Linnaeus, 1758)	AM, MCHS e equipe - V, F	Rio Araguaia - Santa Isabel	Ananás	2010	-6,141202	-48,325941
Caiman crocodilus (Linnaeus, 1758)	AM, MCHS e equipe - V, F	Rio Araguaia - trecho 1	Araguatins	2010	-5,574294	-48,158616
Caiman crocodilus (Linnaeus, 1758)	AM, MCHS e equipe - V, F	Rio Araguaia - trecho 2	Araguatins	2010	-5,784852	-48,291714
Caiman crocodilus (Linnaeus, 1758)	MNHN 1412	Ilha do Bananal 1	Ilha do Bananal	1953	-11,35	-50,66666667
Caiman crocodilus (Linnaeus, 1758)	MPEG 006	Ilha do Bananal 2	Ilha do Bananal	1962	-11,09805556	-50,20083333
Caiman crocodilus (Linnaeus, 1758)	MPEG 136	Rio Araguaia, lha de Sao Vicente	Araguatins	2010	-5,604267	-48,153037
Caiman crocodilus (Linnaeus, 1758)	MZUSP 2343	Cristalândia	Cristalândia	1990	-11,33333333	-50,4
Caiman crocodilus (Linnaeus, 1758)	MZUSP 2106-07-08-09	Ilha do Bananal 3	Ilha do Bananal	1949	-11,33333333	-50,4
Caiman crocodilus (Linnaeus, 1758)	MZUSP	Paranã	Paranã	2000	-12,61694444	-47,88555556
Caiman crocodilus (Linnaeus, 1758)	MZUSP	São Salvador	São Salvador	2001	-12,75	-48,21666667

Caiman crocodilus (Linnaeus, 1758)	Oikos, 2002 - C, V	Fazenda Morro Grande - TO 010	Babaçulândia	2001	-7,091111111	-47,82694444
Caiman crocodilus (Linnaeus, 1758)	Oikos, 2006 - V, F	Porto da Balsa	Caseara	2006	-9,219456472	-49,9166
Caiman crocodilus (Linnaeus, 1758)	Pavan, 2007 - C, V	Floresta de galeria 3	Arguianópolis	2004	-6,5181	-47,5272
Caiman crocodilus (Linnaeus, 1758)	Pavan, 2007 - C, V	Floresta de galeria 5	Babaçulândia	2001	-7,205833333	-47,75527778
Caiman crocodilus (Linnaeus, 1758)	Pavan, 2007 - C, V	Floresta de galeria 6	Filadélfia	2003	-7,341666667	-47,51472222
Caiman crocodilus (Linnaeus, 1758)	Pavan, 2007 - C, V	Floresta de galeria 8	Palmeirante do TO	2004	-7,841944444	-47,99527778
Caiman crocodilus (Linnaeus, 1758)	Pavan, 2007 - C, V	Floresta de galeria 14	Peixe	2000	-12,24944444	-48,41972222
Caiman crocodilus (Linnaeus, 1758)	Pavan, 2007 - C, V	Floresta de galeria 15	São Salvador	2001	-12,46222222	-48,26472222
Caiman crocodilus (Linnaeus, 1758)	Pavan, 2007 - C, V	Floresta de galeria 16	Paranã	2000	-12,61694444	-47,88555556
Caiman crocodilus (Linnaeus, 1758)	Penha et al. 2004 - C, V	Desconhecido 3	Formoso do Araguaia	2002	-11,96666667	-49,78333333
Caiman crocodilus (Linnaeus, 1758)	Poyry, 2012 - V, F	Rio Tocantins 4	Peixe	2012	-12,12472222	-48,48388889
Caiman crocodilus (Linnaeus, 1758)	PSAF, 2001 - V	Aeroporto de Palmas	Palmas	2001	-10,27944444	-48,36194444
Caiman crocodilus (Linnaeus, 1758)	Seplan, 2000 - C, V	Parque Estadual do Cantão 2	Pium	2000	-8,607777778	-48,31472222
Caiman crocodilus (Linnaeus, 1758)	Oikos e MRS, 2005 - C, V	Parque Estadual Monumento Árvores Fossilizadas	Filadélfia	2000	-7,401666667	-47,76722222
Caiman crocodilus (Linnaeus, 1758)	Soares, 2007 - C, V	Centro de Pesquisa Canguçu - Lago do mato verde	Pium	2007	-7,841944444	-47,99361111
Caiman crocodilus (Linnaeus, 1758)	Soares, 2007 - C, V	Rio Javaés	Ilha do Bananal	2007	-9,966666667	-50,03333333
Caiman crocodilus (Linnaeus, 1758)	Themag, 1996 - V	Rio Tocantins - Pa3	Palmas	1995	-10,11666667	-48,38333333
Caiman crocodilus (Linnaeus, 1758)	Themag & Engevix 2000 - C, V	UHE Peixe 1	Peixe	2000	-7,841944444	-47,99527778
Caiman crocodilus (Linnaeus, 1758)	Themag & Engevix 2000 - C, V	UHE Peixe 2	Paranã	2000	-12,24944444	-48,41972222
Caiman crocodilus (Linnaeus, 1758)	Tocantins, 1998 - C, V	Trecho TO-476	Rio da Conceição	1997	-11,46666667	-46,81666667
Caiman crocodilus (Linnaeus, 1758)	Unitins, 1996 - C, V	Rio Formoso	Lagoa da Confusão	1996	-10,73333333	-49,85
Caiman crocodilus (Linnaeus, 1758)	Unitins, 1998 - C, V	Projeto Formoso	Formoso do Araguaia	1998	-11,79277778	-49,61138889
Caiman crocodilus (Linnaeus, 1758)	Villaça, 2004 - C, V	Rio Tocantins - Lj03	Lajeado	2003	-9,806111111	-48,35083333

Caiman crocodilus (Linnaeus, 1758)	Villaça, 2004 - C, V	Rio Tocantins - Br01	Brejinho de Nazaré	2003	-11,04472222	-48,5725
Caiman crocodilus (Linnaeus, 1758)	Villaça, 2004 - C, V	Rio Tocantins - Br10	Brejinho de Nazaré	2003	-11,23861111	-48,48694444
Caiman crocodilus (Linnaeus, 1758)	Villaça, 2004 - C, V	Rio Tocantins - Br02	Brejinho de Nazaré	2003	-11,05194444	-48,55166667
Caiman crocodilus (Linnaeus, 1758)	Villaça, 2004 - C, V	Rio Tocantins - Br03	Brejinho de Nazaré	2003	-11,12222222	-48,52083333
Caiman crocodilus (Linnaeus, 1758)	Villaça, 2004 - C, V	Rio Tocantins - Br04	Brejinho de Nazaré	2003	-11,10027778	-48,53583333
Caiman crocodilus (Linnaeus, 1758)	Villaça, 2004 - C, V	Rio Tocantins - Br05	Brejinho de Nazaré	2003	-11,18416667	-48,50305556
Caiman crocodilus (Linnaeus, 1758)	Villaça, 2004 - C, V	Rio Tocantins - Br07	Brejinho de Nazaré	2003	-11,06916667	-48,54638889
Caiman crocodilus (Linnaeus, 1758)	Villaça, 2004 - C, V	Rio Tocantins - Br08	Brejinho de Nazaré	2003	-11,14194444	-48,52416667
Caiman crocodilus (Linnaeus, 1758)	Villaça, 2004 - C, V	Rio Tocantins - Br09	Brejinho de Nazaré	2003	-11,21555556	-48,48777778
Caiman crocodilus (Linnaeus, 1758)	Villaça, 2004 - C, V	Rio Tocantins - Lj01	Lajeado	2003	-9,799722222	-48,40944444
Caiman crocodilus (Linnaeus, 1758)	Villaça, 2004 - C, V	Rio Tocantins - Lj02	Lajeado	2003	-9,821111111	-48,33333333
Caiman crocodilus (Linnaeus, 1758)	Villaça, 2004 - C, V	Rio Tocantins - Mi01	Miracema	2003	-9,755555556	-48,39138889
Caiman crocodilus (Linnaeus, 1758)	Villaça, 2004 - C, V	Rio Tocantins - Mi02	Miracema	2003	-9,789444444	-48,39194444
Caiman crocodilus (Linnaeus, 1758)	Villaça, 2004 - C, V	Rio Tocantins - Mi03	Miracema	2003	-9,776388889	-48,40777778
Caiman crocodilus (Linnaeus, 1758)	Villaça, 2004 - C, V	Rio Tocantins - Mi04	Miracema	2003	-9,771388889	-48,38027778
Caiman crocodilus (Linnaeus, 1758)	Villaça, 2004 - C, V	Rio Tocantins - Pa01	Palmas	2003	-10,18222222	-48,36555556
Caiman crocodilus (Linnaeus, 1758)	Villaça, 2004 - C, V	Rio Tocantins - Pa02	Palmas	2003	-10,1975	-48,36861111
Caiman crocodilus (Linnaeus, 1758)	Villaça, 2004 - C, V	Rio Tocantins - Pa03	Palmas	2003	-10,12416667	-48,3625
Caiman crocodilus (Linnaeus, 1758)	Villaça, 2004 - C, V	Rio Tocantins - Pa04	Palmas	2003	-10,30555556	-48,36611111
Caiman crocodilus (Linnaeus, 1758)	Villaça, 2004 - C, V	Rio Tocantins - Pa05	Palmas	2003	-10,355	-48,36861111
Caiman crocodilus (Linnaeus, 1758)	Villaça, 2004 - C, V	Rio Tocantins - Pa06	Palmas	2003	-10,31333333	-48,365
Caiman crocodilus (Linnaeus, 1758)	Villaça, 2004 - C, V	Rio Tocantins - Pa07	Palmas	2003	-10,16805556	-48,36222222
Caiman crocodilus (Linnaeus, 1758)	Villaça, 2004 - C, V	Rio Tocantins - PN01	Porto Nacional	2003	-10,10111111	-48,41055556
Caiman crocodilus (Linnaeus, 1758)	Villaça, 2004 - C, V	Rio Tocantins - PN10	Porto Nacional	2003	-10,77944444	-48,41361111
Caiman crocodilus (Linnaeus, 1758)	Villaça, 2004 - C, V	Rio Tocantins - PN02	Porto Nacional	2003	-10,15166667	-48,42638889
Caiman crocodilus (Linnaeus, 1758)	Villaça, 2004 - C, V	Rio Tocantins - PN03	Porto Nacional	2003	-10,33333333	-48,43472222

Caiman crocodilus (Linnaeus, 1758)	Villaça, 2004 - C, V	Rio Tocantins - PN04	Porto Nacional	2003	-10,71888889	-48,44027778
Caiman crocodilus (Linnaeus, 1758)	Villaça, 2004 - C, V	Rio Tocantins - PN05	Porto Nacional	2003	-10,73138889	-48,41472222
Caiman crocodilus (Linnaeus, 1758)	Villaça, 2004 - C, V	Rio Tocantins - PN06	Porto Nacional	2003	-10,81805556	-48,3825
Caiman crocodilus (Linnaeus, 1758)	Villaça, 2004 - C, V	Rio Tocantins - PN07	Porto Nacional	2003	-10,17527778	-48,43555556
Caiman crocodilus (Linnaeus, 1758)	Villaça, 2004 - C, V	Rio Tocantins - PN08	Porto Nacional	2003	-10,32027778	-48,43694444
Caiman crocodilus (Linnaeus, 1758)	Villaça, 2004 - C, V	Rio Tocantins - PN09	Porto Nacional	2003	-10,74277778	-48,44277778
Caiman crocodilus (Linnaeus, 1758)	Vitt et. al., 2005 - C, F	Jalapão	Mateiros	2004	-10,55	-46,41666667
Caiman crocodilus (Linnaeus, 1758)	WPS e equipe - V	Alagado 1	Araguatins	2011	-5,223798	-48,369109
Caiman crocodilus (Linnaeus, 1758)	WPS e equipe - V, F	Alagado 2	Palmeirante do TO	2009	-7,856393	-47,944638
Caiman crocodilus (Linnaeus, 1758)	WPS e equipe - V	Alagado 3	Dois Irmãos	2016	-9,056513	-49,179482
Caiman crocodilus (Linnaeus, 1758)	WPS e equipe - V	Floresta de galeria 2	São Bento	2019	-5,89612	-48,030174
Caiman crocodilus (Linnaeus, 1758)	WPS e equipe - V	Lago azul	Araguaína	2015	-7,212402	-48,220883
Caiman crocodilus (Linnaeus, 1758)	WPS e equipe - V	Lagoa 1	Ananás	2019	-6,317803	-48,083404
Caiman crocodilus (Linnaeus, 1758)	WPS e equipe - V	Lagoa 2	Piraquê	2019	-6,698477	-48,285681
Caiman crocodilus (Linnaeus, 1758)	WPS e equipe - V	Lagoa 3	Colinas	2013	-7,909444	-48,523767
Caiman crocodilus (Linnaeus, 1758)	WPS e equipe - V	Lagoa 4	Marianópolis	2013	-9,634249	-49,564456
Caiman crocodilus (Linnaeus, 1758)	WPS e equipe - V	Lagoa 5	Porto Nacional	2015	-10,230687	-48,592362
Caiman crocodilus (Linnaeus, 1758)	WPS e equipe - V	Lagoa 6	Gurupi	2013	-11,621889	-49,053636
Caiman crocodilus (Linnaeus, 1758)	WPS e equipe - V	Lagoa 7	Gurupi	2015	-11,846082	-48,963693
Caiman crocodilus (Linnaeus, 1758)	WPS e equipe - V	Lagoa 8	Figueirópolis	2015	-12,126391	-49,072403
Caiman crocodilus (Linnaeus, 1758)	WPS e equipe - V, F	Lagoa 9	Talismã	2019	-12,781642	-49,000201
Caiman crocodilus (Linnaeus, 1758)	WPS e equipe - V, F	Lagoa Canabrava	Pedro Afonso	2012	-9,216318	-48,302815
Caiman crocodilus (Linnaeus, 1758)	WPS e equipe - V	Lagoa da Embrapa - Rib. Agua Fria	Palmas	2018	-10,13673	-48,311208
Caiman crocodilus (Linnaeus, 1758)	WPS e equipe - V	Lagoa de captação	Arraias	2018	-12,940443	-46,802305
Caiman crocodilus (Linnaeus, 1758)	WPS e equipe - V	Lagoa Vereda	Couto Magalhaes	2019	-8,541729	-49,138257
Caiman crocodilus (Linnaeus, 1758)	WPS e equipe - V	Lagos	Pium	2018	-10,527662	-49,844052

Caiman crocodilus (Linnaeus, 1758)	WPS e equipe - V	Mata ciliar 1	Porto Nacional	2015	-10,393094	-48,701857
Caiman crocodilus (Linnaeus, 1758)	WPS e equipe - V	Mata ciliar 2	Brejinho de Nazaré	2015	-11,108345	-48,648653
Caiman crocodilus (Linnaeus, 1758)	WPS e equipe - V	Rio Araguaia 2	Araguacema	2011	-8,796611	-49,546752
Caiman crocodilus (Linnaeus, 1758)	WPS e equipe - V, F	Rio Cana-brava	Talismã	2020	-12,794521	-49,020256
Caiman crocodilus (Linnaeus, 1758)	WPS e equipe - V	Rio Manoel Alves	Almas	2009	-11,746881	-47,142488
Caiman crocodilus (Linnaeus, 1758)	WPS e equipe - V	Rio Palmeiras	Dianópolis	2009	-11,964305	-46,748869
Caiman crocodilus (Linnaeus, 1758)	WPS e equipe - V	Rio Piedade	Divinópolis	2019	-9,855196	-49,185162
Caiman crocodilus (Linnaeus, 1758)	WPS e equipe - V	Vereda 2	Palmas	2017	-10,122663	-48,355481
Melanosuchus niger (Spix, 1825)	AM, MCHS e equipe - V, F	Rio Tocantins 1	São Sebastiao	2010	-5,187125	-48,428166
Melanosuchus niger (Spix, 1825)	AM, MCHS e equipe - V, F	Rio Tocantins 2	Sampaio	2010	-5,28575	-47,87807
Melanosuchus niger (Spix, 1825)	AM, MCHS e equipe - V, F	Rio Tocantins 3	Esperantina	2010	-5,336767	-48,594754
Melanosuchus niger (Spix, 1825)	Atizengen e Silva 1999 - V, F	Rio Araguaia - Parque Estadual Serras das Andorinhas	Xambioá	1998	-6,216666667	-48,41666667
Melanosuchus niger (Spix, 1825)	Bioterra, 2005 - C, V	Faz. Dois Rios	Lagoa da Confusão	2005	-10,75722222	-49,89833333
Melanosuchus niger (Spix, 1825)	Consplan, 2004 - C, V	Sete Barracas	São Miguel do TO	2004	-5,541944444	-47,56027778
Melanosuchus niger (Spix, 1825)	FADESP, 1999 - V	Ilha Santa Maria Velha	Couto Magalhaes	1997	-8,303611111	-49,26527778
Melanosuchus niger (Spix, 1825)	FAET, 1999 - V	Projeto Agrícola Campos Lindos	Campos Lindos	1999	-8,0301	-46,921
Melanosuchus niger (Spix, 1825)	Magna 2002 - V	Projeto Sampaio	Sampaio	2002	-5,345833333	-47,92
Melanosuchus niger (Spix, 1825)	Malvasio e Salera-Jr., 2006 - V	Rio Javaes	Ilha do Bananal	2005	-9,966666667	-50,03333333
Melanosuchus niger (Spix, 1825)	AM, MCHS e equipe - V, F	Rio Araguaia	Esperantina	2010	-5,412195	-48,498413
Melanosuchus niger (Spix, 1825)	AM, MCHS e equipe - V, F	Rio Araguaia - Santa Isabel	Ananás	2010	-6,141202	-48,325941
Melanosuchus niger (Spix, 1825)	AM, MCHS e equipe - V, F	Rio Araguaia - trecho 1	Araguatins	2010	-5,574294	-48,158616
Melanosuchus niger (Spix, 1825)	AM, MCHS e equipe - V, F	Rio Araguaia - trecho 2	Araguatins	2010	-5,784852	-48,291714
Melanosuchus niger (Spix, 1825)	MNHN 1414	Ilha do Bananal - Rio Araguaia	Ilha do Bananal	1953	-11,33333333	-50,4
Melanosuchus niger (Spix, 1825)	MZUSP 2374	Lagoa Roma - Rio Tocantins	São ValéRio	2001	-11,29361111	-48,46583333

Melanosuchus niger (Spix, 1825)	Oikos, 2002 - C, V	Lagoa marginal ao Rio Tocantins	Palmeirante do TO	2001	-7,885555556	-47,95138889
Melanosuchus niger (Spix, 1825)	Oikos, 2006 - V, F	Rio Caiapo	Araguacema	2006	-8,9	-49,61666667
Melanosuchus niger (Spix, 1825)	Oikos, 2006 - V, F	Rio Piranhas - TO 437	Araguacema	2006	-8,804722222	-49,42472222
Melanosuchus niger (Spix, 1825)	Pavan, 2007 - C, V	Floresta de galeria 8	Palmeirante do TO	2004	-7,841944444	-47,99361111
Melanosuchus niger (Spix, 1825)	Penha et al. 2004 - C, V	Formoso do Araguaia	Formoso do Araguaia	2002	-11,96666667	-49,78333333
Melanosuchus niger (Spix, 1825)	Rodrigues, 2001 - V	Ilha dos Canoeiros, barra com Rio das Almas	Sao Salvador	1931	-12,24027778	-48,37555556
Melanosuchus niger (Spix, 1825)	Seplan, 2000 - C, V	Parque Estadual do Cantao 1	Pium	2000	-7,341666667	-47,50388889
Melanosuchus niger (Spix, 1825)	Soares, 2007 - C, V	Centro de Pesquisa Cangucu - Lago do mato verde	Pium	2007	-7,841944444	-47,99361111
Melanosuchus niger (Spix, 1825)	Soares, 2007 - C, V	Rio Javaes	Ilha do Bananal	2007	-9,966666667	-50,03333333
Melanosuchus niger (Spix, 1825)	Themag, 1996 - V	Rio Tocantins - Pa3	Palmas	1995	-10,11666667	-48,38333333
Melanosuchus niger (Spix, 1825)	Themag, 2000 - V	Ilha de Serra Quebrada (Rio Tocantins)	Itaguatins	2000	-5,740906	-47,521715
Melanosuchus niger (Spix, 1825)	TD - V, F	Praia da Balsa	Caseara	2018	-9,231388889	-49,96944444
Melanosuchus niger (Spix, 1825)	Unitins, 1996 - C, V	Rio Formoso	Lagoa da Confusão	1996	-10,73333333	-49,85
Melanosuchus niger (Spix, 1825)	Unitins, 1998 - C, V	Projeto Formoso	Formoso do Araguaia	1998	-11,79277778	-49,61138889
Melanosuchus niger (Spix, 1825)	WPS e equipe - V	Rio do Coco	Caseara	2011	-9,346061	-49,970843
Melanosuchus niger (Spix, 1825)	WPS e equipe - V, F	Rio Javaes 2	Lagoa da Confusão	2019	-10,471628	-49,933851
Paleosuchus palpebrosus (Cuvier, 1807)	AM, MCHS e equipe - V, F	Rio Tocantins 1	São Sebastiao	2010	-5,187125	-48,428166
Paleosuchus palpebrosus (Cuvier, 1807)	AM, MCHS e equipe - V, F	Rio Tocantins 2	Sampaio	2010	-5,28575	-47,87807
Paleosuchus palpebrosus (Cuvier, 1807)	AM, MCHS e equipe - V, F	Rio Tocantins 3	Esperantina	2010	-5,336767	-48,594754
Paleosuchus palpebrosus (Cuvier, 1807)	AM, MCHS e equipe - C, V, F	Parque Cesamar	Palmas	2019	-10,20833333	-48,32694444
Paleosuchus palpebrosus (Cuvier, 1807)	CHUNB 42271	37,2km W Mateiros Rio Novo	Mateiros	2000	-10,55916667	-46,75388889
Paleosuchus palpebrosus (Cuvier, 1807)	CHUNB 37540	Desconhecido 2	Dianópolis	2003	-11,59805556	-46,80972222
Paleosuchus palpebrosus (Cuvier, 1807)	CHUNB 47991	Lagoa Bonita	Dianópolis	2003	-11,67222222	-46,65638889
Paleosuchus palpebrosus (Cuvier, 1807)	Consplan, 2004 - C, V	Sete Barracas	São Miguel do TO	2004	-5,541944444	-47,56027778

Paleosuchus palpebrosus (Cuvier, 1807)	Direção, 2006 - C, V	Área Lizarda - Vereda	Lizarda	2006	-9,583333333	-46,81666667
Paleosuchus palpebrosus (Cuvier, 1807)	Direção, 2006 - C, V	Faz Sussuapara - Mata de galeria	Lizarda	2006	-9,526111111	-46,79944444
Paleosuchus palpebrosus (Cuvier, 1807)	Direção, 2006 - C, V	Vereda 1	São Felix do TO	2006	-10,11666667	-46,63333333
Paleosuchus palpebrosus (Cuvier, 1807)	Magna 2002 - C, V	Projeto Sampaio	Sampaio	2002	-5,345833333	-47,92
Paleosuchus palpebrosus (Cuvier, 1807)	AM, MCHS e equipe - V, F	Rio Araguaia	Esperantina	2010	-5,412195	-48,498413
Paleosuchus palpebrosus (Cuvier, 1807)	AM, MCHS e equipe - V, F	Rio Araguaia - trecho 1	Araguatins	2010	-5,574294	-48,158616
Paleosuchus palpebrosus (Cuvier, 1807)	MPEG 134	Rio Tocantins 3	Esperantina	2010	-5,336767	-48,594754
Paleosuchus palpebrosus (Cuvier, 1807)	MZUSP 2369	Guaraí	Guaraí	2001	-8,816666667	-48,48333333
Paleosuchus palpebrosus (Cuvier, 1807)	MZUSP 2173	Ilha do Bananal 3	Ilha do Bananal	1949	-11,33333333	-50,4
Paleosuchus palpebrosus (Cuvier, 1807)	Naturae, 2005 - C, V	Fazenda União - Ribeirão das Lajes	Paranã	2004	-11,31027778	-49,93888889
Paleosuchus palpebrosus (Cuvier, 1807)	Naturae, 2005 - C, V	Fazenda Várzea Grande	São Salvador	2005	-12,41972222	-48,24555556
Paleosuchus palpebrosus (Cuvier, 1807)	Naturae, 2005 - C, V	Foz do Córrego Cipó	São Salvador	2001	-12,32666667	-48,30805556
Paleosuchus palpebrosus (Cuvier, 1807)	Naturae, 2005 - C, V	Foz do Ribeirão Santa Cruz	Paranã	2004	-11,22083333	-46,88555556
Paleosuchus palpebrosus (Cuvier, 1807)	Oikos, 2002 - C, V	Fazenda Marupiara	Guaraí	2001	-8,616944444	-48,31777778
Paleosuchus palpebrosus (Cuvier, 1807)	Oikos, 2002 - C, V	Fazenda Morro Grande - TO 010	Babaçulândia	2001	-7,091111111	-47,82694444
Paleosuchus palpebrosus (Cuvier, 1807)	Oikos, 2002 - C, V	Rio Crixas - TO 070	Aliança do TO	2001	-11,13027778	-48,80333333
Paleosuchus palpebrosus (Cuvier, 1807)	Oikos, 2006 - V	Rio Caiapo	Araguacema	2006	-8,9	-49,61666667
Paleosuchus palpebrosus (Cuvier, 1807)	Oikos, 2006 - V	Rio Piranhas - TO 437	Araguacema	2006	-8,804722222	-49,42472222
Paleosuchus palpebrosus (Cuvier, 1807)	Pavan, 2007 - C, V	Floresta de galeria 3	Arguianopolis	2004	-6,5181	-47,5272
Paleosuchus palpebrosus (Cuvier, 1807)	Pavan, 2007 - C, V	Floresta de galeria 5	Babaçulândia	2001	-7,205833333	-47,75527778
Paleosuchus palpebrosus (Cuvier, 1807)	Pavan, 2007 - C, V	Floresta de galeria 6	Filadélfia	2003	-7,341666667	-47,50388889
Paleosuchus palpebrosus (Cuvier, 1807)	Pavan, 2007 - C, V	Floresta de galeria 11	Guaraí	2001	-8,607777778	-48,31472222
Paleosuchus palpebrosus (Cuvier, 1807)	Pavan, 2007 - C, V	Floresta de galeria 13	Aliança do TO	2001	-11,31032	-48,940778
Paleosuchus palpebrosus (Cuvier, 1807)	Pavan e Dixo, 2004 - V	Represa, cativeiro da CEULP/ ULBRA,	Palmas	2000	-10,26666667	-48,33333333
Paleosuchus palpebrosus (Cuvier, 1807)	Recoder et al., 2011 - C, V	EESGT - campo úmido	Almas	2011	-11,22083333	-46,88555556

Paleosuchus palpebrosus (Cuvier, 1807)	Recoder et al., 2011 - C, V	EESGT - Mata de Galeria	Almas	2011	-11,31027778	-49,93888889
Paleosuchus palpebrosus (Cuvier, 1807)	Villaça, 2004 - C, V	Rio Tocantins - Br1	Brejinho de Nazaré	2003	-11,04472222	-48,5725
Paleosuchus palpebrosus (Cuvier, 1807)	Villaça, 2004 - C, V	Rio Tocantins - Br2	Brejinho de Nazaré	2003	-11,05194444	-48,55166667
Paleosuchus palpebrosus (Cuvier, 1807)	Villaça, 2004 - C, V	Rio Tocantins - Br3	Brejinho de Nazaré	2003	-11,12222222	-48,52083333
Paleosuchus palpebrosus (Cuvier, 1807)	Villaça, 2004 - C, V	Rio Tocantins - Br4	Brejinho de Nazaré	2003	-11,10027778	-48,53583333
Paleosuchus palpebrosus (Cuvier, 1807)	Villaça, 2004 - C, V	Rio Tocantins - Br5	Brejinho de Nazaré	2003	-11,18416667	-48,50305556
Paleosuchus palpebrosus (Cuvier, 1807)	Villaça, 2004 - C, V	Rio Tocantins - Br6	Brejinho de Nazaré	2003	-11,03083333	-48,56083333
Paleosuchus palpebrosus (Cuvier, 1807)	Villaça, 2004 - C, V	Rio Tocantins - Lj1	Lajeado	2003	-9,798888889	-48,35944444
Paleosuchus palpebrosus (Cuvier, 1807)	Villaça, 2004 - C, V	Rio Tocantins - Lj2	Lajeado	2003	-9,821111111	-48,33333333
Paleosuchus palpebrosus (Cuvier, 1807)	Villaça, 2004 - C, V	Rio Tocantins - Mi1	Miracema	2003	-9,755555556	-48,39138889
Paleosuchus palpebrosus (Cuvier, 1807)	Villaça, 2004 - C, V	Rio Tocantins - Mi2	Miracema	2003	-9,788888889	-48,39194444
Paleosuchus palpebrosus (Cuvier, 1807)	Villaça, 2004 - C, V	Rio Tocantins - Pa1	Palmas	2003	-10,18222222	-48,36555556
Paleosuchus palpebrosus (Cuvier, 1807)	Villaça, 2004 - C, V	Rio Tocantins - Pa2	Palmas	2003	-10,1975	-48,36861111
Paleosuchus palpebrosus (Cuvier, 1807)	Villaça, 2004 - C, V	Rio Tocantins - Pa4	Palmas	2003	-10,30555556	-48,36611111
Paleosuchus palpebrosus (Cuvier, 1807)	Villaça, 2004 - C, V	Rio Tocantins - Pa5	Palmas	2003	-10,355	-48,36861111
Paleosuchus palpebrosus (Cuvier, 1807)	Villaça, 2004 - C, V	Rio Tocantins - PN1	Porto Nacional	2003	-10,10111111	-48,41055556
Paleosuchus palpebrosus (Cuvier, 1807)	Villaça, 2004 - C, V	Rio Tocantins - PN11	Porto Nacional	2003	-10,75222222	-48,39944444
Paleosuchus palpebrosus (Cuvier, 1807)	Villaça, 2004 - C, V	Rio Tocantins - PN2	Porto Nacional	2003	-10,15166667	-48,42638889
Paleosuchus palpebrosus (Cuvier, 1807)	Villaça, 2004 - C, V	Rio Tocantins - PN3	Porto Nacional	2003	-10,33333333	-48,43472222
Paleosuchus palpebrosus (Cuvier, 1807)	Villaça, 2004 - C, V	Rio Tocantins - PN4	Porto Nacional	2003	-10,71888889	-48,44027778
Paleosuchus palpebrosus (Cuvier, 1807)	Villaça, 2004 - C, V	Rio Tocantins - PN5	Porto Nacional	2003	-10,73138889	-48,41472222
Paleosuchus palpebrosus (Cuvier, 1807)	Villaça, 2004 - C, V	Rio Tocantins - PN6	Porto Nacional	2003	-10,81805556	-48,3825
Paleosuchus palpebrosus (Cuvier, 1807)	Vitt et. al., 2005 - C, F	Jalapão	Mateiros	2004	-10,55	-46,41666667
Paleosuchus palpebrosus (Cuvier, 1807)	WPS e equipe - V, F	Floresta de galeria 1	Araguatins	2011	-5,796701	-47,845663
Paleosuchus palpebrosus (Cuvier, 1807)	WPS e equipe - V	Floresta de galeria 4	Wanderlândia	2010	-7,045849	-47,868588
Paleosuchus palpebrosus (Cuvier, 1807)	WPS e equipe - V, F	Floresta de galeria 7	Nova Olinda	2009	-7,625016	-48,369543

Paleosuchus palpebrosus (Cuvier, 1807)	WPS e equipe - V	Floresta de galeria 9	Palmeirante do TO	2009	-7,895666	-47,888778
Paleosuchus palpebrosus (Cuvier, 1807)	WPS e equipe - V, F	Floresta de galeria 10	Colinas	2013	-8,25758	-48,270731
Paleosuchus palpebrosus (Cuvier, 1807)	WPS e equipe - V, F	Floresta de galeria 12	Pedro Afonso	2011	-9,190736	-48,163324
Paleosuchus palpebrosus (Cuvier, 1807)	WPS e equipe - V, F	Parque Cesamar	Palmas	2018	-10,20833333	-48,32694444
Paleosuchus palpebrosus (Cuvier, 1807)	WPS e equipe - V	ribeirão próximo a cidade	Babaçulândia	2009	-7,241513	-47,774814
Paleosuchus palpebrosus (Cuvier, 1807)	WPS e equipe - V	Serra do Estrondo	Paraiso do Tocantins	2012	-10,156698	-48,859715
Paleosuchus trigonatus (Schneider, 1801)	Atizengen e Silva 1999 - V	Rio Araguaia - Parque Estadual Serras das Andorinhas	Xambioá	1998	-6,216666667	-48,41666667
Paleosuchus trigonatus (Schneider, 1801)	AM, MCHS e equipe - V	Rio Araguaia	Esperantina	2010	-5,412195	-48,498413
Paleosuchus trigonatus (Schneider, 1801)	AM, MCHS e equipe - V	Rio Araguaia - Santa Isabel	Ananás	2010	-6,141202	-48,325941
Paleosuchus trigonatus (Schneider, 1801)	AM, MCHS e equipe - V	Rio Araguaia - trecho 1	Araguatins	2010	-5,574294	-48,158616
Paleosuchus trigonatus (Schneider, 1801)	AM, MCHS e equipe - V, F	Rio Araguaia, cachoeira do São Bento	Araguatins	2010	-5,430508	-48,347163
Paleosuchus trigonatus (Schneider, 1801)	AM, MCHS e equipe - V, F	Rio Araguaia, ponte BR230	Araguatins	2010	-5,702966	-48,164309
Paleosuchus trigonatus (Schneider, 1801)	MPEG 135	Rio Araguaia, ilha de São Vicente	Araguatins	2010	-5,604267	-48,153037
Paleosuchus trigonatus (Schneider, 1801)	Themag, 2000 - V	Ilha de Serra Quebrada (Rio Tocantins)	Itaguatins	2000	-5,740906	-47,521715
Caiman latirostris (Daudin, 1802)	Pessoa et al., 1972	Zona Rural Almas	Almas	1972	-11,556824	-47,169694
Caiman latirostris (Daudin, 1802)	MZUSP 2136	Ilha do Bananal 3	Ilha do Bananal	1949	-11,33333333	-50,4

Trabajo

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Identification of enteroendocrine cells of *Tropidurus torquatus* and *Salvator merianae* (Squamata: Lacertilia)

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ABSTRACT
The Squamata lizards *Tropiduros torquatus* and *Salvator merianae* are omnivores with different diets and foraging strategies, which requires adequate physiological responses of enteroendocrine cells, since they control digestive events, gastrointestinal epithelium renewal, metabolism and food intake. Thus, we aim to describe the distribution and frequency of enteroendocrine cells in the digestive tract of these two lizard's species. Five individuals of each species were collected, euthanized and the digestive organs removed. Histological sections were submitted to the techniques of Grimelius for argyrophilic cells and Masson-Fontana for argentaffin cells. Both endocrine cells were found in the lining and glandular epithelium, with a pyramidal, oval, rounded, or elongated shape. Argyrophilic cells were observed in both species, predominantly in the stomach, but absent in the cranial esophagus. Argentaffin cells were distributed in almost the entire digestive tract but absent in the cranial portion of the esophagus and small intestine in both species, and with high frequency in the *T. torquatus* pyloric region. The absence of argentaffin cells in the cranial portion of the small intestine is intriguing and may reveal greater neural control over certain secretomotor functions involving this segment. We concluded that the inter-specific and inter-regional variations in the distribution and frequency of these cells may reflect the regulatory functions and mechanisms of each organ, taking also into account the oscillations of the food and nutritional status of animals in the wild.

Key Words: Diet; Argentaffin cells; Argyrophilic cells; Histology; Morpho-physiology; Reptiles.

RESUMO
Os lagartos Squamata *Tropiduros torquatus* e *Salvator merianae* são onívoros com diferentes dietas e estratégias de forrageamento, o que requer respostas fisiológicas adequadas de células enteroendócrinas, uma vez que estas controlam os eventos digestivos, a renovação do epitélio gastrointestinal, o metabolismo e a ingestão alimentar. Portanto, pretendemos descrever a distribuição e frequência de células enteroendócrinas no trato digestivo destas duas espécies de lagartos. Foram coletados cinco indivíduos de cada espécie, eutanasiados e os órgãos digestivos removidos. Seções histológicas foram submetidas às técnicas de Grimelius para células argirófilas e Masson-Fontana para células argentafins. Ambas as células endócrinas foram encontradas no epitélio de revestimento e glandular, com formatos piramidal, oval, arredondado ou alongado. Células argirófilas foram observadas em ambas as espécies, predominantemente no estômago, mas ausentes no esôfago craniano. As células argentafins estiveram distribuídas em quase todo o trato digestivo, mas ausentes na porção cranial do esôfago e intestino delgado em ambas as espécies, e com alta frequência na região pilórica de *T. torquatus*. A predominância das células endócrinas no estômago pode estar relacionada, por exemplo, à maior permanência do alimento neste órgão, ao contrário do esôfago. A ausência de células argentafins na porção cranial do intestino delgado é intrigante e pode revelar maior controle neural sobre certas funções secretomotoras envolvendo este segmento. Nós concluímos que as variações inter-específicas e inter-regionais na distribuição e frequência destas células podem refletir as funções e mecanismos regulatórios de cada órgão, levando-se também em conta as oscilações do estado alimentar e nutricional dos animais na natureza.

Palavras-chave: Dieta; Células argentafins; Células argirófilas; Histologia, Morfofísiologia; Répteis.

Introduction

Enteroendocrine cells constitute a small proportion of the digestive tract epithelium in all vertebrates, however their functions are essential for digestive physiology and organism's homeostasis (Dockray,

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2006; Rodrigues *et al.*, 2005). These cells release peptides or amines to regulate the secretion, absorption, motility and proliferation of digestive tract epithelial cells (Rindi *et al.*, 2004; Gribble and Reimann, 2019). In addition, they are involved with the regulation of appetite and consequent food intake, regulation of hepatic and pancreatic secretions, glucose concentrations and energy metabolism (Gutierrez-Aguilar and Woods, 2011; Moran-Ramos *et al.*, 2012). Enteroendocrine cells can be evidenced by histochemical techniques using silver salts, and are classified into argentaffins, which absorb and reduce silver salts; and argyrophilic, which only absorb silver salts but do not reduce it, requiring an exogenous substance with a reducing capacity (Polak *et al.*, 1993). Enteroendocrine cells have already been identified in all vertebrates, from fish, amphibians and reptiles to birds and mammals (Ku *et al.*, 2000; Lee and Ku, 2004; Santos *et al.*, 2013; Lin *et al.*, 2016; Mazzoni *et al.*, 2018), and in invertebrate taxa some gut cells resemble those that produce gastrointestinal peptides in vertebrates, such as in insects, echinoderms and protochordates (Dimaline and Dockray, 1994; Neves *et al.*, 2003; García-Arrarás *et al.*, 2019). In the primitive group cnidaria regulatory peptides are secreted only by neurons, and the peptide production in endocrine cells probably may have derived from these neuropeptides (Dimaline and Dockray, 1994).

Reptiles are an animal model for studies of the gastrointestinal tract because they have intestinal morphological flexibility, ingest large prey, and have exacerbated physiological and structural responses to ingestion, such as increased intestinal mass, metabolic rate, and nutrient transport rates (Holmberg *et al.*, 2003). For this reason, they are useful for studies on the regulation of digestive processes and the evolution of regulatory mechanisms, including those carried out by enteroendocrine cells (Secor and Diamond, 1998; Starck and Beese, 2002).

Squamata are one of the most diverse groups of terrestrial vertebrates, including lizards, snakes, and amphisbaenians (Pyron *et al.*, 2013). The *Tropidurus torquatus* (Wied-Neuwied, 1820) is an omnivorous, generalist and opportunistic lizard with “sit-and-wait” eating strategies (Bergallo and Rocha, 1994; Carvalho *et al.*, 2007). The tegu *Salvator merianae* (Duméril and Bibron, 1839) is also an omnivorous lizard, but it is an active forager, locating its prey with the aid of its long and bifid tongue (Vitt, 1995). It consumes everything ranging from vertebrates, mollusks, and arthropods to decomposing animals, in addition to vegetables, being important seed dispersers (Castro and Galetti, 2004; Sazima and D'Angelo, 2013). The objective of this study was to describe and compare the distribution and frequency of argyrophilic and argentaffins enteroendocrine cells in *T. torquatus* and *S. merianae* in order to improve knowledge about the digestive physiology of these species, which are omnivorous but differ in diets and feeding strategies.

Materials and methods

Study area and capture of animals

Five individuals, among males and females, of each species studied *T. torquatus* and *S. merianae*, were captured in the municipalities of Viçosa and Guiricema, which are part of the Zona da Mata in the State of Minas Gerais, Brazil (IBAMA: 10504-1). After a period of fasting, the animals were euthanized with pentobarbital overdose (120 mg/kg) injected intraperitoneally, and then measured with a tape measure and weighed on a digital scale. The peritoneal cavity was exposed by a median longitudinal incision in the ventral region and the digestive tract was removed and measured with a tape measure, collecting fragments of the esophagus (cranial and caudal), esophagus-gastric transition, stomach (fundic and pyloric), gastro-intestinal transition, small intestine (cranial, middle and caudal), transition between intestines, and large intestine (colon and rectum). The study was authorized by the Ethics Committee for the use of animals (CEUA, protocol 27/2016).

Histological and histochemical analyzes

The organs fragments were washed in saline solution and fixed in Carson's formalin for 24 hours (Carson *et al.*, 1973) and dehydrated in an increasing series of ethyl alcohol, embedded in histological paraffin (paraplast) and sectioned with a manual rotating microtome (Olympus America Inc. CUT 4055), obtaining sections 5 μm thick. These sections were submitted to silver impregnation: Grimelius for argyrophilic cells (Grimelius and Wilander, 1980) and Masson-Fontana (Barbosa *et al.*, 1984) for argentaffin cells; as positive control, histological sections of monkey's stomach and duodenum, previously tested, were used.

Morphometric analyzes

Argyrophilic and argentaffin cells were quantified using an optical microscope (Nikon E100 LED) with 40X objective lens and 10X eyepiece lens. Ten random areas of the mucosa of four histological sections (20 μm spacing between sections), for each segment of the digestive tube of each animal (n= 10 x 4 x 5= 200) were analyzed. This quantification was represented with a frequency scale: absent; rare, 1 to 2 cells / area; few, 3 to 4 cells / area; many, 5 to 8 cells / area; and elevated, 9 or more cells / area. The area was calculated using the formula: A= π x r2, with r= 0.25 mm (given the characteristics of the objective and ocular lenses), obtaining the value of 0.2 mm^2. The photographic record of the histological sections was performed using an optical microscope (Olympus BX60) coupled with a digital camera (QColor3 DP73 Olympus).

Results

The rostro-cloacal length and the weight of *T. torquatus* and *S. merianae* were 9.7 cm and 110.43 g and 26.8 cm and 441.75 g, respectively. The digestive tube of *T. torquatus* (Fig. 1) measured an average of 9.6 cm in length, representing 99% of its body length; and that of *S. merianae* (Fig. 2) measured 30.8 cm in average length, which represents 115% of its body length.

The digestive tract was composed of the four typical layers: mucosa with lining epithelium, lamina propria and muscularis mucosae; submucosa (without glands); muscular with its circular and longitudinal sublayers; and serosa. The lining epithelium was varied, prismatic pseudo-stratified, with ciliary cells, goblet cells and basal cells in the esophagus; simple prismatic in the stomach; simple prismatic with goblet cells and striated border cells in the small and large intestines. Mucosa´s glands were registered in the caudal esophagus and in different gastric regions, even though they were absent in the small intestine and were scarce in the large intestine. Enteroendocrine cells, when present, were located diffusely through the lining and / or glandular epithelium, with a rounded or oval shape, characteristic of closed type cells, and pyramidal or spindle shape, characteristic of closed type cells; in addition to an uncolored nucleus and cytoplasm marked in brown or black, especially around or below the nucleus (Figs. 3 to 6). The frequency of argyrophilic and argentaffin enteroendocrine cells in the different 159 regions analyzed, for both species, is shown in Figs. 7 and 8.

Although argyrophilic cells were not found in the *T. torquatus* esophagus, few were present in their esophageal-gastric transition (Figs. 3a and 7a), and rare were found in the esophageal glands of *S. merianae* (Figs. 3b and 7b). Rare argentaffin cells have been identified in the caudal esophageal epithelium

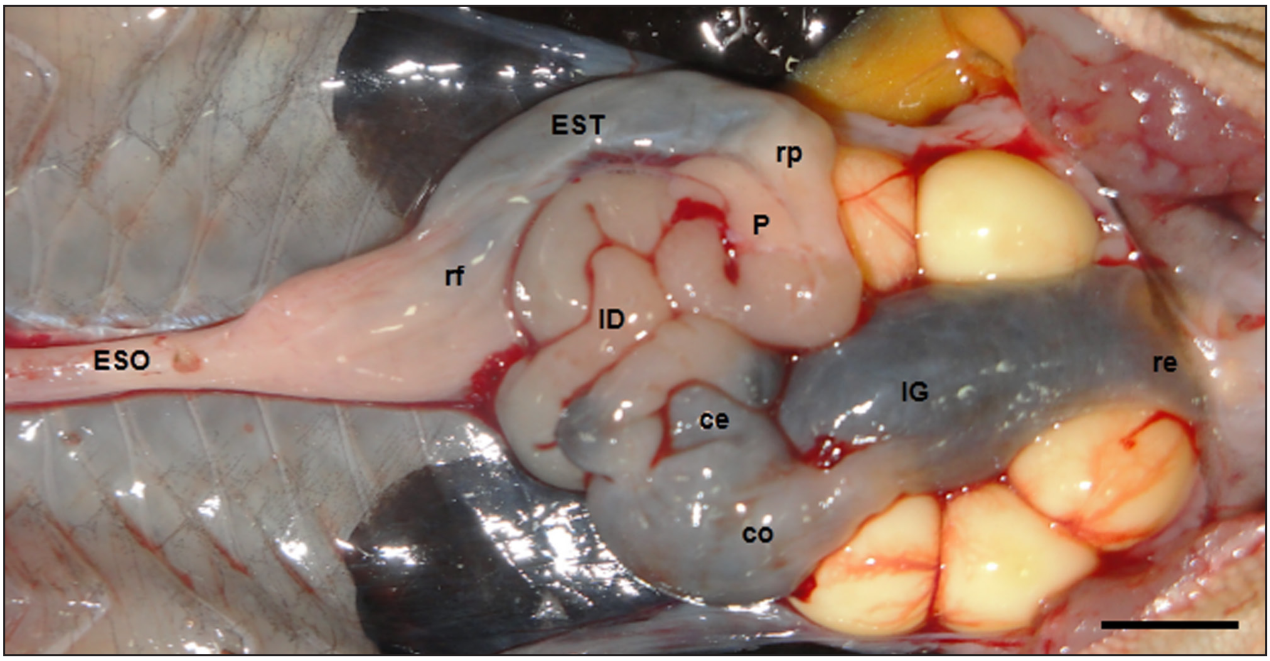


Figure 1. Digestive tube of *Tropidurus torquatus* with indication of its segments: esophagus (ESO), stomach (EST) with the fundic (rf) and pyloric (rp) regions, small intestine (ID), large intestine (IG) with cecum (ce), colon (co) and rectum (re), and pancreas (P). Bar=5 mm.

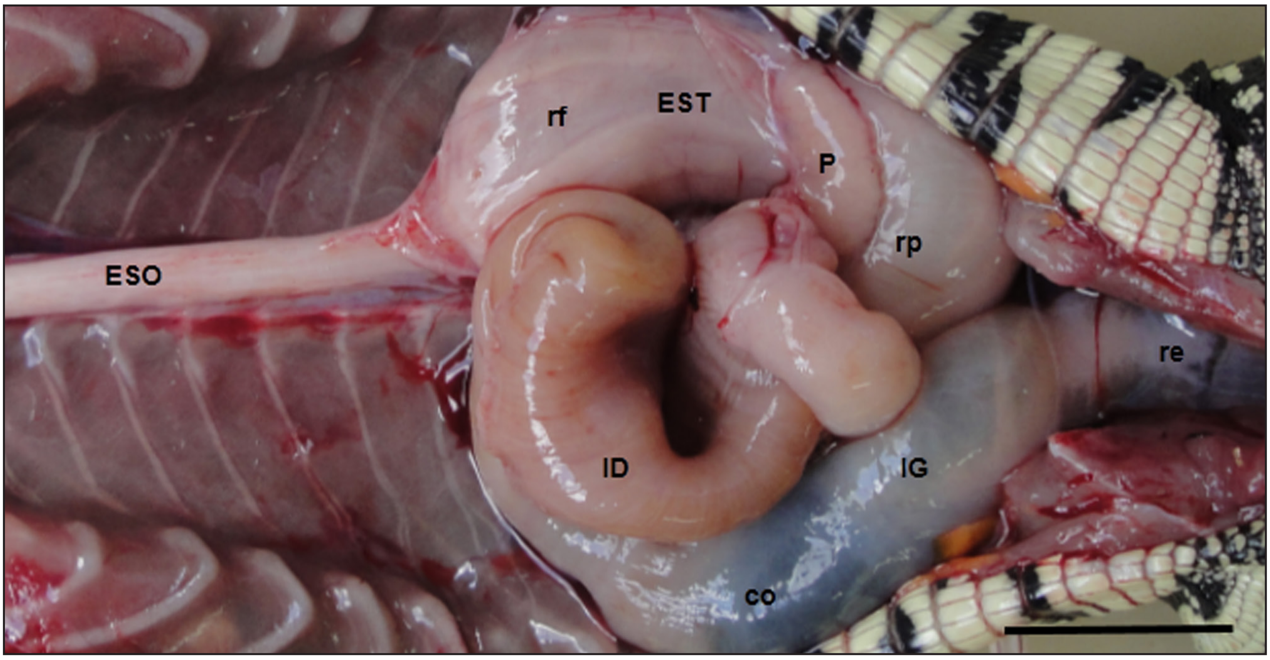


Figure 2. Digestive tube of *Salvator merianae* with indication of its segments: esophagus (ESO), stomach (EST) with the fundic (rf) and pyloric (rp) regions, small intestine (ID), large intestine (IG) with colon (co) and rectum (re), and pancreas (P). Bar=20 mm.

of both species (Figs. 4c and d, 8a and b), however many were seen in the esophageal-gastric transition in *T. torquatus* (Fig. 8a).

Argyrophilic cells were observed in the different regions of the stomach, on the surface, in the pits and especially in the glands of both species (Fig. 4a to d), with variable frequency: in *T. torquatus* there were many in the entire stomach (Fig. 7a); in *S. merianae*, many were found only in the pyloric region, being rare in the fundic region (Fig. 7b). Likewise, argentaffin cells were identified in the lining and glandular epithelium of both species (Fig. 4e to h) with variable frequency: in *T. torquatus* they were few in the fundic region and elevated in the pyloric (Fig. 8a); while in *S. merianae* they were few in the fundic and rare in the pyloric region (Fig. 8b). In the gastro-intestinal transition there were few argyrophilic cells and rare argentaffin cells in both species (Figs. 5a and b, 8a and b).

Rare argyrophilic cells were found in the small intestine of *T. torquatus* and *S. merianae* (Figs. 5c and d, 7a and b). Rare argentaffin cells were also observed in the small intestine of these species, except for the cranial segment in which they were absent (Figs. 5e and f, 8a and b). In the transition between the intestines, argyrophilic cells were many in *T. torquatus* and few in *S. merianae*, although rare in the large intestine of these species (Figs. 6a and b, 7a and b). Argentaffin cells showed the same pattern in the transition and in the large intestine, they were rare in *T. torquatus* and few in *S. merianae* (Figs. 6c to f, 8a and b), and some had an inverted morphological configuration, with granules concentrated in the supra-nuclear region (Fig. 6c).

Discussion

The enteroendocrine cells found in the digestive tract of *Tropidurus torquatus* and *Salvator merianae* presented different formats, which allows categorizing them in "closed type" or "open type". The first one, with a rounded to oval shape, does not communicate with the lumen; and the second one, pyramidal to spindle shape, presents an apical extension communicating with the lumen, although this extension cannot always be seen in histological sections (Latorre *et al.*, 2016). "Closed type" cells are believed to respond to mechanical distension or humoral stimulation, while "open type" cells detect changes in luminal pH or content composition (Ceccarelli *et al.*, 1995; Solcia *et al.*, 2000; Gribble and Reimann, 2016).

The *T. torquatus* and *S. merianae* enteroendocrine cells were argyrophilic and / or argentaffins, with variable distribution and frequency in the different organs of the digestive tract. According to Grimelius and Wilander (1980), all enteroendocrine cells are argyrophilic, except for cholecystokinin

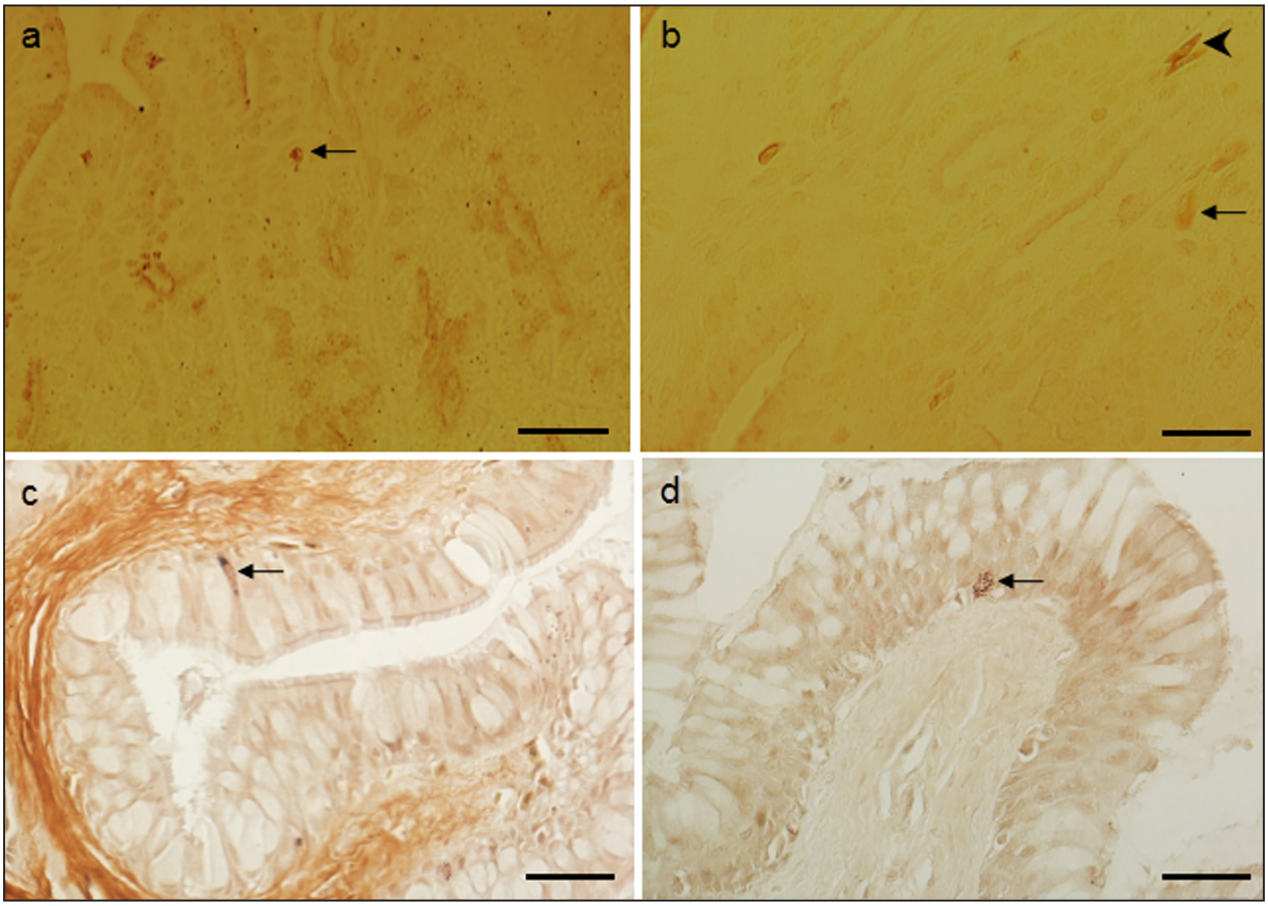


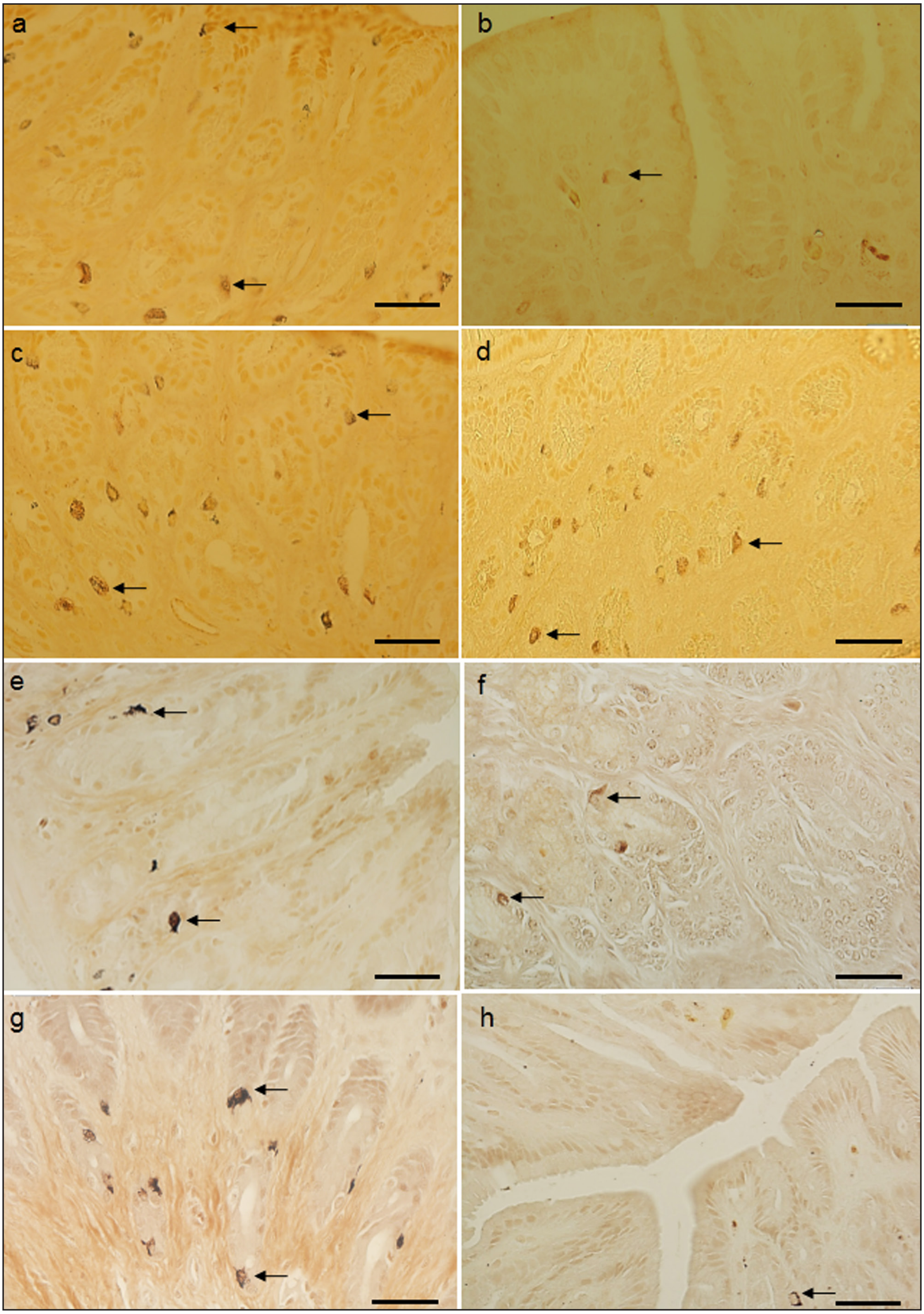
Figure 3. Histological sections of the esophagus of *Tropidurus Torquatus* and *Salvator merianae*, with arrows indicating endocrine cells: argyrophilic (a and b; Grimelius technique) and argentaffin (c and d; Masson-Fontana technique). Esophagus-gastric transition (a) and caudal esophagus (c) of *T. torquatus*. Caudal esophagus of *S. merianae* (b, d). Arrowhead: red blood cell. Bar= 20 µm.

and somatostatin producing cells, while argentaffin cells are serotonin-producing cells, called type I (serotonin and substance P) or type II (serotonin and motilin) enterochromaffin cells.

In our study, we observed enteroendocrine cells in the caudal esophagus of *T. torquatus* and *S. merianae*, especially in the glands that appear in this esophageal region, allowing us to infer that they are involved with the control of glandular secretion. According to several authors, who worked with different reptilian species (Zamith, 1952; Ferri *et al.*, 1976; El-Salhy and Grimelius, 1981; Madrid *et al.*, 1989; Zug, 1993; Pereira *et al.*, 2005; Rodrigues-Sartori *et al.*, 2015), these glands secrete mucus for protection and lubrication of the esophagus, which has a storage function in addition to conduction of food, especially in reptiles that swallow whole prey. Thus, in *T. torquatus* and *S. merianae* the existence of endocrine cells in the caudal esophageal portion may also be associated with the permanence of food in this location, which presents itself as an extension of the stomach for the storage of larger prey. Endocrine cells may also be involved in controlling esophageal motility in reptiles. Serotonin released by argentaffin cells is a paracrine mediator known to stimulate the contraction of smooth muscle in the digestive tract, and which may also be involved in the regulatory mechanism of ciliary movement in the reptilian esophagus (Perez -Tomas *et al.*, 1989).

Among the organs analyzed, the stomach was the one with the largest number of enteroendocrine cells, certainly because it is a place of greater permanence of the food content, being important in the control of gastric secretion and motility, unlike the esophagus. Argyrophilic cells were numerous in the pyloric region of both species studied, as well as argentaffin cells in *T. torquatus*. The argyrophilic and argentaffin cells were present in the stomach of different reptiles, with a predominance in the pyloric glands (Deng and Chu, 2006; Ahmed *et al.*, 2009; Rodrigues-Sartori *et al.*, 2011; Pereira *et al.*, 2015).

Argyrophilic and argentaffin cells were identified in the intestines of both species, except in the cranial portion of the small intestine, in which there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were no argentaffin cells. According to Furness *et al.* (1999), the intrinsic sensory neurons that detect luminal changes can do it directly or indirectly, through the release of chemical mediators by enteroendocrine cells, with serotonin being one of the possible intermediary in this mucosal signal transduction. Since the argentaffin cells release serotonin and other mediators related to motility, it is assumed that certain motor functions involving the cranial intestine are performed directly by the

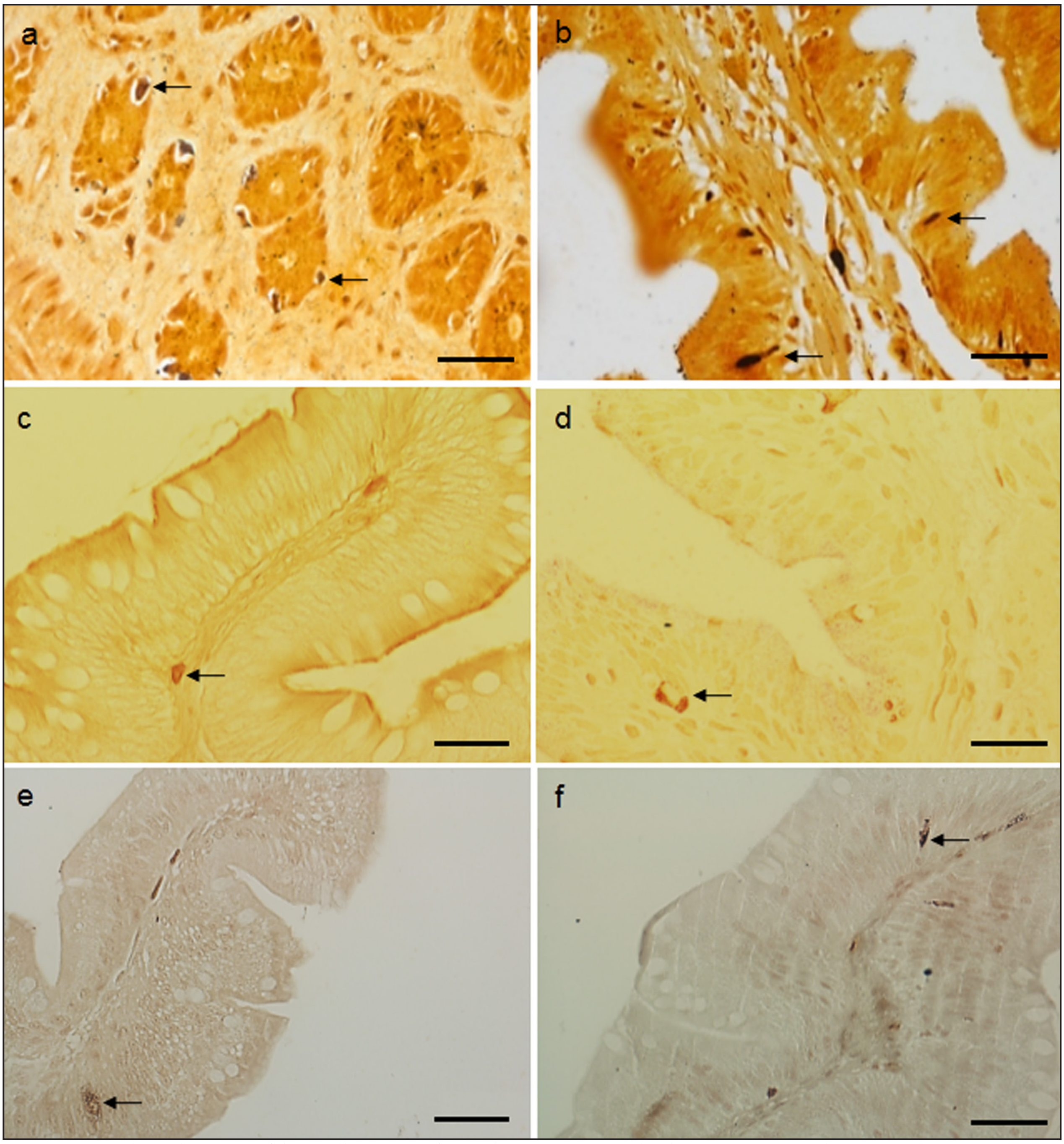

Figure 5. Histological sections of the small intestine of *Tropidurus torquatus* and *Salvator merianae*, with arrows indicating endocrine cells: argyrophilic (a to d; Grimelius technique) and argentaffin (e, f; Masson-Fontana technique). Gastro-intestinal transition (a), and cranial (c) and medium (e) small intestine of *T. torquatus*. Gastro-intestinal transition (b), and caudal (d) and medium (f) small intestine of *S. merianae*. Bar= 20µm.

🡐 **Figure 4**. Histological sections of the stomach of *Tropidurus torquatus* and *Salvator merianae*, with arrows indicating endocrine cells: argyrophilic (a to d; Grimelius technique) and argentafin (e to h; Masson-Fontana technique). Fundic region (a, e) and pyloric region (c, g) of *T. torquatus*. Fundic region (b, f) and pyloric region (d, h) of *S. merianae* (b, f). Bar= 20 µm.

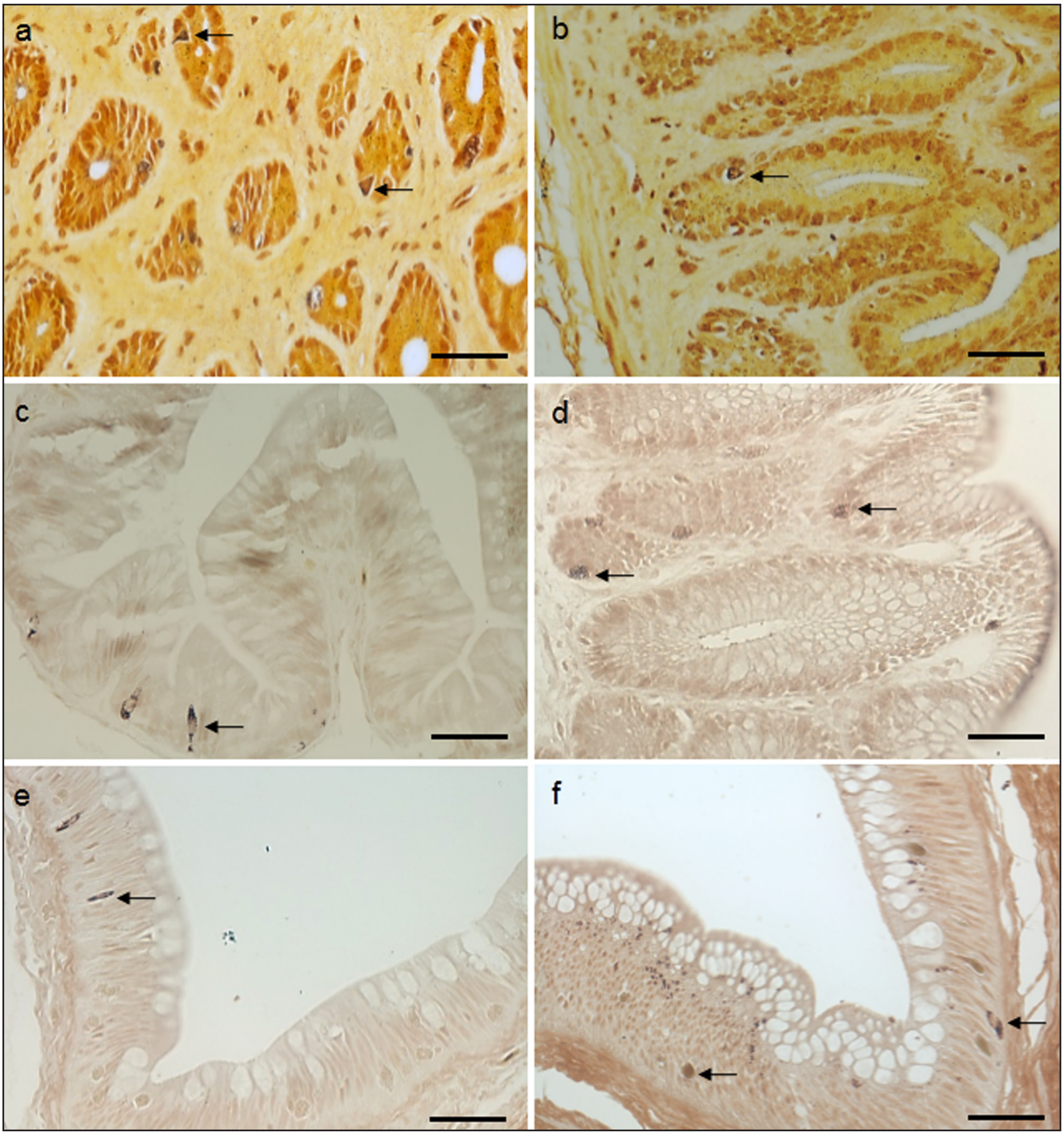

Figure 6. Histological sections of the large intestine of *Tropidurus torquatus* and *Salvator merianae*, with arrows indicating endocrine cells: argyrophilic (a, b; Grimelius technique) and argentaffin (c - f; Masson-Fontana technique). Transition between small and large intestines (a), colon (c) and rectum (e) of *T. torquatus*. Transition between small and large intestines (b), colon (d) and rectum (f) of *S. merianae*. Bar= 20μm.

nervous system.

The low number of enteroendocrine cells in the intestines of the studied reptiles is certainly related to the relatively short intestinal length and the absence or scarcity of glands, that is, it is less complex organ when compared to those of mammals. In the intestines of mammals, enteroendocrine cells are located mainly in the glands, however in many reptiles the intestinal glands or crypts are non-existent (Perez-Tomas *et al.*, 1989; George *et al.*, 1998; Hamdi *et al.*, 2014; Rodrigues-Sartori *et al.*, 2014), or exist only in the large intestine, as in *T. torquatus* and *S. merianae*, and other reptilian species (Luppa, 1977). Thus, argyrophilic and / or argentaffin cells have also been located in the intestines of several reptiles, generally sparse (Ferri *et al.*, 1976; Martin-Lacave *et al.*, 1982;

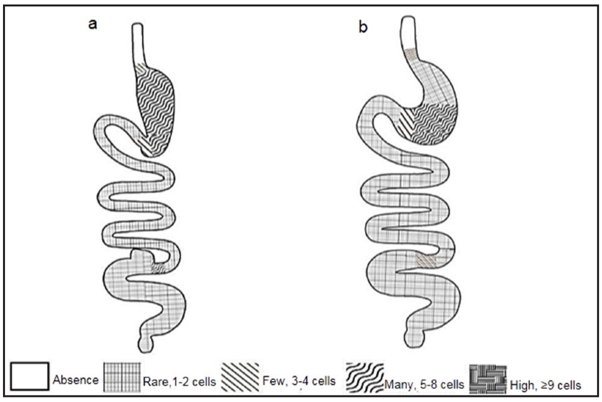

Figure 7. Schematic drawing showing the frequency of argyrophilic endocrine cells (number of cells / 0.2 mm²) in the digestive tract of *Tropidurus torquatus* (a) and *Salvator merianae* (b).

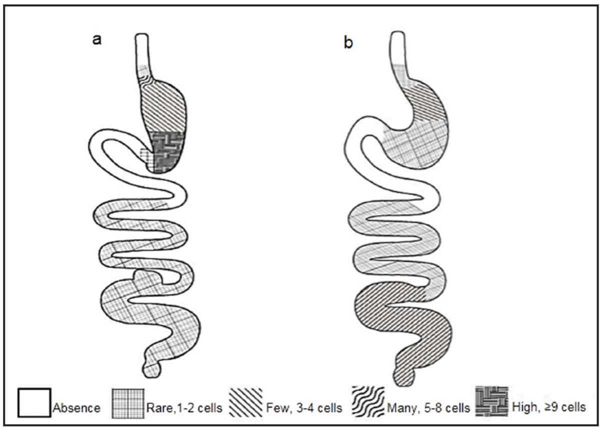

Figure 8. Schematic drawing showing the frequency of argentaffin endocrine cells (number of cells / 0.2 mm²) in the digestive tract of *Tropidurus torquatus* (a) and *Salvator merianae* (b).

D´Este *et al*., 1993; Deng and Chu, 2006; Rodrigues-Sartori *et al*., 2014). In addition, serotonin immunoreactive cells have been identified in the epithelium of the surface and glands in the large intestine of reptiles, suggesting that this mediator has a trophic action on the intestinal epithelium (Perez-Tomas *et al*., 1989; Tarakçi *et al*., 2005; Rodrigues-Sartori *et al*., 2014). Comparing the two species studied, the intestinal crypts are more prominent in *S. merianae*, and perhaps that is why it presented a slightly larger number of argentaffin endocrine cells. On the other hand, many argyrophilic cells were found in *T. torquatus*, in the transition between the intestines, which may be related to the existence of the cecum in this species.

From the general analysis of the species studied, we can see how they differ in the number of argyrophilic and argentaffin cells when compared with mammal species (Shimizu and Nozaki, 1994; Fonseca *et al*., 2002; Bressan *et al*., 2004; Machado-Santos *et al*., 2009; Basile *et al*., 2012; Freitas-Ribeiro *et al*., 2012; Rodrigues-Sartori *et al*., 2018). The lower density of these cells can be compensated by a greater direct neural participation in the control of digestive functions, which we understand as a primitive characteristic given that the nervous system arose before the endocrine in the evolution of homeostatic control systems (Falkmer, 1993). Thus, the discrepancies between reptiles and mammals can be attributed to evolutionary aspects, while the differences between reptilian species can be due not only to phylogenetic but also ecological issues, such as the animal's diet and food status, in view of the flexibility of the digestive tract.

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Trabajo

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Contagem diferencial e morfometria de células sanguíneas nos lagartos *Ameivula ocellifera* (Squamata: Teiidae) e *Tropidurus hispidus* (Squamata: Tropiduridae) do semiárido brasileiro, com análise dos efeitos por hemoparasitos

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ABSTRACT
Differential counting and blood cell morphometry in the lizards *Ameivula ocellifera* (Squamata: Teiidae) and *Tropidurus hispidus* (Squamata: Tropiduridae) from the Brazilian semiarid region, with analysis of the effects by hemoparasites. The lizards *Ameivula ocellifera* and *Tropidurus hispidus* are sympatric species in the Brazilian semiarid region. Despite being considered interesting models for ecological studies, little is known about basic aspects of their biology, such as hematological parameters. Thus, the objective of this work was to perform the differential count of leukocytes and describe the morphometry of erythrocytes and leukocytes in these two species of lizards, with emphasis on hemoparasitism by intracellular protozoa. For this, 30 specimens (15 *A. ocellifera* and 15 *T. hispidus*) were used for analysis. After making the slides, a differential count of 100 leukocytes per slide was performed. In addition, 20 erythrocytes and 15 leukocytes were photographed per slide for morphometric analysis. The measurements were measured for the parameters area, perimeter, maximum and minimum axes, with the ellipse formulas used for area and perimeter. Interspecific and intraspecific differences (parasitized and non-parasitized lizards) were tested for erythrocyte morphometric parameters, erythrocyte nucleus and leukocytes. The differential count showed that heterophiles were the predominant cell type for both species of lizards, while basophils were the least abundant for *A. ocellifera* and eosinophils for *T. hispidus*. As for leukocyte measurements, only the monocyte differed significantly between species, greater in *A. ocellifera*, and intraspecifically only for the minimum axis parameter, greater in parasitized *T. hispidus*. Erythrocytes differed between species in terms of cell and nucleus size, being higher in *T. hispidus*. Among the hemoparasites, found only in *T. hispidus*, *Plasmodium* sp. caused more discrete changes in the erythrocyte, while *Hepatozoon* sp. caused marked macrocytosis and hypochromia in these cells. In general, there does not seem to be a relationship between the differential leukocyte count and hemoparasitism.

Key Words: Caatinga; Erythrocytes; *Hepatozoon*; Leukocytes; *Plasmodium*.

RESUMO
Os lagartos *Ameivula ocellifera* e *Tropidurus hispidus* são espécies simpátricas na região do semiárido brasileiro. Apesar de serem consideradas modelos interessantes para estudos ecológicos, pouco se conhece sobre aspectos básicos de sua biologia, tais como parâmetros hematológicos. Dessa forma, o objetivo deste trabalho foi realizar a contagem diferencial de leucócitos e descrever a morfometria dos eritrócitos e leucócitos nessas duas espécies de lagartos, com ênfase no hemoparasitismo por protozoários intracelulares. Para tanto, 30 espécimes (15 *A. ocellifera* e 15 *T. hispidus*) foram utilizados para análise. Após confecção das lâminas, foi realizada a contagem diferencial de 100 leucócitos por lâmina. Além disso, 20 eritrócitos e 15 leucócitos totais foram

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fotografados por lâmina para as análises morfométricas. As medidas foram aferidas para os parâmetros área, perímetro, eixos máximo e mínimo, com as fórmulas da elipse utilizadas para área e perímetro. As diferenças interespecíficas e intraespecíficas (lagartos parasitados e não parasitados) foram testadas para os parâmetros morfométricos dos eritrócitos, núcleo dos eritrócitos e leucócitos. A contagem diferencial mostrou que heterófilo foi o tipo celular predominante para ambas as espécies de lagartos, enquanto basófilo foi o menos abundante para *A. ocellifera* e eosinófilo para *T. hispidus*. Quanto às medidas dos leucócitos, apenas o monócito diferiu significativamente entre as espécies, maior em *A. ocellifera*, e intraespecificamente apenas para o parâmetro eixo mínimo, maior em *T. hispidus* parasitado. Os eritrócitos diferiram interespecificamente quanto ao tamanho da célula e do núcleo, maiores em *T. hispidus*. Dentre os hemoparasitos, encontrados apenas em *T. hispidus*, *Plasmodium* sp. causou alterações mais discretas no eritrócito, enquanto *Hepatozoon* sp. causou macrocitose acentuada e hipocromia nessas células. De um modo geral, não parece haver relação entre a contagem diferencial de leucócitos e o hemoparasitismo.

Palavras-chave: Caatinga; Hemácias; *Hepatozoon*; Leucócitos; *Plasmodium*.

Introdução

O estudo da hematologia em répteis pode ser complexo devido à morfologia peculiar das células sanguíneas e a impossibilidade de realizar exames automatizados (Rovira, 2010). Além disso, aspectos como variações sazonais, incluindo temperatura, podem alterar o número dos leucócitos, bem como a presença de parasitos/patógenos (Martínez-Silvestre *et al.*, 2011).

Parasitos sanguíneos são frequentemente encontrados em répteis selvagens aparentemente saudáveis e, na maioria das vezes, não sendo considerados como patogênicos (Campbell, 2004; Telford, 2009). No entanto, a presença de parasitos intracelulares pode causar alterações citopatológicas e a destruição das células hospedeiras, além de também induzir a produção de células sanguíneas (Schall, 1996; Silva *et al.*, 2004; Bonadiman *et al.*, 2010). Dentre os hemoparasitos intracelulares podemos destacar as hemogregarinas e os hemosporídeos, ambos do Filo Apicomplexa, como sendo os mais comuns nesses animais (Davies e Johnston, 2000; Stacy *et al.*, 2011; Picelli *et al.*, 2020a).

Componentes da herpetofauna da Caatinga, as espécies de lagartos *Ameivula ocellifera (*Spix, 1825) (Teiidae) e *Tropidurus hispidus (*Spix, 1825) (Tropiduridae) são de ampla distribuição no bioma, generalistas de hábitat e encontradas nos mais variados tipos de Caatinga (Rodrigues, 2003; Ribeiro e Freire, 2011; Albuquerque *et al.*, 2018). Devido ao fato dessas espécies apresentarem padrões de atividade diferentes, bem como modos de forrageio distintos, ambas ocorrem em simpatria (Bergallo e Rocha, 1993; Albuquerque *et al.*, 2018), sendo bons modelos de estudos.

Visto que pesquisas envolvendo as células sanguíneas em lagartos do semiárido são escassas, o objetivo deste trabalho foi realizar a contagem diferencial de leucócitos e descrever a morfometria e morfologia dos eritrócitos e leucócitos nas espécies *A. ocellifera* e *T. hispidus*, além de investigar os efeitos da presença de hemoparasitos.

Material e Métodos

Procedência dos animais

No período de junho a novembro de 2014, um total de 30 lagartos (sendo 15 *T. hispidus* e 15 *A. ocellifera*) foi capturado nos munícipios de Brejo Santo e Mauriti no estado do Ceará, e Floresta, estado de Pernambuco. Os municípios estão localizados no bioma Caatinga, e deste modo, apresentam vegetação xerófila, com variações na fisionomia e florística (Drumond *et al.*, 2004). O clima semiárido é predominante, com escassez e irregularidade das chuvas, além de alta radiação solar, baixa nebulosidade e temperaturas médias elevadas (Prado, 2003).

Os lagartos foram capturados durante as atividades de resgate de fauna silvestre nas áreas de influência da obra do Projeto de Integração do Rio São Francisco com Bacias Hidrográficas do Nordeste Setentrional (PISF). Após a captura manual, os lagartos passaram por triagem nos Centros de Triagens Móveis do Centro de Conservação e Manejo de Fauna da Caatinga (CEMAFAUNA-CAATINGA) e posteriormente foram destinados ao Laboratório de Morfofisiologia do CEMAFAUNA-CAATINGA em Petrolina, Pernambuco, Campus de Ciências Agrárias, Universidade Federal do Vale do São Francisco (UNIVASF).

Preparação dos espécimes

Para cada espécime foram aferidas as medidas do comprimento rostro-cloacal (CRC em mm), com o auxílio de paquímetro digital e a massa corpórea (g) em balança digital. Apenas indivíduos adultos, machos e fêmeas, de ambas as espécies foram utilizados, conforme CRC definido por Vitt (1995). Nesse sentido, para *T. hispidus* adotou-se CRC's a partir de 70 e 71 mm para fêmeas e machos, respectivamente, e CRC's a partir de 55 mm para fêmeas e 56 mm para machos de *A. ocellifera*.

Os lagartos foram contidos fisicamente e, em seguida, foi realizada a coleta sanguínea através da veia coccígea ventral (Sykes e Klaphake, 2015) com o uso de seringa de 1 ml acoplada à agulha 30G 5/16 (8 mm x 0,30 mm), sendo acesso ventral para *A. ocellifera* e lateral para *T. hispidus*, com o animal em decúbito dorsal (Samour *et al.*, 1984). A quantidade de sangue coletada foi de 100 µl por animal.

Imediatamente, após a coleta de sangue, confeccionaram-se esfregaços sanguíneos, sendo quatro lâminas por animal, totalizando 120 lâminas. As extensões sanguíneas foram secas em temperatura ambiente e coradas com *kit* Panótico Rápido, o qual se baseia no princípio de coloração hematológica estabelecida por Romanowsky. Posteriormente, os esfregaços foram montados sob lamínula com o auxílio de bálsamo do Canadá.

Leitura dos esfregaços sanguíneos

O exame dos esfregaços sanguíneos foi em microscópio óptico modelo NOVA 180i-FT, onde foram realizadas a contagem diferencial e a busca por hemoparasitos. A identificação morfológica dos hemoparasitos foi a nível de gênero através da comparação com as descrições reunidas por Telford (2009) e Lainson (2012). Na contagem diferencial, 100 leucócitos por lâmina foram contados, totalizando 400 leucócitos por animal. A objetiva de 100x foi utilizada, separando os leucócitos de acordo com suas características morfológicas em heterófilos, monócitos, linfócitos, eosinófilos e basófilos, obtendo-se o percentual de cada tipo celular no sangue. A diferenciação dos leucócitos foi realizada de acordo com a classificação de Rovira (2010). Para análise morfométrica, as células foram fotografadas com a câmera Industrial Digital Camera, modelo UCMOS05100KPA acoplada ao microscópio óptico, e as imagens foram processadas no software ImagePro-plus® versão 4.5.0.29.

Os parâmetros obtidos foram área (µm²), perímetro (µm) e eixos máximo e mínimo (µm), totalizando 20 eritrócitos e 15 leucócitos totais por lâmina (Casal e Orós, 2007). As fórmulas da elipse foram utilizadas para cálculos de área e perímetro (Área = raio maior x raio menor x π; Perímetro = π x ((3 x ((raio maior/2) + (raio menor/2))) - V((3 x (raio maior/2) + (raio menor/2)) x ((raio maior/2) + 3 x (raio menor/2))))). Os parâmetros também foram mensurados para os respectivos núcleos dos eritrócitos (Zhelev *et al.*, 2006). Nos indivíduos parasitados, o mesmo método foi adotado, porém com os dados tabulados separadamente. Em seguida, os dados foram confrontados entre os espécimes não parasitados e parasitados.

Análise estatística

A análise estatística foi realizada com o auxílio do software Past® versão 3.11. Os parâmetros morfométricos (área, perímetro, eixos máximo e mínimo) foram apresentados na forma de média ± desvio padrão. Os dados foram separados por espécie e para cada espécie foram subdivididos em animais parasitados e não parasitados, com o intuito de investigar diferenças interespecíficas nos parâmetros morfométricos das células sanguíneas, assim como, diferenças intraespecíficas no tamanho dos eritrócitos parasitados e não parasitados. Para tanto, realizou-se o teste de normalidade Shapiro-Wilk e, em seguida, o teste *t* para dados paramétricos e o Mann-Whitney para dados não-paramétricos. Os grupos comparados foram: (i) *Ameivula ocellifera* : *Tropidurus hispidus* não parasitado; e (ii) *Tropidurus hispidus* não parasitado : *Tropidurus hispidus* parasitado. Em todos os testes estatísticos utilizados o nível de significância adotado foi de 5%.

Aspectos éticos e legais

O presente estudo foi executado sob autorizações emitidas pelo Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) (Autorização para atividades de finalidade científica nº. 45033-2) e aprovação pelo Comitê de Ética no Uso de Animais (CEUA) da Universidade Federal do Vale do São Francisco - UNIVASF (Protocolo nº. 0006/100614).

Resultados

Aspectos morfológicos

As células sanguíneas de *T. hispidus* e *A. ocellifera* são mostradas nas Figuras 1 e 2. Os eritrócitos foram observados com formato elíptico e coloração eosinofílica no citoplasma e arroxeada no núcleo, para ambas as espécies (Fig. 1A; Fig. 2A). Eritrócitos anucleados foram observados em *T. hispidus* (Dado não amostrado), tanto em indivíduos parasitados e não parasitados. Além disso, células eritrocitárias em formato de lágrima também foram observadas para ambas as espécies (Dado não amostrado).

Os basófilos de ambas as espécies apresentaram formato circulares, coloração basofílica e grânulos dispersos no citoplasma (Fig. 1B; Fig. 2B). Os tipos celulares heterófilo e eosinófilo foram observados com coloração eosinofílica (Fig. 1C-D; Fig. 2C-D). Principalmente em *T. hispidus*, esses tipos celulares apresentaram-se com granulações citoplasmáticas pouco evidentes. Ainda se tratando dos heterófilos foi observado que para *T. hispidus* houve presença apenas de heterófilos com núcleos lobulados, diferentemente de *A. ocellifera*, em que foram constatados heterófilos com núcleos lobulados e não lobulados.

Os linfócitos também apresentaram formatos circulares, com o citoplasma homogêneo e escasso, e o núcleo ocupando a maior porção celular (Fig. 1E; Fig. 2E).

A maioria dos monócitos nas duas espécies apresentaram coloração azul-acinzentada e vacúolo (Fig. 1F; Fig. 2F). No entanto, em *A. ocellifera* foram encontradas células com coloração mais basofílica e estruturas granulares no citoplasma (Dado não amostrado). Além disso, macrófagos foram observados no sangue periférico somente de *A. ocellifera* (Fig. 3).

Hemoparasitismo

Entre os lagartos amostrados, apenas cinco indivíduos de *T. hispidus* (33%; n = 15) estavam positivos para hemoparasitos. Os espécimes parasitados foram capturados no estado do Ceará (Fig. 4). Dois gêneros de parasitos do Filo Apicomplexa foram identificados (Fig.5): *Plasmodium* sp. (26%; n = 4/15) e *Hepatozoon* sp. (6%; n = 1/15). Estes parasitos foram encontrados infectando apenas eritrócitos e

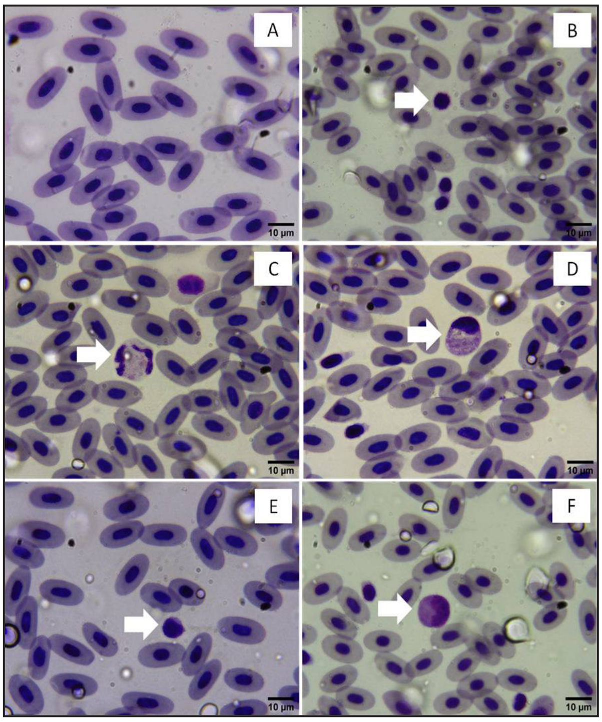

Figura 1. Células sanguíneas de *Tropidurus hispidus*. A: Eritrócitos; B: Basófilo; C: Heterófilo; D: Eosinófilo; E: Linfócito; F: Monócito.

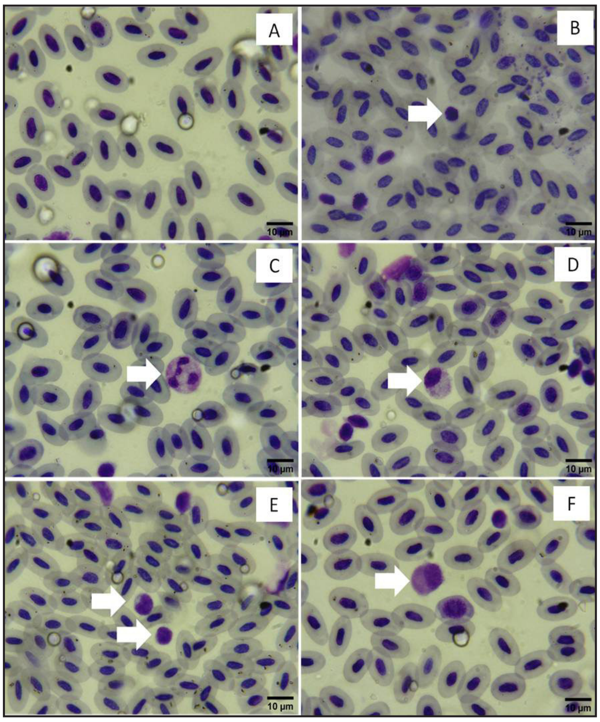

Figura 2. Células sanguíneas de *Ameivula ocellifera*. A: Eritrócitos; B: Basófilo; C: Heterófilos; D: Eosinófilo; E: Linfócitos; F: Monócito.

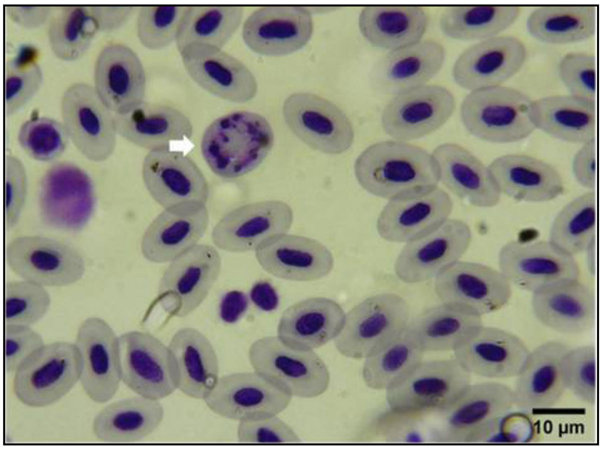

Figura 3. Macrófago no sangue periférico de *Ameivula ocellifera*.

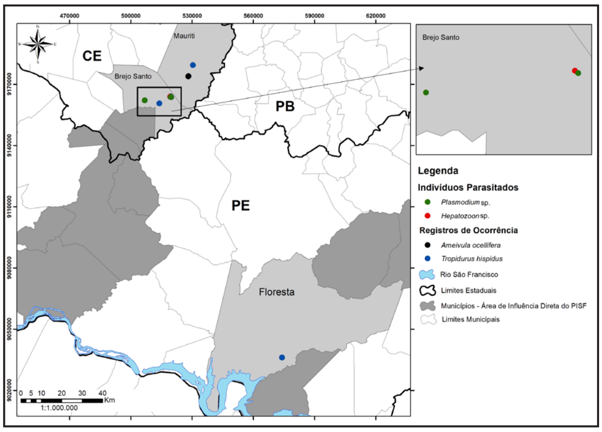

Figura 4. Representação geográfica de parte da região nordeste do Brasil, com registros de ocorrênciados espécimes de *Ameivula ocellifera* e *Tropidurus hispidus* não parasitados e parasitados; estes últimos em destaque ampliado. PISF: Projeto de Integração do Rio São Francisco com Bacias Hidrográficas do Nordeste Setentrional.

não foi observada a ocorrência de co-infecção em nenhum dos lagartos.

Contagem diferencial

A contagem diferencial de leucócitos para ambas as espécies é apresentada na Tabela 1. O tipo heterófilo foi o mais predominante para as duas espécies de lagartos, enquanto o eosinófilo apresentou menor percentual para *T. hispidus* e o basófilo em *A. ocellifera*. Quando comparados os percentuais de leucócitos dos animais parasitados com aqueles não parasitados não foi observada diferença.

Aspectos morfométricos

Os dados morfométricos das células estão apresentados na Tabela 2. Com base nas análises morfométricas dos leucócitos de *A. ocellifera* e *T. hispidus* (não parasitado), não houve diferença significativa entre os parâmetros, exceto para o tipo celular monócito. Este último diferiu no tamanho da área, perímetro, eixos máximo e mínimo, sendo maior em *A. ocellifera*. De forma intraespecífica, apenas o eixo mínimo diferiu para essa célula, sendo maior para *T. hispidus* parasitado.

Sobre os eritrócitos, houve diferença significativa interespecífica entre o tamanho da área, perímetro, eixos máximo e mínimo dos eritrócitos e do núcleo dos mesmos, sendo maiores em *T. hispidus*, com exceção do eixo mínimo do eritrócito que foi maior para *A. ocellifera*.

Houve também diferença intraespecífica significativa para o tamanho dos parâmetros eritrocitários entre *T. hispidus* não parasitado e parasitado, sendo maiores nestes últimos. No entanto, não houve diferença significativa intraespecífica para o tamanho do núcleo dos eritrócitos entre esses dois grupos, excetuando o eixo mínimo, maior em *T. hispidus* não parasitado.

Considerando ainda, os grupos *Tropidurus hispidus* – não parasitado, *Tropidurus hispidus* – *Plasmodium* sp. e *Tropidurus hispidus* – *Hepatozoon* sp., não houve diferença entre o tamanho dos eritrócitos parasitados por *Plasmodium* sp. e as células saudáveis. No entanto houve diferença significativa entre o tamanho do núcleo, onde os parâmetros área e eixo mínimo foram maiores em indivíduos não parasitados. Além disso, foi observado descentralização do núcleo de alguns eritrócitos parasitados, bem como, também em alguns, foi observado enrugamento na borda do núcleo.

Entre as células parasitadas por *Hepatozoon* sp. e as células não parasitadas, houve diferença

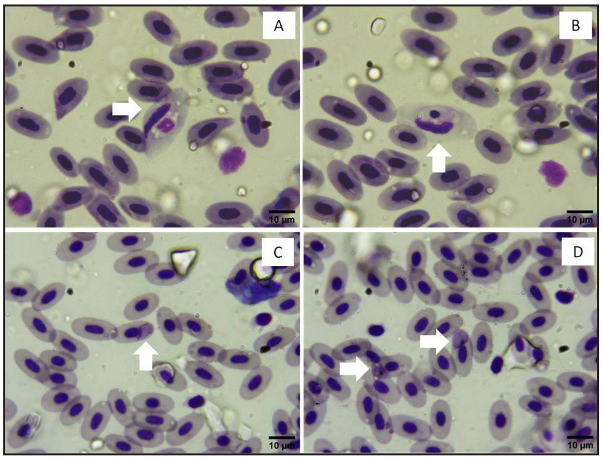

Figura 5. Eritrócitos de *Tropidurus hispidus* parasitados. A - B: Espécie *Hepatozoon* sp.; C - D: Espécie *Plasmodium* sp.

Tabela 1. Dados obtidos na contagem diferencial de leucócitos para *Tropidurus hispidus*, *Tropidurus hispidus – Plasmodium* sp., *Tropidurus hispidus – Hepatozoon* sp. e *Ameivula ocellifera*, representados na forma de média ± desvio padrão.

Espécie	**Tipo celular (%)**				
	Basófilo	**Eosinófilo**	**Heterófilo**	**Linfócito**	**Monócito**
Tropidurus hispidus– não parasitados por parasito intraeritrocitário (n=10)	4,31 ± 2,51	1,08 ± 1,02	53,95 ± 10,85	23,16 ± 5,64	17,31 ± 6,98
Tropidurus hispidus – Plasmodium sp. (n=4)	3,57 ± 1,90	0,71 ± 1,11	55,14 ± 10,09	21,57 ± 5,85	18,85 ± 8,19
Tropidurus hispidus – Hepatozoon sp. (n=1)	3,50 ± 0,70	1,00 ± 1,41	54,50 ± 14,84	25,50 ± 14,69	16,50 ± 3,53
Ameivula ocellifera (n=15)	1,35 ± 1,49	4,45 ± 2,94	41,82 ± 11,67	29,96 ± 8,67	22,40 ± 6,81

significativa entre os parâmetros área, perímetro e eixos máximo e mínimo dos eritrócitos, bem como para área, perímetro e eixo máximo do núcleo; para todos os parâmetros, os maiores valores foram encontrados em indivíduos parasitados. Além disso, foi observada descentralização, lobulação e alogamento do núcleo, hipocromia da célula como um todo e macrocitose acentuada na célula parasitada.

Discussão

Aspectos morfológicos

A presença de grânulos pouco evidentes dos tipos celulares heterófilo e eosinófilo, observados principalmente em *T. hispidus*, pode ser explicada pelo fato de que algumas espécies de lagartos apresentam granulações discretas ou isso também pode ser decorrente da interferência do método de fixação usado nas lâminas (Martínez-Silvestre *et al.*, 2011), o que merece novas investigações com uso de outras formas de fixação.

Outra característica observada foram os grânulos citoplasmáticos em alguns monócitos de *A. ocellifera*. De fato, embora não seja tão comum, pode ser encontrada nesse tipo celular (Harr *et al.*, 2001; Campbell, 2004; Bonadiman *et al.*, 2010). Além disso, há indícios de que a presença de grânulos citoplasmáticos pode estar relacionada com o parasitismo intraleucocitário (Lainson *et al.*, 2003; Silva *et al.*, 2004). Contudo, no presente estudo não foram encontradas estruturas parasitárias nessa espécie de lagarto, portanto, tais grânulos podem estar associados à doenças inflamatórias e infecciosas. Enquanto que a presença de macrófagos no sangue periférico desse lacertílio, provavelmente sejam macrófagos eritrofagocitários, relacionados à imunomodulação e doenças infecciosas ou neoplásicas (Stacy *et al.*, 2011).

Quanto às alterações morfológicas encontradas nos eritrócitos como células anucleadas, em *T. hispidus*, ou em formato de lágrima, em ambas as espécies, estas são ocasionalmente encontradas em répteis (Stacy *et al.*, 2011).

Contagem diferencial

Para os valores obtidos na contagem diferencial de leucócitos, sendo heterófilo e linfócito os mais representativos para ambas as espécies, estes estão de acordo com os elevados percentuais observados em outras espécies de lagartos, bem como o baixo percentual de eosinófilo no sangue periférico (Fisse *et al.*, 2004; Ponsen *et al.*, 2008).

A inexistência de diferenças entre as contagens diferenciais de leucócitos dos animais parasitados e não parasitados, sugere que não há uma relação direta entre o parasitismo e o aumento ou redução de determinados tipos celulares, conforme observado em outras espécies de outros grupos animais. Para os mamíferos, por exemplo, o eosinófilo atua como supressor de infecções parasitárias, podendo induzir à eosinofilia (Mitchell e Johns, 2008). Em aves, há associação entre o aumento do heterófilo no sangue periférico com o parasitismo, bem como, o hemoparasitismo aviário apresenta dentre outros sinais clínicos, a leucocitose (Mitchell e Johns, 2008). No caso dos répteis, a presença de hemogregarinas não é associada à eosinofilia, como observado tanto em crocodilos como em tartarugas (Martínez-Silvestre *et al.*, 2011), provavelmente ocorrendo o mesmo em lagartos, como nos espécimes de *T. hispidus* aqui parasitados.

O parasitismo por *Plasmodium* sp. em lagartos pode causar alterações comportamentais e no fitness (Schall, 1992; Scholnick *et al.* 2010). Além disso, o curso da infecção no sangue pode variar conforme as espécies de parasitos, bem como entre os indivíduos hospedeiros (Schall, 1996). Desse modo, a ausência de diferenças entre as contagens diferenciais de indivíduos parasitados e não parasitados do presente estudo, pode estar associada ao estágio da infecção

Tabela 2. Dados morfométricos das células sanguíneas de *Ameivula ocellifera* e *Tropidurus hispidus* (não parasitado e parasitado, nesse caso por ambos os parasitos intraeritrocitários) e *Tropidurus hispidus – Plasmodium* sp. e *Tropidurus hispidus – Hepatozoon* sp., medidos separadamente. Valores dos parâmetros (Área (μm^2); Perímetro (µm); Eixo máximo: Eixo Máx. (µm); Eixo mínimo: Eixo Mín. (µm)) estão indicados na forma de média ± desvio padrão. O traço (––) significa que a análise não se aplica. Letras maiúsculas e minúsculas indicam diferença estatística significativa.

Tipo celular	Parâmetro	Grupo				
		Ameivula ocellifera (n=15)	*Tropidurus hispidus* não parasitado (n=10)	*Tropidurus hispidus* parasitado (n=5)	*Tropidurus hispidus* *Plasmodium* sp. (n=4)	*Tropidurus hispidus* *Hepatozoon* sp. (n=1)
Eritrócitos	Área	96,49 ± 9,62a	106,29 ± 14,43 AB c D	150,01 ± 78,23 b	103,05 ± 17,88 c	249,58 ± 61,21 d
	Perímetro	36,87 ± 1,79 a	39,82 ± 2,48 AB c D	46,29 ± 11,24 b	39,22 ± 3,42 c	61,29 ± 5,96 d
	Eixo Máx.	14,67 ± 0,84 a	16,44 ± 1,09 AB c D	19,20 ± 4,68 b	16,22 ± 1,54 c	25,50 ± 2,11 d
	Eixo Mín	8,37 ± 0,61 a	8,21 ± 0,81 AB c D	9,42 ± 2,47 b	8,05 ± 0,86 c	12,35 ± 2,22 d
Núcleo–Eritrócitos	Área	14,64 ± 2,01 a	17,64 ± 2,69 A b C D	19,66 ± 7,11 b	16,88 ± 3,91 c	30,20 ± 6,66 d
	Perímetro	14,85 ± 1,02 a	15,79 ± 1,30A b c D	17,60 ± 4,50 b	15,64 ± 2,16 c	25,03 ± 3,06 d
	Eixo Máx.	6,15 ± 0,51 a	6,28 ± 0,67A b c D	7,38 ± 2,43 b	6,32 ± 1,14 c	11,42 ± 1,65 d
	Eixo Mín	3,02 ± 0,32 a	3,57 ± 0,36AB C d	3,39 ± 0,46 b	3,39 ± 0,42 c	3,38 ± 0,60 d
Basófilos	Área	30,39 ± 6,16 a	35,30 ± 12,13 a b	39,09 ± 12,34 b	––	––
	Perímetro	19,47 ± 2,04 a	20,82 ± 3,37 a b	21,92 ± 3,55 b	––	––
	Eixo Máx.	6,41 ± 0,73 a	6,80 ± 1,03 a b	7,17 ± 1,21 b	––	––
	Eixo Mín	5,98 ± 0,61 a	6,44 ± 1,12 a b	6,78 ± 1,04 b	––	––
Eosinófilos	Área	79,33 ± 18,65 a	68,66 ± 14,59 a b	52,79 ± 4,02 b	––	––
	Perímetro	31,42 ± 3,65 a	29,45 ± 3,23 a b	25,76 ± 1,01 b	––	––
	Eixo Máx.	10,39 ± 1,25 a	10,10 ± 1,24 a b	8,50 ± 0,47 b	––	––
	Eixo Mín	9,6 ± 1,13 a	8,60 ± 1,15 a b	7,90 ± 0,16 b	––	––
Heterófilos	Área	106,47 ± 37,29 a	100,65 ± 42,34 a b	95,22 ± 36,12 b	––	––
	Perímetro	36,19 ± 6,27 a	34,99 ± 7,19 a b	33,98 ± 6,71 b	––	––
	Eixo Máx.	12,17 ± 2,10 a	11,79 ± 2,50 a b	11,19 ± 2,25 b	––	––
	Eixo Mín	10,84 ± 2,05 a	10,45 ± 2,19 a b	10,44 ± 2,05 b	––	––
Linfócitos	Área	45,22 ± 11,82 a	46,76 ± 13,61 a b	45,48 ± 13,51 b	––	––
	Perímetro	23,65 ± 3,14 a	24,01 ± 3,69 a b	23,64 ± 3,79 b	––	––
	Eixo Máx.	7,76 ± 1,06 a	7,96 ± 1,22 a b	7,81 ± 1,25 b	––	––
	Eixo Mín	7,29 ± 0,97 a	7,31 ± 1,20 a b	7,23 ± 1,18 b	––	––
Monócitos	Área	107,03 ± 38,10 a	92,20 ± 35,00A b	110,70 ± 49,70 b	––	––
	Perímetro	36,36 ± 6,35 a	33,68 ± 6,04 A b	36,55 ± 8,21 b	––	––
	Eixo Máx.	12,41 ± 2,27 a	11,41 ± 2,17 A b	12,25 ± 3,03 b	––	––
	Eixo Mín	10,68 ± 1,95 a	9,99 ± 1,82 A B	10,99 ± 2,26b	––	––

Valores de Mann-Whitney para os resultados com diferença estatística significativa. **Eritrócitos**: área: z = -16,151, P= 1,12e-58 (a/A), z = -3,343, P = 0,0008 (b/B), z= -8,538, P= 1,363e-17 (d/D); perímetro: z= -25,868, P= 1,52e-147(a/A), z= -3,370, P= 0,0007 (b/B), z= -8,542, P= 1,312e-17(d/D); eixo máximo: z= -31,287, P= 7,101e-215 (a/A), z= -3,283, P= 0,001 (b/B), z= -8,348, P= 1,211e-17 (d/D); eixo mínimo: z= -6,026, P= 1,671e-9 (a/A) z= -3,049, P= 0,002 (b/B), z= -8,023, P= 1,025e-15 (d/D); **Núcleo-Eritrócitos**: área: z= -24,938, P= 2,853e-137 (a/A), z= -2,525, P= 0,011 (c/C), z= -6,196, P= 5,769e-10 (d/D); perímetro: z= -17,237, P= 1,395e-66 (a/A), z= -6,417, P= 1,385e-10 (d/D); eixo máximo: z= -5,362, P= 8,189e-8(a/A), z= -6,421, P= 1,35e-10 (d/D); eixo mínimo: z= -29,585, P= 2,356e-192 (a/A), z= -3,776, P= 0,0001 (b/B), z= -3,291, P= 0,0009 (c/C); **Monócitos**: área: z= -2,905, P= 0,0036 (a/A); perímetro: z= -2,945, P= 0,0032 (a/A); eixo máximo: z= -3,087, P= 0,002 (a/A); eixo mínimo: z= -2,403, P= 0,016 (a/A), z= -2,222, P= 0,026 (b/B).

nesses hospedeiros, bem como à parasitemia e/ou idade do animal (Schall, 1996). Esses fatores, embora não tenham sido testados aqui, são conhecidos por contribuir com a ausência da resposta imune leucocitária perante a presença do parasito.

Aspectos morfométricos das células sanguíneas

Os eixos dos leucócitos se assemelharam aos valores disponíveis na literatura, correspondendo aos intervalos propostos para essas células em répteis (Stacy *et al.*, 2011).

As medidas dos eritrócitos de *T. hispidus* e *A. ocellifera* (Tabela 2), encontram-se dentro do padrão observado para lacertílios, em especial para as médias de eixos máximo, mínimo e área (entre 12,43 a 16,85 µm; 7,51 a 10,21 µm; 73,27 a 130,33 µm^2, respectivamente) (Arikan e Çiçek, 2014).

Os eritrócitos de *T. hispidus* foram maiores do que os de *A. ocellifera*. Esta diferença entre os valores morfométricos interespecíficos era esperada de se constatar por se tratarem de espécies de famílias diferentes. A diferença no tamanho dos eritrócitos entre representantes de famílias distintas ou entre representantes de uma mesma família de lagartos é possível de ocorrer (Arikan e Çiçek, 2014).

Hemoparasitismo

Ambos os gêneros de parasitos encontrados nesse trabalho já foram relatados infectando espécies de lagartos neotropicais das famílias Teiidae e Tropiduridae (Telford 2009; Lainson, 2012; Matta *et al.*, 2018; Picelli *et al.*, 2020 a). Para o bioma Caatinga, *Plasmodium* sp. já foi registrado em *T. hispidus*, *Ameiva ameiva* Linnaeus, 1758 (Teiidae) e *Hemidactylus mabouia* Moreau de Jonnès, 1818 (Gekkonidae) (Harris *et al.*,2019). Enquanto que para *Hepatozoon* sp., apesar de ser um parasito comum em lagartos (Smith, 1996; Picelli *et al.*, 2020 b), este foi o primeiro registro do gênero em *T. hispidus* no semiárido brasileiro. Contudo, é importante ressaltar que apesar da diversidade reptiliana da Caatinga (Costa e Bérnils, 2018), pesquisas envolvendo esses hospedeiros e seus hemoparasitos são pouco exploradas nessa região, visto que grande parte dos estudos se concentram na Amazônia (Picelli *et al.* 2020 b; Picelli *et al.*, 2020 a).

O gênero *Hepatozoon* já havia sido registrado em *Tropidurus torquatus* Wied-Neuwied, 1820 (Tropiduridae) no estado de São Paulo, bioma Mata Atlântica (Rocha-Silva, 1975). Contudo, vale destacar que parte do complexo de espécies *T. torquatus* foi desmembrado (Rodrigues, 1987), originando dentre outras, a espécie *Tropidurus itambere* Rodrigues, 1987 (Tropiduridae), e o local de estudo de Rocha-Silva (1975) coincide com a ocorrência dessas duas últimas espécies de lagartos. Na região da Amazônia Oriental, esse gênero de hemogragrina foi encontrado também em um tropidurídeo pertencente ao grupo *torquatus*, sendo inicialmente identificado como *T. torquatus* (Paperna e Lainson, 2004). No entanto, considerando a revisão do grupo (Rodrigues, 1987) e dada a distribuição geográfica dos espécimes estudados, há possibilidade das espécies de lagartos hospedeiros serem os tropidurídeos *T. hispidus*, *Tropidurus insulanus* Rodrigues, 1987 ou *Tropidurus oreadicus* Rodrigues, 1987. Além disso, o gênero *Hepatozoon* já foi registrado em *Hemidactylus mabouia*, *Phyllopezus pollicaris (*Spix, 1825) (Phyllodactylidae) e *Phyllopezus periosus (*Rodrigues, 1986) (Phyllodactylidae), sendo esse último amostrado no semiárido brasileiro (Harris *et al.*, 2015).

Hemoparasitismo e os parâmetros morfométricos

A ausência de macrocitose e o deslocamento do núcleo no parasitismo por *Plasmodium* sp. conforme relatados nesse estudo, também foram observados no parasitismo de *Plasmodium tropiduri* Aragão & Neiva, 1909 (Apicomplexa: Plasmodiidae) em *Mabuya mabouya* Bonnaterre, 1789 (Squamata: Scincidae) (Lainson e Shaw, 1969). Para essa espécie de lagarto hospedeiro, após sua revisão taxonômica e considerando o local de ocorrência (Hedges e Conn, 2012), podem se tratar das espécies *Copeoglossum nigropunctatum* Spix, 1825 ou *Varzea bistriata* Spix, 1825 (Scincidae). Como efeito do parasitismo, a presença do parasito na célula hospedeira, atrelada ao vacúolo parasitóforo, comum aos organismos do Filo Apicomplexa (Striepen *et al.*, 2007), pode influenciar não apenas no deslocamento do núcleo, mas também na sua morfometria; alterações essas observadas aqui nos parâmetros área e eixo mínimo do núcleo.

Em contrapartida, outras espécies do gênero *Plasmodium* que infectam lagartos causam distorções mais notórias, como macrocitose e/ou arredondamento do eritrócito, além de hipocromia. A exemplo, os parasitismos de *Cnemidophorus* cf. *gramivagus* McCrystal & Dixon, 1987 (Squamata: Teiidae) por *Plasmodium kentropyxi* Lainson *et al.*, 2001 (Apicomplexa: Plasmodiidae) (Matta *et al.*, 2018) e *Plasmodium diploglossi* Aragão & Neiva, 1909 (Apicomplexa: Plasmodiidae) em *Diploglossus fasciatus* Gray, 1831 (Squamata: Anguidae) e

M. mabouya (= *Copeoglossum nigropunctatum* ou *Varzea bistriata*), onde o esquizonte ao aumentar de tamanho compromete o formato da célula do hospedeiro. Embora, nesses casos o núcleo sofra uma leve deformação (Aragão e Neiva, 1909; Lainson e Shaw, 1969), o enrugamento na borda do núcleo de alguns eritrócitos parasitados, pode não ser decorrente do parasitismo, uma vez que eritrócitos não parasitados também apresentem tais modificações (Lainson e Shaw, 1969).

No parasitismo por *Hepatozoon* sp., morfologicamente a célula hospedeira apresentou alterações mais acentuadas, podendo ser característica desse grupo de parasito (Telford, 2009). Em um estudo nas Ilhas Canárias com hemogregarinas do gênero *Karyolysus* em lagartos dos gêneros *Gallotia*, *Tarentola* e *Chalcides*, foi observada distorção dos eritrócitos infectados, tanto no tamanho quanto na forma da célula hospedeira e de seu núcleo. Houve aumento na área dos eritrócitos, acarretando em formatos arredondados ou alongados, bem como alongamento do núcleo devido ao deslocamento e/ou crescimento do parasito (Tomé *et al*., 2019).

As alterações nos parâmetros morfométricos apresentadas no presente trabalho foram observadas no parasitismo por *Hepatozoon* em diferentes hospedeiros (Han *et al*., 2015; Picelli *et al.* 2020 b), revelando alterações significativas nos eritrócitos parasitados. Na mesma perspectiva, diferenças significativas na morfometria de eritrócitos não parasitados e parasitados por *Hepatozoon* em *Caiman crocodilus yacare* Daudin, 1802 (Crocodylia: Alligatoridae) foram observadas, devido às distorções causadas pelo parasito (Bouer *et al*., 2017).

Conclusão

O presente trabalho apresenta os resultados do estudo hematológico nas espécies de lagartos *Ameivula ocellifera* e *Tropidurus hispidus* no Brasil, descrevendo de forma pioneira a morfologia e morfometria das células sanguíneas, além da contagem diferencial de leucócitos. Dessa forma, observou-se similaridade entre a morfologia destas células com aquela dos demais répteis, e a morfometria dos eritrócitos se manteve no intervalo encontrado para os lacertílios. Quanto ao efeito do parasitismo na morfometria, a presença do parasito *Plasmodium* sp. resultou em alterações mais discretas, diferente do parasito *Hepatozoon* sp. que causou alterações tanto na morfometria quanto nos aspectos morfológicos do eritrócito. Além disso, o presente estudo relata o primeiro registro de *Hepatozoon* sp. parasitando *T. hispidus* no bioma Caatinga. Finalmente, o trabalho oferece dados descritivos acerca da hematologia dos lagartos estudados, além de expandir o conhecimento sobre a distribuição de hemoparasitos na herpetofauna Neotropical.

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Trabajo

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Hematological parameters and globulin profile of *Bothrops asper* and *Crotalus simus* snakes (Serpentes: Viperidae), after the main venom gland removal

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ABSTRACT
Snakebite accidents are a neglected tropical disease worldwide. In Costa Rica, the snakes *Bothrops asper* and *Crotalus simus* cause more than the 60% of the snakebite accidents already reported. To fight this neglected human disease, biomedical institutions need to manage live venomous snake collections in order to extract venoms and produce antivenoms. The welfare of the snakes in captivity must be guaranteed, especially in the case of old animals with defective venom production, which is a common situation at the Clodomiro Picado Institute. A surgical removal of the main venom glands was performed in five *B. asper* and *C. simus* with none or low venom production. The hematological parameters and plasma proteins of these individuals were analyzed pre-surgery and for 90 days post-surgery. Surgical procedures did not produce anemia during the post-surgical evaluation, but heterophilia, relative monocytosis and lymphopenia were detected one to three days afterwards. We conclude that the surgical removal of the main venom gland is a safe procedure and did not affect the snake's health. Additionally, the use of electrophoretic determination of the albumin:globulin ratio to assess the health condition of the snakes is discussed. Finally, the relative monocytosis and lymphopenia seems to be linked to continuous handling of the snakes during consecutive days. Monocytes play a key role in the stress response, therefore the relative monocytosis and lymphopenia of leukogram reports should be interpreted in this context.

Key Words: snake globulins, snake hematology, *Bothrops asper*, *Crotalus simus*, globulin profile, venomoid snakes.

Introduction

Costa Rica possess 23 venomous snakes of the 141 species reported to occur in this country, which represent a potential risk to humans, pets, and livestock (Leenders, 2019). Of these, *Bothrops asper* (Terciopelo snake) and *Crotalus simus* (Middle American rattlesnake), are the most important species regarding human snakebite accidents. The first is responsible for 50-60% of all cases reported (Sasa and Vázquez, 2003; Fernández and Gutiérrez, 2008). Thus, it is not surprising that the venoms of these snake species are widely used for antivenom production, like it takes place at the Instituto Clodomiro Picado from Costa Rica (Gutiérrez *et al*., 2014).

In members of the families Elapidae, Viperidae and Atractaspidae, a relevant component of the venom production system are the main venom glands that synthetize, store, and secrete the proteins conforming the venom. The glands differ in shape and size of the lumen, as well as in the distribution of their mucous and serous cells (Jackson, 2007). These glands are covered by connective tissue, which is attached to the compressor muscles; the muscles are responsible for contracting the glands in order to eject the venom when biting (Mackessy, 1991). In viperids, the main gland drains the venom into a primary duct that is typically absent in elapids (Mackessy, 1991; Sakai *et al*., 2012).

It is known that after years of extracting venom for biomedical purposes, the viperid main venom glands become dysfunctional and stop producing

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venom, mainly due to a mild to severe trauma caused by the mechanical pressure applied to the glands during extraction (Giannotti *et al*., 2013; Rodríguez-Abarca *et al*., 2019). This repetitive traumatic injury induces venom leaking that affects the surrounding tissue, and the glands may undergo fibrotic dysplasia (Rodríguez-Abarca *et al*., 2019). For this reason, removal of the main venom glands is desirable as they may affect the welfare of the snakes; these snakes without the main venom gland are called venomoids (Bielli and Silvetti, 2014).

Unfortunately, there is a scarce number of scientific articles dealing with hematology, blood biochemistry and some associated clinical aspects in reptiles, especially in venomous snakes (Giménez *et al*., 2010; Lentini *et al*., 2011; Melillo, 2013; Gómez *et al*., 2016). Performing hematology tests and leukogram readings may help in disease diagnosis and reveal inflammatory responses in snakes, particularly to evaluate post-surgical performance as they are fast and simple methods. Furthermore, there is evidence that heterophil counts in snakes increase after surgical procedures, as do monocytes related to chronic infections, while lymphocytes and basophils increase in response to foreign bodies such as telemetric transmitters (Lentini *et al*., 2011).

Plasma proteins serve a myriad of functions, i.e., maintaining blood osmotic pressure, pH regulation, hormone and drug transport, blood clotting (Melillo, 2013), immune and inflammatory reactions and tissue repair (Thrall *et al*., 2012). Nevertheless, there are still no studies related to *B. asper* and *C. simus* plasma proteins.

In birds and reptiles, the plasma proteins migrate similarly in gel electrophoresis, according to their electric charges. The order of the plasma proteins fractions usually seen in a gel is: albumins, α1-globulins, α2-globulins, β-globulins y γ-globulins, with albumin exhibiting the highest negative charge and the lowest molecular weight, while globulins the low negative charge and higher molecular weight. Furthermore, there is a prealbumin fraction also, with higher negative charge than albumins (Melillo, 2013), with and a hypothetical function of transport, named bisalbumins (Giménez *et al*., 2010).

In snakes, albumins are the predominating plasma proteins, while globulins are at a very low concentration in healthy individuals (Thrall *et al*., 2012; Campbell, 2015). Therefore, it is possible to observe differences in the electrophoretic patterns of plasma proteins in sick and healthy individuals (Zaias and Cray, 2002). The albumin:globulin (A:G) ratio is another tool used to evaluate variations in plasma proteins that can be used to determine dysproteinemia, even when the total protein level is normal (Melillo, 2013). This ratio can also aid to identify hypoalbuminemia, which could be a sign of anorexia, enteropathies, nephropathies, or liver disease (Silva *et al*., 2011), and also hyperalbuminemia as a possible sign of dehydration (Divers, 2000). Nonetheless, it must be considered that the technique has a high variability and value in diagnostics because of the variations related to species, sex, temperature, humidity, photoperiod, nutrition, and season (Melillo, 2013; Silva *et al*., 2011).

Hence, the aim of this study was to determine some electrophoretic and hematological parameters of viperid snakes after a surgical procedure. Since the World Health Organization (WHO) declared the snakebite in the Neglected Tropical Diseases Category A list (Chippaux, 2017), it is extremely important to generate clinical knowledge on how to approach the welfare of snakes kept in captivity for antivenom research and production.

Materials and methods

This study was approved by the "Comité Institucional para el Cuido y Uso de Animales de Laboratorio (CICUA)" of Universidad de Costa Rica (CICUA-033-17 and CICUA-82) and meet the International Guiding Principles for Biomedical Research Involving Animals (CIOMS, 1986). We followed the Legislation for the protection of animals used for scientific purposes (Directive 2010/63/EU, Commission Implementing Decision 2012/707/EU, Recommendation 2007/526/EC), and Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines for designing and conducting all experiments involving animals; and "Ley 7317: Ley de Conservación de la Vida Silvestre" legislation for Costa Rica.

Snakes.

Bothrops asper and *Crotalus simus* individuals we studied belong to the snake collection from the Instituto Clodomiro Picado's Serpentarium. They were kept in 62x32x23 cm acrylic cages with aluminum lids, at an environmental humidity of 80-90%. The environmental temperature was between 22-26°C and 28-30°C for *B. asper* and *C. simus* respectively. The snakes were fed every 10 days with four adult (+22g body weight) albino mice (*Mus musculus*, strain CD-1). Two groups of five adult snakes were conformed for both species, corresponding one to the control group and another one in which the venom gland was surgically removed (venomoid group). The venomoid group included adult individuals of both species which were not able to produce more venom, or whose venom yield production had been very low for several consecutive venom extraction procedures.

Venom glands removal surgery.

For the excision of venom glands in *B. asper* and *C. simus* individuals were anesthetized with 4 mg/kg of Propofol and 10mg/kg of Ketamine; two analgesics were used: Tramadol 5mg/kg of and Meloxicam 0.2 mg/kg. Drugs were administered intramuscularly except for Propofol, which was injected intravenously.

The surgery consisted in making an incision between the oral and labial mucosa lining, starting at the eye's medial canthus, and ending at the mandibular commissure. After that, the superficial adductor muscle was separated from the glandular joint in the caudal region of the gland; then, the square ligament that gives support to the posterior region of the gland was cut. Once separated, the gland was extracted and its conduct communicating with the accessory gland was ligated with absorbable suture (Vycril 4-0®). Finally, the initial incision was closed with non-absorbable suture (Vycril 4-0®).

After the surgery, the following medications were injected intramuscularly daily: Ceftriaxone 50 mg/kg for five days, Enrofloxacin 10 mg/kg for 10 days, Ketoprofen 2mg/kg for five days and Tramadol 5mg/kg for three days.

Blood extraction and handling.

Blood was extracted from the caudal vein with a 22-gauge in a 3 mL syringe, before surgery, immediately afterwards, and also 3, 30, 60 and 90 days later. The amount of blood extracted was 1 mL, but not surpassing the 0.8% of the individual's body weight, as recommended by Allender *et al.* (2006). Blood samples were kept in tubes with heparin, and immediately used for the estimation of hemoglobin and hematocrit, as described elsewhere (Thrall *et al.*, 2012; Campbell, 2015). Then, plasma was separated from cells by centrifugation and frozen at -20°C until used. Five healthy venom producing snakes of both species were used as negative controls and submitted to the same conditions of handling and blood withdrawal.

Hematological analysis

The hematological tests were performed manually by two independent technicians following the methods described by Salakij (2002), in which blood smears are air dried before staining with Wright stain. Leukocytes were classified as heterophils, eosinophils, basophils, monocytes, and lymphocytes according to Thrall *et al.* (2012). All tests were made in duplicates, and values are expressed as mean ± standard deviation (SD).

Analyses of plasma proteins.

To determine plasma protein concentration, 2 µL of each sample were loaded and measured at 280 nm in a NanoDrop 2000 Spectrophotometer. Protein electrophoresis (SDS-PAGEs) were carried out at 7.5% under non-reducing conditions and run at 180V. The wells were loaded with 6 µL of sample at a plasma protein concentration of 1.5 µg/µL (9 µg of protein per well), and 3 µL of molecular weight marker (Precision Plus Protein Dual Color Standard, Bio-Rad) were used. Gels were photographed, and edited in a Bio-Rad ChemiDoc XRS, used also to generate a report with the relative density percentages for each band on every lane. These relative percentages were used to calculate the albumin:globulin (A:G) ratio, with the globulin relative values obtained by the sum of the bands corresponding to α- (~75 kDa), β- (~150 kDa) and γ-globulins (~250 kDa), while the albumins are just one band at ~50 kDa (Thrall *et al.*, 2012; Melillo, 2013; Campbell, 2015).

Statistical analysis.

A repeated-measure ANOVA was performed to assess the variation of the hematological parameters through time, as well as the comparisons between venomoids and control snakes in both species; we used it also to test for differences in the albumin:globulin ratio of both species during the study. The sphericity assumption was tested by Mauchly's test, and when the assumption was not achieved, the Greenhouse-Geisser correction factor was used. All values with $p<0.05$ were considered as statistically significant.

Results

Hematological analysis.

Within group differences in the hematocrit were observed for both *B. asper* (F= 4.923, df= 5, p= 0.004) and *C. simus* (F= 3.342, df= 5, p= 0.013), during the experiment. When the hematocrit value of venomoid

B. asper was compared against its control group, no differences were observed (F= 2.509, df= 5, p= 0.064); the same occurred with *C. simus* (F= 1.853, df= 5, p= 0.125) (Table 1). Venomoid snakes of both species showed a decrease in the hematocrit content until day 60, then a slightly recover until the end of the study was recorded (Table 1).

Regarding the hemoglobin concentration, the venomoid group of *B. asper* did not show differences when compared through time (F= 1.017, df= 5, p= 0.434), nor when compared against control snakes (F= 0.994, df= 5, p= 0.447). Conversely, the hemoglobin content in *C. simus* venomoids showed differences through time (F= 17.151, df= 5, p< 0.001), as well as when compared against its controls (F= 12.239, df= 5, p< 0.001) (Table 1). A three-fold increase after one day post-surgery was recorded, but a decrease occurred by day 30 after surgery. Interestingly, this hemoglobin concentration of venomoid *C. simus* 30 days after surgery did not coincide with the hematocrit data (Table 1).

Regarding leukocytes, the relative percentage of monocytes for *B. asper* varied during the experiment (F= 3.877, df= 5, p= 0.019), but not when compared with control snakes (F= 1.697, df= 5, p= 0.196). The basophils did not vary during time (F= 1.683, df= 5, p= 0.199), nor between control and venomoid snakes (F= 1.814, df= 5, p= 0.171). The eosinophils did not show differences neither during the experiment (F= 0.688, df= 5, p= 0.674), nor between control and operated snakes (F= 0.941, df= 5, p= 0.483). Similar variation presented the heterophils during time (F= 2.399, df= 5, p= 0.087), and between control and venomoid snakes (F= 2.168, df= 5, p= 0.113). The lymphocytes varied during the experiment (F= 4.821, df= 5, p= 0.008), but did not show significant differences when the control and venomoid snakes were compared (F= 2.358, df= 5, p= 0.091) (Table 2).

In venomoids *B. asper*, a relative monocytosis is evident at day one that is concurrent with an inverse relative lymphopenia. However, high levels of monocytes and lymphocytes were maintained during the study; as well as a moderate relative increase in heterophils was found in venomoids *B. asper* (Table 2). Whereas in control *B. asper* a relative monocytosis and an inverse relative lymphopenia was recorded at day three, as well as an increase in heterophils (Table 2).

Regarding the relative percentage of monocytes in *C. simus*, they varied during the experiment (F= 12.174, df= 5, p< 0.0001), but did not differ when the venomoid and control rattlesnakes were compared (F= 2.135, df= 5, p= 0.081). The basophils of venomoids did not vary during the experiment (F= 2.009, df= 5, p= 0.098), nor when compared with the control snakes (F= 0.225, df= 5, p= 0.950). The eosinophils did also not vary with time (F= 1.740, df= 5, p= 0.148), nor when they were compared with those of control snakes (F= 0.803, df= 5, p= 0.555). The heterophils showed a differential behavior during the study (F= 3.485, df= 5, p= 0.010), and also when compared with the control group (F= 2.754, df= 5, p= 0.031). Similarly, the lymphocytes varied through time (F= 9.629, df= 5, p< 0.0001) and when compared with controls (F= 2.597, df= 5, p= 0.040) (Table 2).

In summary, a relative monocytosis and an

Table 1. Hematocrit and hemoglobin values of *Bothrops asper* and *Crotalus simus* snakes. A comparison between control group (CG) and surgically venom gland removed or venomoid group (VG) is provided. All values are shown as mean ± SD.

Hematological Parameter	Days	*B. asper* VG	*B. asper* CG	*C. simus* VG	*C. simus* CG
Hematocrit (%)	0	22.8 ± 3.0	21.8 ± 5.7	25.4 ± 1.8	22.8 ± 1.2
	1	20.5 ± 7.8	22.1 ± 5.1	21.5 ± 2.4	15.6 ± 5.3
	3	17.0 ± 2.8	15.9 ± 9.1	15.6 ± 4.0	23.0 ± 3.8
	30	15.3 ± 3.2	18.8 ± 3.6	19.2 ± 6.1	22.4 ± 2.5
	60	15.0 ± 2.6	17.7 ± 3.2	20.1 ± 5.5	27.1 ± 5.9
	90	18.2 ± 3.7	11.5 ± 3.3	22.3 ± 5.8	22.3 ± 3.6
Hemoglobin (g/dl)	0	7.8 ± 1.8	5.7 ± 1.9	10.7 ± 0.5	10.2 ± 2.7
	1	9.7 ± 6.0	6.1 ± 4.0	28.9 ± 11.9	8.1 ± 1.3
	3	6.1 ± 1.2	6.9 ± 2.9	4.3 ± 1.4	7.4 ± 0.6
	30	6.2 ± 0.3	6.4 ± 2.1	2.1 ± 1.8	3.9 ± 1.8
	60	6.8 ± 3.9	6.1 ± 2.4	8.7 ± 3.7	9.3 ± 3.0
	90	7.0 ± 0.7	7.0 ± 3.2	8.5 ± 4.2	7.3 ± 1.5

Table 2. Leukogram values of *Bothrops asper* and *Crotalus simus* snakes. A comparison between control group (CG) and surgically venom gland removed or venomoid group (VG) is provided. All values are shown as mean ± SD.

Leukocyte cell type (%)	Days	*B. asper* VG	*B. asper* CG	*C. simus* VG	*C. simus* CG
Monocytes	0	19 ± 9	13 ± 2	27 ± 5	19 ± 3
	1	40 ± 2	26 ± 14	47 ± 19	41 ± 8
	3	48 ± 5	22 ± 11	45 ± 13	31 ± 10
	30	9 ± 4	18 ± 16	16 ± 4	25 ± 6
	60	15 ± 8	10 ± 4	19 ± 10	24 ± 15
	90	17 ± 10	14 ± 9	20 ± 7	22 ± 11
Basophils	0	0 ± 1	0 ± 0	1 ± 1	1 ± 2
	1	3 ± 4	1 ± 1	2 ± 2	1 ± 2
	3	5 ± 6	1 ± 1	1 ± 1	1 ± 1
	30	1 ± 2	1 ± 1	1 ± 1	1 ± 1
	60	3 ± 4	1 ± 1	1 ± 1	1 ± 1
	90	2 ± 2	0 ± 1	0 ± 1	0 ± 1
Eosinophils	0	0 ± 0	0 ± 0	0 ± 1	0 ± 1
	1	0 ± 0	2 ± 4	3 ± 6	1 ± 2
	3	0 ± 0	1 ± 1	2 ± 3	0 ± 0
	30	1 ± 1	0 ± 0	2 ± 2	0 ± 1
	60	0 ± 0	0 ± 0	1 ± 1	0 ± 0
	90	0 ± 1	0 ± 0	0 ± 0	0 ± 0
Heterophiles	0	5 ± 5	7 ± 5	3 ± 4	6 ± 9
	1	13 ± 8	15 ± 10	7 ± 8	7 ± 5
	3	29 ± 14	13 ± 13	26 ± 16	8 ± 5
	30	19 ± 15	11 ± 15	16 ± 11	10 ± 5
	60	15 ± 1	5 ± 3	22 ± 13	10 ± 6
	90	5 ± 4	9 ± 11	12 ± 10	8 ± 5
Lymphocytes	0	75 ± 12	80 ± 6	68 ± 5	71 ± 7
	1	44 ± 24	56 ± 19	40 ± 25	49 ± 14
	3	19 ± 3	65 ± 25	27 ±8	59 ± 9
	30	69 ± 17	70 ± 20	65 ± 10	64 ± 8
	60	67 ± 10	84 ± 6	57 ± 7	64 ± 15
	90	76 ± 11	77 ± 15	68 ± 12	69 ± 16

inverse relative lymphopenia was observed at day one in venomoid *C. simus*. Moreover, a moderate increase in the relative percentage of heterophils during the study was registered; as well as high levels of monocytes and lymphocytes (Table 2). In control *C. simus*, the same phenomenon of relative monocytosis and an inverse relative lymphopenia was observed at day three, as well as an increase in heterophils (Table 2).

SDS-PAGE and albumin:globulin ratio.

The A:G ratio of *Bothrops asper* did not differ during the study (F= 0.269, df= 5, p= 0.923) nor when compared with control snakes through time (F= 0.328, df= 5, p= 0.889) (Table 3).The A:G ratio of venomoid *C. simus* significantly varied during the experiment (F= 3.217, df= 5, p= 0.017), but when compared to controls no differences were observed (F= 2.268, df= 5, p= 0.069) (Table 3).

The electrophoretic profile of a venomoid and a control *B. asper* and *C. simus* snakes is shown in Figs. 1 and 2. Although variable, the A:G ratios for either venomoid or control *B. asper* snakes maintained a 1.5 proportion, whereas, the ratio on the venomoid *C. simus* varied at 30 days after surgery but not in the controls during the whole experiment.

Table 3. The albumin:globulin ratio values of *Bothrops asper* and *Crotalus simus* snakes. A comparison between control group (CG) and surgically venom gland removed or venomoid group (VG) is provided. All values are shown as mean ± SD.

Group	0 days	1 day	3 days	30 days	60 days	90 days
***B. asper* VG**	2.23 ± 1.30	1.49 ± 0.19	1.27 ± 0.01	2.02 ± 1.97	1.94 ± 0.73	2.09 ± 1.01
***B. asper* CG**	1.07 ± 0.50	0.99 ± 0.50	1.03 ± 0.50	1.08 ± 0.47	1.34 ± 0.87	1.34 ± 0.90
***C. simus* VG**	1.68 ± 0.47	1.67 ± 1.01	1.65 ± 0.53	2.59 ± 1.45	1.74 ± 0.41	1.73 ± 0.41
***C. simus* CG**	1.60 ± 0.55	1.65 ± 0.36	1.50 ± 0.28	1.65 ± 0.51	1.37 ± 0.38	1.60 ±0.80

Discussion

The generation of valuable clinical information in snakes, especially in venomous species, is a difficult task. Besides, baseline information available to aid in diagnosis is scarce. Nevertheless, recent efforts in this regard have been conducted in snake collections established for biomedical purposes (Thrall *et al.*, 2012; Melillo, 2013; Campbell, 2015; Gómez *et al.*, 2016; Rodríguez-Abarca *et al.*, 2019).

It was demonstrated that manual venom extraction in crotaline viperids (i.e., genus *Bothrops* and *Crotalus*), affects the glandular tissues and the venom yield (Giannotti *et al.*, 2013; Rodríguez-Abarca *et al.*, 2019). This damage leads to a fibrotic tissue generation, switching from normal glandular tissue to a high content of collagen scar tissue. Moreover, this process could be harmful for the snake's homeostasis, with the occurrence of edema, hemorrhage, and necrosis (Giannotti *et al.*, 2013; Rodríguez-Abarca *et al.*, 2019). Additionally, it may cause indirect effects such as pro-inflammatory activity due to venom leakage (Clissa *et al.*, 2001; Texeira *et al.*, 2003; Moreira *et al.*, 2012).

In general terms, hematocrit of *B. asper* and *C. simus* snakes were within the 95% reference interval (RI) reported by Gómez *et al.* (2016). However, the hematocrit values of *B. asper* venomoids tend to decrease after day one as well as in its control counterparts. Whereas *C. simus* venomoids showed a decrease in hematocrit value at day three and day 30, control *C. simus* snakes showed a decrease at day one (Table 1). This phenomenon could be related to the environmental (physical) conditions in which both snake species were kept. *Crotalus simus* snakes were maintained at higher temperatures and low relative humidity as compared to *B. asper*, which would have favored an increase in the metabolic rate and consequently an enhanced erythropoietic response (Thrall *et al.*, 2012; Campbell, 2015). The

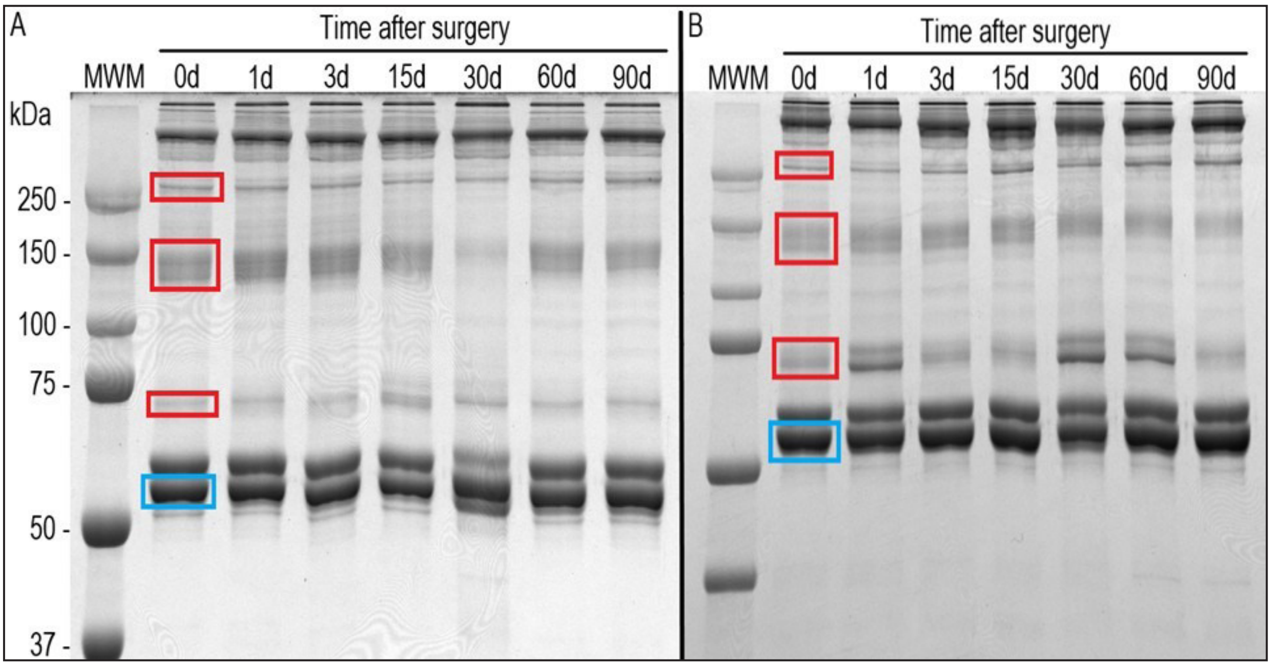

Figure 1. SDS-PAGE profile of *Bothrops asper* blood serum samples taken at different time intervals after main venom glands removal (A), and comparison with a control individual (B). Light-blue square marks the protein bands corresponding to albumins; red squares mark the protein bands corresponding to globulins.

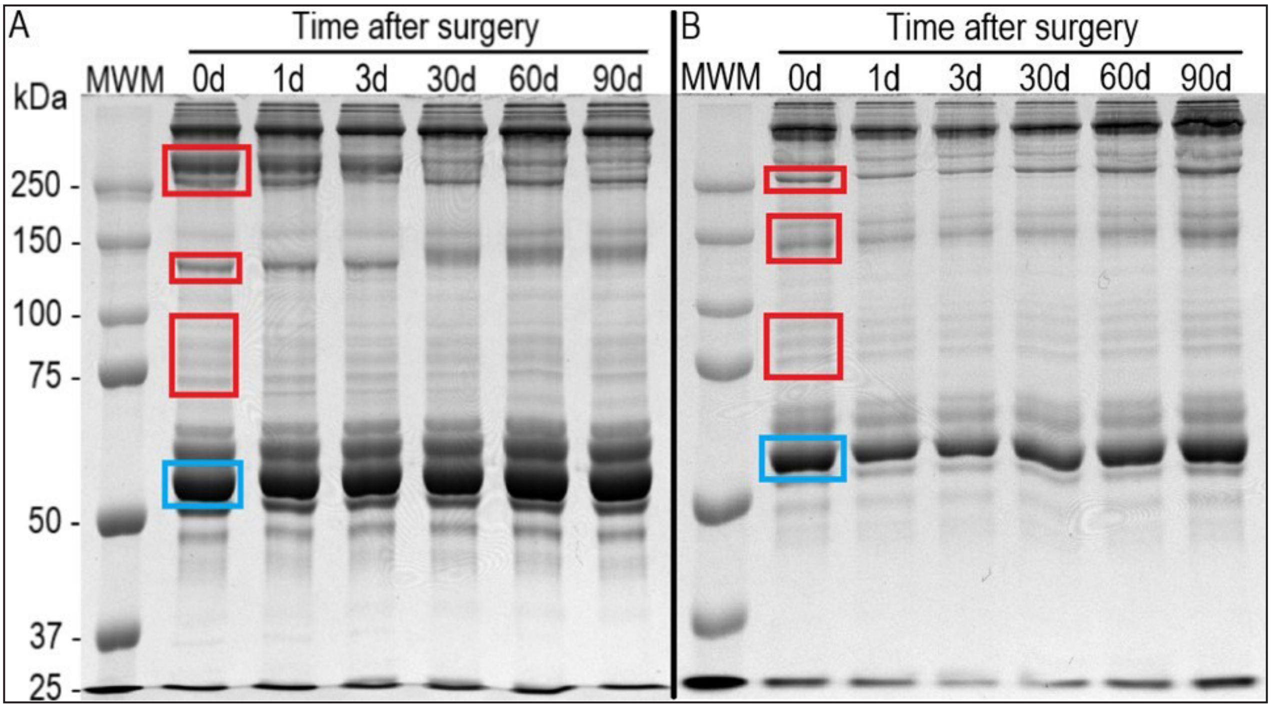

Figure 2. SDS-PAGE profile for *Crotalus simus* blood serum samples taken at different time intervals after main venom glands removal (A), and comparison with a control individual (B). Light-blue square marks the protein band corresponding to albumins; red squares mark the protein bands corresponding to globulins.

hematocrit levels of snakes could change depending on the environmental conditions (Brischoux *et al.*, 2011; Thrall *et al.*, 2012), and one major abiotic factor affecting snake metabolism is temperature. Hemoglobin displays lower oxygen affinity at higher temperatures (Weber and Campbell, 2011), and this may have induced a faster production of red blood cells in *C. simus*. Additionally, it is not possible to consider a post-surgical anemia since the variations in the hematocrit values were not different through the time in both species; *B. asper* (p= 0.064) and *C. simus* (p= 0.125).

The hemoglobin content for *B. asper* and *C. simus* were within the 95% reference interval (RI) described by Gómez *et al.* (2016). Nonetheless, venomoid *C. simus* showed a slightly high hemoglobin content pre- surgery, by day zero. An outlier value was registered at day one in *C. simus* venomoids, which could be due to a methodological artifact. However, a further research on this particular feature is needed.

The scarce variation of hemoglobin content in both species suggests that the surgical procedure did not produced anemia. However, there is a discrepancy between hematocrit and hemoglobin values in *C. simus* at days three and 30. Unlike other vertebrates, reptile rubricytes mature in peripheral blood (Stacey *et al.*, 2011; Thrall *et al.*, 2012; Campbell, 2015), instead in the bone marrow. A possible explanation could be the detection of recently produced rubricytes by the hematocrit, but these immature cells bear hemoglobin content (Thrall *et al.*, 2012; Campbell, 2015).

Regarding the leukocytes, the basophils and the eosinophils values were within the 95% RI reported by Gómez *et al.* (2016) for both snake species. However, a relative heterophilia, and an inverse relative monocytosis and lymphopenia was evident for at least three days, regardless the species. Because there were no drastic variations of basophils and eosinophils, we can assume a relatively good health condition in the snakes studied. Since basophilia may be associated to blood microparasites and viral infections, while eosinophilia is a common response to macroparasites and non-specific immune stimulations (Thrall *et al.*, 2012; Campbell, 2015).

On the other hand, heterophilia is regarded as a normal response to inflammation caused by infection (i.e., bacteria, parasites), or other tissue injuries including necrosis (Thrall *et al.*, 2012; Campbell, 2015). The heterophilia observed in *B. asper* although is evident, was not different between venomoids and controls (p= 0.113). In contrast, the phenomenon is evident when venomoid and control *C. simus* snakes are compared (p= 0.031), supporting the general idea of heterophilia caused by tissue damage and inflammatory processes after surgical procedures.

Lymphopenia has been associated with malnutrition and with excess of endogenous or exogenous corticosteroids (Thrall *et al.*, 2012; Campbell, 2015). The decrease observed in both, *B. asper* and *C. simus* during the whole study especially at day three as no differences were observed between *B. asper* venomoids and controls (p= 0.091), the lymphopenia was likely caused by the continuous handling of the snakes in both groups (Arguedas *et al.*, 2018). Additionally, *C. simus* snakes seem to be more sensitive to continuous handling because venomoids showed a more marked decrease than controls (Table 2).

The monocytes increase with antigenic stimulation and their increment is suggestive of chronic infections (Thrall *et al.*, 2012; Campbell, 2015). Monocytosis is also involved in granulomas and giant cell formation and is specifically associated with granulomatous responses to bacterial infections (Thrall *et al.*, 2012; Campbell, 2015). Although monocytosis was evident in both species after surgery, *B. asper* (p= 0.019) and *C. simus* (p< 0.0001), there were no significant differences within species (p= 0.196; p= 0.081; respectively). We argue again for an effect of continuous handling of snakes (Arguedas *et al.*, 2018), rather than being caused by the surgery.

The albumin:globulin (A:G) ratio of the electrophoretic report, could give a general idea on the health state of the individual. The A:G ratio for both snake species could be considered as stable, without a hyper-stimulation of globulin production like in an immune response, that was not the case due to a surgical intervention. Regarding the assessment of health condition, the A:G ratio is a good indicator since α-globulin hyperglobulinemia has been associated with necrosis, whereas a decrease in α-globulins indicates malnutrition, enteropathy and/or chronic renal disease (Campbell, 1996; Thrall *et al.*, 2012; Divers, 2000; Campbell, 2015).

Additionally, hyper- and hypoalbuminemia are indicators of poor health condition in snakes. Low levels of serum albumins may be indicative of liver failure, chronic malnutrition, glomerular diseases, and end-stage renal disease; also, it is present in other gastrointestinal, liver, and cardiovascular diseases (Mader, 2006). Hyperalbuminemia is typically associated with dehydration and diets with excess of protein. There is direct association of hyperalbuminemia with renal disease, although it may be present while dehydration is present in patients with renal failure (Thrall *et al.*, 2012; Campbell, 2015).

Measurements of A:G ratio may be clinically relevant, especially as an aid to detect post-surgical alterations of snake's homeostasis. This kind of alteration was observed in *Iguana iguana* (Zaias and Cray, 2002), in which healthy and sick animals had marked differences in their serum protein electrophoretic pattern.

The A:G ratio slightly decreased in venomoid *B. asper* snakes at day one and day three, whereas in venomoid *C. simus* snakes, slightly increased at day 30. It must be noted that the environmental conditions in which *C. simus* snakes were kept (specially temperature), would have induced temporarily slight dehydration in some specimens (Divers, 2000). However, other unidentified factors associated to surgery may be involved in the case of *B. asper*. Slight dehydration is not necessarily clinically relevant information in our conditions since no other clinical alterations were detected.

Conclusion

The surgical method used to remove the main venom gland and the post-surgical treatment did not affect the overall health condition in two snake species. The procedures did not alter the hematocrit, hemoglobin value, and the percentage of eosinophils and basophils, providing the first insights into hematological and blood biochemistry parameters in two neotropical viper snakes after surgical procedures.

The heterophilia we observed could be attributed to a natural event caused by the surgically removal of tissue that cause damage and inflammation in the mouth cavity and surrounding musculature.

The relative monocytosis and relative lymphopenia seems to be directly linked to a continuously handling of the snakes and evidence stress. Monocytes may play a key role in the stress response of *B. asper* and *C. simus*, and presumably pit vipers and snakes in general.

Finally, the determination of A:G values in both species indicated no major metabolic affection of individuals post- surgery. The good recovery process under stable rearing conditions in our experiment (i.e., temperature, relative humidity, diet, and water supply) indicates adequate post-surgical handling of the snakes (i.e., antibiotics and analgesics).

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Cranial characters in *Caiman crocodilus* (Crocodylia: Alligatoridae) with emphasis on the subspecies distributed in Colombia

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ABSTRACT

Studies on the biology of *Caiman crocodilus* have drawn attention to its biology with emphasis on systematic, taxonomy and ecology. However, anatomical aspects, such as skull characteristics, have not been studied in detail throughout its geographic range. In this study the skull characteristics for *C. crocodilus* subspecies, *C. c. fuscus, C. c. chiapasius, C. c. crocodilus,* and *C. c. apaporiensis* were analyzed using geometric morphometrics and descriptive morphology, including geographic and ontogenetic variation. Variation in skull morphology was found between the subspecies analyzed. Trans-Andean subspecies, *C. c. fuscus* and *C. c. chiapasius,* exhibit brevirostrine skulls but they are different in the contact between frontal and nasal bones and the size of the palatine process of the maxilla; therefore, populations in Colombia correspond to *C. c. fuscus* and, *C. c. chiapasus* is not distributed in Colombia. Although cis-Andean subspecies, *C. c. apaporiensis* and *C. c. crocodilus,* have longirostrine skulls, both subspecies differ in the shape of the skull and in osteological characters; then, adults of *C. c. apaporiensis* present frontal and nasal in contact and, V-shape maxillary-premaxillary suture; while *C. c. crocodilus* specimens exhibit a high geographic and ontogenetic variation, supporting the hypotheses that there at least two clades of such subspecies for Colombia. These morphological differences should be considered in future systematics studies and policies on global conservation of the different *C. crocodilus* subspecies.

Key Words: Caimaninae; Skull Variation; Geometric Morphometrics; Ontogeny; Geographic Variation.

Introduction

Caiman crocodilus is the only alligatorid distributed throughout most Latin America, from México to Perú and Brazil (Medem, 1983; Velasco and Ayarzagüena, 2010). The wide distribution of this species, along with its genetic and morphological variation, has led to the recognition of four subspecies: *C. crocodilus crocodilus, C. c. apaporiensis, C. c. chiapasius* and *C. c. fuscus* (King and Burke, 1989; Venegas-Anaya *et al.*, 2008; Fig. 1). The recognition of different subspecies has important implications for regional and global conservation policies, and the proposal to maintain the four subspecies has been broadly discussed, (Busack and Pandya, 2001; Venegas-Anaya *et al.*, 2008; Balaguera-Reina *et al.*, 2020). In fact, there are extraction and hunting pressures over *Caiman crocodilus* in Colombia, which are reported from the Late Pleistocene to the present (Balaguera-Reina and González-Maya 2009, 2010; De la Ossa-Lacayo and De la Ossa, 2015; Balaguera-Reina 2019, Morcote-Ríos *et al.*, 2021). Therefore, the delimitation of taxonomic units will allow better planning of the conservation efforts for each subspecies.

The presence of four subspecies in Colombia has been recognized for several decades. Medem (1962) distinguished *C. c. chiapasius* in the Pacific region from *C. c. fuscus* in the Caribbean and Andean region based on skull shape, lepidosis, and

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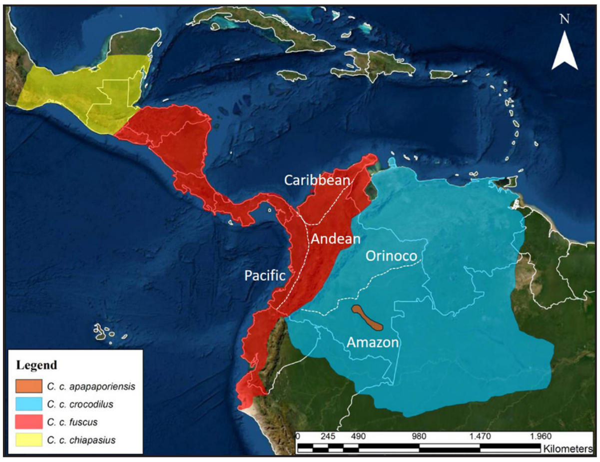

Figure 1. Geographic distribution of *Caiman crocodilus* with natural regions of Colombia.

skin coloration, highlighting differences in the last two characteristics. Subsequently, Medem (1981) published drawings of the skulls and recorded different body measurements and scale counts for the four subspecies.

Bocourt (1876) described *C. c. chiapasius* from Mexico based on five specimens, using coloration and scalation, but the geographical distribution was not discussed (Schmidt, 1928; Medem, 1962; Busack and Pandya, 2001; Venegas-Anaya *et al.*, 2008; Escobedo-Galván *et al.*, 2011, 2015). Venegas-Anaya *et al.* (2008) used mitochondrial DNA from Central American populations to indicate that *C. c. chiapasius* occur in Mexico, Guatemala, and northern El Salvador, excluding the subspecies from Colombia. This taxonomic classification was later supported by Jiménez (2016), who also used mitochondrial DNA, and suggested the populations in the Pacific region of Colombia were *C. c. fuscus*.

The classification of *C. c. apaporiensis* as a subspecies (Medem, 1955) was questioned by Bloor (2013), who suggested it was the same evolutionary significant unit (ESU) as *C.c. crocodilus,* based on mitochondrial DNA. Recently, Balaguera-Reina *et al.* (2020), via mitochondrial molecular markers, suggested that *C. c. apaporiensis and C. c. crocodilus* are the same ESU. However, Escobedo-Galván *et al.* (2015) analyzed the skull of *C. c. apaporiensis* and described osteological and morphometric characters that distinguish it from *C. c. crocodilus*, which warrants further investigation into whether both subspecies should be considered as such.

On the other hand, Angulo-Bedoya *et al.* (2019) analyzed the intraspecific variation of ontogenetic skull development in *C. c. crocodilus, C. c. fuscus,* and *C. c. apaporiensis* in Colombia using geometric morphometrics. They confirmed the pattern observed by Escobedo-Galván *et al.* (2015), where *C. c. apaporiensis* differs from *C. c. crocodilus*, and these two subspecies, in turn, differ from *C. c. fuscus*.

Morphological variation in Colombian populations of *C. crocodilus* has not been completely studied (Busack and Pandya, 2001; Escobedo-Galván *et al.*, 2015). Detailed descriptions of the skull are only available for *C. c. apaporiensis*, a questionable subspecies with a restricted geographic range in Colombia (Escobedo-Galván *et al.*, 2015; Fig. 1), while *C. c. fuscus* and *C. c. crocodilus* have a wide distribution (Fig. 1). Here, we analyze the skulls of three subspecies of *C. crocodilus* present in Colombia, the ontogenetic development patterns in the skull, and the variability between populations. These data may provide useful morphological characters for taxonomic and evolutionary studies, which are essential for crocodilian conservation policies in Latin America.

Materials and methods

We analyzed 158 skulls of *C. crocodilus* stored in the Reptile Collection at the Instituto de Ciencias Naturales at Universidad Nacional de Colombia, and photographs of three specimens of *C. c. chiapasius* stored in the Amphibian and Reptile Collection at the National Museum of Natural History of the United States (USNM) (Table 1; Appendix I).

First, we performed a morphometric analysis in a subset of the specimens, composed by 53 skulls of adult specimens of the four subspecies of *C. crocodilus* and compared our results to the patterns described in previous studies (Escobedo-Galván *et al.*, 2015, Angulo-Bedoya *et al.*, 2019). The analysis was performed over photographs of the dorsal and ventral views of the skulls. Nine landmarks were used in dorsal view and 15 landmarks were used in ventral view (Fig. 2; Table 2) based on the protocols of Fernandez Blanco *et al.* (2014) and Okamoto *et al.* (2015). Landmarks were digitized using TPSUtil (Rohlf, 2018) and TPSDig2 (Rohlf, 2017). In order to avoid increasing the degrees of freedom in the analyses (Okamoto *et al.*, 2015) and due to the bilateral symmetry of the skull, landmarks were only digitized on the left side of the skull for each view.

For the geometric morphometric analyses, a Generalized Procrustes Analysis (GPA) and other

Table 1. Analyzed specimens of *Caiman crocodilus*.

Subspecies of *C. crocodilus*	Total analyzed specimens	Cranial element analyzed in all specimens by region and state	Geometric morphometrics analyzed adult specimens by state	Source
C. c fuscus	52 Colombia	**Caribbean region** Bolívar (4) Córdoba (19) Magdalena (1) **Pacific region** Chocó (16) Cauca (10) **Andean region** Caldas (1) Tolima (1)	Bolívar (2) Córdoba (12) Chocó (6)	ICN
C. c. chiapasius	3 Mexico	Chiapas (3)	Chiapas (3)	USNM
C. c. apaporiensis	44 Colombia	**Amazon region** Vaupés (44)	Vaupés (15)	ICN
C. c. crocodilus	62 Colombia	**Amazon region** Amazonas (1) Caquetá (2) Guainía (1) Guaviare (2) Putumayo (1) Vaupés (7) **Orinoco region** Casanare (2) Meta (46)	Caquetá (1) Guaviare (2) Putumayo (1) Vaupés (2) Casanare (1) Meta (8)	ICN

statistical and graphic analysis were performed using the software MorphoJ 1.06d (Klingenberg, 2011) and Past 3.20 (Hammer *et al.*, 2001). A Principal Component Analysis (PCA) was performed to detect patterns of change between subspecies and between the two populations of *C. c. fuscus* analyzed, in order to determine if both populations correspond to the same subspecies, as proposed by Jiménez (2016). We used a MANOVA and the Wilk's lambda and Pillai's trace statistics to analyze differences between groups. Subsequently, a paired comparison between subspecies was conducted using the Bonferroni correction for each view. One *C. c. crocodilus* specimen, ICN 8726, showed deterioration in the occipital region of the dorsal view so it was excluded from the morphometric analysis for that view.

We analyzed three morphological skull characteristics in all the specimens: 1) contact between the frontal and nasal bones, 2) the shape of the premaxillary-maxillary suture in the ventral view, and 3) the variability in the length/width ratio of the palatine process of the maxilla in the four subspecies, based on characters used for distinguishing *C. latirostris* and *C. yacare* (Fernandez Blanco *et al.*, 2018). The three skull characters were examined and compared across geographic distributions (particularly the Colombian populations; Table 1) and through post-hatchling ontogenetic stages to assess the variation among *C. crocodilus* subspecies and to determine the taxonomic utility of the characters. Individuals of each subspecies in Colombia were categorized according to the five geographic regions of the country (Fig. 1). We also used four age classes based on the total length (TL= from the tip of the tail to the tip of snout), according to Reserva de la Biosfera la Encrucijada (2011) which were: hatchlings (between 41 - 80 cm TL); juveniles (between 81 - 120 cm TL); subadults (between 121 - 160 cm TL); and

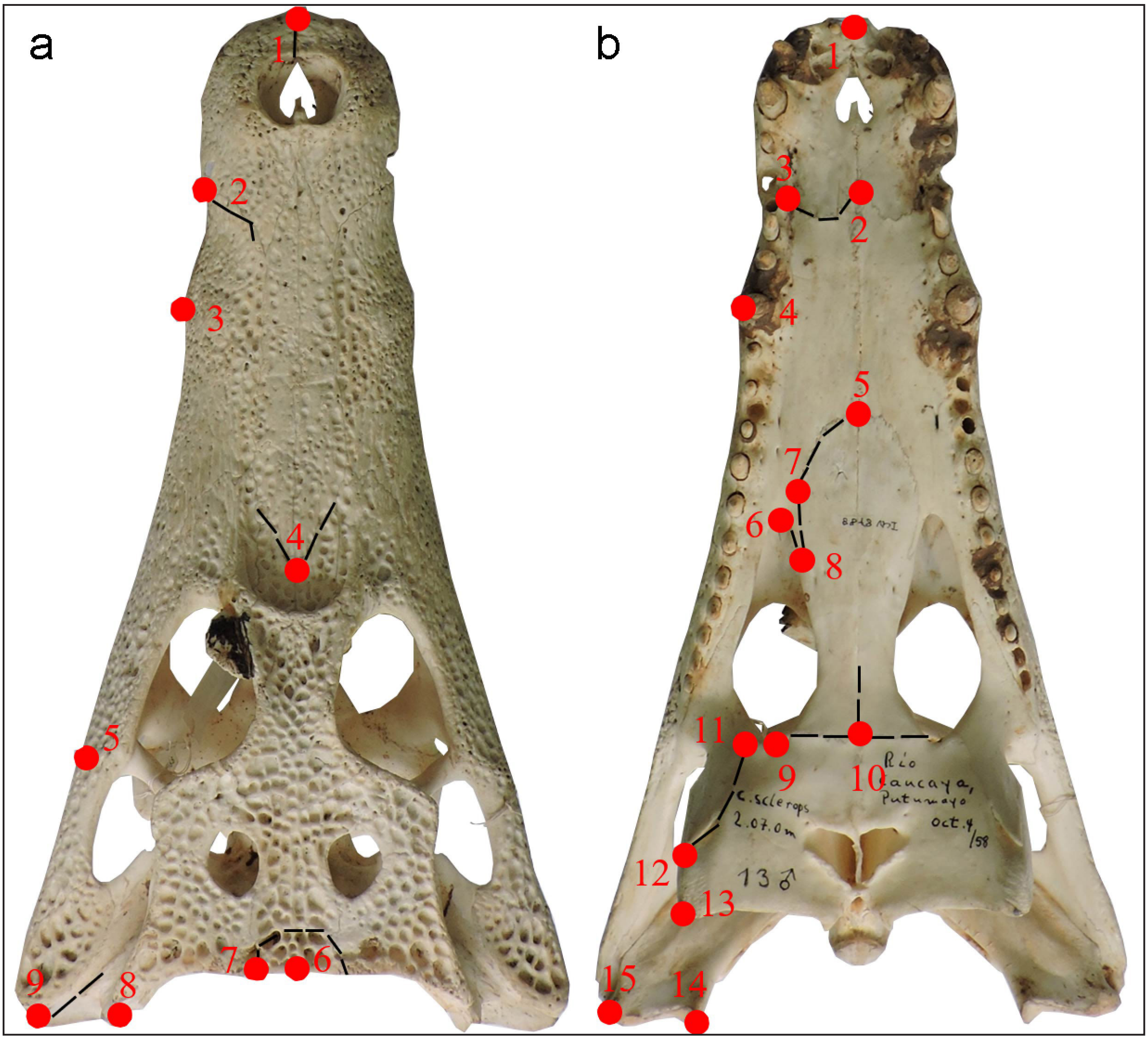

Figure 2. *C. crocodilus* (ICN 8788). Distribution of landmarks. (a). Dorsal view. (b). Ventral view.

adults (161 cm TL or more). Five specimens were excluded from the contact between the frontal and nasal bones analysis, due to the poor condition of the skull or difficulties in the visualization of the suture (noted as unidentified in Tables 5 - 8).

The shape of the ventral premaxillary-maxillary suture was determined by its orientation from the midline to the lateral border of the palate. On the other side, the variation in the palatine process of the maxilla was determined by comparing the length/width ratio of the palatine process in a qualitative way; thus, it ranged from a process as long as the width at the base (i.e., forming a wider and stouter palatine process) to two, three, or four times longer than the width at the base (i.e., forming a narrower palatine process).

For the skull characteristics, the Mexican specimens were analyzed for comparison with the Colombian specimens. We adopted the nomenclature reported by Romer (1956) for bony elements. Maps were built with ArcGIS 10.5 (ESRI, 2017).

Results

Geometric Morphometric analysis

In the PCA for the dorsal view, the first two Principal Components (PCs) accounted for 71.55% of the total variance. Subspecies were separated along the PC1 (Fig. 3a) due to the width of the snout, with *C. c. apaporiensis* and *C. c. crocodilus* specimens located mainly in the negative values of this component due to both subspecies having a longirostrine skull

Table 2. Description of landmarks in dorsal and ventral views.

View	Number	Landmark Definition	Type
Dorsal	1	Most anterior point of the premaxilla.	I
	2	Contact between premaxilla and maxilla in lateral view.	I
	3	Maximum width point of the snout, posterior to the premaxilla-maxilla contact.	II
	4	Anterior contact point between the nasals.	I
	5	Left side of the skull at the posterior point of the orbital bar.	II
	6	Middle point of the posterior margin of the supraoccipital.	II
	7	Contact between supraoccipital and squamosal bones at the posterior margin of the skull table.	I
	8	Most posterolateral point of the squamosal.	II
	9	Most posterolateral point of the quadrate, where it contacts the quadratojugal.	I
Ventral	1	Most anterior point of the premaxilla.	I
	2	Contact between maxilla and premaxilla at the sagittal plane.	I
	3	Contact between maxilla and premaxilla at the lateroventral margin.	I
	4	Lateroventral point of the fourth mandibular tooth.	I
	5	Most anterior point of contact between the palatines.	I
	6	Most anterior point of the suborbital fenestra.	II
	7	Most posterolateral point of the palatine process of the maxilla.	II
	8	Most distal point of the palatine process of the maxilla.	II
	9	Most posterior point of the suborbital fenestra.	II
	10	Contact between palatine and pterygoid at sagittal plane.	I
	11	Apical end of the anterior process of the pterygoid.	II
	12	Most anterior point of the descending process of the ectopterygoid.	II
	13	Most posterior point of the pterygoid wings.	II
	14	Most posteromedial point of the quadrate.	II
	15	Most posterolateral point of the quadrate.	II

(Fig. 3b). In contrast, *C. c. fuscus* specimens from the Caribbean and Pacific regions were located in the positive values of the PC1, having a wider and more robust snout (Fig. 3c), a typical configuration of a brevirostrine skull.

For the ventral view, the first three PCs accounted for 63.8% of the total variance. Subspecies were separated along the PC1 due to the width of the snout (Fig. 4a), with *C. c. fuscus* specimens from the Caribbean and Pacific regions having a brevirostrine skull (Fig. 4b), whereas *C. c. apaporiensis* and *C. c. crocodilus* were grouped into the positive values of the PC1, showing a longirostrine skull (Fig. 4c).

We performed a MANOVA using the loadings of the first two and three PCs in the dorsal and ventral view, respectively. Multinormality was tested (p <0.05) for both views. MANOVA results were statistically significant for the dorsal and ventral views (Table 3). The comparison test using the Bonferroni correction did not show statistical differences neither between the Caribbean and Pacific populations of *C. c. fuscus* nor between this subspecies and *C. c. chiapasius* for both views (Table 4). There were no statistically significant differences in the skull shape between *C. c. apaporiensis* and *C. c. crocodilus* in the dorsal view, but there was a slight statistically significant difference in ventral view (Table 4). Finally, there were no statistical differences in the skull between *C. c. crocodilus* and *C. c. chiapasius* in ventral view, although there were significant differences in dorsal view (Table 4).

Cranial element analysis

There was morphological variation in the contact of the frontal and nasal bones in the subspecies of *C. crocodilus* (Table 5). In *C. c. fuscus* specimens, the frontal and nasal bones were in contact (i.e., they were not separated by prefrontal bones) (Fig. 5a; Table 5). We observed this characteristic in 50 skulls (Table 5), including specimens from the Andean,

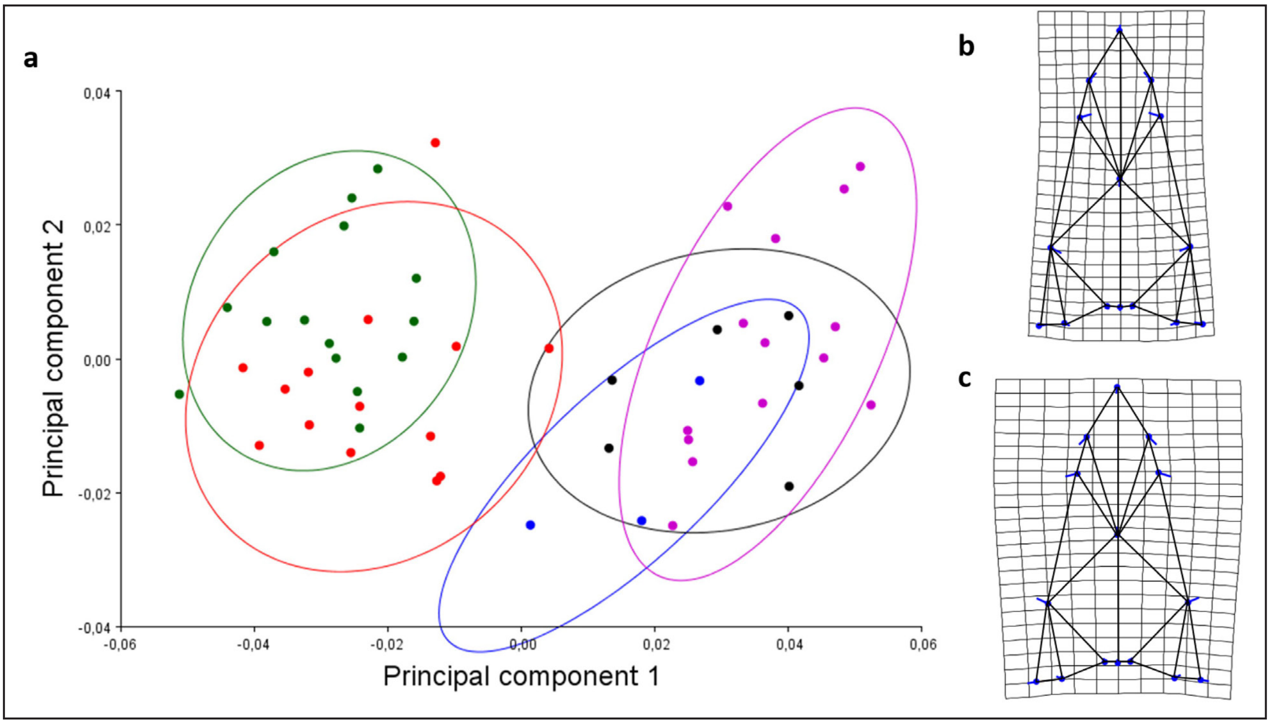

Figure 3. (a). Principal Component Analysis, dorsal view. Scatterplot of the PC1 vs PC2. *C. c. apaporiensis* (green), *C. c. crocodilus* (red), *C. c. chiapasius* (blue), C. *c. fuscus* Pacific region (black), *C. c. fuscus* Caribbean region (purple). (b). Transformation grid of the maximum negative value in the PC1. (c). Transformation grid of the maximum positive value in the PC1.

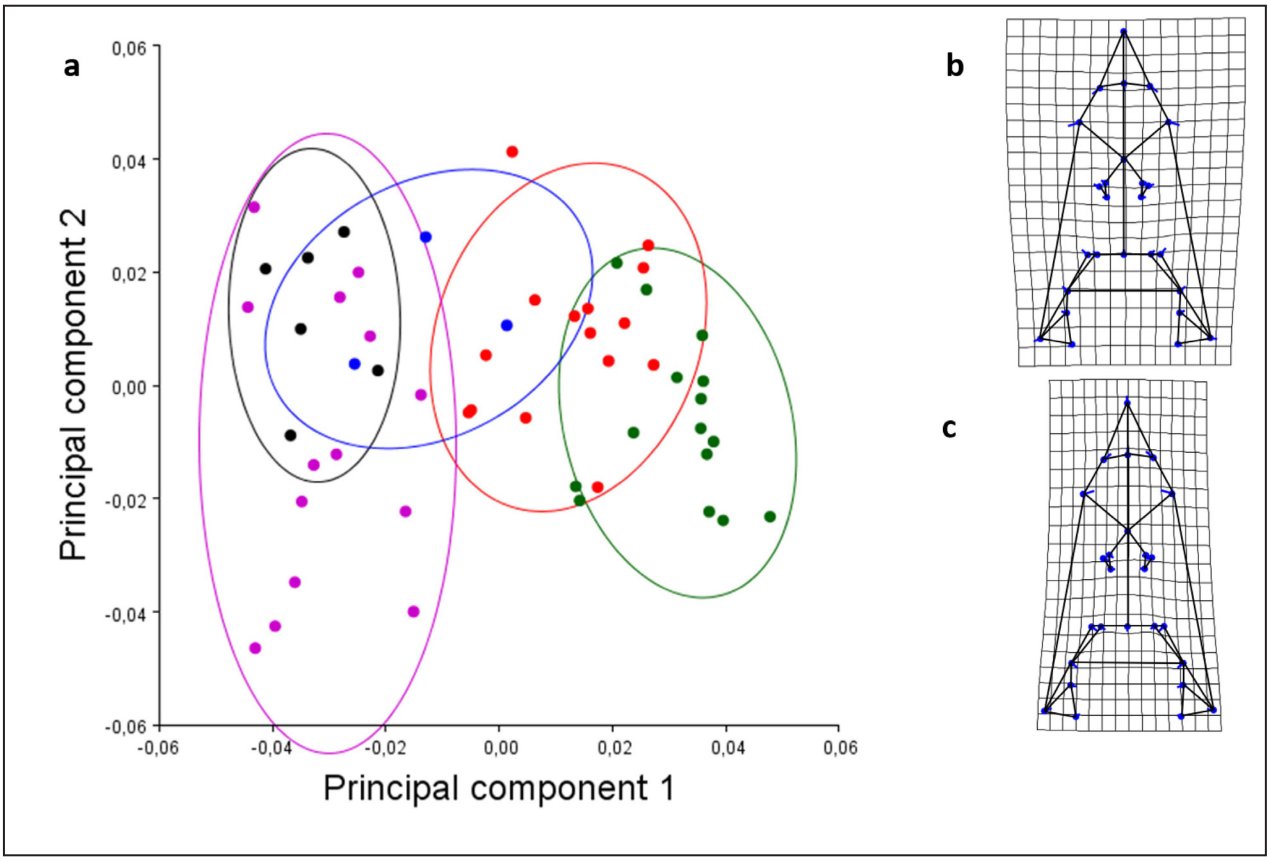

Figure 4. (a). Principal Component Analysis, ventral view. Scatterplot of the PC1 vs PC2. *C. c. apaporiensis* (green), *C. c. crocodilus* (red), *C. c. chiapasius* (blue), *C. c. fuscus* Pacific region (black), *C. c. fuscus* Caribbean region (purple). (b). Transformation grid of the maximum negative value in the PC1. (c). Transformation grid of the maximum positive value in the PC1.

Table 3. MANOVA test results for landmark configurations in dorsal and ventral views of the subspecies of *Caiman crocodilus*.

	Wilk's lambda		**Pillai's trace**	
Dorsal	**p-value**	1,227 E-18	**p-value**	3,702 E-10
	F	22,49	**F**	10,2
Ventral	**p-value**	2,199 E-17	**p-value**	5,309 E-08
	F	13,53	**F**	5,715

Table 4. MANOVA pairwise comparisons using Bonferroni's correction. Results of the dorsal landmark analysis (above diagonal) and ventral landmark analysis (below diagonal).

	C. c. apaporiensis	*C. c. chiapasius*	*C. c. crocodilus*	*C. c. fuscus* Pacific	*C. c. fuscus* Caribbean
C. c. apaporiensis	-	0,000837	0,851*	1,966 E-07	9,234 E-13
C. c. chiapasius	0,00197	-	0,00802	3,316*	0,427*
C. c. crocodilus	0,00513	0,1451*	-	3,355 E-06	4,56 E-11
C. c. fuscus Pacific	1,0002 E-07	2,406*	9,863 E-06	-	4,621*
C. c. fuscus Caribbean	3,885 E-12	1,274*	4,949 E-09	9,7625*	-

* indicates non-significant differences between groups (*p* >0,05).

Caribbean, and Pacific populations, as well as all ontogenetic stages from hatchlings to adults (Table 6). In the three specimens of adult *C. c. chiapasius*, the frontal and nasal bones were not in contact (Fig. 5b; Table 5).

In the 41 subadult and adult specimens of *C. c. apaporiensis*, the frontal and nasal bones were not in contact (Fig. 5c; Table 5), although they were in contact in a juvenile specimen (Table 7). Most of the specimens of *C. c. apaporiensis* were from the west Vaupés state, municipality of Apaporis, near the type locality of the subspecies; and only three specimens were from Ajajú river (Table 7).

Contact between the frontal and nasal bones varied in *C. c. crocodilus* specimens, both within and between populations. In a sample of 62 specimens from the Amazon and Orinoco regions of Colombia, 45 specimens had contact between the frontal and nasal bones, while 16 specimens did not have contact between these bones (Table 5). Different age groups showed both contact and no contact between the frontal and nasal bones (Table 8). Specimens from populations in the Amazon and Orinoco regions also showed both contact and no contact between the frontal and nasal bones (Fig. 6a; Table 8). However, all the specimens from populations at the east of the Amazon region showed contact between the frontal and nasal bones (Fig. 6a; Table 8).

We found the following variations in the shape of the premaxillary-maxillary suture: almost horizontal (Fig. 7a), U-shaped (Fig. 7b), V-shaped (Fig. 7c), and J-shaped (Fig. 7d). The ventral premaxillary-maxillary suture was U-shaped in 47 *C. c. fuscus* specimens; however, this suture was almost horizontal in two juveniles and a subadult specimen from Cauca state, in the Pacific region (Tables 5 and 6). The U-shaped suture was also observed in the three *C. c. chiapasius* specimens (Fig. 7b; Table 5). The ventral premaxillary-maxillary suture was V-shaped in 43 *C. c. apaporiensis* specimens (Fig. 7c; Table 5) and one subadult *C. c. apaporiensis* specimen from Ajajú river (Vaupés state) had the U-shaped suture (Tables 5 and 7).

The four different morphologies of the ventral premaxillary-maxillary suture were observed in 62 *C. c. crocodilus* specimens (Table 5). The U-shaped suture was the most common pattern (N=33), followed by V-shaped (N=11), almost horizontal (N= 9), and J-shaped (N=8, Fig. 6b). Variation was observed in specimens of different ontogenetic stages and even within each ontogenetic stage (Table 8). Regarding the geographic distribution, there was no evident pattern associated with this characteristic in *C. c. crocodilus* (Table 8) however, the U-shaped suture was the most common in most localities (Fig. 6b).

For *C. c. fuscus*, 26 specimens showed a palatine process as long as the width at the base (Fig. 8a; Table 5), whereas 26 specimens showed a process two times longer than the width at the base. In this subspecies, all the hatchlings showed a process two times longer than the width at the base, while juveniles, subadults, and adults showed more variation

Table 5. Variation of the analyzed characters in the subspecies of *C. crocodilus*. Frontal-Nasal bones: FN (in contact); F-N (no contact). Ventral premaxillary-maxillary suture shape: U (U-shaped); V (V-shaped); J (J-shaped); H (almost horizontal). Palatine process: as long as the base width (1X), twice as long as the width of the base (2X), three times longer than the width of the base (3X), four times longer than the width of the base. Unidentified (?).

Character Subspecies	Snout shape	Frontal-Nasal bones contact	Ventral premaxillary-maxillary suture shape	Palatine process length
C. c. fuscus	Brevirostrine	50 FN	3 H	1x (26)
		2?	49 U	2x (26)
C. c. chiapasius		3 F-N	3 U	3x (3)
C. c. apaporiensis	Longirostrine	1 FN	1 U	1x (1)
		41 F-N	43 V	2x (39)
		2?		3x (2)
				4x (2)
C. c. crocodilus		45 FN	9 H	1x (12)
		16 F-N	33 U	2x (35)
		1?	8 J	
			11 V	3x (15)
			1?	

in the length/width ratio (Table 6). There was no geographical pattern that distinguished populations, since both wide and narrow palatine processes were identified in all regions (Table 6). In all the three specimens of *C. c. chiapasius*, the palatine process was almost three times longer than the width at the base (Fig. 8b; Table 5).

The *C. c. apaporiensis* specimens showed all the configurations for this character. Most specimens (N=39) had a palatine process almost twice as long as the width at the base (Fig. 8c; Table 5), whereas the remaining configurations were observed only in five specimens (Table 7). There were no clear ontogenetic trends for this morphological character (Table 7).

In the *C. c. crocodilus* specimens, the palatine process was as long as the width at the base (N=12), two times longer than the width at the base (N=35), and three times longer than the width at the base (N=15, Table 5). There was an ontogenetic trend toward a progressively longer palatine process relative to the width at the base in subadults and adults (Table 8). We did not observe any geographic variation associated with this characteristic (Fig. 6c), since all characteristics were recorded in most populations from both the Orinoco and Amazon regions (Fig. 6c; Table 8).

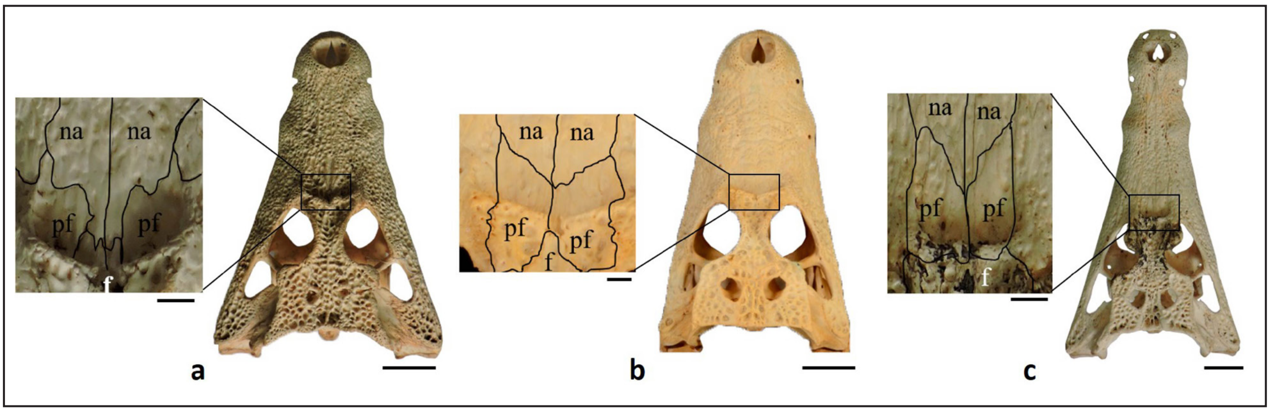

Figure 5. Nasal and frontal bones contact. (a). C. *c. fuscus* (ICN 8886) with frontal and nasals bones in contact. (b). C. *c. chiapasius* (USNM 115335) with frontal and nasals bones separated by prefrontals. (c) C. *c. apaporiensis* (ICN 8730) with frontal and nasals bones separated by prefrontals. Abbreviations: f, frontal; na, nasal, pr, prefrontal. Scale bar equals 5 cm for complete skulls; 1 cm for miniatures.

Table 6. Geographic and ontogenetic variation in *C. c. fuscus*. Frontal-Nasal bones: FN (in contact); F-N (no contact). Ventral premaxillary-maxillary suture shape: U (U-shaped); V (V-shaped); J (J-shaped); H (almost horizontal). Palatine process: as long as the base width (1X), twice as long as the width of the base (2X), three times longer than the width of the base (3X), four times longer than the width of the base. Unidentified (?).

Subspecies	**Region**						
C. c. fuscus		Pacific		Caribbean			Andean
	Ontogenetic stage	Cauca	Chocó	Córdoba	Bolívar	Magdalena	Caldas
Frontal-Nasal bones contact	Hatchling (9)		8FN	1FN			
	Juvenile(14)	5FN	2FN	5FN	2FN		
	Subadult (18)	5FN	2FN	6FN 1?	2FN	1FN	1FN
	Adult (11)		4FN	6FN	1?		
Ventral premaxillary-maxillary suture shape	Hatchling (9)		8U	1U			
	Juvenile(14)	2H 3U	2U	5U	2U		
	Subadult (18)	1H 4U	2U	7U	2U	1U	1U
	Adult (11)		4U	6U	1U		
Palatine process length	Hatchling (9)		8(2x)	1(2x)			
	Juvenile (14)	4(1x) 1(2x)	1(1x) 1(2x)	3(1x) 2(2x)	2(1x)		
	Subadult (18)	5(1x)	2(2x)	3(1x) 4(2x)	1(1x) 1(2x)	1(2x)	1(2x)
	Adult (11)		1(1x) 3(2x)	6(1x)	1(2x)		

Discussion

Regarding the geometric morphometric analyses, *C. crocodilus* subspecies showed skull configurations falling into two groups, the longirostrine type and the brevirostrine type (Figs. 3 and 4). Our results are similar to those described by Escobedo-Galván *et al.* (2015) and Angulo-Bedoya *et al.* (2019) for Colombian subspecies, under the taxonomic classification of Venegas-Anaya *et al.* (2008). Therefore, "trans-Andean" subspecies, *C. c. fuscus* and *C. c. chiapasius*, distributed west of the Andes at the Andean, Caribbean, and Pacific regions in Colombia have a brevirostrine skull; while the "cis-Andean" subspecies, *C. c. apaporiensis* and *C. c. crocodilus*, distributed in the Orinoco and Amazon regions have a longirostrine skull.

In the trans-Andean populations, Medem (1962, 1981) reported differences between Pacific and Caribbean populations of *C. crocodilus* and proposed the presence of *C. c. chiapasius* in the country based on skull shape, lepidosis, and coloration. Our morphometric analyses showed no differences between the Pacific and Caribbean populations in Colombia, which is in accordance with the results reported by Jiménez (2016), as the Caribbean, Andean, and Pacific populations of *C. crocodilus* correspond to *C. c. fuscus*, supporting the taxonomic classification of Venegas-Anaya *et al.* (2008). On the other hand, our results showed that *C. c. chiapasius* and *C. c. fuscus* did not differ in the shape of their skulls in either the dorsal or ventral views.

The skulls of *C. c. apaporiensis* and *C. c. crocodilus* did not differ in dorsal view but they did

Table 7. Geographic and ontogenetic variation in *C. c. apaporiensis*. Frontal-Nasal bones: FN (in contact); F-N (no contact). Ventral premaxillary-maxillary suture shape: U (U-shaped); V (V-shaped); J (J-shaped); H (almost horizontal). Palatine process: as long as the base width (1X), twice as long as the width of the base (2X), three times longer than the width of the base (3X), four times longer than the width of the base. Unidentified (?).

Subspecies		**Amazon region**	
C. c. apaporiensis		Vaupés state: Apaporis	Guaviare state: Ajajú River
	Ontogenetic stage		
Frontal-Nasal bones contact	Juvenile (2)	1FN 1F-N	1F-N
	Subadult (14)	12F-N 1?	
	Adult (28)	27F-N 1?	
Ventral premaxillary-maxillary suture shape	Juvenile (2)	2V	1U
	Subadult (14)	13V	
	Adult (28)	28V	
Palatine process length	Juvenile (2)	2(2x)	1(2x)
	Subadult (14)	12(2x) 1(3x)	
	Adult (28)	1(1x) 24(2x) 1(3x) 2(4x)	

in ventral view (Table 4), according to the results of Angulo-Bedoya *et al.* (2019), which applied a similar methodology as it was used in this study, and Escobedo-Galván *et al.* (2015) which used traditional morphometry. These two subspecies showed a similar skull shape, but they differed in other morphological characteristics analyzed here. They are also distributed in different geographical regions with diverse ecological conditions.

The analysis of skull elements in *C. crocodilus* highlight the importance of studying morphological changes and patterns to distinguish subspecies (Table 5). Differences in skull morphology have been widely studied, due to its application in the taxonomic and systematic study of crocodilians (Mook, 1921; Brochu, 1999, 2001; Pierce *et al.*, 2008; Clarac *et al.*, 2016; Fernandez Blanco *et al.*, 2018; Foth *et al.*, 2018). In this study, we could see that the contact between the frontal and nasal bones allows us to distinguish trans-Andean subspecies. In *C. c. chiapasius* specimens, the two bones were separated but in *C. c. fuscus* specimens, the two bones were in contact (Table 5).

On the other hand, cis-Andean subspecies also showed variation in the contact between frontal and nasal bones. In the adult forms of *C. c. apaporiensis*, the frontal and nasal bones were separated (Table 7), which is a diagnostic cranial character for this subspecies. However, in *C. c. crocodilus,* both conditions were documented in specimens from the Orinoco and western Amazon populations, with the east Amazon populations tending to have frontal and nasal bones in contact (Fig. 6; Table 8). This geographic pattern is in accordance with Jiménez (2016), who used molecular markers to propose the existence of two clades for *C. c. crocodilus* in Colombia, the western one in the Orinoco region and the Andean piedmont and the eastern one in the Amazon region. This classification is also supported by Roberto *et al.* (2020), who actually reported three lineages for the

Table 8. Geographic and ontogenetic variation in *C. c. crocodilus*. Frontal-Nasal bones: FN (in contact); F-N (no contact). Ventral premaxillary-maxillary suture shape: U (U-shaped); V (V-shaped); J (J-shaped); H (almost horizontal). Palatine process: as long as the base width (1X), twice as long as the width of the base (2X), three times longer than the width of the base (3X), four times longer than the width of the base. Unidentified (?).

Subspecies		**Region**							
		Orinoco		**Amazon**					
		Casanare	Meta	Caquetá	Putumayo	Guaviare	Guainía	Vaupés	Amazonas
Frontal-Nasal bones contact	Hatchling (1)		1FN						
	Juvenile (23)	1FN	12FN 3F-N	1FN		1F-N		4FN	1FN
	Subadult (22)	1FN	11FN 5F-N 1?	2FN	1F-N		1FN		
	Adult(16)		5FN 6F-N			1FN		4FN	
Ventral premaxillary-maxillary suture shape	Hatchling (1)		1H						
	Juvenile (23)	1?	1H 8U 2J 4V	1V		1V		3U 1V	1U
	Subadult (22)	1U	3H 9U 5J 1V			1U 1V	1U		
	Adult(16)		3H 6U 2V			1U		1H 2U 1J	
Palatine process length	Hatchling (1)		1(1x)						
	Juvenile (23)	1(2x)	3(1x) 12(2x)	1(2x)		1(1x)		4(2x)	1(2x)
	Subadult (22)	1(1x)	4(1x) 9(2x) 4(3x)	2(2x)	1(2x)		1(2x)		
	Adult(16)		2(1x) 3(2x) 6(3x)			1(3x)		4(3x)	

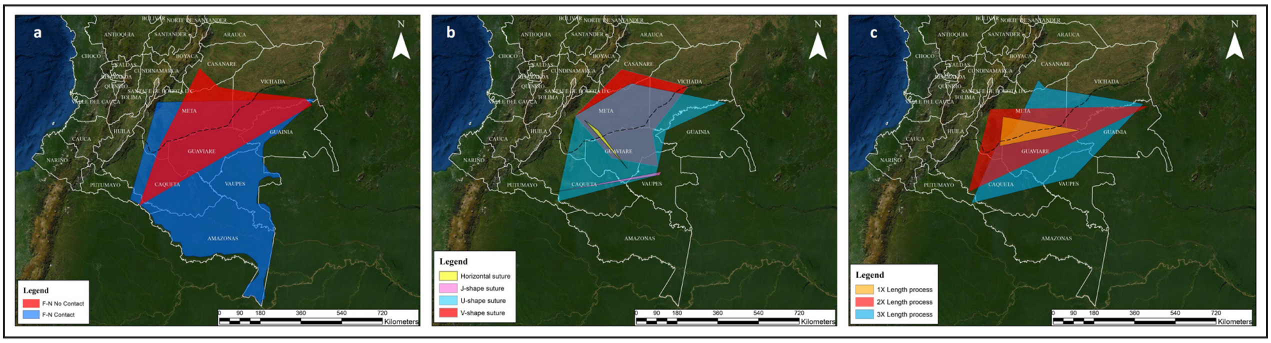

Figure 6. Geographic distribution of the characters in *C. c. crocodilus* in Colombia. (a). Frontal-Nasal contact. (b). Premaxillary-maxillary suture shape. (c). Palatine process length. Discontinuous line in the maps divide Orinoco (northern) and Amazon (southern) regions.

Amazon and Orinoco regions, forming *C. crocodilus sensu stricto*.

A similar pattern of variation was observed in the shape of the ventral premaxillary-maxillary suture. Cis-Andean *C. c. crocodilus* showed four different shapes for this characteristic, the U-shaped suture being the most common. In contrast, *C. c. apaporiensis* showed a V-shaped suture in adults, providing a new characteristic to distinguish this subspecies from *C.c. crocodilus*, along with the characteristics described by Escobedo-Galván *et al.* (2015). Conversely, the trans-Andean subspecies *C. c. chiapasius* and *C. c. fuscus* share a U-shaped suture, which could be associated with a morphological change from longirostrine skulls in cis-Andean subspecies to brevirostrine skulls in trans-Andean subspecies (Venegas-Anaya *et al.*, 2008; Jiménez, 2016). It is important to examine differences in diet between the subspecies, as different morphologies may represent local adaptations to prey items and postnatal dietary shifts (Ayarzagüena, 1984; Monteiro and Soares, 1997).

Cis-Andean subspecies show enormous variation in the length/width ratio of the palatine process (Table 5). However, in trans-Andean subspecies, we found that *C. c. chiapasius* differs from *C. c. fuscus*

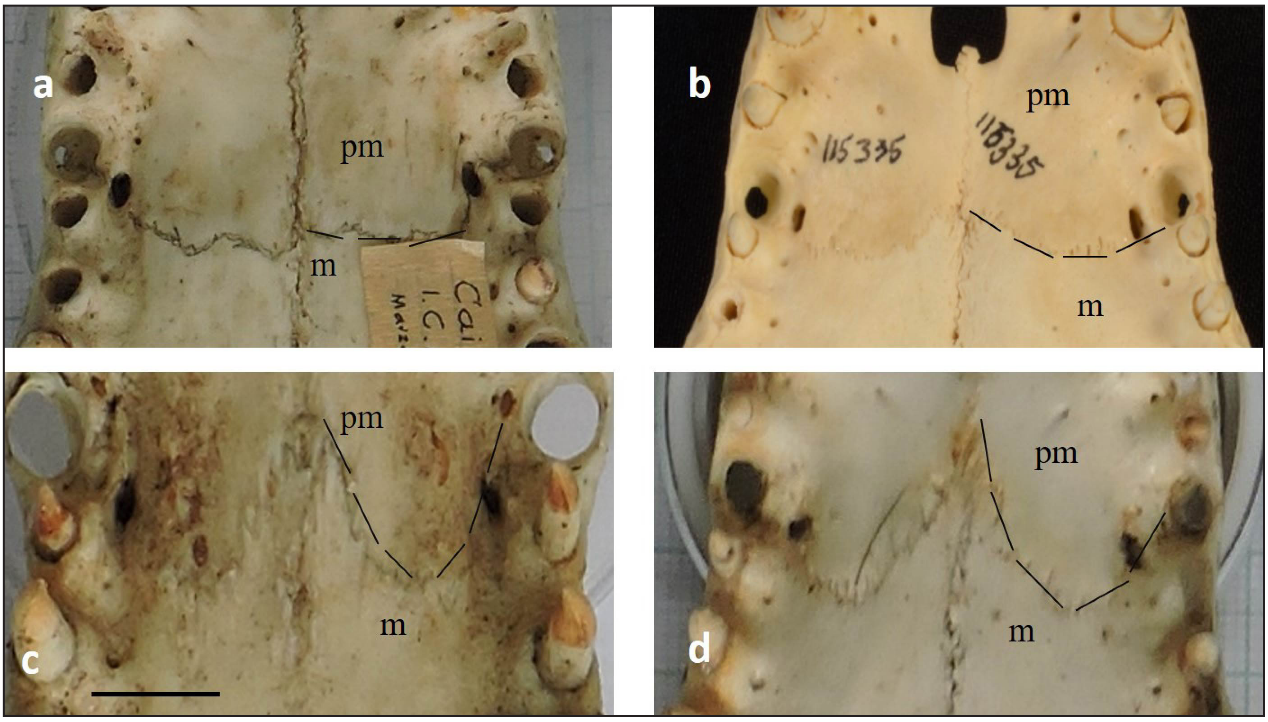

Figure 7. Ventral premaxillary-maxillary suture shape. (a). Almost horizontal suture in *C. c. crocodilus* (ICN 1813); (b). U-shaped suture in *C. c. chiapasius* (USNM 115335); (c). V-shaped suture in *C. c. apaporiensis* (ICN 8730); (d). J-shaped suture in *C. c. crocodilus* (ICN 8825). Abbreviations: m, maxilla; pm, premaxilla. Scale bar equals 1 cm.

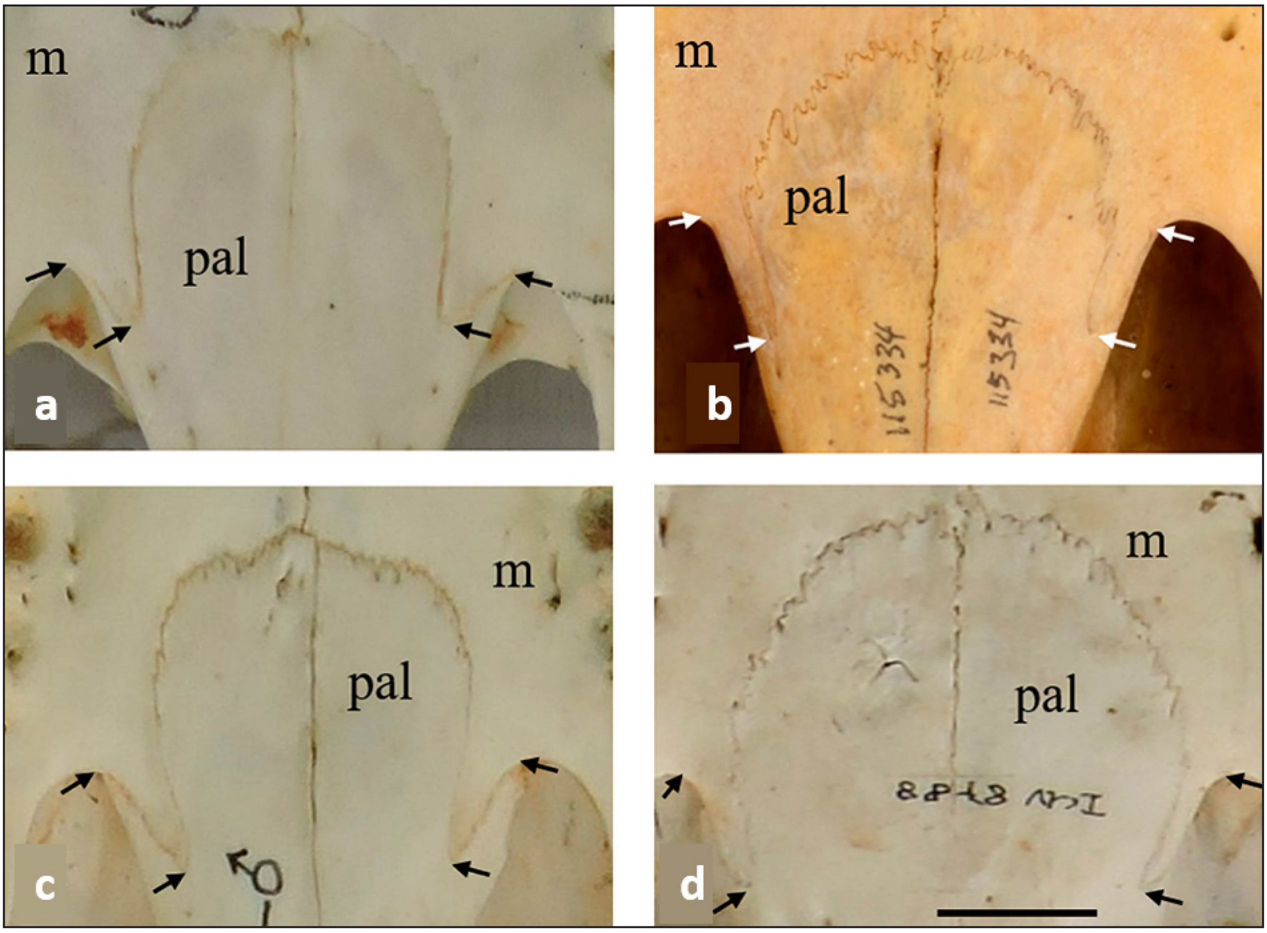

Figure 8. Palatine process of the maxilla in ventral view, arrows show the anterior and posterior portion of the process for each subspecies. (a) *C. c. fuscus* (ICN 1834). (b) *C. c. chiapasius* (USNM 115334). (c) *C. c. apaporiensis* (ICN 8738). (d) *C. c. crocodilus* (ICN 8788). Abbreviations: m, maxilla; pal, palatine. Scale bar equals 1 cm.

in the palatine process. *C. c. chiapasius* has a process three times longer than the base width, while *C. c. fuscus* has a process one or two times longer than the base width.

The three cranial characteristics examined in this study showed minimal variation across the geographic distributions for *C. c. apaporiensis* and *C. c. fuscus*. However, there was a lot of variation for Colombian populations of *C. c. crocodilus,* (Fig. 6; Table 5), which could be in accordance with the wide genetic variation reported for the populations throughout Amazonia, vicariant events of dispersion, and/or different hybridization processes with *C. yacare* (Brazaitis *et al.*, 1998; Farias *et al.*, 2004; Vasconcellos *et al.*, 2006; Hrbek *et al.*, 2008, Jiménez, 2016; Roberto *et al.*, 2020). Therefore, morphological and genetic variation could reveal hidden speciation patterns, similar to those of other alligatorid species in Amazonia (Vasconcellos *et al.*, 2008; Muniz *et al.*, 2017; Bittencourt *et al.*, 2019).

C. c. crocodilus specimens showed significant variation in the three characters throughout its postnatal development (Table 8), which has already been reported for *C. yacare* and *C. latirostris* (Fernandez Blanco *et al.*, 2018) and other crocodilians (Brochu, 1999; Watanabe and Slice, 2014). Therefore, we recommend considering cranial morphological variation during ontogeny to distinguish crocodilian subspecies because, ontogenetic changes in the skull could reveal important anatomical differences, useful for taxonomic classification.

Despite Busack and Pandya's (2001) proposal for maintaining *C. crocodilus* as a unique taxonomic entity (i.e., discouraging the division into subspecies), our results show enough skull morphological differences between the four *C. crocodilus* subspecies, supporting the findings reported by Venegas-Anaya *et al.* (2008), Escobedo-Galván *et al.* (2015), Jiménez (2016), and Angulo-Bedoya *et al.* (2019). Therefore, we argue that morphological analyses of the skull in

C. crocodilus can provide useful information for the taxonomic division of the subspecies.

These morphological analyses reveal that *C. c. apaporiensis* has a different skull shape and other skull elements (i.e., the separation between the frontal and nasal bones; the form of the premaxillary-maxillary suture in ventral view) that distinguish this subspecies from the others in the *C. crocodilus* complex. As in other crocodilians, those differences could be related to geographic distribution and diet (Ayarzagüena, 1984; Muniz *et al.*, 2017; Bittencourt *et al.*, 2019). However, Balaguera-Reina *et al.* (2020) proposed that *C. c. apaporiensis* is not a distinct subspecies from *C. c. crocodilus*, based on mitochondrial DNA. McCurry *et al.* (2017) analyzed the convergent evolution of longirostrine skulls and found that species with riparian habits eat mostly fish and small prey. They also suggested a scenario where biomechanical constraints had shaped the evolution of those ecomorphological patterns, as reported by Fernandez Blanco *et al.* (2018) for *C. yacare* and *C. latirostris*. Thus, the anatomical, ecological, and geographical peculiarities of *C. c. apaporiensis* should be considered in future systematic analysis and conservation policies.

In conclusion, although trans-Andean subspecies *C. c. chiapasius* and *C. c. fuscus* showed a similar skull shape, with a brevirostrine snout and a similar ventral premaxillary-maxillary suture shape, they differ in frontal-nasal contact and length of the palatine process, characters that may be useful in their distinction as taxonomic units. Additionally, all populations with a trans-Andean distribution in Colombia correspond to *C. c. fuscus*. Although cis-Andean subspecies present longirostrine skulls, additional characters support the taxonomic identity for *C.c. apaporiensis.* Variation in the skull characters of *C.c. crocodilus* supports the hypothesis that there are at less two clades in its distribution in Colombia. Our data on skull morphology, ontogeny, and geographic variation provide support for the recognition of *C. crocodilus* subspecies and highlights the importance of an evolutionary perspective in conservation policies, especially in a region with serious conservation challenges.

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Appendix I

***Caiman crocodilus apaporiensis.* ICN.** Colombia: Vaupés: 8669; 8680; 8684; 8690; 8693; 8694; 8701; 8702; 8703; 8705; 8706; 8710; 8712; 8720; 8724; 8727; 8730; 8731; 8732; 8735; 8736; 8738; 8740; 8741; 8743; 8746; 8747; 8750; 8752; 8754; 8762; 8766; 8778; 8779; 8824; 8826; 8827; 8839; 8864; 12393; 12395; 12399; 12400; 12404.

***Caiman crocodilus chiapasius.* USNM.** México: Chiapas 115334; 115335; 115336.

***Caiman crocodilus crocodilus.* ICN.** Colombia: Amazonas: 8836. Caquetá: 8818; 8825. Casanare: 8798; 8840. Guainía: 8880. Guaviare: 8790; 8822. Meta: 1782; 1786; 1788; 1789; 1790; 1791; 1793; 1796; 1813; 1819; 1825; 1826; 1829; 1835; 1836; 1837; 8726; 8794; 8800; 8808; 8811; 8814; 8817; 8819; 8838; 8841; 8842; 8845; 8846; 8847; 8858; 8860; 8862; 8872; 8877; 8884; 8897; 12383; 12390; 12398; 12401; 12402; 12403; 12416; 12418; 12419. Putumayo: 8815. Vaupés: 8681; 8793; 8801; 8813; 8830; 8869; 12394.

Caiman crocodilus fuscus: ICN. Colombia: Bolívar: 1834; 8771; 8849; 8856. Caldas: 1856. Cauca: 8685; 8688; 8689; 8691; 8699; 8707; 8708; 8709; 8711; 8716. Chocó: 1784; 1785; 1798; 1811; 1822; 1843; 1844; 1845; 1846; 1847; 1855; 8799; 8857; 8870; 8873; 12384. Córdoba: 1797; 1814; 1821; 1823; 1827; 1828; 1832; 1833; 1838; 8700; 8756; 8802; 8823; 8863; 8867; 8871; 8883; 8886; 12389. Magdalena: 8821. Tolima: 12385.

Nota

Cuad. herpetol. 35 (1): 147-150 (*2021*)

Predation on *Abronia graminea* (Squamata: Anguidae) and *Sceloporus bicanthalis* (Squamata: Phrynosomatidae) by *Lanius ludovicianus* (Laniidae, Aves) in Veracruz, Mexico

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ABSTRACT
Here we document the first case of predation on the lizards *Abronia graminea* and *Sceloporus bicanthalis*, presumably by the Loggerhead Shrike, *Lanius ludovicianus*, in west-central Veracruz, Mexico

Key Words: Diet, impalement behavior, Loggerhead Shrike, predator-prey interactions.

Predation can be an important factor in the evolution of species' life history traits (e. g. Steen *et al.*, 2014). However, is rarely observed in the wild and few studies have confirmed the identity of predators and preys either by direct observation of predation events, or by traces of the interaction, such as traces of prey in feces (e.g. Vásquez-Cruz and Reynoso-Martinez, 2020) or stomach dissection of the predator (e.g. Vásquez-Cruz, 2020), and parts of the prey not consumed and left by predators. (e.g. Silva *et al.*, 2018). Identification of the species is necessary for a better understanding of the dynamics in predator-prey relationships.

Sceloporus bicanthalis and *Abronia graminea* are lizard species endemic to Mexico. *Sceloporus bicanthalis* is a medium-sized terrestrial species (adult snout-vent length [SVL] ca. 60 mm) belonging to the family Phrynosomatidae (Canseco-Márquez and Gutiérrez-Mayén, 2010). It occurs in open areas of rocky grassland in forests or xeric scrub in the states of Hidalgo, Mexico, Oaxaca, Puebla, and Veracruz (Ramírez-Bautista *et al.*, 2014), and is considered common within its restricted distribution (Flores-Villela and Santos-Barrera, 2007a). *Abronia graminea* is a larger, mostly arboreal species (adult SVL ca. 110 mm) belonging to the family Anguidae. It is distributed in the highlands of central Veracruz, southwestern Puebla, and northern Oaxaca, and inhabits a wide array of mesic forest types but often dominated by oak (*Quercus* spp.) (Canseco-Márquez and Gutiérrez-Mayén, 2010). The species is endangered by deforestation, climate change, and illegal harvest for pet trade, and is protected by Mexican law under the category Special Protection (IUCN Red List of Threatened Species, Flores-Villela and Santos-Barrera, 2007b; SEMARNAT 2010).

The Loggerhead Shrike (*Lanius ludovicianus*) is a passeriform bird in the family Laniidae with a wide distribution in North America, from southern Canada to northeastern Oaxaca in Mexico (Miller, 1931; Vázquez *et al.*, 2009). It has a carnivorous diet that includes invertebrates, amphibians, reptiles, rodents, and small birds (Jensen, 2013; Cogalniceanu, *et al.*, 2015). Its foraging behavior includes impaling its prey on thorns, branches, and even barbed wire, both to secure the prey for consumption and also for longer-term storage (Craig, 1978). Here we report for the first case of the predation on *Sceloporus bicanthalis* and *Abronia graminea* lizards presumably by the Loggerhead shrike (*Lanius ludovicianus*) in the central west of Veracruz, Mexico.

On 4 December 2019, we found seven lizards impaled on plant thorns, within a 50 m radius of each other, ca. 3 airline km north of Acultzingo

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(18°44'30.7"N, 97°19'43.2"W, WGS 84, elev. 2520 m), Veracruz, Mexico. The area is characterised by pine-oak forest remnants, *Pinus patula* plantations, open areas of cattle pasture, and corn crops. We recorded two males and three females adults of *Sceloporus bicanthalis* and a skull of *Abronia graminea* impaled on thorns of unidentified shrubs at a height of 110–210 cm above the ground (Fig. 1A, B). We also found a male of *Sceloporus bicanthalis* on a terminal leaf spine of a maguey (*Agave* sp.) at a height of 150 cm above the ground (Fig. 1C).

Although we did not observe the predation events, we suggest that these *Sceloporus bicanthalis* and *Abronia graminea* were predated by *Lanius ludovicianus*, since it is a species observed in the study site (Fuentes-Moreno pers. comm., Howell and Webb, 1995) and whose foraging behavior includes the impaling of its prey (Yosef and Pinshowb, 2005). No other bird species in the study area exhibits this type of foraging behavior. Additionally, many species of lizards have been previously documented as preys of *L. ludovicianus* and were predated by impalement. Prey species include *Phrynosoma modestum* (Reid and Fulbright, 1981), *Anniella pulchra* (Fisher, 1901), *Anolis carolinensis*, *Plestiodon inexpectatus*, *Scincella lateralis, Aspidoscelis sexlineata* (Tyler, 1991; Yosef and Grubb, 1993), *Phrynosoma cornutum* (Tyler, 1991; Lemos-Espinal *et al*., 1998), *Sceloporus edbelli* (Lemos-Espinal *et al*., 2001), *Uma inornata* (Barrows, 2006), *Ophisaurus ventralis* (Clarke *et al*., 2011), *Dipsosaurus catalinensis* (Carbajal-Márquez *et al*., 2012), *Phrynosoma braconnieri* (Arias-Balderas *et al*., 2012), *Anniella grinnelli*, *Uta stansburiana* (Herr and Papenfuss, 2017), *Phrynosoma hernandesi* (Cairns *et al*., 2017), *Anolis sagrei* (Simpson *et al*., 2019), *Phrynosoma mcallii* (e. g. Duncan, 1994; Lara-Resendiz *et al*., 2019), *Sceloporus aeneus* and *Plestiodon brevirostris* (Gómez-Campos *et al*., 2019). *Lanius ludovicianus* inhabits open areas where it usually hunts (Howell and Webb, 1995), and many of the lizard species known in its diet are terrestrial and inhabit open areas (e.g. species of Phrynosomatidae and Scincidae families), suggesting that feeding on *Sceloporus bicanthalis* may be recurrent, while predation on *Abronia graminea* could be a fortuitous case since it is an arboreal species (Canseco-Márquez and Gutiérrez-Mayen, 2010). Refuge availability for prey and abundance of both prey and predator species could influence these predator-prey interactions (e.g. Sih, 1987; Kumar, 2006; Das and Samanta, 2019).

Our observations are the first documented records of predation on *Abronia graminea* and add information about *Sceloporus bicanthalis* predators since only have been reported a snake species, *Crotalus intermedius*, as a predator (Schramer et al., 2020). These types of records are important for a better understanding of predator-prey dynamics, especially in reptiles species with secretive habits (e. g. Wiseman *et al*., 2018), threatened with extinction and restricted distribution, as *Abronia graminea*.

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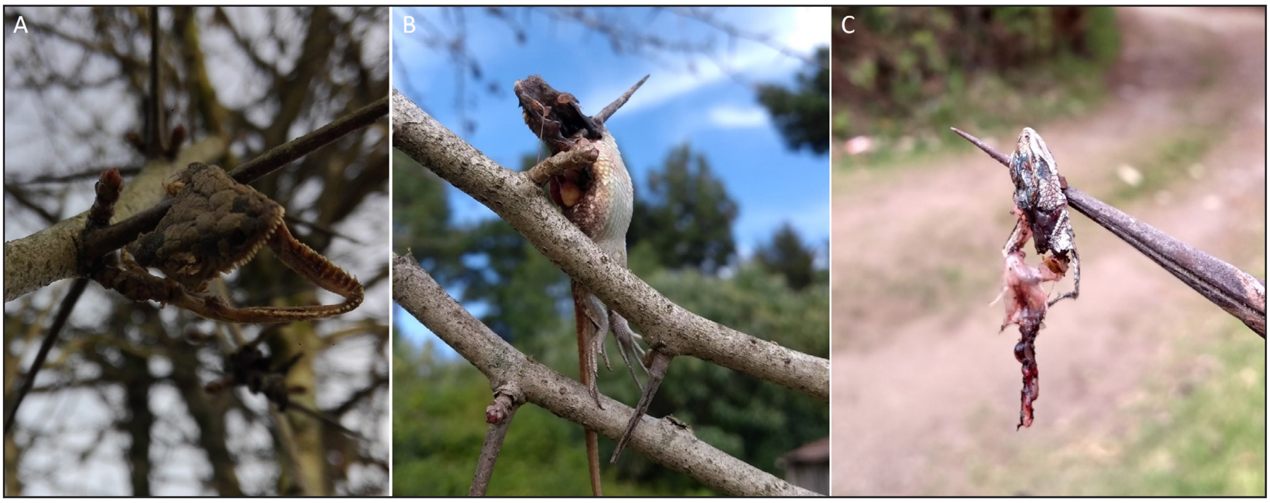

Figure 1. Impaled and predated lizards of *Sceloporus bicanthalis* and *Abronia graminea* in Acultzingo, Veracruz, Mexico. (A) *Abronia graminea* on thorn bush, (B) female *Sceloporus bicanthalis* on thorn bush, and (C) male *S. bicanthalis* on spine of *Agave* sp.

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Nota

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First record of *Eustrongylides* sp. (Nematoda: Dioctophymatidae) larvae parasitizing snakes in the Neotropical region

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ABSTRACT

Eustrongylides uses piscivorous birds as definitive hosts, oligoquets as intermediate hosts and fish as second intermediate hosts. However, amphibians and reptiles can act as secondary or paratenic hosts. The following work presents the first record of *Eustrongylides* larvae in snakes in the Neotropics. The coelomic cavity of 101 snakes belonging to *Helicops infrataeniatus*, *Philodryas aestiva, Philodryas olfersii, Philodryas patagoniensis, Erythrolamprus poecilogyrus*, *Erythrolamprus jaegeri, Atractus reticulatus, Dipsas ventrimaculatus, Thamnodynastes strigatus, Phalotris lemniscatus* and *Bothrops alternatus* from southern Brazil were examined. Six *H. infrataeniatus* were parasitized with larvae of *Eustrongylides* sp. The encounter of these larvae only in this specie can be related to the aquatic habit of the snake, which allows ecological interactions with the definitive and intermediate hosts of the *Eustrongylides* cycle. The occurrence of these parasites in a Neotropical snake is registered for the first time, contributing to the knowledge of the helminth diversity associated to Brazilian snakes.

Key Words: *Helicops infrataeniatus*; Helminths; Parasitism; Reptiles; Serpentes.

Eustrongylides (Jägerskiöld, 1909) (Nematoda: Dioctophymatidae) was proposed for Dioctophymatidae species that cause tumors on the wall of the proventriculus, mainly from piscivorous birds (Measures, 1988). Some species were reported in Gaviiformes, Podicipediformes, Pelecaniformes, Anseriformes, Charadriiformes, Ciconiiformes and Passeriformes (Measures, 1988). *Eustrongylides* consists of three species: *Eustrongylides tubifex* Jägerskiöld, 1909, *Eustrongylides ignotus* (Jägerskiöld, 1909) and *Eustrongylides excisus* (Jägerskiöld, 1909) (Melo *et al.*, 2016).

Regarding the geographical distribution of these parasites, *Estrongylides ignotus* has the widest distribution, occurring in the Nearctic, Neotropical and Australian regions. *Eustrongylides excisus* occurs in Europe and *E. tubifex* is present only in the Holartic and Neotropical regions (Measures, 1988).

The life cycle of these parasites is not well known (Xiong *et al.*, 2009), however, some studies indicate that these helminths use piscivorous birds as definitive hosts, oligoquets as intermediate hosts and usually fish as intermediate second hosts, with amphibians and reptiles as intermediate or paratenic hosts (Measure, 1988; Friend and Franson, 1999; Melo *et al.*, 2016). *Eustrongylides* species may occasionally parasitize humans, thus demonstrating zoonotic potential (Xiong *et al.*, 2009; Eiras *et al.*, 2016).

Little is known about the parasitism of *Eustrongylides* species in wild snake populations, but larvae of these parasites have been reported in some Colubridae from North America, Europe and Asia,

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such as: *Nerodia sipedon* (Linnaeus, 1758), *Natrix natrix* (Linnaeus, 1751) and *N. tessellata* (Laurenti, 1768) (Bursey, 1986; Biserkov, 1995; Mihalca, 2007; Yildirimhan *et al.*, 2007). In acrochordid snakes from Australia there are also reports of the occurrence of these parasites (Jones, 1978). In captivity, larvae of *Eustrongylides* have been observed in some colubrid snakes such as *Coluber constrictor* (Linnaeus, 1758), *Masticophis flagellum* (Shaw, 1802), *Nerodia sipedon*, *Pituophis melanoleucus* (Daudin, 1803), *Thamnophis sirtalis* (Linnaeus, 1758), as well as in the viperid snakes *Bothrops atrox* (Linnaeus, 1758) and *Agkistrodon contortrix* (Linnaeus, 1766) in North America (Winsor, 1948 *in* Bursey, 1986). In Brazil, while these nematodes have been reported in some hosts, such as birds (Vicente *et al.*, 1995; Bernardon *et al.*, 2017), amphibians (Melo *et al.*, 2016) and fish (Isaac *et al.*, 2004; Meneguetti *et al.*, 2013; Carvalho *et al.*, 2017), we know of no reports of their occurrence in snakes. The following work presents the first record of *Eustrongylides* larvae in wild snakes from the Neotropical region.

One hundred and one specimens belonging to 11 species of snakes were examined: *Helicops infrataeniatus* Jan, 1865 (n=65), *Philodryas aestiva* (Duméril, Bibron & Duméril, 1854) (n=1), *P. olfersii* (Lichtenstein, 1823) (n=4), *P. patagoniensis* (Girard, 1858) (n=6), *Erythrolamprus poecilogyrus* (Wied-Neuwied, 1825) (n=10), *E. jaegeri* (Günther, 1858) (n=2), *Atractus reticulatus* (Boulenger, 1885) (n=1), *Dipsas ventrimaculatus* (Boulenger, 1885) (n=3), *Thamnodynastes strigatus* (Günther, 1858) (n=2), *Phalotris lemniscatus* (Duméril, Bibron & Duméril, 1854) (n=1) (Dipsadidae) and *Bothrops alternatus* (Duméril, Bibron & Duméril, 1854) (n=6) (Viperidae).

The snakes came from the municipalities of Capão do Leão (31° 45’ 48” S - 52° 29’ 02” W), Pelotas (31° 46’ 19 “S - 52° 20’ 33 “W), Rio Grande (32° 02’ 06 “S - 52° 05’ 55 “W), Encruzilhada do Sul (30° 32’ 38 “S - 52° 31’ 19 “W) and Dom Pedrito (30° 58’ 58 “S - 54° 40’ 23 “W), Rio Grande do Sul. Fifty of them were collected dead on roads from March 2017 to June 2019. The collection of specimens were licensed by Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio No. 38913). Four snakes were donated by the Núcleo de Reabilitação da Fauna Silvestre e Centro de Triagem de Animais Silvestres of the Federal University of Pelotas (NURFS-CETAS/UFPel), where they died after a rehabilitation attempt. The other hosts were donated by the Vertebrate Laboratory of the Federal University of Rio Grande (n= 36) and Vertebrate Zoology Laboratory of the Federal University of Pelotas (n= 11), where the specimens were fixed in formalin and conserved in 70°GL alcohol.

The hosts were necropsied for analysis of the coelomic cavity. The larvae were fixed in AFA, conserved in 70°GL alcohol and clarified with Amann lactophenol for morphological identification according to Measures (1988). The parasitological indices were calculated according to Bush *et al.* (1997). The specimens were deposited in the helminth collection of the Laboratory of Wild Animal Parasitology (CHLAPASIL n° 831-836). The photomicrographs were done in an Olympus® BX 41 microscope with attached camera system and the images were prepared in Adobe Photoshop® CS5.

Among the total hosts examined, six (5.9%) were parasitized by fourth stage larvae of *Eustrongylides* sp. (Fig. 1), which were found in the coelomic cavity of *Helicops infrataeniatus* (Fig. 2), with a prevalence of 9.2% and mean intensity of infection of 1.83 helminths/host. Female larvae were larger than male larvae, which measured 75-115 mm and 65-80 mm length, respectively.

Helicops infrataeniatus is a non-venomous aquatic snake that can reach about one meter in length. It is distributed in southern Brazil, Paraguay, Uruguay and Argentina (Quintela and Loebmann, 2009; Abegg and Entiauspe-Neto, 2012). The diet of the species includes mostly fish and amphibians (Aguiar and Di-Bernardo, 2004), which were possibly the source of infestation of the snakes in this study.

The transmission of *Eustrongylides* species occurs through the trophic chain involving aquatic organisms such as oligochaetes (primary intermediate hosts), fish, anurans, snakes (secondary and/or paratenic intermediate hosts) and birds (definitive hosts). Bird infection occurs by ingestion of the infective fourth-stage larva present in the secondary and/or paratenic intermediate hosts (Measure, 1988; Moravec, 1994; Friend and Franson, 1999).

Larvae are often found parasitizing several fish species in Brazil, such as *Hoplias malabaricus* (Bloch, 1794) (Characiformes), *Cichla piquiti* Kullander & Ferreira, 2006 and *Plagioscion squamosissimus* (Heckel, 1840) (Perciformes) (Isaac *et al.*, 2004; Martins *et al.*, 2009; Meneguetti *et al.*, 2013; Carvalho *et al.*, 2017). Amphibians were also reported as hosts of *Eustrongylides* larvae in northern Brazil (Melo *et*

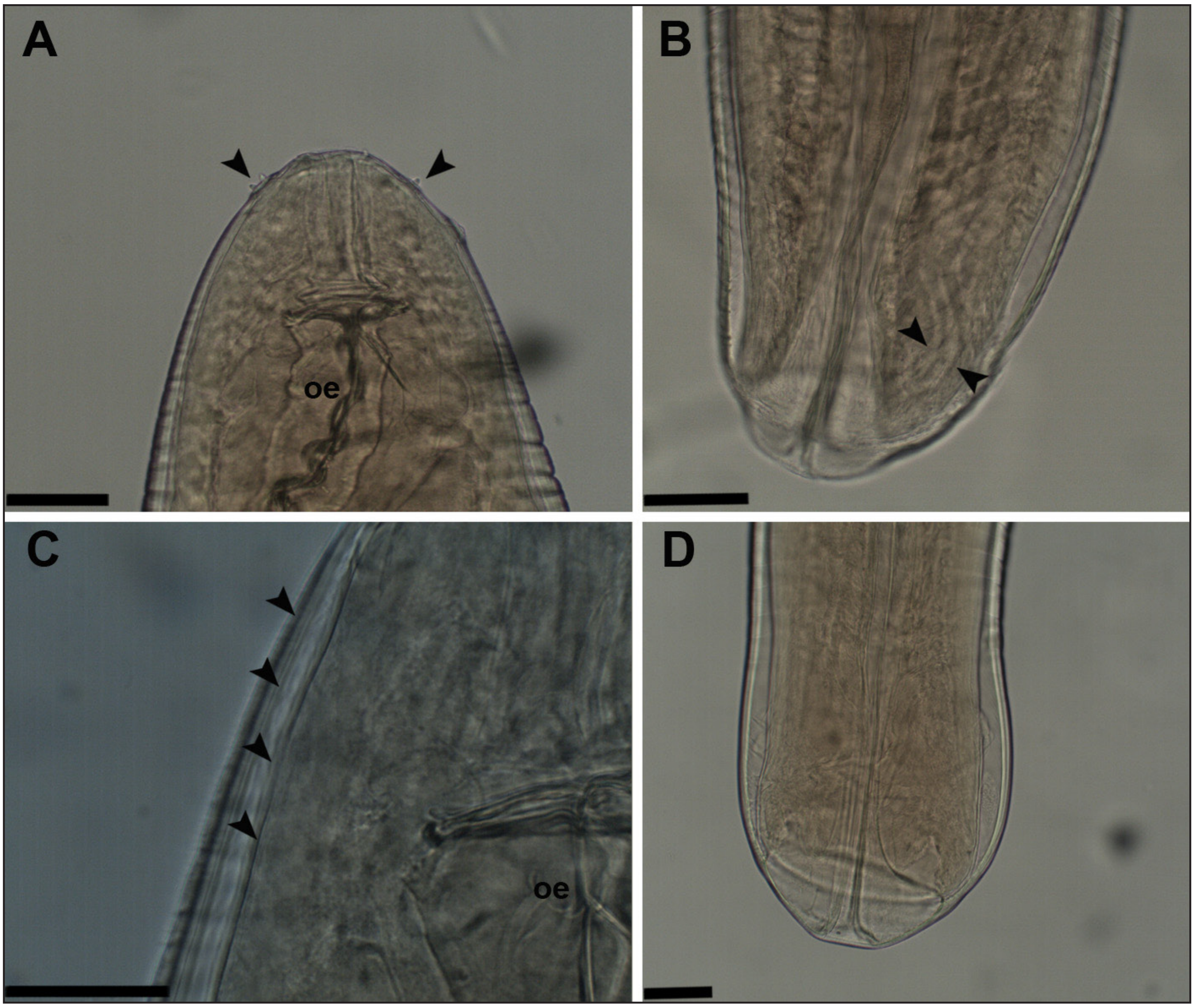

Figure 1. Fourth stage larvae from *Eustrongylides* sp. (Enoplida: Dioctophymatidae) found in the coelomic cavity of *Helicops infrataeniatus* Jan, 1865 (Serpentes: Dipsadidae) in the extreme south of Brazil. A - Anterior extremity of female larva (Arrows indicate the cephalic papillae; *oe*: esophagus; bar – 100 µm). B - Posterior extremity of the female larva (Arrows indicate the primordial vulvar; bar - 100µm). C - Arrows indicate cuticle layers (*oe*: esophagus; bar - 12,5 µm). D - Posterior end of the male larva (bar – 60 µm).

al., 2016). There are no previous records of larvae of this nematode associated with snakes in Brazil; however, there are records in North America (Bursey, 1986), Europe (Mihalca *et al.*, 2007; Biserkov, 1995; Yildirimhan *et al.*, 2007) and Australia (Lichtenfels and Lavies, 1976; Jones, 1978).

Snakes can include in their diet of large variety of vertebrates (Voris and Murphy, 2002; Abegg *et al.*, 2005; Costa *et al.*, 2009), enabling the transmission of parasites through complex trophic networks (Marcogliese, 2004). Herons, such as *Syrigma sibilatrix* (Temminck, 1824) (Ardeidae) feed on *Helicops infrataeniatus* as observed by Franz *et al.* (2007) in Campo Belo do Sul in the state of Santa Catarina, Brazil. In the region of our present study, *Eustrongylides ignotus* was reported in herons *Ardea cocoi* Linnaeus, 1766 and *Nycticorax nycticorax* (Linnaeus, 1758) (Ardeidae) (Bernardon *et al.*, 2017), and larvae of this nematode were found in siluriform fish, *Callichthys callichthys* (Linnaeus, 1758), as well as in *Hoplias malabaricus* (Carolina Silveira Mascarenhas, com. pess.). The record of fourth stage larvae of *Eustrongylides* sp. in *H. infrataeniatus* suggests that the species may act as a trophic bridge in the parasite cycle, since the snake has aquatic habits, feeds on fish and serves as a food resource for birds such as herons. These records highlight the importance of the region for maintenance of the *Eustrongylides* species life cycle and warns about the presence of a nematode with zoonotic potential (Eiras *et al.* 2016).

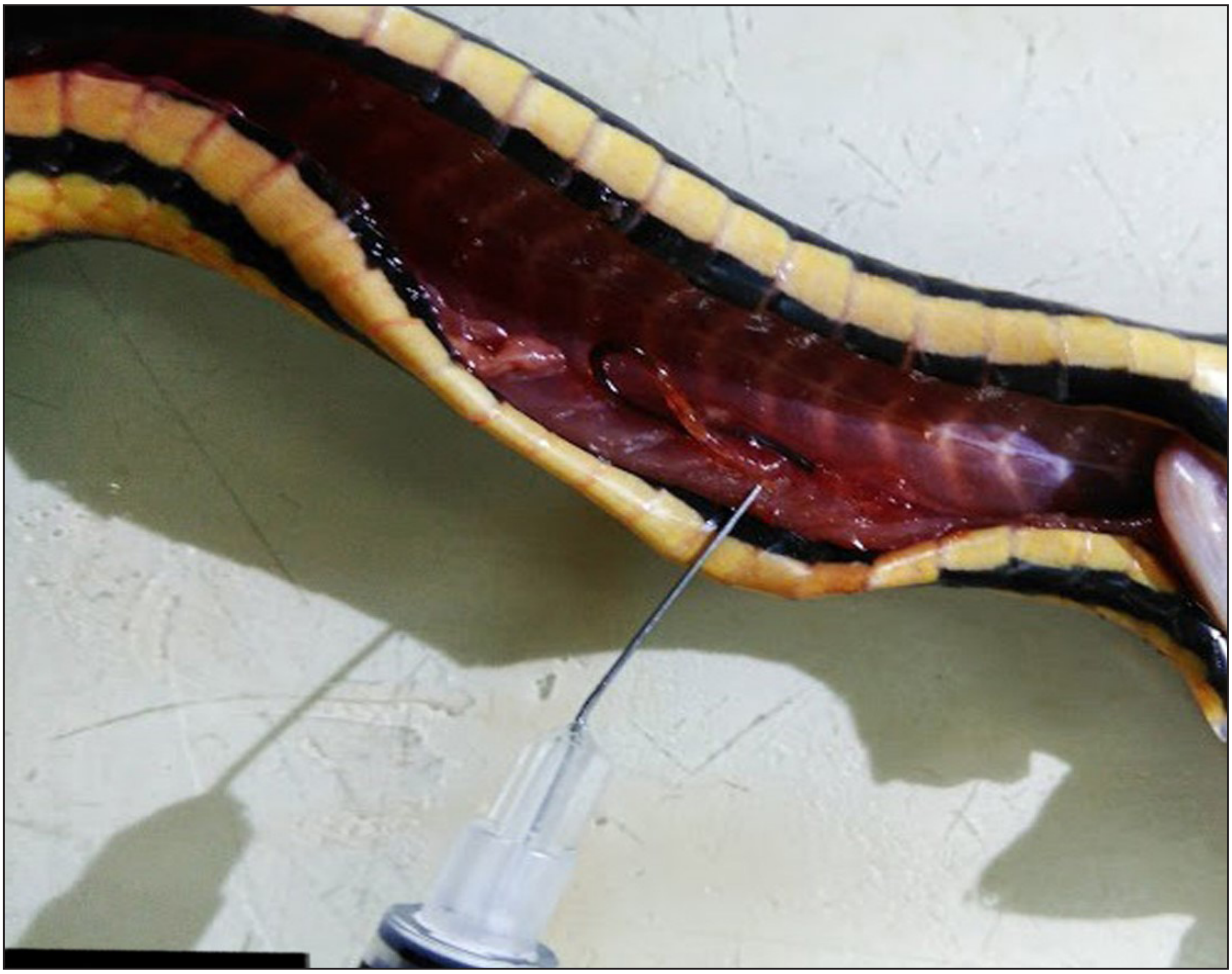

Figure 2. *Eustrongylides* sp. fourth stage larva (Enoplida: Dioctophymatidae) found in the coelomic cavity of *Helicops infrataeniatus* Jan, 1865 (Serpentes: Dipsadidae) in the extreme south of Brazil (Bar – 12 mm).

It is important to point out that the other snakes used in this study present predominantly terrestrial habits, such as *Bothrops alternatus, Thamnodynastes strigatus, Philodryas patagoniensis*, or semi-aquatic habit as *Erythrolamprus jaegeri* and *E. poecilogyrus* (Achaval and Olmos, 2003), which may hinder or even make completely impossible the transmission of this nematode. However, future studies should be conducted to complement the results presented herein to help in the understanding of parasitic relationships involving *Eustrongylides* species and snakes in the Neotropical region.

The occurrence of *Eustrongylides* larvae in *Helicops infrataeniatus* registered for the first time, is the second finding of helminths parasitizing this species in Brazil, since there is a previous record of parasitism by *Opisthogonimus serpentis* Artigas, Ruiz & Leão 1943 (Digenea: Opsitogonimidae) (Fernandes and Kohn, 2014).

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Nota

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Axanthism in *Pithecopus gonzagai Andrade et al., 2020* (Anura: Phyllomedusidae)

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ABSTRACT
The coloration pattern in wild animals is an important characteristic for survival as well as for intra and interspecific communication, being aberrant colorations rarely observed in vertebrates. Axanthism is the lack of yellow pigmentation, and considered an infrequent anomaly, for which cases have been described in amphibians. We describe herein a case in the frog *Pithecopus gonzagai*, a species characterized by predominantly arboreal habits and widely distributed in northeastern Brazil. This is the first report of an axanthic adult specimen of *P. gonzagai*, found in the municipality of Juazeiro do Norte, Ceará, Brazil.

Key Words: Mutation; Pigment; Color aberrancy; monkey frog; Caatinga.

Coloration plays an important role in many aspects of the natural history of animals, and is often an important feature for inter and intra-specific communication (Silva *et al.,* 2020; Araújo *et al.,* 2020). Intimately linked to animal fitness, color aberrancies rarely occur in nature (Ribeiro and Gogliath, 2012; Silva *et al.,* 2020), possibly due to natural selection, since aberrant individuals may become more visible to predators (Bensch *et al.,* 2000; Rees, 2004). Axanthism is one of these uncommon aberrancies, and refers to the lack of yellow pigmentation in an individual, producing a particular phenotype in populations, which can consist of conspicuous blue, gray or abnormally dark specimens (Chilote and Moreno, 2019).

In amphibians, the most frequently described colour anomalies are albinism and leucism (Lunghi et al., 2017). Although considered less frequent, cases of animals with axanthism have been described for a large number of amphibians, such as *Bufo bufo* (Kolenda *et al.,* 2017), *Dryophytes japonicus* (Maslova *et al.,* 2018), *Melanophrynicus estebani* (Chilote and Moreno, 2019), *Dendropsophus minutus* (Araújo *et al.,* 2020) and *Smilisca baudinii* (Vásquez-Cruz and Fuentes-Moreno, 2020).

Here we study the specie *Pithecopus gonzagai* is characterized by predominantly arboreal habits, and can be distinguished from other similar species by its smaller size and color pattern, composed mainly of lemon green, which covers the entire back of the animal, white on the abdomen, and orange with black stripes on the inner portions of the hind legs and front (Caramaschi, 2006; Andrade et al., 2020). The recently described species is distributed throughout the northeastern region of Brazil to the north of the São Francisco River, occupying most of

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the Caatinga and Atlantic Forest biome of the states of Alagoas, Pernambuco, Paraíba, Rio Grande do Norte Piauí and Ceará (Caramaschi, 2006; Roberto & Loebmann, 2016; Da Silva *et al*., 2020; Andrade et al., 2020).

On July 23, 2020, we found an adult axanthic specimen of *P. gonzagai* in the rural area of the municipality of Juazeiro do Norte, northeast Brazil (-7.1625383 S, -39.2808628 W). The individual presented grayish coloration on most of the body, but its usual coloration between the lower and forelimbs was preserved (Fig. 1A). The typical color pattern of the species is usually a vibrant green, with orange bars on the lower thighs (Fig. 1B). The referred specimen was observed in activity during the sunset around 5:30 pm, photographed, and returned to the site of collection.

Axanthic individuals of species with typically green coloration (resulting from the combination of yellow and blue pigments), usually present a darker, blue color pattern (Jablonski *et al.,* 2014; Martínez-Silvestre *et al.,* 2016). Consequently, axanthism is often confused with melanism as observed by Vásquez-Cruz and Fuentes-Moreno (2020). However, the individual of *P. gonzagai* we observed presented mainly grayish color. Similar observations about darker overall coluration in cases of axanthism have also been reported for axanthic snakes, that are usually called black albinos (Borteiro *et al.,* in press).

The causes that induce axanthism are poorly understood, some factors seem to be related to endogamy, environmental stress, nutritional deficiencies, and pollution (Bensch *et al.,* 2000; Chętnicki *et al.,* 2007; Jablonski *et al.,* 2014). Additionally, the frequency of rare phenotypes could also be an indicator of putative low genetic variability in natural populations (Guevara *et al*., 2011). This could be an explanation of our study case, since the specimen was found in an anthropized area that although rural, is surrounded by urbanization, which can somehow isolate small local populations.

Even though this chromatic aberration was reported for several species of amphibians around the world, this is the first account concerning a free-living individual of *P. gonzagai*. Future investigations are pertinent in an attempt to uncover the adaptive importance this phenotype, and the consequences on individual fitness.

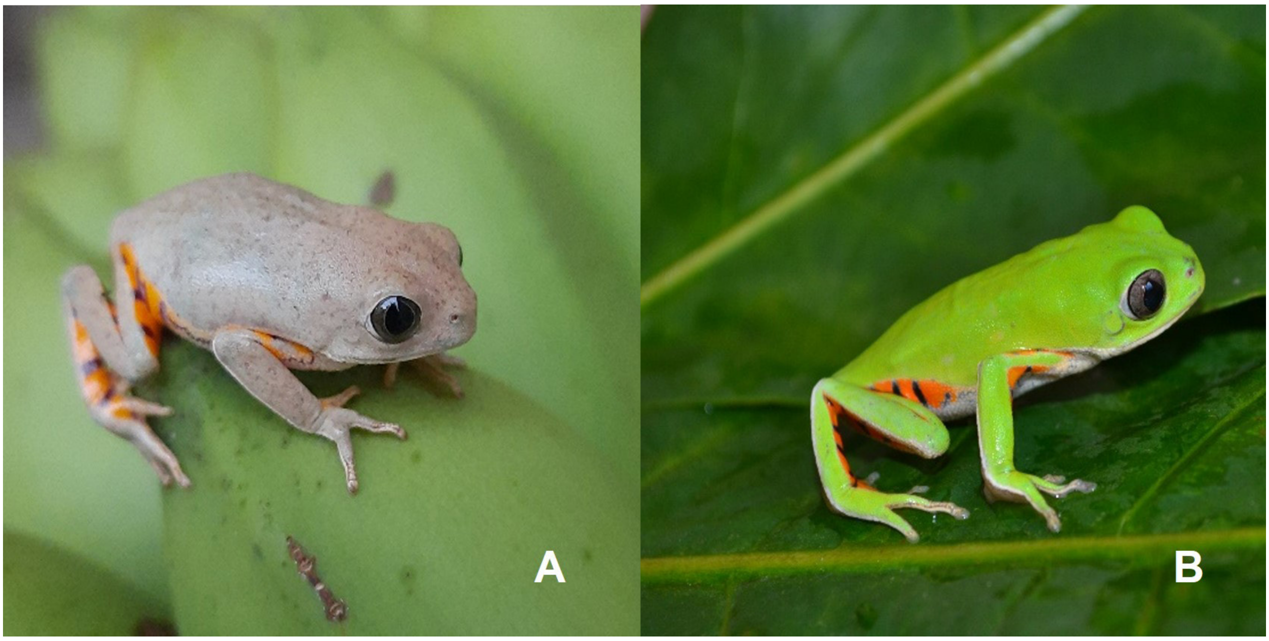

Figure 1. Comparative dorsolateral views of Axanthism (A) and normal (B) adults of *Pithecopus gonzagai* recorded in the municipality of Juazeiro do Norte, northeast Brazil. (Photos: Mascarenhas, W.).

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Nota

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Defensive repertory of *Pseudoeryx plicatilis* (Linnaeus, 1758) in the Pantanal, Mato Grosso do Sul, Brazil

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ABSTRACT
Defensive behavior is an important mechanism for the survival of organisms, and understanding the different strategies improves our knowledge regarding prey-predator relationships. Herein, based on field observation, we describe a sequence of behaviors displayed by the South American pond snake *Pseudoeryx plicatilis* from the Pantanal floodplains in midwestern Brazil. The individual displayed a repertoire of five different defensive behaviors, without an evident sequence pattern recognized. Two of the displays are new for the species, and one for the tribe. The new defensive repertory described may suggest animals oriented by vision as main predators of *P. plicatilis*.

Key Words: Natural History; Aquatic Snake; Behavior; Dipsadidae; Hydropsini.

Snakes have implemented a diverse repertoire of defensive behaviors depending on the situation, in order to avoid predators and increase their probability of survival (Greene, 1988). Although there are knowledge gaps, new information elucidating the defensive behaviors of snakes have been extensively reported (e.g. Martins, 1996; Araújo and Martins, 2006; Marques *et al.*, 2017; Fiorillo *et al.*, 2018). Moreover, understanding the defensive strategies is important to encompass the prey-predator relationship along with the evolutionary processes involved in these interactions (Tozetti *et al.*, 2009). The effectiveness of these strategies depends on the surrounding environment along with the characteristics and the level of threat of the stimuli (Shine *et al.*, 2000; Shepard, 2007; Tozetti *et al.*, 2009).

In the Pantanal floodplains several species of water snakes occur, including the rare *Pseudoeryx plicatilis* (Strüssmann and Sazima, 1993; Marques *et al.*, 2005). This species is widespread in South America, occurring from southwestern Paraguay, north of Argentina and east of Bolivia, through the Humid Chaco Region, and along the Midwestern and North of Brazil, Peru, Colombia, Venezuela and Guiana Shield in the Amazon basin (Scartozzoni *et al.*, 2010). Despite its wide distribution, there are few observations on its natural history (Schargel *et al.*, 2007; Kaefer and Montanarin, 2010). Herein, we describe a sequence of behaviors displayed by the South American pond snake *Pseudoeryx plicatilis* from the Pantanal floodplains.

On 02 September 2016, around 21h, at Base de Estudos do Pantanal (19º34'36" S; 57º01'09" W, 92 m elev.), in Corumbá municipality, Mato Grosso do Sul state, western Brazil, we collected an adult male (TL 982mm) of *Pseudoeryx plicatilis* (Fig. 1a) actively foraging in a swamp area at night and stored in a 100 liters bucket with aquatic vegetation and water to minimize stress, the snake did not try to bite or display any defensive behavior. On the next day, around 8:30h, while the specimen was being manipulated with a snake hook in order to take photographs, the individual displayed five distinct defensive behavior displays by hiding its head and the tail (Fig. 1b); dorsally-ventrally flattening of the full body (Fig. 1b-c); making a knot of itself (knot-

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ting) (Fig. 1d-e); turning the ventral region facing upward (invertbody) (Fig. 1f); and performing erratic movements. We did not observe sequence patterns during these displays, perhaps a random choice of defensive strategy and all behaviors were observed several times. The specimen was collected and deposited at Universidade of Mato Grosso do Sul (UFMS), under the voucher ZUFMS-REP 3623.

On the Hydropsini tribe *Pseudoeryx plicatilis* share defensive behaviors with other water snakes, such as: dorsally-ventrally flattening, hiding the head and cloacal discharge (Marques *et al.*, 2005; Santos-Costa *et al.*, 2015) and some strategies are reported only for *Helicops* (e.g. bite, constriction and rotate the body (Martins and Oliveira, 1998)). Dorsal-ventrally flattening is a common behavior in *Helicops* representatives (Marques *et al.*, 2005, 2017; Marques and Sazima, 2004). However, *H. pastazae* and *H. carinicaudus* showing a subtle difference than we reported here, with compression of the first third of the body versus the full body in *P. plicatilis* (García-Cobos and Gómez-Sánchez, 2019; pers.

Figure 1. Defensive behaviors displayed by the observed male of (a) *Pseudoeryx plicatilis* (ZUFMS-REP 3623; TL 982mm) in the Pantanal of Mato Grosso do Sul state, western Brazil: (b) hiding the head and tail, (c) dorsally-ventrally flattening of the full body, (d) making a knot in itself, (e) making a knot in itself, and (f) turning the ventral region facing upward.

obs.). This strategy is common in terrestrial snakes (Greene, 1979; Martins *et al*., 2008), and makes the individual bigger than it is, and may intimidate visual predators, such birds, natural water snake eaters (see Sazima, 2007; Tozetti *et al*., 2009, 2011).

Many snakes hide the head as anti-predator mechanism (e.g. Marins and Oliveira, 1998; Carbajal-Márquez *et al*., 2018) and in *Helicops* genus is reported for several species (Melgarejo and Meneghel, 1980; Marques & Sazima; 2004; Marques *et al*., 2017). During our observation we reported the individual hiding the head and tail at the same time, that strategy may suggest predators that attack on the both ends of the body (see Buaso *et al*., 2006). Erratic movements might be another way to protect the head from attack and demonstrate physical vigor. Such behavior is reported for two other Hydropsini, *H. angulatus* and *H. hagmanni* (Martins, 1996; Tozetti *et al*., 2009).

Cloacal discharges have been reported to almost all hydropsins, including *Pseudoeryx plicatilis* (Marques *et al*., 2005, 2017), however we did not record at this field observation. It might be because such behavior appears as a response just when reaching the last stage of the sequence of predation (see Vitt and Caldwell, 2014. p.324), when the predator captures, or handling, the snake, being a chemical response to discourage the aggressor (see Egler *et al*., 1996; Tozetti *et al*., 2009). In addition, two new behaviors for *Pseudoeryx plicatilis* were reported here, invertbody and knotting. Invertbody is a visual strategy and might show a contrast color venter similar to other water snakes (e.g. *Hydrodynastes gigas* and *Helicops infrataeniatus*) that occur in the Pantanal floodplains and are very aggressive (Marques *et al*., 2005). That behavior may be a response for continuous stimulus (Angarita-Sierra, 2015) and occurs in synergy with other displays (Greene, 1988). A knotting is an uncommon behavior in snakes that is usually associated with ecdysis process and ectoparasites prevention (Pickwell, 1971; Lillywhite, 1989). Nonetheless, during our observation the snake does not appear to be in ecdysis process, nor with ectoparasites. Additionally, this behavior may prevent predator attacks due to a larger body part. Pickwell (1971) suggests that a sea snake, *Hydrophis platurus* (Linnaeus, 1766) uses this strategy against sea eagles as a secondary defense strategy.

Pseudoeryx plicatilis is a rare snake with scarce field observations, this species might has a complex defensive repertory as reported here, which indicate that animals oriented by vision (i.e., birds) as its main predators. Nevertheless, this observation does not allow us to make consistent conclusions, and more field, and even captive, observations may clarify if these behaviors have a hierarchical decision making pattern, if the species has a different response for different aggressors, as well as how each defensive technique works in prey-predator interactions.

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Nota

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Functional morphology of the tongue of lizard *Salvator merianae* (Reptilia: Squamata)

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ABSTRACT
The reptilian tongue has morphological and functional variations according to the feeding mechanisms and habitat of the species. This study aimed to understand the morphofunctional characteristics of the tongue of the lizard *Salvator merianae*, through anatomical, histological and scanning electron microscopy analyses. This study involves five adult animals, which were collected in the municipality of Viçosa, Minas Gerais, from Brasil. The tongue of *S. merianae* was protractile and bifid, important to drive molecules from the environment to the vomeronasal organ and thus identify odors and locate prey, besides being strongly muscular and with keratinized and pigmented stratified epithelium for protection. Mucous secretion was present as an important tool for lubrication and protection in view of the protrability of the tongue and the great presence of bacteria. Morphological specialization of the *S. merianae* tongue reflect its direct participation in food assimilation, with squamiform papillae for the mechanical function of maintaining prey in the oral cavity and contributing to swallowing. Taste buds were not found, which excludes the tongue participation in the taste, although it has olfactory sensory function in association with the vomeronasal organ.

Key Words: Histology; Digestive System; Mouth; Lacertilia.

Salvator merianae (Duméril and Bibron, 1839) is a species of lizard belonging to the Teiidae family, found in Argentina, Uruguay, Paraguay and all regions of Brazil (except the Amazon Forest) (Vanzolini *et al*., 1980; Péres Júnior, 2003). This species occurs in open areas of the Cerrado, edges of gallery forests and within more open forests. It has daytime habits, is heliophile and active throughout the day, spending most of the time looking for prey, which is found with the help of its long, bifid and protractile tongue (Vitt, 1995). It has an omnivorous feeding habit, which includes vertebrates, vegetables, mollusks, arthropods and even carrion in their diet (Ávila-Pires, 1995).

The main role of the tongue is facilitating feeding, whether for catching prey, transporting food during swallowing and, or, sensory function (Schwenk, 2000). Reptiles can live in fresh water, sea water and on land, with very variable temperature and humidity levels. The reptilian tongue presents morphological and functional variations between species, reflecting differences in habitat, feeding habit and behavior, that is important for the survival of reptiles in different environments (Iwasaki, 2002; Koca *et al*., 2007).

Thus, this study aimed to understand the morphofunctional characteristics of the tongue of *S. merianae*, through macro and microscopic analysis. Five adult (four females and one male) animals of this species were used. The animals were collected in the municipality of Viçosa, in the Zona da Mata Mineira region, from Brasil (IBAMA license: 10504-1). The experiment was conducted in accordance with the “Ethical Principles for the Use of Laboratory Animals” (Brazilian College of Animal Experimentation - COBEA, 1991) andit was approved by the Ethics Committee for the Use of Animals of the Universidade Federal de Viçosa (protocol 27/2016). The animals were euthanized with an intraperitoneally injected overdose of pentobarbital. After euthanasia, the tongue fragments were collected, washed in saline solution and fixed in Carson’s formalin (Carson *et al*., 1973) for 24 hours. The fragments were analyzed for anatomical description, with the use of a stereoscopic microscope (Olympus SZ40). The tongue was divided into three regions for histological analysis: anterior (apical), middle and posterior (basal) thirds. Then, fragments of these regions were

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submitted to histological processing (Tolosa *et al.*, 2003): dehydrated in a growing ethyl series, included in glycol-methacrylate resin and sectioned by a rotating microtome (RM2055, Leica), which provided 3 μm thick semi-serial cross-sections. The sections were stained with toluidine blue in 1% sodium borate, for histological description, and subjected to the following histochemical methods: Alcian blue (AB) pH 2.5 and pH 0.5, aiming to detect acid mucins and sulfated acid mucins, respectively (Bancroft and Stevens, 1996); periodic acid of Schiff (PAS) for neutral mucins (Pearse, 1968); Sudam black for lipids (McManus and Mowry, 1960) and Ponceau's xylidine (PX) (Kiernan, 1990) for the identification of general proteins. The observation and photographic record of the sections were performed in a light microscope coupled with a digital camera (Olympus BX53).Fragments from the three regions were also used for scanning electron microscope analysis. They were dehydrated in an ethanol series, critical point driedby the carbon evaporator (Quorum Q150 T), placed in stubs and then metallized with gold (Balzers Union FDU 010 Modular Metallizer). Next, the material was analyzed using a scanning electron microscope (Leo, 1430VP).

As anatomical results, we verified that the tongue of the lizard *S. merianae* is elongated, protractile, with a tapered and bifurcated apex, gradually becoming wider and thicker after the apex. The apex has a lateral fold along its bifurcations, and its texture is almost smooth (Fig. 1A). The lingual dorsum protrudes laterally to connect to the venter, which is slightly narrower, cylindrical and bipartite, with a deep central groove and folds arranged obliquely. The middle region is pigmented and irregular on the dorsal surface, with a scaly texture that extends to the posterior region (Fig. 1B).

The tongue protractile and bifurcated allows animals to capture chemical substances from both the environment and the potential prey and lead these substances to the vomeronasal organ, a small tubular structure located in the postero-inferior portion of the cartilaginous nasal septum, close to the union with the vomer, which has an auxiliary olfactorial function (Burghardt, 1970). Some studies indicate the occurrence of a co-evolutionary process between the tongue and the vomeronasal organ in the irradiation of squamates,which is correlated to the shape, elongation and bifurcation of the tongue (Cooper, 1994; Cooper, 1995a; Cooper, 1997a; Cooper, 1997b; Filoramo and Schwenk, 2009).

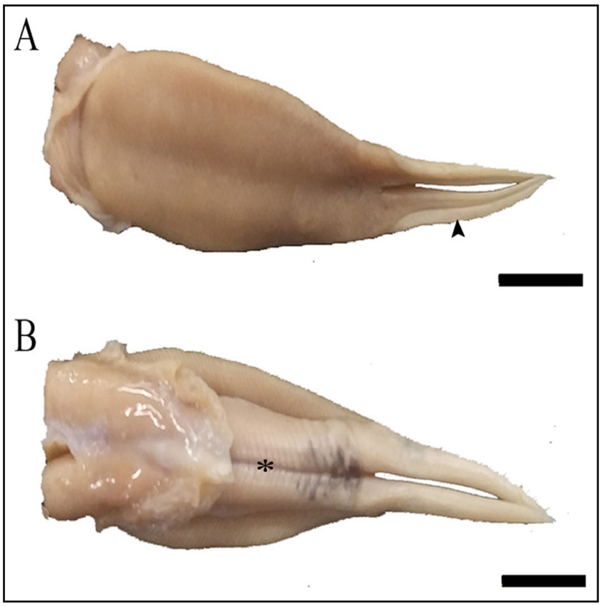

Figure 1. Macroscopic images of *Salvator merianae* tongue. A) Dorsal face of the tongue, showing the bifurcated apex with lateral folding (arrowhead). B) Ventral face of the tongue, with deep central groove (*). Bar: = 1cm

As histological results, we observed that the tongue of *S. merianae* is a muscular organ highly innervated and vascularized. The epithelium is stratified squamous, with two thick bands of keratin in the tongue apex, where a large amount of loose connective tissue and little skeletal muscle tissue are also observed (Fig. 2A). The epithelial type of the lizard's tongue, with layers of keratin and pigmentation, provides important protection in view of the protractile ability of this organ. In lizards, differences in relation to the histological structure of the tongue epithelium have been reported between species in different habitats (Schwenk, 1985; Schwenk, 1986; Smith, 1988; Schwenk, 1989; Iwasaki, 1990; Toubeau *et al.*, 1994; Iwasaki, 2002). One of them refers to the level of epithelial keratinization, which may vary according to the humidity levels of the environment, with greater keratinization in the tongue of animals from dry and hot environments (Iwasaki, 1990; Iwasaki 2002). The *S.merianae* lizard inhabit dry environments with high temperatures (Winokur, 1988), thus, the keratinization is extremely important to protect the protractile tongue of these animals. In some groups of lizards, such as *Gekko japonicus* and *Takydromus tachydromoides*, differences were observed in the keratinization of the epithelium between the regions of the tongue. The epithelial cells in the tongue apex are keratinized; those of the posterior of the tongue are not keratinized; and those in the

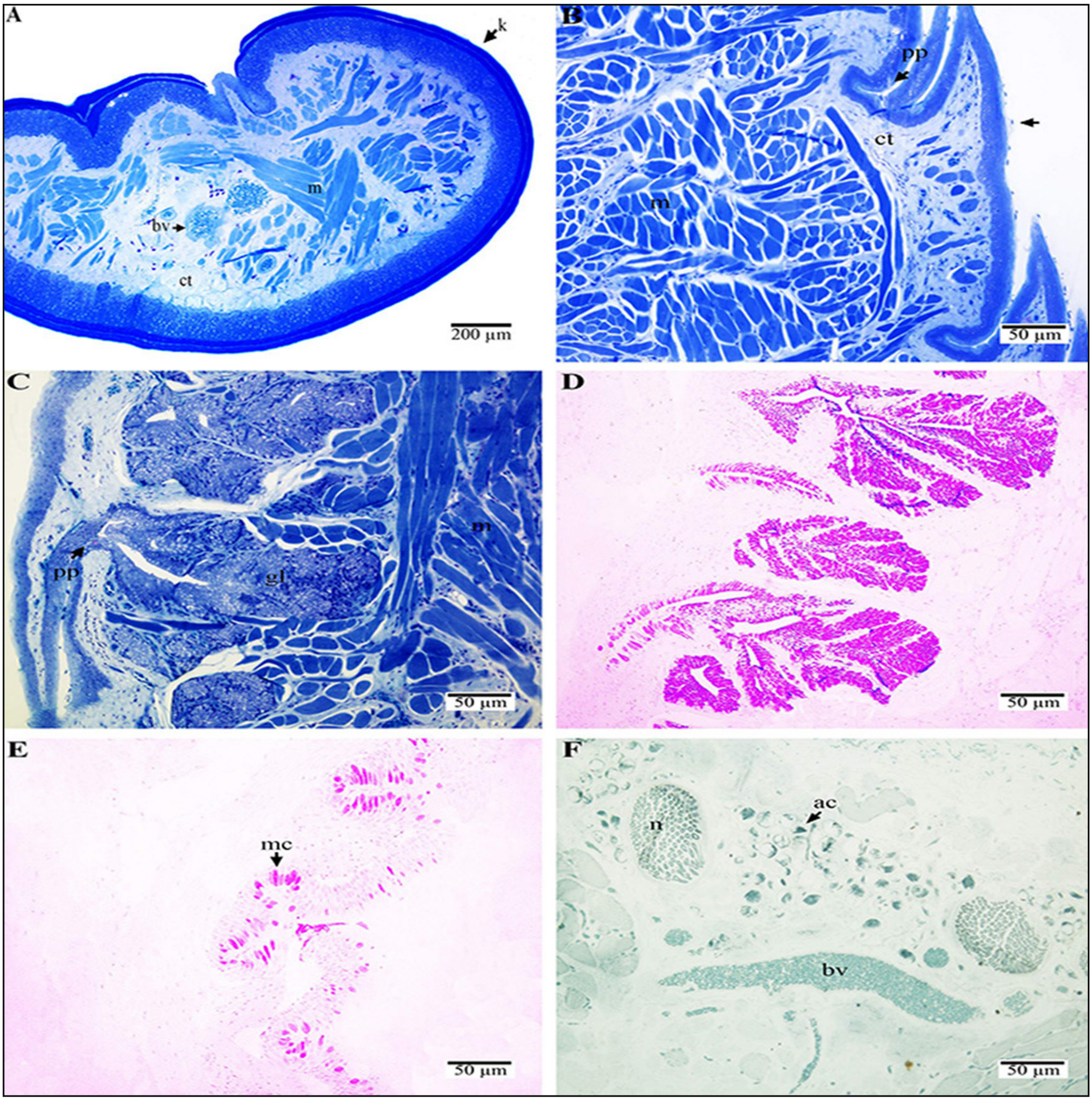

Figure 2. Light microscopy images of *Salvator merianae* tongue. A) Apex of the tongue with stratified squamous epithelium and two keratin bands (k). Blue toluidine staining. B) Middle region of the tongue, with well-developed musculature (m) and squamiform papillae (pp) of stratified squamous epithelium, with bacteria (arrow) on the surface. Blue toluidine Staining. C) Posterior region of the tongue, with well-developed musculature (m) and squamiform papillae (pp), among which there are branched tubular mucous glands (gl). Blue toluidine staining. D) Posterior region of the tongue, with PAS-positive branched tubular mucous glands. Schiff periodic acid Staining (PAS). E) Posterior end of the tongue, of prismatic epithelium with PAS-positive mucous cells (mc). Schiff periodic acid staining (PAS). F) Posterior region of the tongue, with adipose cells (ac) and nerve bundles (n). Sudam Black coloring. bv: blood vessel; ct: connective tissue.

intermediate region show some transition (Iwasaki and Miyata, 1990; Iwasaki, 1990), which partly corroborates the results found in *S. merianae*, in view of the presence of keratin in the apical region (and noton basal region) of its tongue, which is more exposed to the environment.

In the middle and posterior regions of the *S. merianae* tongue, there are squamiform papillae on the dorsal surface, where bacteria are frequently observed (Fig. 2B and C). Tongue papillae of lizards are described as filiform, with the ends having varied shapes: rounded, conical, cylindrical, fan-shaped or squamiform (Iwasaki, 1990; Darwish, 2012), depending on the species and region of the tongue.

These differences are due to the feeding habit and the way the species handles food (Pianka, 1986). Squamiform filiform papillae, such as those found in *S. merianae*, assist in the mechanical function of maintaining prey in the oral cavity, also contributing to swallowing (Schwenk, 2000; Silva, 2015). The same papillary shape has been described for the *Anthrosaura* genus, which also belongs to the Teiidae family (Hoogmoed and Avila-Pires, 1992), which may have a phylogenetic correlation. Since no taste buds were observed in the tongue papillae of *S. merianae*, these papillae have a purely mechanical function, without involvement with the sense of taste. The absence of taste buds may reflect the generalist feeding habit of this species and the greater importance of the olfaction for food selection, with the participation of the tongue transferring molecules from the environment to the vomeronasal organ.

In the middle and posterior regions of the *S. merianae* tongue, the muscle tissue is predominant, arranged in different directions and in a spaced way (Fig. 2B and C), which allows for wide-ranging movements forthe capture of odorous molecules, handling and swallowing of prey (Silva, 2015). In the posterior region of *S. merianae* tongue, there are branched tubular mucous glands that flow between the papillae, with PAS-positive and AB-PX-negative secretion (Fig. 2D). Further posteriorly, at the pharyngeal end, the epithelium becomes ciliated prismatic, with PAS-positive and AB-PX-negative mucous cells (Fig. 2E). Mucous secretion is important due to its lubricating action, prevention of damage to the epithelium, aid in swallowing and defense against harmful bacterial colonization (Arellano *et al.*, 1999; Radaelli *et al.*, 2000; Domeneghini *et al.*, 1998). Neutral mucus (PAS staining) is denser and thicker than acidic mucus (AB staining) and therefore seems to have a great capacity to form protective barriers (Beamish *et al.*, 1972). Therefore, neutral mucus (PAS-positive) observed in *S. merianae* is especially important in view the great exposure of its protractile tongue, the great amount of bacteria in its mouth and the generalist diet of this species. On the other hand, no serous cells or granules were observed in the *S. merianae* tongue, considering the PX-negative reaction. Therefore, there is no secretion of enzymes or other proteins, which differs from the observed in some species of reptiles, such as the *American chameleon* and *Anolis carolinensis*, whose tongue epithelium presents cells with mucous and serous granules (Rabinowitz and Tandler, 1986).

Tongue base of *S. merianae* presents many adipocyte cells and nerve bundles intermingling muscular tissue, as evidenced by Sudan black staining (Fig. 2F). The rich vascularization, innervation and adiposity detected in the tongue of this species are consistent with the high activity of this organ. The adipose tissue found in the *S. merianae* tongue may be an energy reserve to be used during the hibernation period in the coldest months (Derickson, 1976; Abe, 1983). In the Southeastern Brazil, teiú lizards remain active during the hot and humid season (August to April), and retract in shelters during the cold and dry months (May to July) (Abe, 1983).

Scanning electron microscopy analysis corroborate the observations made in the anatomical and histological studies. There are small undulations at the apex of the tongue that may be involved with the adhesion of molecular odoriferous, and the scaly papillae starting right after the apical bifurcation (Fig. 3A). The surface of the tongue has flattened polygonal cells, making it difficult to see its limits, especially at the apex where there are layers of keratin, which leave the surface looking like rough plates (Fig. 3B). The papillae extend along the tongue dorsum and its surface is flattened (Fig. 3C). The lateroventral portion of the tongue has no papillae, but there are folds arranged obliquely, with a flattened surface similar to that of the back (Fig. 3E). The posterior end of the tongue also has no papillae. Its surface has a velvety appearance, due to the presence of cells with numerous apical projections, which certainly represents the transition to the ciliated epithelium of the pharynx (Fig. 3F).

This study described the morphology of the tongue of the lizard *Salvator merianae* and the functional implications in the face of the feeding habit and behavior. The tongue of *S. merianae* is protractile and bifid, with great exposure to the environment, requiring protection mechanisms, such as keratinized pigmented stratified epithelium and mucus secretion. The great presence of bacteria reflects the generalist feeding habit of this species. The *S. merianae* tongue plays a direct role in the acquisition of food, presenting itself as a robust structure, extremely muscular and full of squamiform lingual papillae on the dorsal surface, which are involved in the apprehension and swallowing of prey. The absence of taste buds on the tongue of this species reveals that this organ plays no role in tasting, but is crucial for olfaction in cooperation with

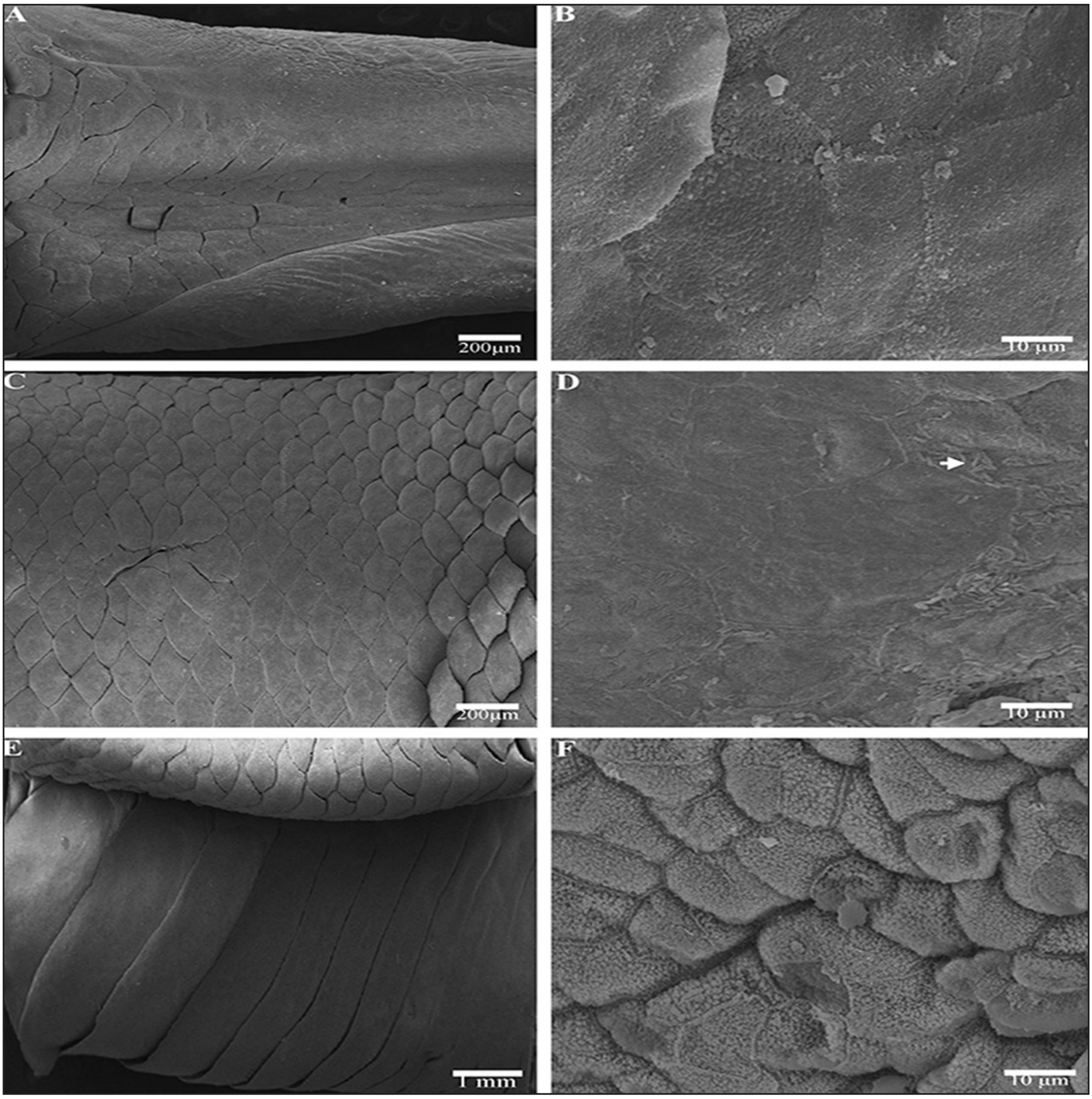

Figure 2. Scanning microscopy images of *Salvator merianae* tongue. A) Dorsal surface of the apical region. B) Detail of the epithelium of the apical region, with a flattened aspect. C) Dorsal surface of the middle region, with squamiform papillae. D) Detail of the papillae lining epithelium, with a flattened aspect and presence of bacteria (arrow). E) Lateroventral surface of the tongue, with oblique folds. F) Posterior end of the tongue, with a velvety surface due to the presence of cells with numerous apical projections.

the vomeronasal organ, which favors their foraging behavior for locating prey.

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Nota

Cuad. herpetol. 35 (1): 171-176 (*2021*)

Anfibios de una Reserva Natural del departamento Las Colonias, Provincia de Santa Fe, Argentina

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ABSTRACT
In this work, we report the amphibian fauna composition of a natural protected area located near Esperanza city, Province of Santa Fe, Argentina. It opened to the public in 2015, with yet no baseline inventory of amphibian species. Additionally, we evaluated the presence of the amphibian parasitic fungus *Batrachochytrium dendrobatidis* (*Bd*). We carried out eleven samplings from October 2015 to March 2016 in three environmental units: native forest, exotic forest, and floodplain grasslands. The sampling methods we used were live pit-fall traps with drift fences, auditory encounters, and visual encounters. Sixteen species belonging to 9 genera and 4 families were recorded. October was the month with the highest specific richness. *Bd*-specific real-time Taqman qPCR made on skin swabs made on 11 species were all negative. Even small, the amphibian species identified in the protected area represent ca. 30% of the anurans already reported for Santa Fe, and 9% of the Argentinean batracofauna.

Key Words: Anurans; Richness; Protected Area; β-diversity; Conservation.

En Argentina, como en el resto del planeta, los anfibios son un grupo de vertebrados especialmente amenazados (Vaira *et al*., 2012; IUCN, 2020). Los factores más importantes que influyen en su riqueza, abundancia, estado de salud e interacciones son: la destrucción, alteración y fragmentación de hábitat, el cambio climático, la contaminación, el aumento de la radiación UV-B, el impacto provocado directa e indirectamente por especies introducidas, las enfermedades emergentes como la quitridiomicosis (generada por el hongo *Batrachochytrium dendrobatidis -Bd-*) y la sobreexplotación, que incluye la colecta de anfibios como alimentos, mascotas, y con fines medicamentosos (Halliday, 2008; Collins, 2010; Ghirardi *et al.,* 2011; 2014a; 2014b; Vaira *et al*., 2012; Rohde, 2013; García-Feria *et al.,* 2017; Vázquez *et al.,* 2020).

Para implementar planes de conservación y generar un uso sustentable de los recursos naturales de una determinada región es necesario, en principio, conocer la diversidad biológica y las implicancias que pueden tener las actividades antrópicas sobre los sistemas biológicos (Villarreal *et al.,* 2004). Una de las estrategias de conservación es la creación de áreas naturales protegidas, que son espacios geográficos claramente definidos, reconocidos, dedicados y gestionados para conseguir la conservación a largo plazo de la naturaleza y de sus servicios ecosistémicos y sus valores culturales asociados (Dudley, 2008).

La Provincia de Santa Fe cuenta con 22 áreas naturales protegidas (Biasatti *et al.,* 2016), siendo una de ellas La Reserva Natural de la Escuela de Agricultura, Ganadería y Granja “Médico Veterinario Martín Rodolfo De la Peña” (RN). La misma es un área natural de 33 hectáreas protegida por la Universidad Nacional del Litoral (UNL) desde el año 1979 (Biasatti *et al.,* 2016). Está ubicada 5 km al norte de la ciudad de Esperanza, sobre los márgenes del

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Río Salado (31º 23´S; 60º 55´O), en el departamento Las Colonias, Provincia de Santa Fe (Biasatti *et al.*, 2016) (Fig. 1). Ésta RN fue creada con el objetivo de preservar especies y diversidad genética representativa de la ecorregión del espinal, y plantea la conservación del área como refugio para la flora y fauna, en una región profundamente transformada por la actividad agrícola intensiva.

Los trabajos de inventariado de especies realizados hasta la fecha en la RN se restringen a plantas, abejas, hormigas y aves (De la Peña *et al.*, 2003; Exner *et al.*, 2004; De la Peña, 2005a; 2005b; 2006; Vittar y Cuezzo, 2008; Dalmazzo, 2010; Arrabal *et al.*, 2012), pero aún no se ha inventariado la diversidad de anfibios que alberga. Por lo tanto, el objetivo del presente estudio fue inventariar las especies de anfibios presentes en diferentes ambientes de la RN. Complementariamente, nos propusimos evaluar la presencia de *Bd* en los individuos encontrados, ya que este hongo ha sido un patógeno capaz de producir declinaciones poblacionales de anfibios y ha sido registrado en la Provincia de Santa Fe (Gutiérrez *et al.*, 2010; Delgado *et al.*, 2012; Ghirardi *et al.*, 2014a; 2014b).

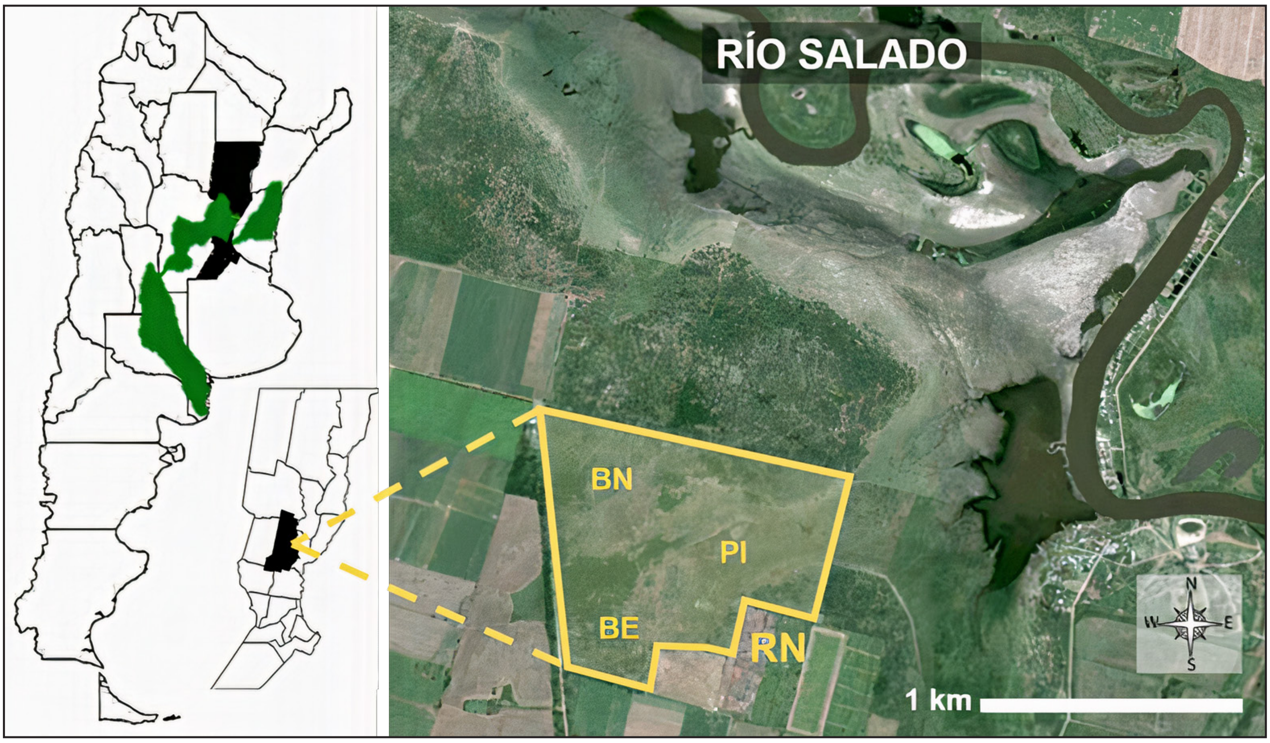

Figura 1. Localización geográfica del área de muestreo. En verde se indica la ecorregión del Espinal, en negro los límites de la Provincia de Santa Fe. En amarillo se resaltan los límites de la Reserva Natural “Médico Veterinario Martín Rodolfo de la Peña” (RN). Dentro de la RN se indican los sitios muestreados correspondientes al bosque nativo (BN), bosque exótico (BE) y pastizal inundable (PI).

Se realizaron 13 salidas de campo entre septiembre de 2015 y marzo de 2016, correspondiente a los meses de primavera y verano. En la primera salida a campo se realizó un reconocimiento de los ambientes presentes y, apoyados por estudios previos sobre la vegetación de la RN (Exner *et al.*, 2004), se definieron tres para su muestreo: bosque nativo (dominado por algarrobo *Prosopis* sp. y chañar *Geoffroea decorticans*, junto a una variada vegetación herbácea nativa); bosque exótico (con dominancia del árbol exótico invasor acacia negra: *Gleditsia triacanthos*); y pastizal inundable (en las zonas bajas más cercanas al río Salado).

El relevamiento de las especies de anfibios se realizó en los tres ambientes mediante (i) búsquedas activas de relevamiento por encuentro visual, (ii) escuchas de cantos reproductivos de adultos, (iii) colocación de baterías con trampas pozo vivas con cercos de derivación, y (iv) uso de copos para la captura de renacuajos en los cuerpos de agua (Heyer *et al.*, 1994; Crosswhite, 1999; Lips, 2001; Ryan *et al.*, 2002; Angulo *et al.*, 2006). El relevamiento por encuentro visual y escuchas de cantos reproductivos fue realizado entre las 19 y las 23 hs por tres observadores en cada campaña (esfuerzo de captura de 44 hs/persona en el total de las campañas, totalizando un esfuerzo de búsqueda de 132 hs). La búsqueda activa implicó mover la vegetación, los troncos caídos, la hojarasca, caminar en las lagunas y por sus márgenes revisando los microhábitats de refugio y reproducción de los anfibios, para encontrar la mayor cantidad de ejemplares posible.

Durante la segunda salida a campo se colocaron dos baterías de trampas pozo vivas con cercos de derivación en cada ambiente (Heyer *et al.*, 1994). Las mismas se instalaron cercanas a cuerpos de agua (entre 10 y 30 m) y consistían en una malla plástica de 5 m de largo y 40 cm de alto. Para las trampas pozo se colocaron recipientes de 30 cm de diámetro y una capacidad de 20 L en cada extremo de la malla, y en su interior una esponja húmeda para permitir la supervivencia de los individuos hasta su encuentro en el próximo muestreo.

Los 11 muestreos restantes se realizaron luego de precipitaciones que pudieran suponer un aumento en la actividad de los anfibios y facilitar su registro (Tabla 1) (López *et al.*, 2011). Las trampas se revisaron en las primeras horas de la tarde. Los individuos encontrados y capturados fueron identificados a través de la clave propuesta en Ghirardi y López (2017). Además, para análisis de presencia de *Bd* se realizó un hisopado de piel (con hisopos Puritan ®) frotando suavemente en los flancos del tórax, el vientre, la superficie ventral de muslos y en las superficies palmar y plantar de algunos de los individuos, siguiendo la metodología de Hyatt *et al.* (2007).

Tabla 1. Fechas de los muestreos y datos de precipitaciones acumuladas durante los 10 días previos a las salidas.

Fechas de muestreos	N° sp. registradas	Precipitaciones
15/10/2015	3	5 mm
21/10/2015	4	6 mm
30/10/2015	15	46 mm
06/11/2015	5	51 mm
18/11/2015	10	30 mm
04/12/2015	8	44 mm
07/01/2016	6	97 mm
01/02/2016	5	20 mm
15/02/2016	8	101 mm
02/03/2016	7	45 mm
30/03/2016	7	22 mm

Se hisoparon 40 individuos de 11 especies para evaluar la presencia de *Bd* (Tabla 2). Las muestras se procesaron mediante la técnica de PCR en tiempo real (Taqman qPCR, Applied Biosystems ®) de acuerdo al método de Boyle *et al.* (2004) en la Escuela Universitaria de Medicina Veterinaria de la Universidad Estatal de Carolina del Norte (EEUU). El límite de detección utilizado para los análisis de PCR fue de 0,5 equivalentes de zoosporas de *Bd*.

Los datos de riqueza y diversidad de cada ambiente fueron analizados con el programa EstimateS 9.1. Además, se evaluaron diferencias espaciales (entre los diferentes sitios) y temporales (entre los diferentes muestreos). La riqueza entre ambientes fue analizada con un PERMANOVA, usando el método Jaccard, con el paquete vegan (Oksanen *et al.,* 2015), mientras que la variación temporal de la riqueza se estudió con un test de Friedman, utilizando el paquete PMCMR (Pohlert, 2014); todos los análisis de realizaron con el programa R Studio (R Core Team, 2020).

La riqueza de anfibios registrada en la RN fue de 16 especies, pertenecientes a nueve géneros, agrupados en cuatro familias: Leptodactylidae (7 spp.), Hylidae (5 spp.), Bufonidae (3 spp.) y Microhylidae (1 sp.) (Tabla 2). Todas las especies presentaron poblaciones reproductivas en la RN (*i.e.* se registraron individuos de diferentes etapas del ciclo de vida y/o amplexos).

La riqueza fue de diez especies en el pastizal inundable, doce en el bosque exótico y quince en el bosque nativo (Tabla 2). *Rhinella arenarum* sólo se registró en bosque exótico, mientras que *Melanophryniscus* sp.*, Leptodactylus mystacinus* y *Pseudopaludicola falcipes* sólo se encontraron en el bosque nativo. El resto de las especies fueron registradas en al menos dos de los tres ambientes.

El ensamble de especies no difirió significativamente entre los ambientes (diversidad β: F=1,18; p=0,25). No obstante, sí varió temporalmente entre los 11 muestreos realizados (test Friedman: X^2=11,93; p=0,03), siendo octubre el mes con mayor riqueza (R=15). Por otro lado, no se registró la presencia de *Bd* en los individuos hisopados.

Los resultados demuestran que la RN "Médico Veterinario Martín Rodolfo de la Peña" presenta un elenco de especies representativo de la anfibiofauna de la Provincia de Santa Fe. La taxocenosis registrada en el presente estudio representa 30,8% de los anfibios de la Provincia de Santa Fe (Ghirardi y López, 2017) y aproximadamente el 9% de la batracofauna

Tabla 2. Lista de las especies de anfibios encontradas en la RN. Entre paréntesis se consigna el número de ejemplares hisopados para análisis de presencia de *Bd*. Con un asterisco se indica la presencia de cada especie en los diferentes ambientes muestreados.

Especie	Bosque nativo	Bosque exótico	Pastizal inundable
Hylidae			
Scinax squalirostris (1)	*	*	*
S. nasicus (1)	*	*	
Dendropsophus nanus (10)	*	*	*
D. samborni	*	*	*
Boana pulchella (1)	*		*
Microhylidae			
Elachisocleis bicolor	*	*	
Leptodactylidae			
Leptodactylus chaquensis (6)	*	*	*
L. gracilis	*	*	*
L. latinasus (2)	*	*	*
L. mystacinus (1)	*		
L. latrans (9)	*	*	*
Physalaemus albonotatus (4)	*	*	*
Pseudopaludicola falcipes	*		
Bufonidae			
Rhinella arenarum		*	
R. fernandezae (1)	*	*	*
Melanophryniscus sp. (4)	*		

Argentina (Akmentins *et al.,* 2018).

Quince de los anuros encontrados en la RN se encuentran en la categoría de Preocupación Menor según la última categorización argentina y la Lista Roja de IUCN (Vaira *et al.,* 2012; IUCN, 2020). Aún no se ha definido la identidad taxonómica de la población de *Melanophryniscus* que habita en la RN (Baldo *et al.* 2012), y en general las especies de este género se listan como Vulnerables en la categorización de Argentina (Vaira *et al.,* 2012), por lo que, a futuro, consideramos importante caracterizar esta población, identificar posibles factores que signifiquen amenazas y realizar un seguimiento en la RN.

La menor riqueza observada en el pastizal inundable puede estar relacionada con los desbordes periódicos del río Salado y la consecuente inundación del área, que obliga a algunos animales a moverse a otros paisajes para alimentarse y refugiarse. Por otro lado, la escasa cobertura vegetal de leñosas (no se registró la presencia de estrato arbóreo ni arbustivo), puede significar una menor cantidad de refugios para los anfibios arborícolas (Zocca *et al.*, 2014; Guderyahn *et al.*, 2016; Figueiredo *et al.*, 2019). Sumado a lo anterior, el estrato herbáceo predominante del pastizal inundable está formado por comunidades de plantas halófilas (Exner *et al.,* 2004), que sugieren la presencia de un suelo salino que puede ser evitado por las especies encontradas en la reserva (Albecker y McCoy, 2017). La gran riqueza observada al comienzo de la primavera (octubre) probablemente esté asociada al aumento de la actividad reproductiva de los anfibios durante el principio de la temporada cálida y el inicio de la estación de mayores precipitaciones (López *et al.,* 2011).

Con respecto a los análisis de *Bd*, es interesante destacar que no hubo registros positivos en los ejemplares analizados. Sin embargo, aún con estos resultados no podemos afirmar de manera definitiva la ausencia del quitridio en la RN, por lo que el seguimiento en las cuatro estaciones del año y a largo plazo de presencia de *Bd* en las poblaciones de anfibios que habitan la reserva sigue siendo importante.

De las 22 áreas protegidas que presenta la Provincia de Santa Fe, sólo el Parque Nacional Islas de Santa Fe (PN) cuenta con un inventario publicado de las especies de anfibios (Sánchez *et al.*, 2020). El PN está localizado al sudeste de la Provincia (32º16'S, 60º43'O), comprende 4.096 ha. sobre el valle aluvial del río Paraná, en la ecorregión Delta e Islas del Paraná. Sánchez *et al.* (2020) reportan 14 especies de anfibios en el PN, riqueza similar a la registrada en este trabajo para la RN (16 especies), pero compartiendo solo 7 especies entre ambas reservas (*Scinax nasicus, Dendropsophus nanus, D. sanborni, Boana pulchella, Elachistocleis bicolor, Leptodactylus latrans, Rhinella fernandezae*). Esto probablemente se debe a que corresponden a diferentes ecorregiones más que a la distancia entre ambas reservas (~105 km).

Este trabajo constituye el primer registro formal de las especies de anfibios de la Reserva Natural "Médico Veterinario Martín Rodolfo de la Peña", el segundo listado de anfibios para las áreas naturales protegidas de la Provincia de Santa Fe. Representa además una base para monitoreos y futuros estudios de historia natural de su anfibiofauna, así como también brinda una herramienta importante para el manejo y la conservación de la diversidad del área natural protegida estudiada.

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Distribution extension of *Amphisbaena lumbricalis* Vanzolini, 1996 with its first predation record by snake *Erythrolamprus viridis* (Günther, 1862)

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ABSTRACT
We expand the geographical distribution of *Amphisbaena lumbricalis* throughout the first record for a semiarid Caatinga area in the Paraíba state, northeastern Brazil. Furthermore, we report the first predation report of *A. lumbricalis* by the snake *Erythrolamprus viridis*. Our findings contribute with information about the habitats occupied by a hitherto considered "Data Deficient" species, beyond to reveal the potential of *E. viridis* to prey upon reptiles.

Key Words: Geographical Distribution; Predator-Prey Interaction; Caatinga.

Data on geographic distribution is essential to support conservation actions and effective species management. Although representing about 20% of the world's reptiles and amphibians, the fossorial herpetofauna is still poorly known (Measey, 2006). Fossorial reptiles have received relatively little attention, and the lack of knowledge about these animals is usually attributed to their secretive habits, which leads to several sampling difficulties (Frey, 2006; Filho *et al.*, 2013). Amphisbaenia is one of the major groups of fossorial reptiles, and like any secretive species, studies on several aspects, such as natural history, ecology, and distribution, are still scarce (Pearse and Pogson, 2000; Macey *et al.*, 2004).

Currently, Amphisbaenia congregates approximately 200 described species, from six families (Amphisbaenidae, Bipedidae, Blanidae, Cadeidae, Rhineuridae, and Trogonophidae) with a wide geographical distribution, since the Neotropical region, sub-Saharan Africa, parts of the Mediterranean region, Baja California, and Florida (Vitt and Caldwell, 2014; Longrich *et al.*, 2015; Uetz *et al.*, 2020). Amphisbaenidae, the most diverse family (ca. 170 species), is distributed throughout South America and Africa (Gans, 2005; Vidal *et al.*, 2008; Uetz *et al.*, 2020). Around 40% (80 species) of all amphisbaenids occur in Brazil, and 27 species along the semiarid Caatinga (Almeida *et al.*, 2018; Costa and Bérnils, 2018; Ribeiro *et al.*, 2018, Uetz *et al.*, 2020).

Amphisbaena lumbricalis Vanzolini, 1996 was described to the Xingó Hydroelectric Power Plant located in the lower São Francisco River, which divides the states of Alagoas and Sergipe in the Northeast region from Brazil (Vanzolini, 1996). Initially, its distribution was in Delmiro Gouveia and Piranhas's municipalities in Alagoas state, and in Canindé de São Francisco in Sergipe state (Vanzolini, 1996). In 2015, it was recorded for Traipu municipality in Ala-

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goas state (100 km to its southeast site), in an ecotone between Atlantic Forest and Caatinga environments, including a non-sandy area (Galdino *et al.*, 2015). Subsequently, it was extended to the Pernambuco state, for the municipalities of Custódia, Floresta and Sertânia associated with several soil types (Tavares *et al.*, 2017). The last distribution update was recorded to the Reserva Biológica Guaribas (hereafter REBIO Guaribas) in the Mamanguape municipality, Paraíba state, an integral protection unit inserted in the Atlantic Forest domain (Mesquita *et al.*, 2018). Herein, we expand the geographical distribution of *A. lumbricalis* throughout the first record for a Caatinga area in the Paraíba state, beyond to report the first predation case of *A. lumbricalis* by the snake *Erythrolamprus viridis* (Günther, 1862).

During a snake ecology study in several herpetological collections in the northeastern Brazil, we found one specimen of *A. lumbricalis* in examining gut contents of a specimen of *Erythrolamprus viridis* housed in the herpetological collection of the Universidade Federal da Paraíba – CHUFPB. The predator (CHUFPB 17237) was an adult male with 403 mm of snout-vent length collected at São Mamede municipality, Paraíba state (Fig. 1). The predator's identity was determined by a combination of following meristic characters: 19/19/17 dorsal, 185 ventral, divided subcaudal, and eight supralabial scales. The prey consisted in the anterior region of an individual of *A. lumbricalis* with 57.2 mm of length, and 3.2 mm of diameter, which was housed in the scientific collection Coleção Herpetológica do Semiárido – CHSA at Universidade Federal Rural do Semi-Árido. Despite this material (CHSA R 1714) was in an advanced degree of deterioration, we were able to precisely identify the target species by its slender body diameter and typical cephalic scutelation. In comparison with other slender am-

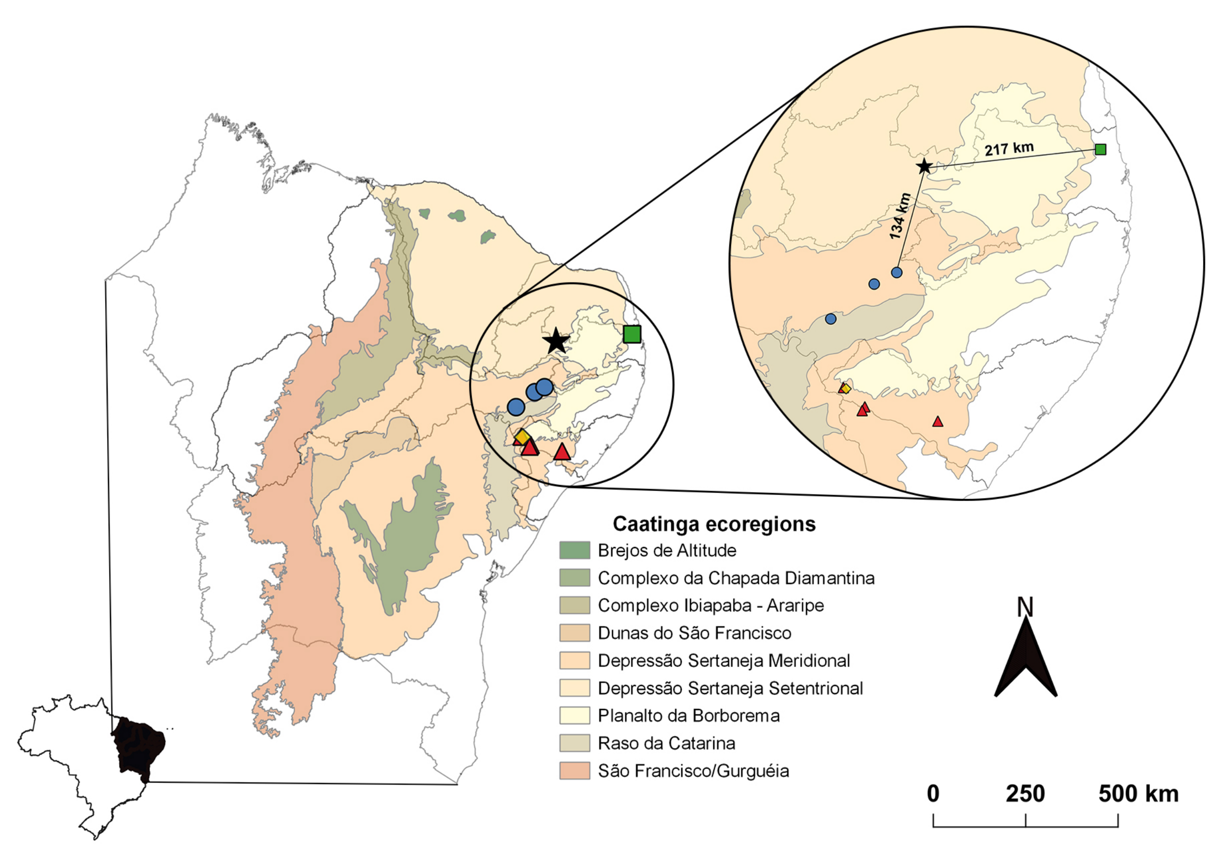

Figure 1. Updated distribution map of *Amphisbaena lumbricalis*. Black star constitutes the new record to São Mamede municipality in Paraíba state. Colorful symbols correspond to the previously known records: yellow diamond (type-locality; Xingó - Alagoas; Vanzolini, 1996), red triangles (Delmiro Gouveia, Piranhas and Traipu - Alagoas; Canindé do São Francisco - Sergipe; Galdino *et al.*, 2015), blue circles (Custódia, Floresta, Sertânia - Pernambuco; Tavares *et al.*, 2017), and green square (Mamanguape - Paraíba; Mesquita *et al.*, 2018). Caatinga ecoregions follows Silva *et al.* (2017).

phisbaenian species, the size and shape of posterior edge of frontal scales allow to distinguish *A. lumbricalis* from *A. hastata* Vanzolini, 1991 (greater angle between two smaller frontal scales) and *A. heathi* Schmidt, 1936 (smaller angle between two larger frontal scales); the shape of anterior edge of frontal scales allows to distinguish *A. lumbricalis* from *A. ignatiana* Vanzolini, 1991 (arched anterior edge and greater angle between two frontal scales); and the size and shape of prefrontal scales allow to distinguish *A. lumbricalis* from *A. carvalhoi* Gans, 1965 (smaller and shorter prefrontal scales).

Table 1. Detailed information of all known geographical records of *Amphisbaena lumbricalis*. Acronyms: CHSAR - Coleção Herpetológica do Semiárido, MFCH - Coleção de Herpetologia do Museu de Fauna da Caatinga, MUFAL - Museu de História Natural da Universidade Federal de Alagoas, MZUSP - Museu de Zoologia da Universidade de São Paulo, and RT Coleção Herpetológica da Universidade Federal da Paraíba em Rio Tinto.

Municipality	State	Ecoregion	Latitude	Longitude	Voucher	Source
Hydroelectric of Xingó, on the left bank of the Rio São Francisco (type locality)	Alagoas	Depressão Sertaneja Meridional	-9.400	-37.967	MZUSP 79433 MZUSP 79434-79490 MZUSP 79419-79432	Vanzolini, 1996
Delmiro Gouveia	Alagoas	Depressão Sertaneja Meridional	-9.386	-37.997	MUFAL 2790	Galdino *et al.*, 2015
Piranhas	Alagoas	Depressão Sertaneja Meridional	-9.600	-37.759	MUFAL 2785, 2786, 2787, 2802, 2803, 2804, 2805; 2493	Galdino *et al.*, 2015
Traipu	Alagoas	Depressão Sertaneja Meridional	-9.759	-36.948	MUFAL 9071; 9812	Galdino *et al.*, 2015
Canindé do São Francisco	Sergipe	Depressão Sertaneja Meridional	-9.642	-37.789	MUFAL 2777; 2778; 2779; 2780; 2781; 2782; 2783; 2784; 2789; 2796; 2797; 2798; 2800; 2806; 2807; 2808; 2809; 2811; 2812; 2813; 2814; 2815; 2816; 2817; 2818; 2819	Galdino *et al.*, 2015
Custódia	Pernambuco	Depressão Sertaneja Meridional	-8.237	-37.655	MFCH 2183	Tavares *et al.*, 2017
Floresta	Pernambuco	Raso da Catarina	-8.624	-38.137	MFCH 2180	Tavares *et al.*, 2017
Sertânia	Pernambuco	Depressão Sertaneja Meridional	-8.109	-37.404	MFCH 2163	Tavares *et al.*, 2017
Mamanguape	Paraíba	Atlantic Forest	-6.742	-35.142	RT 0267	Mesquita *et al.*, 2018
São Mamede	Paraíba	Depressão Sertaneja Setentrional	-6.932	-37.097	CHSAR 1714	Present study

Our record of *A. lumbricalis* to São Mamede municipality in Paraíba state extends its distribution (Fig. 1, Table 1) by 134 km Northeast from the nearest previously recorded locality (Sertânia municipality, Pernambuco state), and by 217 km W from the northeast known occurrence site (Mamanguape municipality, Paraíba state). This latter was the first record of *A. lumbricalis* to Paraíba state, in the REBIO Guaribas, despite there is no detailed information on the habitat in which voucher specimens were collected (Mesquita *et al.* 2018). However, REBIO Guaribas is inserted in the Atlantic Forest domain, including complex vegetation formations throughout its range (Barbosa *et al.*, 2011; Melo and Vieira, 2017; Costa-Lima and Alves, 2018), as the *Tabuleiros*, and Stational Semidecidual Forest (Oliveira-Filho and Carvalho, 1993; Mesquita *et al.*, 2018). Therefore, our findings constitute the second record of *A. lumbricalis* for the Paraíba state and the first record for the semiarid Caatinga in this state.

This new site is a typical Caatinga area located in the Depressão Sertaneja Setentrional ecoregion (Fig. 1, Table 1), constituted by hyperoxerophilous vegetation and deciduous forest patches. Thus, we contributed with information about the mesohabitats occupied by *A. lumbricalis*, which still remains categorized as "Data Deficient " for conservation purposes (Mott, 2010).

Regarding the predation record, the genus *Erythrolamprus* belongs to an anuran-eating snakes guild, whose the most species from Caatinga can be considered anuran specialists (Vitt, 1983). One of them is *Erythrolamprus viridis*, a small diurnal and terrestrial snake whose ecology remains poorly studied. A pioneering study described that *E. viridis* feed on frogs, treefrogs, and toads (Vitt and Vangilder, 1983), but recently, it was recorded that it can also prey lizards (Mesquita *et al.*, 2013). An *Amphisbaena* recorded in the digestive tract of *E. viridis* expands its prey list, reinforcing its ability to preying reptiles. Although this event might be considered fortuitous, further studies on ecology of *E. viridis* are required to a better understanding of its dietary preferences. However, due to their secretive habits, these occasions provide a unique opportunity to acquire natural history data on such species guild.

Despite its fossorial habits, amphisbaenians are preyed by a sort of predators, as such as many birds (Folly *et al.*, 2015; Hayes *et al.*, 2015), mammals (Oliveira *et al.*, 2004; Soibelzon *et al.*, 2007) and snakes (Marques and Sazima, 1997; Maschio *et al.*, 2010). Many fossorial snakes commonly prey on amphisbaenians, like elapids (Zamprogno and Sazima, 1993; Cisneros-Heredia, 2005) and Elapomorphini species (Duarte, 2006; Caramaschi and Niemeyer, 2012). However, the consumption of worm lizards by terrestrial snakes is much scarcer. Dixon (1989) reported this behavior for the Xenodontini *Erythrolamprus poecilogyrus* (Wied-Neuwied, 1825) and Barbo and Marques (2003) for the Echinantherini *Taeniophallus affinis* (Günther, 1858). Our finding for *E. viridis* reveals its potential on preying amphisbaenians, allowing us to consider that this behavior is less rare than expected between Xenodontini snakes.

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Novedad zoogeográfica

Cuad. herpetol. 35 (1): 183-185 (*2021*)

First formal records of *Stenocercus doellojuradoi* (Freiberg, 1944) (Squamata, Tropiduridae) from the Paraguayan Chaco

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Localities— Paraguay, Department of Boquerón, Mariscal Estigarribia District, locality of Pirizal (-61.092264; -23.031885, 180 m a.s.l; Fig. 1). Specimens were collected on July 22 of 2014, processed and deposited in the national herpetological collections of the Museo Nacional de Historia Natural del Paraguay (MNHNP), and the Colección Zoológica de la Facultad de Ciencias Exactas y Naturales (CZ-CEN) according to national regulations (MNHNP 12824, CZ-CEN 1165). Subsequently, a revision of the collection of the Museo Nacional de Historia Natural del Paraguay was made. An additional individual of *Stenocercus doellojuradoi* (MNHNP 2696) was found, collected on August 19 of 1982, (-22.183333; -59.35) at the Department of Boqueron, Loma Plata District, locality of Estancia Pozo Azul.

Comments— Active diurnal and nocturnal searches were performed on July 2014, to detect amphibians and reptiles on the ground at the edge of an unused road. Two individuals of *Stenocercus doellojuradoi* were found, both in the forest edge near the road. The ecosystem corresponds to xerophytic open forest with an understory of bromeliads near the Pilcomayo basin, in the Dry Chaco ecoregion

Figure 1. Individual of *Stenocercus doellojuradoi* (Freiberg, 1944). Locality of Pirizal, Mariscal Estigarribia District, Department of Boquerón, Paraguay. July 2014.

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(Dinerstein *et al.*, 1995). The records provided here increase the species distribution in over 300 km eastward to the nearest known locality in Formosa, Argentina (Fig. 2).

The genus *Stenocercus* Duméril & Bibron (1837) is one of the most speciose and widespread among the Tropiduridae family, occurring from the Andean mountains, adjacent lowlands of Venezuela and Colombia, to central Argentina (Torres-Carvajal, 2000; 2005; 2007). *Stenocercus doellojuradoi* (Freiberg, 1944) was described from La Rioja (Argentina), and was formerly assigned to the genus "*Proctotretus*" (Frost, 1992) with *S. azureus* and *S. pectinatus*. It can be distinguished from other similar species by its dorsal coloration pattern composed of longitudinal cream blotches, pink ventrolateral body edges as well as notched and smooth ventral and gular scales. Also, by not having the mental in contact with the first pair of sublabials and the presence of a small subhorizontal antehumeral fold (Freiberg, 1944; Torres-Carvajal, 2007). The species is currently known from Catamarca, Córdoba, La Rioja, San Juan, San Luis, Santiago del Estero, Chaco, Formosa, and Tucumán Provinces in Argentina (Ávila *et al.*, 2013; Cei, 1993; Laspiur and Acosta, 2006). The presence of *S. doellojuradoi* in the Paraguayan Chaco is mentioned by Trutnau *et al.* (2016), yet a precise location nor reference material was provided. Herein we present the first Paraguayan records of the species with voucher specimens.

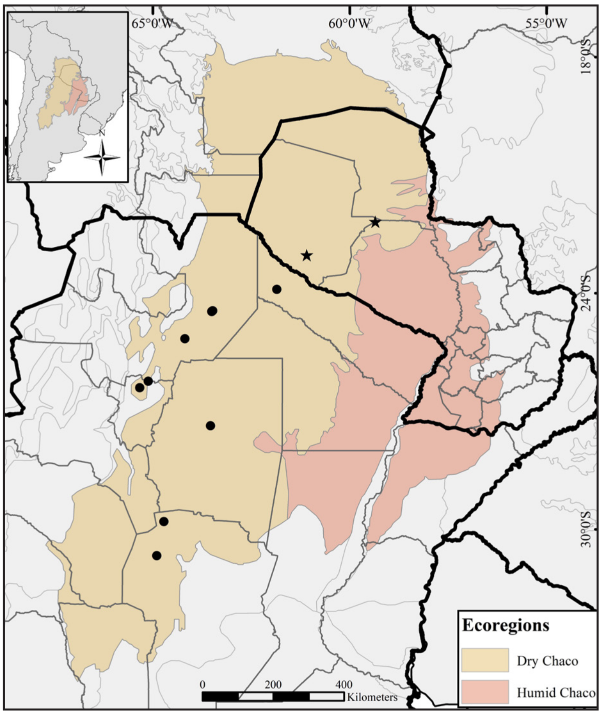

Figure 2. Map showing known distribution points for the species. The stars correspond to the new localities.

According to Cei (1993), Freiberg (1944) and Torres-Carvajal (2007), the three individuals correspond mostly to *Stenocercus doellojuradoi*. Nevertheless, some differences were found including: the mental scale marginally in contact with the sublabials on the specimens collected in Pirizal. But the most noticeable discrepancy was found on the specimen of the locality of Pozo Azul, that beside presenting mucronate and smooth scales in the ventral region as expected also presents scales with rounded edges and small terminal keels in the gular region. The individuals from both localities present the antehumeral fold, which according to Torres-Carvajal (2007) is not present in this species; however, Freiberg (1944) and Cei (1993) mention that it is, but it might not be obvious since it is covered by scales projected backwards. These divergent characteristics could be related to the fact that the records correspond to the extremes of the species distribution, therefore, deeper morphological and molecular studies are necessary, with a larger number of specimens.

The Paraguayan Chaco is currently a deforestation hotspot with almost a third of its forest surface converted to agriculture and cattle areas (Baumann *et al.*, 2017) and protected areas cover only 9.1% of the distribution of the endemic species of this ecoregion (Nori *et al.*, 2016). As well as the loss of habitat, the lack of knowledge of the actual distribution of the species in Paraguay, the morphological variations found in the specimens and the possible presence of other species of the same genus in the Paraguayan Chaco (Ávila *et al.*, 2013, Torres *et al.*, 2000) demand further studies on the *Stenocercus* principally in regions bordering Argentina and Bolivia.

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Novedad zoogeográfica

Cuad. herpetol. 35 (1): 187-188 (*2021*)

Nuevo registro de *Tantilla melanocephala* (Linnaeus 1758) (Reptilia: Serpentes: Colubridae) en Paraguay

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Localidad- República del Paraguay, Departamento de Misiones, Estancia Santa María a 12 km de la ciudad de San Ignacio (26°50'10"S, 57°10' 09"W). Fecha: 14 de mayo de 2020. Colectado por Alvaro Vera. Depositado en el Instituto de Investigación Biológica del Paraguay (IIBP-H-5659).

Comentarios- *Tantilla melanocephala* es una serpiente semifosorial de tamaño pequeño ampliamente distribuida en Centro y Sudamérica, presente desde el sur de Guatemala hasta Perú, Bolivia, noreste de Argentina, Brasil, Paraguay y Uruguay (Peters y Orejas-Miranda 1970; Cunha y Nascimento, 1993; Cacciali *et al.,* 2016). Esta es una especie poco conocida en Paraguay, con escasos registros en colecciones biológicas (Motte *et al.,* 2009; Cacciali 2009; Cacciali *et al.,* 2016).

T. melanocephala se distingue de todas las otras especies del género por los siguientes caracteres: **(1)** 15-15-15 hileras dorsales; **(2)** 125-177 escamas ventrales; **(3)** 41-92 escamas subcaudales; **(4)** generalmente 7/7 supralabiales (3-4 contactan con la órbita); **(5)** 6/6 infralabiales (4 contactan con geneiales); **(6)** escama loreal ausente; **(7)** 1 preocular y 2 postoculares, **(8)** 4 geneiales con surco mental; **(9)** adultos en promedio 225 mm SVL y juveniles 110-112 mm SVL **(10)** color dorsal pardo a pardo rojizo. En las escamas vertebrales posee puntos de color negro formando una línea vertebral a veces muy difusa, la cabeza es negra con dos manchas laterales blancas. Posee un collar postparietal negro y el vientre es blanco inmaculado (Wilson y Mena, 1980; Marques y Puorto, 1998; Giraudo 2001).

La especie fue previamente registrada en el Departamento Central (25°17'S, 57°37'W) y Departamento San Pedro (24°36'S, 56°44'W). Cacciali y Brusquetti (2005) mencionan un ejemplar de *T. melanocephala* en Estancia Toro Mocho, Departamento de Boquerón (23°38'S, 60°24'W). Posteriormente, Cacciali *et al.* (2016) citan este último registro erróneamente para el Departamento Presidente Hayes, por lo que se actualiza la distribución correcta verificando el registro original de Cacciali y Brusquetti (2005) en el Departamento de Boquerón (Fig. 1).

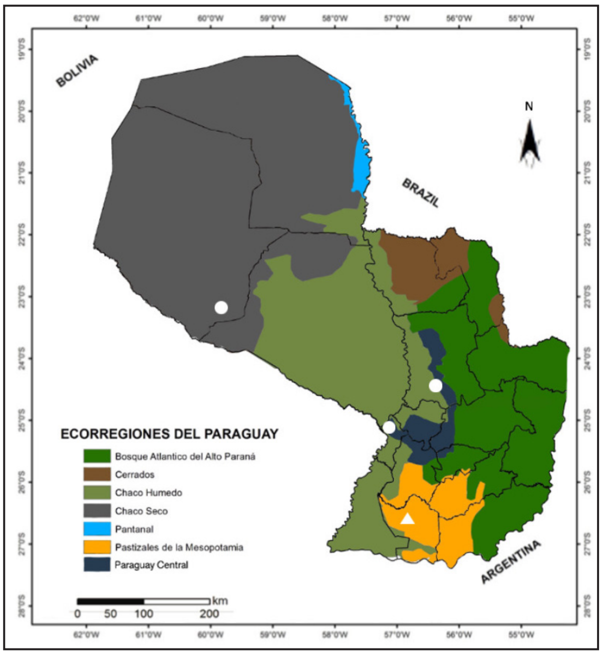

Figura 1. Mapa de distribución de *Tantilla melanocephala* en Paraguay. Ecorregiones siguen a Cacciali (2010) y Del Castillo & Clay (2005). Circulos: localidades previas conocidas. Triángulo: nuevo registro.

En este trabajo presentamos el primer registro de *T. melanocephala* para el Departamento de Misiones, extendiendo así 180 km hacia el sur su área de ocurrencia previamente conocida. El ejemplar IIBP-H-5659 fue encontrado en la ciudad de San Ignacio, Estancia Santa María (26°50'10"S, 57°10'09"W) siendo el primer registro en Paraguay para la ecorregión praderas de la Mesopotamia, donde predominan los pastizales naturales con pequeños bosques. El individuo fue hallado muerto, probablemente a consecuencia de maquinaria agrícola funcionando en el lugar, en la fecha 14 de mayo de 2020. El ejemplar (Fig. 2) presenta una coloración marrón rojiza, con

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Figura 2. Vista lateral, dorsal y ventral de *Tantilla melanocephala* (IIBP-H-5659). Foto: Alvaro Vera

una línea vertebral oscura de una escama de ancho que inicia en un collar nucal negro de tres escamas de longitud. El vientre es blanco inmaculado. Debido al estado en que se encontraba el ejemplar, no pudo ser examinado de manera detallada por lo que no se mencionan caracteres merísticos ni morfométricos.

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Novedad zoogeográfica

Cuad. herpetol. 35 (1): 189-194 (*2021*)

New occurrence records of *Lepidodactylus lugubris* (Duméril & Bibron, 1836) (Squamata: Gekkonidae) for the amazon and atlantic forest in Brazil

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Locality: Brazil, Pará, Capitão Poço, 1°45’7.1676” S; 47°3’50.5728” W. Annelise Batista D´Angiolella, 11/02/2018. MPEG 32963. Osvaldo Rodrigues da Cunha Herpetological Collection, Museu Paraense Emílio Goeldi (MPEG). Brazil, Pará, Breves, 1°40’56.222” S; 50°28’58.933” W. Lywouty Reymond, 06/24/2020. Specimen photographed and released. Brazil, Bahia, Salvador, 12°59’56.983” S; 38°31’4.969” W. Ben Phalan and Luciana Leite, March, and June 2019. Specimens were filmed, photographed and these observations were archived on the citizen science site iNaturalist.

Comments: The mourning gecko, *Lepidodactylus lugubris* (Duméril and Bibron, 1836), is native to southwest Pacific, and has been introduced to a range of tropical and subtropical localities from the Seychelles to Latin America (Bauer and Henle, 1994; Ineich, 1999; Uetz *et al*., 2016). It is a nocturnal species, with a generalist diet (e.g., Ota, 1994; Savage, 2002; Nafus, 2012) usually found near urban environments, inside houses or other buildings (e.g., Hoogmoed and Avila-Pires, 2015; Señaris *et al*., 2017; Behm *et al*., 2019). Its dispersal is likely to be related to human activities, such as transport of horticultural plants and cargoes on ships (Lever, 2003; Powell *et al*., 2011; Powell and Henderson, 2012; Krysko and Mackenzie-Krysko, 2016). Currently, it is known to have spread to Mexico, Central America, and parts of South America, besides some remote regions such as the Galapagos and Easter Islands (Hoogmoed and Avila-Pires, 2015).

In South America, there are records in Colombia (Moreno-Arias *et al.,* 2006; Daza *et al*., 2012; Giovanny Montes *et al*., 2012; Rubio-Rocha *et al*., 2012; Sierra *et al*., 2012), Ecuador (Fugler, 1966; Schauenberg, 1968; Torres-Carvajal, *et al*., 2014), Venezuela (Guerreiro and Graterol, 2011), Surinam (Bauer *et al*., 2007) and northern Brazil (Hoogmoed and Avila-Pires, 2015). For Brazil, seven published records are known, all from the city of Belém, State of Pará, in the Brazilian Amazon (Hoogmoed and Avila-Pires, 2015). The present note extends the known distribution of the introduced gecko *L. lugubris* in Brazil, presenting two other records for the Amazon region, including the first insular record in Brazil, and the first record for the species in the Atlantic Forest in Northeastern Brazil (Fig. 1 and Table 1).

In November 2nd, 2018, an individual of *L. lugubris* was collected by Annelise B. D’Angiolella, in Capitão Poço city, northeast mesoregion of the State of Pará. The capture occurred around 7 PM while the animal was feeding on leftover sugar-based candy inside a dumpster, in a tree of approximately two meters height, located in a residential neighborhood (1°45’7.1676” S; 47°3’50.5728” W). When it was captured, the specimen had its tail curled laterally, a behavior that has also been reported for other gekkos, such as *Hemidactylus frenatus* and *Hemidactylus garnotti* (Dame and Petren, 2006). After collected, the specimen was fixed in formaldehyde 10%, preserved in alcohol 70% and deposited at the Oswaldo Rodrigues da Cunha Herpetological Collection of the Museu Paraense Emilio Goeldi, under the voucher MPEG 32963.

Between March and June 2019, at least three individuals (distinguished by size and state of tail regeneration) of *L. lugubris* (Fig. 2a) were seen by Ben Phalan and Luciana Leite inside their fourth-floor apartment in the city of Salvador, Bahia (12°59’56.983” S 38°31’4.969” W). At least one of

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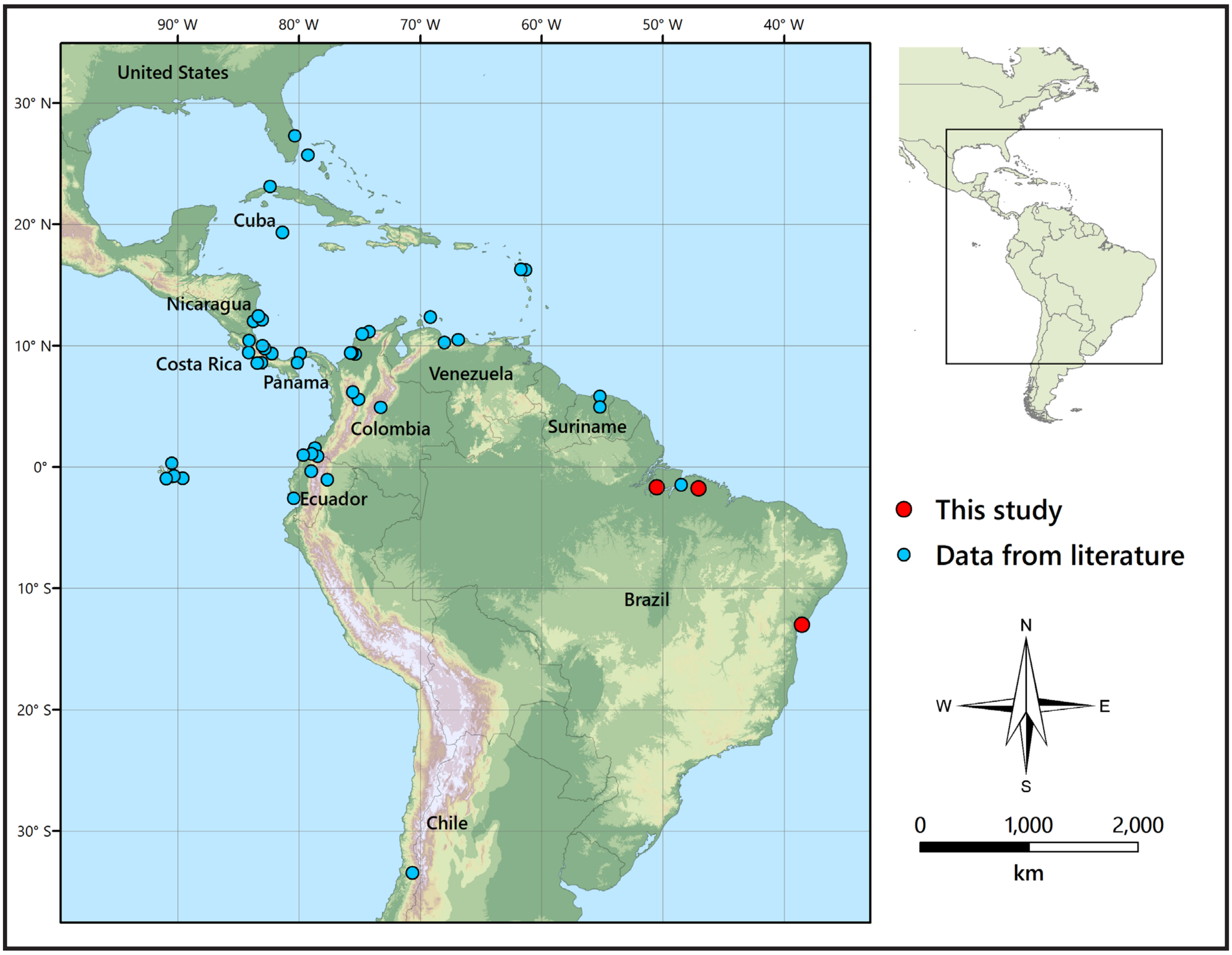

Figure 1. Map of distribution of the gecko *Lepidodactylus lugubris* in the New World. Blue circles represent previous records and red circles represent the new records of Capitão Poço and Breves (State of Pará) and Salvador (State of Bahia), in the Amazon and Atlantic Forest Biome, respectively.

them inhabited a crevice underneath a wooden table during the day, moving in the evenings to a fruit bowl where she was recorded feeding from ripe bananas (available online at https://youtu.be/IJC2RXnNUf0). Individuals were also observed capturing and eating a moth-fly (Diptera: Psychodinae - image available on inaturalist at the link below). and licking condensed droplets of water from a glass bottle. Vocalizations were heard (and recorded) on one occasion, when one called intermittently from a potted bromeliad. This recording and documentation of all other observations are archived on the citizen science site iNaturalist (available online at https://www.inaturalist.org/observations?d1=2019-03-01&d2=2019-06-30&place_id=20640&subview=table&taxon_id=104226).

The third report was performed on June 24th of 2020 by Lywouty Reymond Nascimento in Breves, southwest of Marajó Island, in Pará State. The specimen was seen around 5 PM while initiating its foraging activity inside a room in the residence (1°40’56.222” S; 50°28’58.933” W). When captured, the lizard performed the classical agonistic vocalization and jumping behavior (Señaris *et al.*, 2017). This specimen was photographed (Fig. 2b) and released.

Individuals from Capitão Poço, Breves and Salvador are in accordance with the morphological description of the species made by Hoogmoed and Avila-Pires (2015) and Señaris *et al.* (2017).

Our records expand the distribution of *L. lugubris* in Brazil by 162 km (Capitão Poço), 221.36 km (Breves) and 1,780 km (Salvador) from the first record (Belém). Breves, in Marajó Island, represents the first insular record for the species in Brazil and Salvador is the first record of this species in the Atlantic Forest biome.

The abundance of *L. lugubris* in gardens and houses was suggested to be related to the availability

Table 1. List of records of the gecko *Lepidodactylus lugubris* showed in Figure 1, including location, georeference, and source.

Location	Country	Longitude	Latitude	Reference
Capitão Poço	Brazil	-1.751.991	-47.064.048	This study
Salvador	Brazil	-1.299.916	-38.518.047	This study
Breves	Brazil	-1.682.284	-50.483.037	This study
Belém	Brazil	-1.457.069	-48.496.061	Hoogmoed & Avila-Pires (2015)
Paramaribo	Suriname	5.828.789	-55.180.914	Bauer *et al.* (2007)
Brownsberg plateau	Suriname	494.405	-55.170.631	Hoogmoed & Avila-Pires (2015)
South of El Rodadero	Colombia	11.167.658	-74.230.444	Hoogmoed & Avila-Pires (2015)
Barranquilla	Colombia	10.983.333	-74.816.667	Palacio Sierra *et al.* (2012)
Sincelejo	Colombia	93.025	-75.383.889	Montes *et al.* (2012)
Coveñas	Colombia	9.425.278	-756.225	Montes *et al.* (2012)
Cispata Bay	Colombia	940.355	-75.763.211	Moreno-Arias *et al.* (2007)
Santa Maria	Colombia	4.893.517	-73.282.033	Moreno-Arias *et al.* (2006)
Vereda Puente Lindo	Colombia	5.571.889	-75.123.778	Rubio-Rocha *et al.* (2012)
Medellin	Colombia	6.190.167	-75.581.778	Rubio-Rocha *et al.* (2013)
Tumaco	Colombia	1.544.167	-78.698.056	Pinto-Erazo (2020)
Puerto Misahualli	Ecuador	-1.032.967	-77.669.347	Torres-Carvajal *et al.* (2014)
Alluriquin	Ecuador	-0.322908	-78.995.275	Torres-Carvajal *et al.* (2014)
Lita	Ecuador	0.879125	-78.471.439	Torres-Carvajal *et al.* (2014)
Esmeraldas	Ecuador	1.089.333	-78.989.878	Fugler (1966)
Borbon	Ecuador	0.968917	-7.965.165	Schauenberg (1968)
Puerto Baquerizo Moreno	Ecuador	-0.901119	-89.611.003	Olmedo & Cayot (1992)
Puerto Ayora	Ecuador	-0.743539	-9.031.075	Olmedo & Cayot (1991)
Marchena	Ecuador	0.291483	-90.492.992	Jiménez-Uzcátegui (2014)
Puerto Villamil	Ecuador	-0.957153	-90.967.003	Olmedo & Cayot (1990)
Playas Canton	Ecuador	-2.600.000	-80.433.333	Cuadrado *et al.* (2020)
Colón	Panama	9.353.014	-79.903.719	Fugler (1966)
El Valle de Antón	Panama	8.600.903	-80.129.619	Köhler (2002)
Bocas del Torro	Panama	9.340.692	-82.240.606	Lotzkat (2010)
Golfito	Costa Rica	8.604.261	-83.113.378	Savage (2003)
Osa Peninsula	Costa Rica	8.564.414	-83.465.369	Savage (2004)
Cahuita	Costa Rica	9.738.306	-82.840.839	Mayer (2010)
Quepos	Costa Rica	9.432.039	-84.161.181	Savage (2002)
Limón	Costa Rica	9.991.139	-83.035.489	Hoogmoed & Avila-Pires (2015)
La Virgen of Sarapiquí	Costa Rica	1.041.691	-8.412.477	Jiménez & Abarca (2014)
Bluefields	Nicaragua	12.015.267	-83.760.161	Henderson *et al.* (1976)
Great Corn Island	Nicaragua	12.173.253	-83.052.247	Henderson *et al.* (1977)
Pearl Key	Nicaragua	12.471.406	-83.381.392	Villa (1993)
Port St Lucie	U.S.A.	27.289.111	-8.036.475	Krysko *et al.* (2011)
Grande-Terre	French West Indies	1.624.545	-6.129.411	Lorvelec *et al.* (2011)
Basse-Terre	French West Indies	1.631.739	-6.169.778	Parmentier *et al.* (2013)
Havana	Cuba	23.117.056	-82.402.278	Bosch & Paéz (2017)
Caracas	Venezuela	10.488.694	-66.880.889	Señaris *et al.* (2017)
Carabobo	Venezuela	10.270.972	-68.001.556	Guerrero & Graterol (2011)
Curaçao	Antilhas Holandesas	123.632	-691.547	Behm *et al.* (2018)

Cayman islands	Mar das Caraíbas	19.352.306	-81.381.111	Goetz & Burton (2018)
Santiago	Chile	-33.444.683	-70.648.408	Urra *et al.* (2020)
Sapodilla Bay	Bahamas	2.174.089	-7.228.417	Ruhe & Ruhe (2019)
Marsh Harbour	Bahamas	26.544.272	-77.049.793	Giery *et al.* (2019)
Island of North Bimini	Bahamas	25.727.001	-79.295.807	Krysko & Krysko (2016)

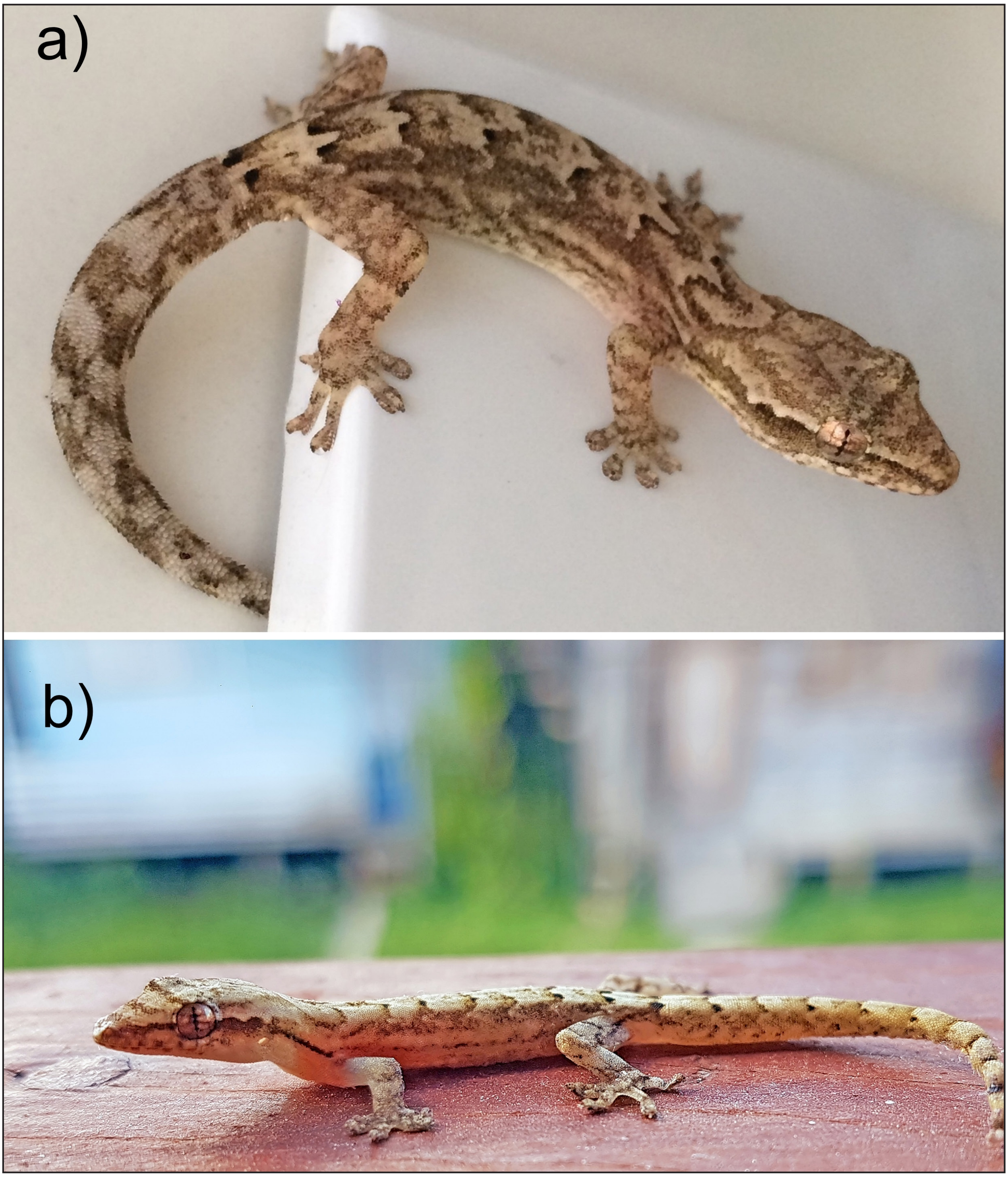

Figure 2. Individuals of *Lepidodactylus lugubris* collected in the present study. a) in the municipality of Salvador, Bahia State, Brazil, photographed by Ben Phalan and b) in the municipality of Breves, Marajó Island, Pará State, Brazil, photographed by Lywouty Reymond Nascimento.

of food resources, especially fruit flies (*Drosophila* spp.) and ripe fruits (Señaris *et a*., 2017). Our observations corroborate the consumption of both insect prey and sugary substances by this species, with its generalist diet as a possible cause for the species' success in colonizing new areas.

We believe our records represent independent colonizations, with specimens arriving in Capitão Poço in auto-vehicles coming from Belém, while the colonization of Breves and Salvador may have occurred by ships, since both represent port cities, similar to others already colonized by the species. Breves is the capital of Marajó Island and the access to the city is primarily made by ships or by planes (least frequent and inaccessible for most people), with no roads connecting the city to the continent. This record reinforces our hypothesis of ship colonization, with the most likely vector for arrival of these individuals being potted plants or soil. Introductions of *L. lugubris* have been described as accidental or as a consequence of the pet trade, so the latter alternative cannot be discarded for the new records presented here.

The fast expansion experienced by this species across the Brazilian territory, leads us to believe that *L. lugubris* has already spread to other states, or even biomes, across north and northeast of Brazil, even though its occurrence is yet to be documented in these regions.

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