

## Sexual dimorphism in *Amphisbaena nigricauda* (Reptilia, Squamata, Amphisbaenidae) from Southeastern Brazil

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**ABSTRACT.** *Amphisbaena nigricauda* Gans, 1966 is a small, poorly known amphisbaenid endemic to the *restinga* of the states of Espírito Santo and Bahia, Brazil. We analyze 178 specimens collected in Vitória municipality, state of Espírito Santo, Brazil, to investigate whether this species show sexual dimorphism in pre-cloacal pores and in morphological characters. Sex was determined by a ventral incision and direct inspection of gonads. A PCA analysis was performed to generate a general body size measurement. A T test and the non-parametric Mann-Whitney test were used to assess whether this species show sexual dimorphism on five morphometric and five meristic characters, respectively. Sex could not be determined in 36 specimens because they were mutilated in the posterior portion of their bodies. The diagnosis of the species is redefined based on this sample size: the smallest number of body annuli changes from 222 to 192, the number of dorsal and ventral segments in an annulus in the middle of the body changes to 9–11/13–16 (instead of 10/16), and the autotomic tail annulus lies between annulus 7–10 (instead of 6–9). The number of tail annuli remained within the known range of variation of the species (19–24). None of the 80 females analyzed showed pre-cloacal pores, whereas within males 59 out of 62 specimens displayed four and two specimens displayed five pre-cloacal pores. A single male did not possess pre-cloacal pores, but showed irregular scales on its cloacal region. Sex-based difference based on presence or absence of pre-cloacal pores as well as males with wider head was seen in other Neotropical amphisbaenids. However, a pattern of body size differences between males and females has not been identified so far in the few amphisbaenid species studied in this regard. Further studies on this taxonomic group are still needed to elucidate the existence of general patterns of sexual dimorphism and to identify the selective pressures driving these patterns.

**KEYWORDS.** Amphisbaenia, Espírito Santo, restinga, sexual dimorphism.

**RESUMO.** Dimorfismo sexual em *Amphisbaena nigricauda* (Reptilia, Squamata, Amphisbaenidae) do Sudeste do Brasil. *Amphisbaena nigricauda* Gans, 1966 é uma pequena anfisbênia pouco conhecida endêmica da restinga dos estados do Espírito Santo e Bahia, Brasil. Analisamos 178 indivíduos coletados no município de Vitória, Espírito Santo, Brasil, para verificar se esta espécie apresenta dimorfismo sexual nos poros pré-cloacais e em caracteres morfológicos. O sexo foi determinado por uma incisão ventral e inspeção direta das gônadas. Uma análise de PCA foi realizada para gerar uma medida geral do tamanho do corpo. O teste-T e o teste de Mann-Whitney não paramétrico foram utilizados para avaliar se esta espécie apresenta dimorfismo sexual em cinco caracteres morfométricos e cinco merísticos, respectivamente. O sexo não pode ser determinado em 36 indivíduos porque eles estavam mutilados na parte posterior do corpo. O diagnóstico da espécie é redefinido com base nesta amostra: o menor número de anéis corporais alterou de 222 para 192, o número de segmentos dorsal e ventral em um anel no meio do corpo mudou de 9-11/13-16 (ao invés de 10/16), e o anel autotômico na cauda encontra-se entre 7-10 (ao invés de 6-9). O número de anéis caudais permaneceu dentro do intervalo de variação conhecida para a espécie (19-24). Nenhuma das 80 fêmeas analisadas apresentou poros pré-cloacais, enquanto que, dentro os 62 machos, 59 apresentaram quatro e dois indivíduos cinco poros pré-cloacais. Um único macho não tinha poros pré-cloacais, mas apresentava escamas irregulares em sua região cloacal. Diferença sexual quanto à presença ou ausência de poros pré-cloacais, assim como machos apresentando cabeças mais largas que a fêmeas, foi observada em outros anfisbênidos neotrópicos. No entanto, um padrão de diferenças de tamanho corporal entre machos e fêmeas não tinha sido identificado até agora nas poucas espécies de anfisbênias estudadas. Outros estudos com este grupo taxonômico ainda são necessários para elucidar a existência de padrões gerais de dimorfismo sexual e identificar as pressões seletivas que resultam estes padrões.

**PALAVRAS-CHAVE.** Anfisbênias, Espírito Santo, restinga, dimorfismo.

Amphisbaenia is a monophyletic group (TOWNSEND *et al.*, 2004) of fossorial reptiles (Squamata) represented by 184 taxa (UETZ, 2014). Among the six families (VIDAL *et al.*, 2008), the Amphisbaenidae include the majority ( $n = 169$ , 91.8%) of amphisbaenian species (UETZ, 2014) distributed mainly in Africa, Central and South America (STRÜSSMANN & MOTT, 2009). Brazil harbors the highest diversity of amphisbaenians worldwide (MOTT & VIEITES, 2009) with 69 species currently catalogued in the country (BÉRNILS & COSTA, 2012; ROBERTO *et al.*, 2014).

Due to amphisbaenians' fossorial habits, collecting specimens is frequently difficult. Consequently, the descriptions of many species are based on a limited number of specimens and almost all aspects of their basic biology

and ecology are poorly known (VEGA, 2001; NAVEGA-GONÇALVES, 2009). The typically small number of known specimens also limits knowledge of morphological variation in many species, including sex-based differences.

The morphometric studies of amphisbaenids that have been performed indicate that sexual dimorphism may be common. WEBB *et al.* (2000) compared total length and head width of two African species *Monopeltis anchietae* (Bocage, 1873) and *Zygaspis quadrifrons* (Peters, 1862) (Amphisbaenidae), observing that females of *Monopeltis* have wider head than males and females of *Zygaspis* have larger body than males. Conversely, GIL *et al.* (1993) and MARTIN *et al.* (2012) observed that males of *Blanus cinereus* (Vandelli, 1797) (Blanidae) and *Trogonophis*

*wiegmanni* Kaup, 1830 (Trogonophiidae) respectively have wider head than females. For *Bipes biporus* (Cope, 1894) (Bipedidae), head shape does not differ between sexes (KEARNEY, 2003). For Neotropical amphisbaenids, sex-based morphological differences were investigated for *Amphisbaena alba* Linnaeus, 1758 (COLLI & ZAMBONI, 1999); *Amphisbaena kingii* (Bell, 1833) and *Amphisbaena munoai* Klappenbach, 1960 (BALESTRIN & CAPPELLARI, 2011); *Amphisbaena ibijara* Rodrigues, Andrade & Lima, 2003 and *Leposternon polystegum* (Duméril, 1851) (GOMES *et al.*, 2009); *Leposternon wuchereri* (Peters, 1879) and *Leposternon microcephalum* Wagler, 1824 (FILOGONIO *et al.*, 2009); and *Amphisbaena prunicolor* (Cope, 1885) (MACIEL *et al.*, 2012). These studies generally found that females are larger than males (exception to *Leposternon microcephalum* whereas total length do not differ, and *L. polystegum* whereas males are larger than females), following the same pattern found in other squamate reptiles (SHINE, 1994). Another sexual dimorphism detected in amphisbaenids is related to the presence/absence of pre-cloacal pores: in contrast to males some female amphisbaenids such as *Amphisbaena kingii* (VEGA, 2001), *A. dubia* L. Müller, 1924, *A. heathi* Schmidt, 1936 (VANZOLINI, 2002), *A. uroxena* Mott, Rodrigues, Freitas & Silva, 2008 and *A. anaemariae* Vanzolini, 1997 (MOTT *et al.*, 2011) do not show pores.

*Amphisbaena nigricauda* Gans, 1966 is a small and slender amphisbaenid (maximum Snout Vent Length= 105

mm) endemic to the states of Espírito Santo and Bahia, Brazil (DIAS & ROCHA, 2005; SILVA-SOARES *et al.*, 2011). The type series consists of three specimens (a female, a juvenile and a male) collected in 1964 at Sooretama Refuge (now the *Reserva Biológica de Sooretama* – Fig. 1) in the municipality of Linhares (19°00'S, 40°08'W), state of Espírito Santo, Brazil. A large series of this species was obtained in the municipality of Vitória, Espírito Santo State by one of the authors (JLG) - one of the specimens from this series was used to extend the species known distribution ca. 135 km southeast (SILVA-SOARES *et al.*, 2011). Here, we use this series of 178 specimens to analyze variation in morphological characters and to assess sex-based differences in this poorly known species.

## MATERIAL AND METHODS

All specimens of *Amphisbaena nigricauda* were collected from July 2005 to November 2006 when vegetation was suppressed by bulldozers during expansion of airport facilities in the *restinga* (coastal sand-dune vegetation) of *Praia de Camburi* (20°16'16"S, 40°16'35"W), Vitória, Espírito Santo, Brazil (Fig. 1). Vitória municipality comprises a main area located on an island within a bay, and a northern portion located on the mainland, where *Camburi* is located. The climate is *Aw Tropical* (KÖPPEN, 1936) with an annual mean temperature of 23°C and precipitation of

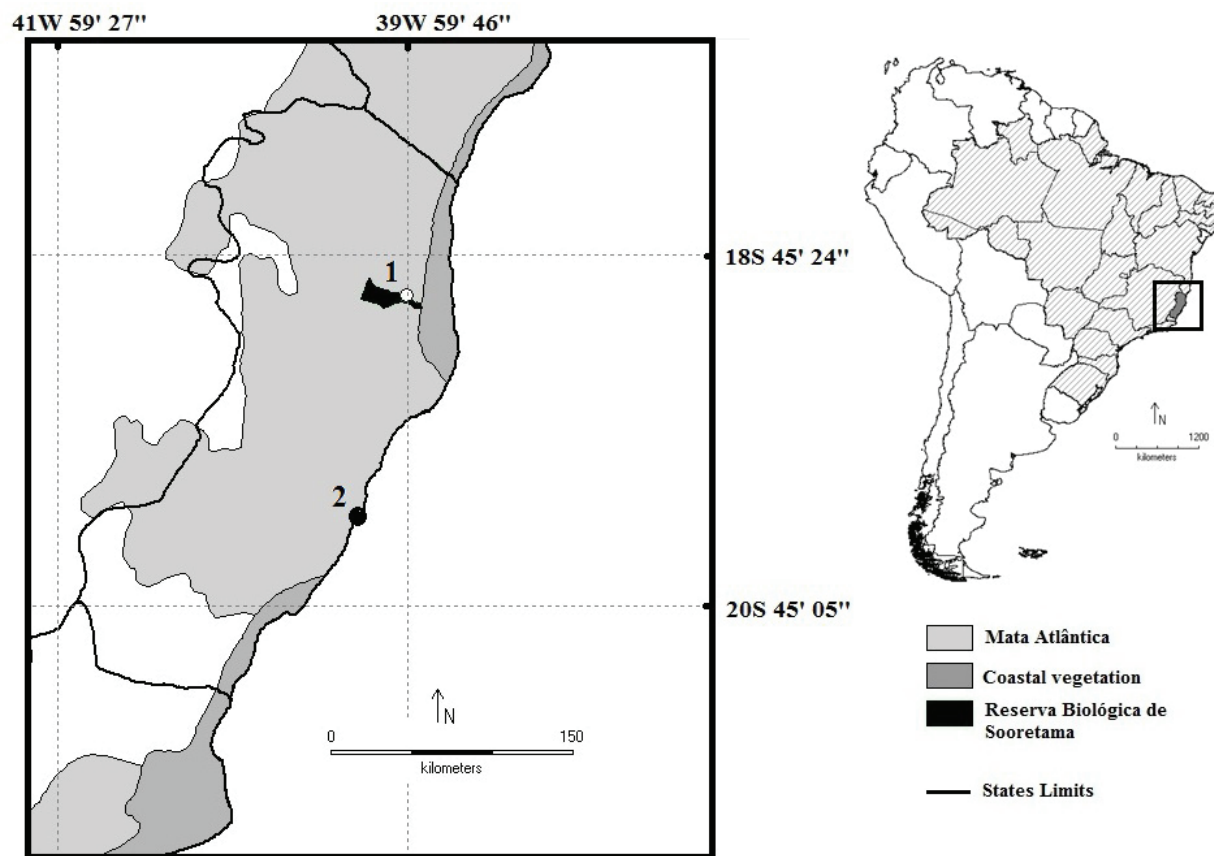


Fig. 1. Geographic distribution of *Amphisbaena nigricauda* Gans, 1966: 1, type locality, Linhares municipality; 2, Vitória municipality. Both records are located in the state of Espírito Santo, Brazil. Squared polygon in the South American inset map highlights the state of Espírito Santo, Brazil.

around 1,230 mm (SILVA-SOARES *et al.*, 2011).

Specimens obtained were kept under observation for one hour; individuals in apparently healthy conditions were released, and those damaged were euthanized with xylocaine, fixed in 5% formalin, and transferred to 70% alcohol. Specimens are housed either at *Coleção Zoológica de Vertebrados da Universidade Federal de Mato Grosso, campus Cuiabá* (CZV-UFMT), Cuiabá, state of Mato Grosso, Brazil, and *Museu de Biologia Professor Mello-Leitão* (MBML), Santa Teresa, state of Espírito Santo, Brazil (Appendix). Collection permits were granted by the Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis – IBAMA/ES: NUFAUNA no. 38/06 and 10/05, processes 2281/05-22 and 3471/06.

Counts of body annuli were made along the left side of the ventral surface following GANS & ALEXANDER (1962). The following meristic data taken for each specimen: body annuli (BA), intercalated annuli (IA), annuli interrupted by the cloacal region (AIC), tail annuli (TA), autotomic annuli (AA), dorsal/ventral segments in an annulus at midbody (D/V), and number of pre-cloacal pores (PP). Morphometric data were taken using a metric tape or a digital paquimeter (accuracy of 0.01 mm) and consisted of snout-vent length (SVL), tail length (TL), head width (HW), head height (HH), and total length (LT).

Sex was determined by gonad inspection through a ventral incision of ca. 5 cm. Although precise data on collecting was not available, we determined the reproductive stage (mature or immature) of each specimen, the position of testes and the number of eggs or follicles. Males with convoluted vas deferens and females with either ovarian follicle larger than one millimeter or oviduct eggs were considered reproductive, all other individuals were considered non-reproductive. All data were taken by one of the authors (FANSL) using a stereomicroscope.

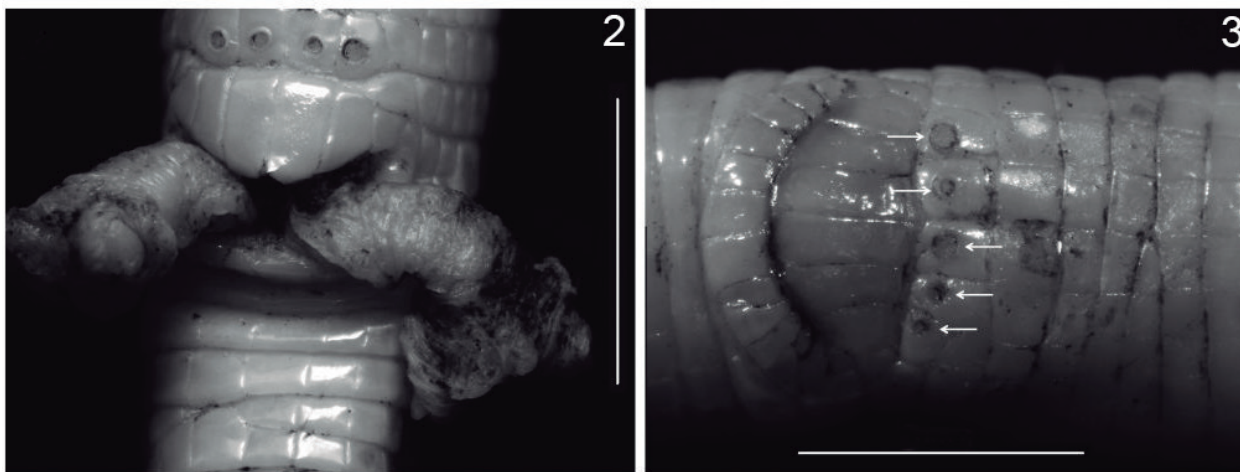
The presence or absence of pre-cloacal pores was analyzed in all non-mutilated specimens. Within this sample, differences between sexes were analyzed by two statistical approaches including only specimens

with intact tails. The continuous morphometric data was analyzed by T tests, whereas the discrete meristic data were analyzed by non-parametric Mann-Whitney tests. Since the morphometric variables were expected to be highly correlated, they were first subject of a Principal Component Analysis (PCA), and further tests were performed with the most informative PCA axes rather than raw data. In order to account for the possible correlation between meristic data, the non-parametric Spearman correlation was calculated between tail annuli, dorsal and ventral segments in an annulus, the number of annuli interrupted by the cloacal region and the number of body annuli. All data analysis was performed using R environment (R CORE TEAM, 2013).

## RESULTS

A total of 178 specimens of *Amphisbaena nigricauda* were analyzed, 36 of which were excluded because they were mutilated. Based on these specimens the morphological diagnosis of *A. nigricauda* is redefined: the smallest number of body annuli changes from 222 to 192, the number of dorsal and ventral segments in an annulus in the middle of the body changes from 10/16 to 9–11/13–16, and the autotomic tail annulus lies between annulus 7 and 10 instead of 6–9. The number of tail annuli (19–22) remained within the range of the known variation (19–24) of the species. The largest specimen known for the species becomes a male of 168 mm (MBML 3092) where previously it was 105 mm (paratype AMNH 97205). Specimens larger than 134 mm in total lengths showed darker brown coloration in the posterior part of their bodies than smaller ones, which have a more uniform brown color.

Eighty females and 62 males (Appendix) of *A. nigricauda* were sufficiently intact for evaluation of presence/absence of pre-cloacal pores. However, only 23 females and 19 males had intact tails and the dimorphism sexual analyses included only these specimens. Males possessed four (n=59) or five (n=2) rounded pre-cloacal pores (Figs 2, 3) - there was one exception (MBML 3082),



Figs 2, 3. Cloacal region of males of *Amphisbaena nigricauda* Gans, 1966 from Vitória municipality, state of Espírito Santo, Brazil: 2, MBML 3131 with four round pre-cloacal pores and an everted hemipenes; 3, MBML 3096 with five pre-cloacal pores (white arrows). Bars: 3 mm.



where pores were absent in irregular pre-cloacal scales. Pores were absent in all females, although in one female specimen (MBML 3119) pale scars of pores could be seen in the pre-cloacal region.

The first axis of the PCA performed with the morphometric data accounted alone for 79.4% of data variability, and all variable loadings (i.e., the correlation between variables and the axis) were at least 0.84 and were positively correlated with the axis. Thus, this first axis was considered as a general measure of body size and further used in a Welch T test for heterogeneous variances (homogeneity of variances was rejected by a Levene test) to verify if there is a sexual body size dimorphism in this species. Males were found to have larger values at the first PCA axis than females ( $t=3.04$ ,  $df=34.95$  and  $p=0.004$ ), as can be seen in Fig. 4, indicating that males are generally larger. The average total body size difference between sexes was 104.7 mm, with males being 7.5% larger than females. Males also have wider heads than females: on average, males head width was 10% larger than females (2.5 mm). The average measurements and differences between sexes are shown in Tab. I.

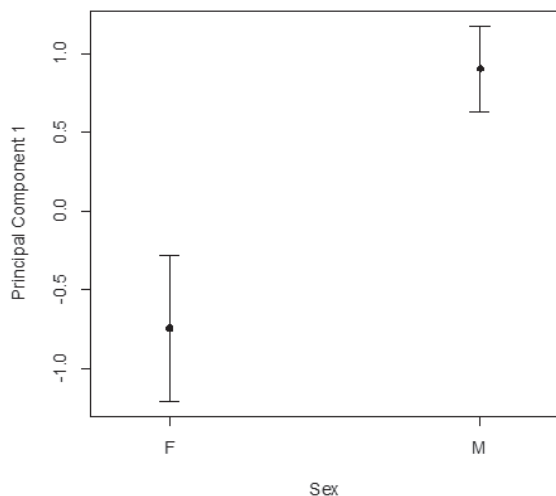


Fig. 4. Average (dots) and standard errors of the first axis of a PCA considering all five morphometric variables of *Amphisbaena nigricauda* Gans, 1966. The axis accounts for 79.4% of data variability and positively represents all morphometric measurements. Males are statistically larger than females when compared by a Welch T test ( $t=3.04$ ,  $df=34.95$  and  $p=0.004$ ).

Tab. I. Average morphometric measurements (in mm) and average differences (raw and percentage differences) from males and females of *Amphisbaena nigricauda* Gans, 1966 (SVL, snout-vent length; TL, tail length; HW, head width; HH, head height; LT, total length).

	Males	Females	Average difference	
			Raw differences	Percentage difference
SVL	131.58	123.04	8.54	6.94
TL	16.26	14.13	2.13	15.09
HW	2.73	2.48	0.25	10.05
HH	2.36	2.22	0.14	6.39
LT	148.74	138.26	10.48	7.58

In contrast, meristic variables were not highly correlated, as can be seen by the Spearman correlations (Tab. II). Differences between sexes within those variables were then analyzed one by one, but no statistically significant results were found by the Mann-Whitney tests performed: AIC, annuli interrupted by the cloacal region ( $W=248.5$ ,  $p=0.36$ ), TA, tail annuli ( $W=216$ ,  $p=0.95$ ), D, dorsal segments in an annulus at midbody ( $W=201$ ,  $p=0.39$ ), V, ventral segments in an annulus at midbody ( $W=255.5$ ,  $p=0.85$ ) and BA, body annuli ( $W=189$ ,  $p=0.46$ ).

Tab. II. Spearman rank correlation between the meristic data of *Amphisbaena nigricauda* Gans, 1966 (AIC, annuli interrupted by the cloacal region; TA, tail annuli; D/V, dorsal/ventral segments in an annulus at midbody; BA, body annuli).

	AIC	TA	V	D	BA
AIC	1				
TA	-0.046	1			
V	0.002	0.108	1		
D	-0.051	-0.136	0.376	1	
BA	0.133	-0.024	0.081	-0.202	1

Of the 80 females without evident pre-cloacal pores, 26 (32.5%) had vitellogenic follicles (either primary or secondary), 12 had one or two eggs (15%) and 42 (52.5%) did not have any follicle or egg and were considered not reproductive. Among males, 44 (70.9%) had the right testicle more cranial than the left testicle, two (3.2%) had left testicles more cranial, and in five of them (8.0%) the testes were symmetrical in position. Thirty-six males (58%) had convoluted vas deferens and 26 (42%) had not.

## DISCUSSION

GANS (1966) defined the diagnostic characters of *Amphisbaena nigricauda* based on the three available specimens as: body annuli 222–226; tail annuli 19–24; autotomic tail annulus 6–9 darker than remaining tail annuli; dorsal/ventral segments at midbody 10/16; intercalated annuli absent; brownish coloration with dorsum slightly darker than ventral region. Our analysis of an additional 178 specimens of *A. nigricauda* provides a more accurate identification of diagnostic characters, expands the known morphological range and indicates sex-based differences in body size. Moreover, although GANS (1966) does not mention the presence of intercalated annuli, these are frequently found in the species - 112 specimens (62.9%) had intercalated annuli.

GANS (1966) analysis of three specimens of both sexes found four and five rounded pre-cloacal pores. Only two male specimens in our analysis displayed five pores and none of the 80 analyzed females had pores.

Sexual dimorphism in body size has been observed in a few amphisbaenian species, although no clear trend has emerged: females can be larger than males, sexes can have similar sizes or males can be larger than females (as we observed for *A. nigricauda*). Given the scarcity of data,

the causes of this variability are uncertain. Larger females may reflect increased fecundity while larger males may be a consequence of male-male competition (combat bouts) for access to females. Similar sizes may reflect constraints imposed by a fossorial lifestyle and more research is still needed to clarify this.

As with body size, there is considerable inter-species variation in sexual dimorphism in head width. Males can have wider head [as seen in *Blanus cinereus* (GIL *et al.*, 1993), *Leposternon microcephalum* and *L. wuchereri* (FILOGONIO *et al.*, 2009), *Trogonophis wiegmanni* (MÁRTIN *et al.*, 2012), and *A. nigricauda* (this study)], narrower head [*Monopeltis anchietae* (WEBB *et al.*, 2000)] or even similar head width [*Amphisbaena alba* (COLLI & ZAMBONI, 1999), *Zygaspis quadrifrons* (WEBB *et al.*, 2000), *Bipes biporus* (KEARNEY, 2003), *Amphisbaena ibijara* and *Leposternon polystegum* (GOMES *et al.*, 2009)]. However, once again, the selective pressures driving these differences are poorly understood. Amphisbaenians are head-first burrowers (GANS, 1969) and head width may be associated either with digging capabilities (speed of burrowing) or with intersexual niche divergence (a larger head may allow the capture of larger prey) or even sexual interactions (male-male combat). There is some evidence that *Blanus cinereus* individuals (a round-headed species) with longer and narrower heads are able to burrow faster (LÓPEZ *et al.*, 1997). NAVAS *et al.* (2004) suggested that the amount of force produced during digging in a shovel-headed amphisbaenian species, *Leposternon microcephalum*, is positively correlated with head width and body length. However, COLLI & ZAMBONI (1999) found no association between prey size and head width in *Amphisbaena alba* and BALESTRIN & CAPPELLARI (2011) found no intersexual differences in volume and diet consumption of *Amphisbaena kingii* and *Amphisbaena munoai* - although the authors did not measure head widths. As reproductive behavior has been rarely observed in amphisbaenians, little can be said regarding the role of male-male competition driving sexual dimorphism.

NAVEGA-GONÇALVES (2009) studied the internal anatomy of six Neotropical amphisbaenids and found that right testicle is more cranial than the left in *Amphisbaena vermicularis*, *A. kingii*, *A. anomala*, *A. bedai*, *A. cuiabana* and *Leposternon microcephalum*. MOTT *et al.* (2011) found the same pattern in *Amphisbaena uroxena*. In the present study we found a higher prevalence of males with the right testicle more cranial than the left. The asymmetrical disposition of elongated paired testes (generally moved forwards) may be because to the need to accommodate the gonads in the elongated body of amphisbaenians (NAVEGA-GONÇALVES, 2009). Further studies on sexual dimorphism on Amphisbaenia are still needed to elucidate if there are general patterns and to identify the selective pressures driving these patterns.

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## APPENDIX

Specimens of *Amphisbaena nigricauda* Gans, 1966 examined [F, female (n = 80); M, male (n = 62); N/D, sex not determinate, mutilated specimens (n = 36); SVL, snout-vent length; TL, tail length].

Institution number	Length (mm)		Annuli			Segments		Number of pores	sex
	Body (SVL)	Tail (TL)	Body	Intercalated	Tail	Dorsal	Ventral		
UFMT 9141	105	4	215	5	8	15	11	-	N/D
UFMT 9142	130	7	218	7	8	15	10	0	F
UFMT 9143	111	5	211	9	8	14	9	0	F
UFMT 9144	-	15	-	-	21	15	10	0	F
UFMT 9145	123	16	212	10	20	14	9	0	F
UFMT 9146	85	10	204	13	22	13	10	0	F
UFMT 9147	100	4	216	2	8	14	9	-	N/D
UFMT 9148	90	12	215	3	21	15	10	0	F
UFMT 9150	0	0	.	.	20	14	9	4	M
UFMT 9151	125	5	218	1	8	15	10	4	M
UFMT 9152	132	15	220	1	21	15	11	4	M
UFMT 9153	-	-	-	-	-	15	11	-	N/D
UFMT 9154	-	-	-	-	-	-	-	-	N/D
UFMT 9155	-	-	-	-	-	-	-	-	N/D
UFMT 9156	127	15	209	1	21	15	9	0	F
UFMT 9157	133	16	208	3	21	15	11	0	F
UFMT 9158	125	7	208	1	8	15	11	0	F
UFMT 9159	132	15	210	3	22	15	10	0	F
UFMT 9160	138	17	218	0	19	15	11	0	F
UFMT 9161	110	13	217	0	21	14	9	0	F
UFMT 9162	128	15	209	2	22	15	10	0	F
UFMT 9163	113	14	212	0	22	15	11	0	F
UFMT 9164	90	4	210	1	8	15	10	0	F
UFMT 9166	128	17	215	1	21	15	9	4	M
UFMT 9167	135	16	208	4	21	15	9	4	M
UFMT 9168	145	6	215	6	8	15	10	4	M
UFMT 9169	109	13	208	2	22	15	9	4	M
UFMT 9170	125	15	213	2	22	16	10	4	M
UFMT 9171	130	15	208	1	21	15	10	4	M
MBML 3022	137	6	205	10	8	14	11	-	N/D
MBML 3023	135	16	211	8	21	15	10	0	F
MBML 3024	124	15	212	1	22	15	10	4	M
MBML 3025	119	13	207	2	22	15	10	0	F
MBML 3026	135	14	210	0	20	15	10	0	F
MBML 3027	118	5	206	4	7	14	10	0	F
MBML 3028	74	8	212	2	21	-	-	-	N/D

## APPENDIX (cont.)

MBML 3029	121	16	206	10	22	14	9	4	M
MBML 3030	131	5	210	0	8	15	11	0	N/D
MBML 3031	126	15	211	0	21	15	9	4	M
MBML 3032	146	5	208	1	7	15	10	0	F
MBML 3033	145	27	205	3	22	15	11	4	M
MBML 3034	137	5	217	1	8	15	11	0	F
MBML 3035	135	7	217	0	8	15	11	4	M
MBML 3036	134	6	212	1	7	15	11	0	F
MBML 3037	137	6	216	3	8	15	10	0	F
MBML 3038	136	7	211	3	8	16	11	4	M
MBML 3039	144	17	211	0	22	15	11	0	F
MBML 3040	127	5	205	4	8	15	9	0	F
MBML 3041	116	3	214	6	8	15	11	0	F
MBML 3042	132	5	200	6	8	15	10	0	F
MBML 3043	142	17	219	4	22	15	11	0	F
MBML 3044	122	5	192	19	7	15	11	0	F
MBML 3045	150	17	213	0	21	15	11	0	F
MBML 3046	135	16	220	2	21	15	10	0	F
MBML 3047	142	16	208	2	21	15	11	4	M
MBML 3048	134	6	206	3	8	15	11	0	F
MBML 3049	138	7	212	2	8	13	9	4	M
MBML 3050	115	15	218	5	22	15	11	0	F
MBML 3051	92	3	205	1	8	15	9	0	F
MBML 3052	140	5	202	3	8	15	9	4	M
MBML 3053	112	5	209	3	8	14	11	4	M
MBML 3054	121	5	213	1	8	15	9	4	M
MBML 3055	152	5	220	0	8	15	11	4	M
MBML 3056	149	6	218	1	8	15	11	4	M
MBML 3057	118	11	216	6	22	15	11	-	N/D
MBML 3058	132	16	210	2	21	15	11	4	M
MBML 3059	124	6	209	1	8	15	11	4	M
MBML 3060	127	15	208	5	21	15	10	0	F
MBML 3061	-	-	-	-	-	-	-	-	N/D
MBML 3062	-	15	-	-	22	-	-	4	M
MBML 3063	-	15	210	2	21	13	9	4	M
MBML 3064	-	-	-	-	-	-	-	-	N/D
MBML 3065	-	-	-	-	-	-	-	-	N/D
MBML 3066	-	14	-	-	21	-	-	4	M
MBML 3067	-	-	-	-	-	-	-	-	N/D
MBML 3068	-	-	-	-	-	-	-	-	N/D
MBML 3069	-	4	-	-	8	-	-	-	N/D
MBML 3070	-	-	-	-	-	-	-	-	N/D
MBML 3071	-	-	-	-	-	-	-	-	N/D
MBML 3072	-	-	-	-	-	-	-	-	N/D
MBML 3073	-	-	-	-	-	-	-	-	N/D
MBML 3074	-	17	-	-	22	-	-	4	M
MBML 3075	141	5	211	-	8	15	11	0	F
MBML 3076	135	15	204	13	20	15	10	0	F
MBML 3077	145	18	218	2	23	15	11	4	M
MBML 3078	134	6	212	8	8	15	10	4	M
MBML 3079	127	5	216	0	7	15	11	0	F
MBML 3080	140	7	216	1	9	15	11	0	F
MBML 3081	130	5	214	4	8	15	11	0	F
MBML 3082	131	14	206	15	20	15	11	0	M
MBML 3083	135	15	220	2	21	15	11	0	F
MBML 3084	112	4	217	0	7	15	11	0	F
MBML 3085	128	5	215	1	8	15	11	0	F
MBML 3086	139	6	216	0	8	15	11	4	M
MBML 3087	147	5	217	3	7	15	11	0	F

## APPENDIX (cont.)

MBML 3088	126	5	201	16	8	15	11	0	F
MBML 3089	133	5	218	0	8	15	11	0	F
MBML 3090	118	4	216	6	8	15	11	0	F
MBML 3091	142	8	215	1	9	15	11	4	M
MBML 3092	146	18	212	0	23	15	11	4	M
MBML 3093	137	15	212	7	21	15	11	0	F
MBML 3094	132	17	207	3	22	15	11	0	F
MBML 3095	131	5	211	1	8	15	11	0	F
MBML 3096	135	17	216	1	22	15	11	5	M
MBML 3097	135	6	209	7	8	15	11	0	F
MBML 3098	137	7	220	0	8	15	9	4	M
MBML 3099	138	16	219	2	22	15	11	4	M
MBML 3100	127	15	215	1	22	15	9	4	M
MBML 3101	114	7	216	5	22	15	11	0	F
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MBML 3103	125	5	222	0	8	-	-	0	F
MBML 3104	111	13	213	2	21	15	11	0	F
MBML 3105	136	16	217	3	21	15	11	0	F
MBML 3106	130	6	216	0	8	15	11	4	M
MBML 3107	131	15	216	3	21	15	11	4	M
MBML 3108	123	5	216	0	8	15	11	4	M
MBML 3109	136	6	220	9	8	14	11	4	M
MBML 3110	137	17	207	1	22	15	11	4	M
MBML 3111	137	17	219	1	23	15	11	4	M
MBML 3112	133	7	215	1	8	15	11	4	M
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MBML 3114	121	15	213	4	21	-	-	4	M
MBML 3115	126	16	216	2	22	15	10	4	M
MBML 3116	135	5	217	1	8	14	11	0	F
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MBML 3118	134	6	212	2	8	15	11	4	M
MBML 3119	136	17	217	1	21	15	11	0	F
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MBML 3123	130	15	214	3	22	15	11	4	M
MBML 3124	100	4	213	2	8	-	-	0	F
MBML 3125	126	5	205	5	8	15	11	4	M
MBML 3126	104	4	214	5	7	15	11	0	F
MBML 3127	90	11	215	3	22	15	11	0	F
MBML 3128	140	17	215	2	22	15	11	4	M
MBML 3129	126	6	212	0	8	15	11	0	F
MBML 3130	131	4	215	7	7	15	11	0	F
MBML 3131	121	14	208	11	21	14	11	4	M
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MBML 3135	107	13	209	19	21	15	11	0	F
MBML 3136	90	3	226	1	7	14	10	0	F
MBML 3137	126	14	219	0	20	15	10	4	M
MBML 3138	95	12	214	4	22	15	11	0	F
MBML 3139	125	5	212	5	8	15	11	0	F
MBML 3140	136	18	216	1	22	15	11	4	M
MBML 3141	132	16	212	1	22	15	9	0	F
MBML 3142	-	16	-	-	22	-	-	0	F
MBML 3143	-	-	-	-	-	-	-	-	N/D
MBML 3144	-	-	-	-	-	-	-	-	N/D
MBML 3145	-	15	-	-	22	-	-	0	F
MBML 3146	-	6	-	-	9	-	-	4	M



## APPENDIX (cont.)

MBML 3147	-	6	-	-	8	-	-	4	M
MBML 3148	-	-	-	-	-	-	-	-	N/D
MBML 3149	-	-	-	-	-	-	-	-	N/D
MBML 3150	-	-	-	-	-	-	-	-	N/D
MBML 3151	-	6	-	-	8	-	-	4	M
MBML 3152	-	-	-	-	-	-	-	-	N/D
MBML 3153	-	-	-	-	-	-	-	-	N/D
MBML 3154	-	-	-	-	-	-	-	-	N/D
MBML 3155	-	15	-	-	21	-	-	5	M
MBML 3156	-	-	-	-	-	-	-	-	N/D
MBML 3157	-	-	-	-	-	-	-	-	N/D
MBML 3158	-	-	-	-	8	-	-	4	M
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MBML 3160	-	-	-	-	-	-	-	-	N/D
MBML 3161	-	5	-	-	8	-	-	-	N/D
MBML 3162	-	4	-	-	8	-	-	0	F
MBML 3163	-	6	-	-	7	-	-	0	F
MBML 3164	-	-	-	-	-	-	-	-	N/D
MBML 3165	-	-	-	-	-	-	-	-	N/D
MBML 3166	-	14	-	-	20	-	-	-	N/D
MBML 3167	-	16	209	16	21	-	-	0	F
MBML 3168	132	4	211	8	8	15	10	0	F
MBML 3169	143	6	224	3	8	15	10	0	F
MBML 3170	-	-	-	-	-	-	-	-	N/D