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Notes on sexual dimorphism, diet and reproduction of the false coral snake *Oxyrhopus rhombifer* Duméril, Bibron & Duméril, 1854 (Dipsadidae: Pseudoboini) from coastal plains of Subtropical Brazil

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Abstract. Herein, we provide information on diet, sexual dimorphism and reproductive biology of the false coral snake *Oxyrhopus rhombifer* from the southernmost Brazilian coast, Pampa Biome, a region under influence of subtropical climatic domains. The analysis of 142 specimens revealed a marked sexual dimorphism, with significant differences for all the characters analyzed. The diet of the species is composed by small rodents and squamate reptiles, the latter consisting mainly of lizard tails, probably autotomized. Males reached sexual maturity at smaller sizes (SVL) than females (354 mm vs 451 mm, respectively). Secondary follicles were found mainly in spring months and one single female presented 3 oviductal eggs in January (summer). Our results add to the knowledge on biology of snakes in the Pampa morphoclimatic domain.

Keywords. Feeding habits, reproductive biology, Pampa Biome, Squamata, Xenodontinae.

Investigating the natural history of species is essential for understanding ecological processes at different levels and provides crucial information for species conservation (Greene, 1993; Gaiarsa et al., 2013). Regarding Neotropical snakes, the last decades have experienced an increment in the number of studies concerning diverse traits of natural history and sexual dimorphism, but these are still fragmented and incipient, considering the group's high diversity (Barbo et al., 2011; Gaiarsa et al., 2013).

The *Oxyrhopus* genus comprises 14 species of medium-sized pseudoboines distributed from southeastern Mexico to central Argentina (Lynch, 2009; Uetz et al., 2017). Most *Oxyrhopus* species exhibit a coral snake mimetic pattern (Savage and Slowinski, 1992), responsible for the popular name 'false coral snakes'. *Oxyrhopus rhombifer* (Fig. 1) is the southernmost distributed *Oxyrhopus*, ranging from southeastern Brazil to central Argentina (province of Buenos Aires) (Giraudo, 2001). There are few scattered data available *O. rhombifer* diet and reproductive biology (Vidal, 2002; Maschio et al., 2003, 2004; Sawaya et al., 2003, 2008; Gaiarsa et al., 2013) and no data on its sexual dimorphism. Herein, we examined size at sexual maturity, female reproductive cycle, feeding habits and sexual dimorphism of *O. rhombifer* from southernmost Brazilian coastal environments.

Specimens were collected along a stretch of ca. 470 km of the coastal plain of the Rio Grande do Sul state (30°15'44"S, 50°28'03"W; 33°40'27"S, 53°30'52"W), southern Brazil, between August 2008 and October 2017. Sampling method consisted in active search performed by two researchers in 77 field trips with an average duration from two to 11 hours (average of four hours).

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Fig. 1. *Oxyrhopus rhombifer* (unvouchered) from Rio Grande, Rio Grande do Sul state, southern Brazil.

Searches were conducted on foot and by car at low speed (20-40 km/h) during day and night periods. This region is inserted in the Pampa biome, under "subtemperate humid" and "temperate humid" climatic domains according to the regional classification by Maluf (2000). Seasons are well-defined, with monthly average air temperature ranging from 13 °C in the winter (July) to 22 °C in the summer (January). Average rainfall is 1,271 mm and rain is distributed mainly from June to October (Climate-Data, 2019). Predominant vegetation types are shrub grassland, and other phytophysiognomies with less coverage include the coastal peat and sandy forests (*restinga* forests) and psamophyte formations of coastal dunes.

Specimens were euthanized through intraperitoneal injection of pentobarbital sodium 50 mg/ml, in accordance with guidelines from international protocols. This procedure had been authorized in our laboratory since 2006 and it is in accordance with the institutional committee for the use of animals in research (CEUA-FURG). Collection was permitted by the Brazilian environmental agency (ICMBio process nº 43658-1). The snakes had to be euthanized for the analyses of the reproductive condition and digestive tract content and for the availability of specimens and tissues for the projects "Taxonomic revision and systematics of *Oxyrhopus rhombifer* (Serpentes: Dipsadidae)" and "Male reproductive cycle of *Oxyrhopus rhombifer* (Serpentes: Dipsadidae) in Southern Brazil". *Oxyrhopus rhombifer* is classified as Least Concern by IUCN (Arzamendia et al., 2019). All specimens are deposited in the herpetological collection of Universidade Federal do Rio Grande (CHFURG) (Appendix).

Sex was determined through verification of the presence or absence of hemipenes via subcaudal incision. The following measurements were taken from each specimen, with a digital caliper to the nearest 0.01 mm: snout-vent length (SVL), tail length (TL), head length (HL), head width (HW), inter-ocular length (IOL), inter-nostril length (INL). We examined the existence of sexual dimorphism in the following characters: 1) SVL; 2) tail proportion in relation to body length (ratio TL/SVL); 3) number of ventral scales; 4) number of subcaudal scales; 5) head length in proportion to body length (ratio HL/SVL); 6) head width in proportion to head length (ratio HW/HL); 7) inter-ocular length in relation to head length (ratio IOL/HL); 8) inter-nostril length in relation to head length (ratio INL/HL). All database was checked for normality through Shapiro-Wilk tests, which detected normal distributions only for SVL, TL/SVL, and number of subcaudal scales. For these characters, the existence of significance differences between males and females was examined with a Student's *t* test. For the other characters (HL/SVL, HW/HL, IOL/HL, INL/HL and number of ventral scales) we applied a Mann-Whitney test aiming to verify significant differences between the sexes. Only mature specimens were examined for sexual dimorphism in SVL, TL/SVL, HL/SVL, HW/HL, IOL/HL and INL/HL. Mature and immature specimens were pooled for the analysis on sexual dimorphism in the number of ventral and subcaudal scales. Statistical tests were performed in software PAST v.3.25 (significance $P < 0.05$). (Hammer et al., 2001).

A ventral incision was made from the esophageal region to around 5 mm above the cloaca and the digestive and reproductive tracts were externalized for analysis. All stomach and gut contents were removed and identified to the lowest possible taxonomic category. Whenever possible, the direction of prey intake (head first or tail first) was annotated. Numeric abundance (N%) of all consumed taxa was determined as the ratio between the absolute number of prey from one taxon and the sum of prey of all identified taxa (Corrêa et al., 2016).

The following reproductive data were recorded from females: total number of ovarian follicles; number of follicles in secondary vitellogenesis (secondary follicles are defined as enlarged follicles with yolk accumulation; diameter ≥ 9 mm, based on the scatterplot of the largest follicles of all females and on coloration [Almeida-Santos et al., 2014]); diameter of the largest secondary follicle; total number of eggs; occurrence of celomatic fat storage; and largest diameter of the largest egg. Females were considered mature when showing at least one of the following characteristics: 1) secondary follicles; 2) oviductal eggs; and 3) folded oviducts, indicating recent oviposition. Males were considered mature when presented coiled and opaque *ductus deferens*, indicating the presence of sperm. The female reproductive cycle was evaluated by: 1) analysis of the distribution of females

Table 1. Summary statics of the variables analyzed for sexual dimorphism in *Oxyrhopus rhombifer* from southern Brazilian coast (Rio Grande do Sul state) and results of Student'*t* and Mann-Whitney tests performed on the variables. Analyses on SVL, TL/SVL, HL/SVL, HW/HL, IOD/HL and IND/HL were performed in mature specimens. Analyses on number of ventral and subcaudal scales were performed on both mature and immature specimens. See main text for abbreviations; n = number of specimens.

Variable	Males	Females	test value	P value
SVL	445 ± 59 (354-597) n = 52	616 ± 92 (451-758) n = 22	9.58	1.701^{E-14}
TL/SVL	0.276 ± 0.023 (0.226-0.358) n = 52	0.189 ± 0.012 (0.161-0.215) n = 22	16.35	5.902^{E-26}
HL/SVL	0.034 ± 0.003 (0.026-0.041) n = 49	0.032 ± 0.003 (0.027-0.039) n = 22	2.94	0.0032
HW/HL	0.557 ± 0.065 (0.478-0.779) n = 49	0.520 ± 0.061 (0.429-0.675) n = 22	2.38	0.017
IOD/HL	0.0346 ± 0.036 (0.296-0.455) n = 49	0.0317 ± 0.006 (0.246-0.368) n = 22	2.56	0.010
IND/HL	0.202 ± 0.028 (0.153-0.289) n=49	0.184 ± 0.022 (0.138-0.226) n = 22	2.40	0.016
Nº ventral scales	180 ± 6 (162-197) n = 78	192 ± 9 (160-212) n = 51	7.74	9.76^{E-15}
Nº subcaudal scales	65 ± 3 (58-71) n = 81	57 ± 3 (50-63) n = 50	16.06	2.19^{E-22}

Table 2. Absolute number (n) and numeric abundance (N%) of prey items found in digestive tracts of *Oxyrhopus rhombifer* from the southern Brazilian coast (Rio Grande do Sul state), frequency and percentage (between parentheses) of prey intake directions of identified taxa, namely: head first (hf), tail first (tf) and not determined (ND).

Prey	n	N%	hf	tf	ND
Sauropsida: Squamata					
Ophiodes sp. (Anguidae)	2	8.3			2 (100)
Aspronema dorsivittatum (Scincidae)	1	4.2	1 (100)		
Cercosaura schreibersii (Gymnophthalmidae)	7	29.2	3 (42.9)	1 (14.2)	3 (42.9)
Lizard not identified	1	4.2			1 (100)
Snake not identified	1	4.2			1 (100)
Mammalia: Rodentia					
Deltamys kempi (Cricetidae: Akodontini)	1	4.2	1 (100)		
Oligoryzomys flavescens (Cricetidae: Oryzomyini)	3	12.5	3 (100)		
small rodent not identified	8	33.3			8 (100)

carrying secondary follicles and oviductal eggs along the months of the year; 2) analysis of the annual profile resultant from the plot of the largest follicles and eggs of each female (Mesquita et al., 2013; Almeida-Santos et al., 2014). Fecundity was determined by the number of eggs in the oviduct (real fecundity) and the number of secondary follicles (potential fecundity) in the ovarium (Mesquita et al., 2013). The correlation between female SVL and potential fecundity was examined with a simple linear regression analysis (Mesquita et al., 2013).

A total of 141 *O. rhombifer* specimens (89 males, 52 mature and 37 immature; 53 females, 22 mature, 31 immature) were analyzed. Females presented higher mean values of SVL and number of ventral scales. Males presented higher mean values for TL/SVL, HL/SVL, HW/HL, IOL/HL, INL/HL, and number of subcaudal scales (Table 1). Summary statistics for all the variables analyzed for sexual dimorphism are presented in Table 1. Significant differences between sexes were found for all the variables tested (Table 1).

Twenty-four specimens contained 24 prey item each. Prey was comprised of rodents (N% = 50.0) and Squamata reptiles (N% = 50.0) (Table 1). Eight out of nine prey in which the direction of intake could be determined were head-first consumed (Table 2). Eleven out of 12 rodents identified were consumed by mature individuals. Lizards were consumed by both mature and immature individuals. The single snake identified was consumed by an immature individual. Half of the squamate prey consisted of tail fragments of Scincomorpha and Anguidae lizards.

The smallest female exhibiting secondary follicles presented 451 mm of SVL, while the smallest male exhibiting coiled/opaque *ducti deferentes* presented 354 mm of SVL. Secondary follicles were found mainly from

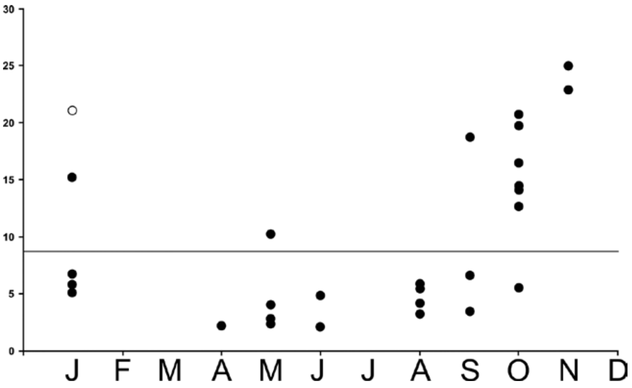

Fig. 2. Monthly variation in diameter (mm) of the largest follicles (black circles) and egg (white circles) of *Oxyrhopus rhombifer* from the southern Brazilian coast (Rio Grande do Sul state). The horizontal line indicates size from which follicles were considered as being in secondary vitellogenesis (secondary follicles).

September to November (spring). A single female presented secondary follicles in January (early summer) and another one showed secondary follicles in May (middle autumn) (Fig. 2). Potential fecundity varied from two to 13 (x = 7.9 ± 3.4 SD; n = 11). One single female, euthanized in January, presented eggs in the oviduct (three eggs). This same female also presented secondary and primary follicles in the ovaries, indicating the possible occurrence of more than one clutch during the cycle. This female did not present expanded oviducts, which indicates that there was no previous egg laying, and the three oviductal eggs may correspond to a single oviposition event. A greater fat storage was observed in mature females during initial and intermediate secondary vitellogenesis while low celomatic fat deposition was found in females in advanced secondary vitellogenesis. We did not find any significant, albeit positive, correlation (r = 0.07; P = 0.84) between female SVL and potential fecundity. An aggregation of three similar-sized males and one larger female, intertwined, but not involved in copulation, was observed in October (early spring).

Our results indicated that sexual dimorphism is very well marked in *O. rhombifer* from southern Brazilian coastal plain, considering that all the variables herein tested were significantly different between sexes. The dimorphism in body size (SVL) is noteworthy, with females significantly larger and showing higher number of ventral scales. Males, however, presented tail proportionally longer, higher number of subcaudal scales, head proportionally longer and wider and greater interocular and inter-nostril measurements. The larger body in females and the tail proportionally longer in males is a well-known aspect in dipsadid snakes (Aguiar and Di-Bernardo, 2005; Balestrin and Di-Bernardo, 2005; Mesquita et al., 2013; Quintela et al., 2017; Quintela and Loebmann, 2019a, b), including the congener *Oxyrhopus trigeminus* (Alencar et al., 2012). Mature females of *Oxyrhopus guibei* also showed SVL significantly larger than mature males (Pizzatto and Marques, 2002). The longer body size in females in species without male-male combat is associated with fecundity so that a larger body can accommodate a larger offspring. Thus, females experience the fertility selection, in which larger bodies take advantage (Shine, 1994). In relation to head dimensions, studies have demonstrated that sexual dimorphism in head traits of snakes are related to differences in the maximum size of preys consumed by different sexes (Shine, 1989; Pearson et al., 2002; Shetty and Shine, 2002; Vincent et al., 2004). A broader analysis of the feeding habits of the species, based on a larger sample of preyed items, could reveal if in fact males feed on larger prey. Another hypothesis to be considered is the use of the head by males for the immobilization of females during mating.

Oxyrhopus rhombifer feeds on small mammals and squamate reptiles in the studied area, which is in agreement with data from other regions where the species occurs (Vidal, 2002; Maschio et al., 2003, 2004; Sawaya et al., 2008; Gaiarsa et al., 2013) and from other *Oxyrhopus* species (Alencar et al., 2012; Gaiarsa et al., 2013). However, the high occurrence of solely lizard tail fragments in the analyzed digestive tracts (25% of all prey items, 50% of Squamata items; Tab. 1), possibly autotomized to avoid predation, is remarkable. Caudal autotomy is a very common defensive mechanism in lizards (Clause and Capaldi, 2006), considering that solely tail fragments have been found in tracts of other xenodontine species such as *Philodryas aestiva* and *P. patagoniensis* (Quintela, pers. comm.).

Females attained sexual maturity at much larger sizes than males, in agreement with *O. guibei* (Pizzatto and Marques, 2002), *O. trigeminus* (Alencar et al., 2012) and other xenodontine genera (Pizzato et al., 2008; Mesquita et al., 2013; Rebelato et al., 2016; Quintela et al., 2017, Quintela and Loebmann, 2019a, b). The small number of mature females in our sample, however, did not allow further analysis or conclusions about the reproductive cycle and fecundity. The occurrence of females showing secondary follicles in spring may indicate a seasonal reproductive cycle, a pattern already recorded for other xenodontines in the studied region (Oliveira et al., 2011; Rebelato et al., 2016; Quintela et al., 2017; Quintela and Loebmann, 2019a, b). A marked greater accumulation of celomatic fat in females during initial and intermediate secondary vitellogenesis when compared to females in advanced secondary vitellogenesis also indicates a capital-breeding strategy (Bonnet et al., 1998), where the consumption of lipidic energy sources occurs along the reproductive cycle (Almeida-Santos et al., 2014). The only data on real fecundity (three oviductal eggs) showed a clutch size smaller than those observed in *O. rhombifer* from other regions (four to 17; Pontes and Di-Bernardo, 1988; Yanosky et al., 1996; Gallardo and Scrocchi, 2006; Gaiarsa et al., 2013), but within the clutch size variation found for *O. guibei* (three to 20; Pizzato and Marques, 2002) and *O. trigeminus* (one to 11; Alencar et al., 2012). Sampling and examination on a greater number of females and histological analyses of male gonads, already in progress, will soon allow a more profound understanding of *O. rhombifer* reproductive biology in the southern Brazilian coast.

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APPENDIX

Specimens examined and deposited in the herpetological collection of Universidade Federal do Rio Grande (CHFURG).

Oxyrhopus rhombifer: Brasil: Rio Grande do Sul: Palmares do Sul (CHFURG 1267, 2548); Tavares (CHFURG 4320, 4321); Rio Grande, Quinta (CHFURG 2015), Parque Marinha (CHFURG 833, 4580), Vila Carreiros (CHFURG 2540, 2963), Distrito Industrial (CHFURG 1313, 1314, 1704, 1846, 1851, 1886, 1888, 1899, 1901, 1904, 1906, 1907, 1908, 1909, 1915, 1959, 1961, 1962, 2385, 3156, 3328, 4561, 5070, 5078, 5243), Senandes (CHFURG 832), Área de Proteção Ambiental da Lagoa Verde (CHFURG 1926, 1963, 1964, 1965, 2975, 3436), Cassino (CHFURG 1120, 1900, 1902, 1905, 3239, 4570, 4582, 4583, 5169, 5170, 5171, 5172, 5174, 5175, 5176, 5177, 5178, 5276, 5404, 5957, 5958, 5959, 5960, 5961, 5962, 5963, 5964), Barra (CHFURG 1910, 3076, 3078, 3079, 3090, 3208, 3274, 3328, 3329, 4419, 4444, 4567, 4578, 4579, 4581, 4584, 4671, 4680, 4687, 4688, 4789, 4790, 4820, 4891, 4902, 4918, 4920, 4921, 5071, 5072, 5073, 5074, 5075, 5076, 5077, 5078, 5079, 5080, 5081, 5082, 5083, 5173, 5201, 5238, 5239, 5240, 5241, 5242, 5243, 5244, 5245, 5246, 5247, 5249, 5250, 5285, 5633, 5966), Estação Ecológica do Taim (CHFURG 1500); Santa Vitória do Palmar (CHFURG 5376).