Natural history of the threatened coral snake Micrurus altirostris (Serpentes: Elapidae) in Argentina

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Natural history of the threatened coral snake *Micrurus altirostris* (Serpentes: Elapidae) in Argentina

*Historia natural de la serpiente de coral amenazada* *Micrurus altirostris* *(Serpentes: Elapidae)* *en Argentina*

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Abstract

The genus *Micrurus* comprises nearly 80 endemic species in the American continent. Knowledge on its natural history is based on populations from Brazil and Uruguay, making it necessary to intensify ecological research in Argentina. We present data on morphology, diet, and reproduction for a threatened Argentine population of *M. altirostris* from the Atlantic Forest biodiversity hotspot. Ecological data were obtained from collected specimens and scientific collections. Females attained sexual maturity at a longer body size than males, but the latter reached a larger size, which is related to male-male combat, a behavior reported in this study. We report a female with 3 oviductal eggs, and 2 ovipositions of 4 and 6 eggs in the late spring-early summer period. Males seemed to have a seasonal reproductive cycle. The diet of *M. altirostris* was based on elongated reptilians and was mostly similar to the diet of populations from Brazil and Uruguay. We reported 4 new prey types for *M. altirostris* and confirm the consumption of reptile eggs for the genus *Micrurus*. Our results contribute to a better understanding of the natural history of *M. altirostris*, providing valuable information for designing strategic conservation plans.

Keywords: Argentina; Diet; *Micrurus altirostris*; Morphology; Natural history; Reproduction

Resumen

El género *Micrurus* comprende cerca de 80 especies endémicas del continente Americano. El conocimiento sobre su historia natural está basado en poblaciones de Brasil y Uruguay, siendo necesario intensificar los estudios ecológicos en Argentina. Presentamos datos de morfología, dieta y reproducción de *M. altirostris* en Argentina, poblaciones amenazadas del hotspot bosque Atlántico. Los datos ecológicos fueron obtenidos del análisis de especímenes colectados y de colecciones científicas. Las hembras maduran con un tamaño corporal mayor al de los machos, pero éstos alcanzan...
Introduction

Coral snakes belonging to the genus *Micrurus* (Wagler, 1824) are endemic and highly venomous elapids found in the American continent (Roze, 1996). The genus comprises nearly 80 species. However, some of those species’ taxonomy is poorly resolved due to both lack of information and morphological similarity among them (Giraudo et al., 2015; Silva & Sites, 1999; Uetz et al., 2017). Coral snakes constitute a relevant issue for public health due to their high toxicity; and thus, it is important to know about their biology and natural history. Even though they have potent neurotoxic venoms, the cases of envenomations caused by *Micrurus* are relatively rare due to the reclusive nature of these snakes and the fact that they generally inhabit sparsely populated areas (Corrêa-Netto et al., 2011). Cambell and Lamar (2004) stated that coral snakes are greatly diminished in many areas altered by humans. Usually, when forest areas are cleared, their habitats become drier, and thus moisture-associated species (such as *M. altirostris*) and their prey are either reduced in number or extirpated. The natural history of *Micrurus* is quite unknown because most current works in the literature are focused on a few species (Campbell & Lamar, 2004; Roze, 1996). There are hardly any studies on the ecology of *Micrurus* (Almeida-Santos et al., 2006; Ávila et al., 2010; Marques, 2002; Marques et al., 2006, 2013), whereas most of them have addressed systematic aspects and information about its venom, morphology, and taxonomy (Camargo et al., 2011; Morales-Mondoñedo, 2010; Silva & Aird, 2001; Silva & Sites, 1999, 2001).

*Micrurus altirostris* is a medium-sized coral snake with populations distributed across southeastern Brazil, Paraguay, Uruguay, and Northeastern Argentina (Silva & Sites, 1999). In the latter country, it shows a restricted distribution associated with humid habitats of Atlantic Forest and extends to the south by the riparian humid forests of the Uruguay River. Additionally, *M. altirostris* has an isolated population in the riparian humid forest of the Middle Paraná River, which is considered the southernmost expansion of the impoverished Atlantic Forest (Arzamendia & Giraudo, 2009; Giraudo, 2001; Giraudo et al., 2012; Scroechi, 1990; Vuoto, 2000). The Atlantic Forest is a biodiversity hotspot considered as one of those with the highest conservation priority in the world due to both its elevated rate of habitat loss and the fact that only 7-8% of the original surface remains (Galindo-Leal & Camara, 2003; Myers et al., 2000).

The natural history of *M. altirostris* in Argentina is scarce because of its fossorial habits, low frequency of capture, the danger of its venom, and the difficulty in manipulating specimens (Oliveira et al., 2005). There exist some data about reproduction and activity patterns of *M. altirostris* based on studies related to Brazilian populations (Marques et al., 2006, 2013). Additionally, there are some data about the ecological traits of *M. altirostris*, but this information is mostly anecdotal and incomplete since it is based on small samples from Brazilian and Uruguayan populations (Carreira-Vidal, 2002; Silva, 1995; Silva & Aird, 2001). In Argentina, *M. altirostris* was categorized as a vulnerable species owing to its relatively restricted distribution, which is strongly associated to the remnants of the humid Atlantic Forest. More than 60% of these habitats have been lost due to the high and still increasing deforestation rate (Giraudo et al., 2003). The poor state of conservation of *M. altirostris* is further affected by high human persecution and road kills (Giraudo et al., 2012). For all these reasons, Giraudo et al. (2012) suggested intensifying ecological research in order to broaden knowledge about the biology of this coral snake species in Argentina. In this study, we present new and basic information about the natural history of *M. altirostris* in Argentina, including morphology, sexual dimorphism, diet, and reproductive biology so that it can be considered when designing conservation strategies for this species.

Materials and methods

The study area is located in the Northeast of Argentina and comprises Misiones, Corrientes, and Entre Ríos provinces. This area is characterized by subtropical-temperate climate, with a seasonality mainly marked by temperature. Average annual temperatures range from 22 °C in the north to 17 °C in the south. Annual precipitation...
averages from 1,000 to 1,800 mm (Arzamendia et al., 2015). The study area has great environmental diversity due to the influence of the Paraná and Uruguay rivers and the confluence of Paranaense or Atlantic Forest, Chacoan, Espinal, and Pampas phytogeographic provinces (Arzamendia & Giraudo, 2009).

Data were obtained by analyzing a total of 74 specimens of *M. altirostris*. We sampled the study area from January 1991 to April 2016, mainly by active search and road sampling in different habitats (Bellini et al., 2014). Road sampling allowed us to cover large areas and detect both roadkills and alive snakes. We also conducted time-constrained searches and opportunistic encounter surveys (Scott, 1994; Valdujo et al., 2002). The sampling was carried out at day and night hours. For each specimen, we recorded the exact location (using a Garmin eTrex Legend Global Positioning System device). Snakes were measured with a flexible ruler to the nearest millimeter and subsequently released at the capture site. We preserved both recently road-killed and intentionally killed snakes that were in good conditions for collecting dietary, reproductive, and morphological data. All collected specimens were housed in the collection of Instituto Nacional de Limnología (INALI, Santa Fe, Argentina). The biological material was supplemented with original data from specimens deposited in the scientific collections of Museo de Ciencias Naturales y Antropológicas Profesor Antonio Serrano (MER), Laboratorio de Genética Evolutiva, Instituto de Biología Subtropical CONICET-UUniversidad Nacional de Misiones (LBG), and Museo de la Plata (MLP).

We recorded the following variables: sex; snout-vent length (SVL), tail length (TL), head length (HL from the tip of the snout to the angle of the jaw), and trunk length (TRL), using a flexible ruler to the nearest millimeter; body mass, using a Pesola scale to the nearest 0.1 g; number of ventral (VEN) and subcaudal (SC) scales, using Gans’ (1964) technique; length, width, and thickness of the right testis; diameter of deferent duct close to cloaca; observation of deferent duct (convoluted or non-convoluted); and length, width, and thickness of ovarian follicles (in primary or secondary vitellogenesis) or oviductal eggs. All measurements were made to the nearest 0.1 mm using digital calipers (CONNOR).

Males were considered mature if they had convoluted deferent ducts (Leite et al., 2009; Pizzatto, Jordão et al., 2008; Shine, 1978). Females were considered mature if they had follicles in secondary vitellogenesis, oviductal eggs, or folded oviductus (Pizzatto, Cantor et al., 2008).

In males, increased size and mass of the testes probably reflect spermiogenesis (Pizzatto, Cantor et al., 2008), so the volume of the testis was calculated using the ellipsoid formula (Pleguezuelos & Feriche, 1999). Male reproductive cycle was determined by comparing variations in the volume of testes and diameter of deferent duct from one season to another, using analyses of covariance (Ancova), since both variables covary with SVL (Almeida-Santos et al., 2006; Pizzatto, Cantor et al., 2008; Pizzatto, Jordão et al., 2008). Due to the questionable accuracy of the testicles volume measure for a real evaluation of male reproductive cycle (Almeida-Santos et al., 2014), sections of testicles and deferent duct were performed. Since most specimens were run over, and the condition of their organs was not adequate, 3 specimens were dissected to obtain histological samples of the proximal region of testes and proximal, medial, and distal region of deferent ducts. Only right-side organs were extracted, thus preserving contralateral testes and deferent ducts. Samples were embedded in paraffin and sectioned at 5 μm thickness with a rotating microtome (Thermo Scientific Rotary Microtome HM 325); then, samples were stained with hematoxylin and eosin (Loebens et al., 2018). Slides were observed using an optical microscope (Leica DM 2500).

The presence of sexual dimorphism was evaluated according to the number of ventral and subcaudal scales and SVL and between sexes by means of a nonparametric Mann-Whitney U test. As TL and body mass covariate with SVL and HL covariates with TRL, an analysis of covariance (Ancova) was used. The index of sexual size dimorphism (SSD) was calculated as (mean SVL of the larger sex) / (mean SVL of the smaller sex) – 1 (Shine, 1994). This index is expressed by positive values if females are the larger sex and in negative values if males are the larger sex.

Neither juvenile nor neonatal specimens were included in the analysis of sexual dimorphism of measurements (SVL, TL, body mass), except for lepidosis variables because they do not vary with sexual maturity. All raw data were tested for normality and homoscedasticity with Shapiro-Wilks and Levene’s tests (Zar, 1999). Digestive tracts of each specimen were dissected to analyze their contents and identify prey items. Prey items were identified to the lowest possible taxonomic group by comparing them with material from the reference collection of the Instituto Nacional de Limnología and guides in the literature. Number and direction of ingestion were recorded from the obtained prey. All statistical analyses were performed by using Infostat software version 2008 (Di Rienzo et al., 2008) with α < 0.05.

Results

Of the 74 individuals analyzed, 57 were males (77.1%, 54 adults and 3 juveniles) and 17 were females (22.9%, 8 adults and 9 juveniles). Females attained sexual maturity
(SVL = 570 mm) at a larger body size than males (SVL = 320). Sexual dimorphism was only recorded for the number of ventral scales (U = 776.5; \( p = 0.0057 \)), females proving to have a greater amount of scales (Table 1). The sexual size dimorphism index for adults was SSD = 0.0064, also showing that males and females presented no differences in body size.

Considering macroscopic data, males are reproducitively active throughout the whole year, since we found convoluted deferent ducts throughout all seasons; and significant differences were not found in testicular volume between seasons (F = 1.28, df = 3, \( p = 0.3193 \)). Neither were differences observed in deferent duct diameter between seasons (F = 0.59, df = 3, \( p = 0.6288 \)). At the microscopic level, 2 different developmental stages were characterized by cellular changes in testes of *M. altirostris*: 1) recrudescence in January, with spermatogonia and spermatocytes in testicles and spermatozoa in deferent duct, and 2) spermiogenesis in February and December, with spermatozoa in testicles and deferent duct. Male-male combat was registered only once on October 7, 1994 in a secondary forest in the province of Misiones.

In addition to the low number of females being studied, only 2 specimens with vitellogenic follicles were found: 1 collected in September (14.25 mm long) and another one in August (13.7 mm long) and another specimen with 3 oviductal eggs (38.2 - 40.75 mm long and 1.4 - 11.6 mm wide) in January. Additionally, 2 events of oviposition were recorded: one of 6 eggs in November, and another of 4 eggs within 90-minute intervals in December.

From the total collected specimens (74), 27 (36%) had stomach content. In the field, 2 species of snakes were recorded as prey items of *M. altirostris* (Table 2). Three different major prey items were identified: Scolecophidia, the most frequent prey category (41.4%), Alethinophidia (27.6%), and Amphisbaenia (20.7%). Also, 2 specimens with a reptile egg in their digestive tract were recorded (Table 2). In every case, head-first ingestion was identified.

**Discussion**

Males of *M. altirostris* were more frequently found than females, representing 77.1% of our sample. These results agree with those obtained by Silva and Sites (1999), Di Bernardo et al. (2007), and Marques et al. (2013), who found 60 - 81% males and 21 - 34% females. The higher activity of males could be due to the courtship ritual (male-male combat). Females would have more secretive habits, which will potentially reduce their vulnerability to predators and increase the chances of survival and, therefore, the amount of mating, which would ensure the reproductive success of the species (Marques et al., 2006).

*Micrurus altirostris* females attain sexual maturity at longer body sizes than males. This pattern has been reported for Pseudoboini (Pizzatto, 2005), Xenodontini (Pizzatto, Jordão et al., 2008), Tachymenini (Bellini et al.,...
Table 2
Diet of *Micrurus altirostris* in the studied population from Argentina and compared with that of populations from Brazil (Silva Jr., 1995; Silva Jr. & Aird, 2001) and Uruguay (Carreira-Vidal, 2002). Number of prey items (N), Frequency (F). * Prey items prey recorded by direct observation.

<table>
<thead>
<tr>
<th>Population</th>
<th>Argentina</th>
<th>Brazil</th>
<th>Uruguay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prey taxon</td>
<td>N</td>
<td>F (%)</td>
<td>N</td>
</tr>
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<td>Amphisbaenia</td>
<td></td>
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<tr>
<td>Family Amphisbaenidae</td>
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<td>3.5%</td>
<td></td>
</tr>
<tr>
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<td>28.6%</td>
<td></td>
</tr>
<tr>
<td><em>Amphisbaena sp.</em></td>
<td>1</td>
<td>25%</td>
<td></td>
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<tr>
<td>Unidentified Amphisbaenidae</td>
<td>5</td>
<td>17.2%</td>
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</tr>
<tr>
<td>Subtotal Amphisbaenia</td>
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<td>20.7%</td>
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</tr>
<tr>
<td>Serpentes</td>
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<td></td>
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</tr>
<tr>
<td>Scolecophidia</td>
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<td></td>
</tr>
<tr>
<td>Family Anomalepididae</td>
<td></td>
<td></td>
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<td><em>Liophthalmus beui</em></td>
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<td>3.5%</td>
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<tr>
<td>Family Typhlopidae</td>
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<td>3.5%</td>
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<tr>
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<td></td>
<td></td>
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<tr>
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<td>34.4%</td>
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<tr>
<td>Subtotal Scolecophidia</td>
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<td>41.4%</td>
<td>2</td>
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<tr>
<td>Alethinophidia</td>
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<td><em>Atractus reticulatus</em></td>
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<td>6.8%</td>
<td>1</td>
</tr>
<tr>
<td><em>Atractus sp.</em></td>
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<td>3.5%</td>
<td></td>
</tr>
<tr>
<td><em>Tomodon ocellatus</em></td>
<td>1</td>
<td>3.5%</td>
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<td><em>Erytrolamprus sp.</em></td>
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<td></td>
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<tr>
<td><em>Sibynomorphus sp.</em></td>
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<td>25%</td>
<td></td>
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<tr>
<td><em>Phalotris bilineatus</em></td>
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<td>14.3%</td>
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<tr>
<td><em>Bothrops jararaca</em></td>
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<td>Subtotal Alethinophidia</td>
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<tr>
<td><em>Ophiodes striatus</em></td>
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<td>14.3%</td>
<td></td>
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<tr>
<td>Family Gymnophthalmidae</td>
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<td></td>
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<tr>
<td><em>Cercosaura schreiberi</em></td>
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<td>14.3%</td>
<td></td>
</tr>
<tr>
<td>Subtotal Sauria</td>
<td>2</td>
<td>28.6%</td>
<td></td>
</tr>
<tr>
<td>Reptile egg</td>
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<td>6.8%</td>
<td></td>
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<tr>
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</tr>
<tr>
<td>Total</td>
<td>29</td>
<td>100%</td>
<td>4</td>
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</tbody>
</table>
2013, 2014), Colubrinae (Leite et al., 2009), Crotalinae (Valdujo et al., 2002), and Hydrodynastini (Giraudo et al., 2014). The evidence indicates that sexual maturity in females may be delayed in relation to males due to the high energy cost involved in reproduction. Reproduction in females is not worthwhile until they gain higher probabilities of reproductive success (larger litter size). In addition, early maturity of males increases their chance of mating (Madsen & Shine, 1994; Shine, 1994, 2003).

The male-male combat observed in October is a new record in *M. altirostris* behavior and was also registered in Brazilian populations but in the months of April and May (Almeida-Santos et al., 1998; Marques et al., 2006; Marques et al., 2013). For other species, in which male-male combat was also registered, males were the largest sex (Shine, 1978, 1994). Despite this trend, *M. altirostris* exhibits sexual dimorphism in the number of ventral scales, females being those with a greater number. Marques et al. (2013) analyzed only morphometric characters (SVL and TL) and males were observed to have longer bodies and tails, which does not match our data. This difference between results could be due to the disparity between sample sizes (our sample included more males than females).

Males probably have a seasonal reproductive cycle, since 2 stages of development of *M. altirostris* testes were detected: recrudescence in summer and spermatogenesis in spring and summer. Males of *M. altirostris* do not present seasonal variation of deferent duct diameter, unlike what was found for *M. corallinus* (Almeida-Santos et al., 2006). For the latter species, a correlation was found between increased sizes of the distal portion of the deferent duct and sperm storage, which was confirmed by using histological techniques (Almeida-Santos et al., 2006).

Our records of the female reproductive cycle agree with those reported by Marques et al. (2013), who recorded females with vitellogenic follicles between June and January. However, our data on gravid females and oviposition (December to January) extends the period reported by Marques et al. (2013) (November). Since mating occurs at the end of summer and early autumn, and ovulation occurs during spring, Marques et al. (2013) suggested that *M. altirostris* females could be able to store sperm during winter. For that reason, further histological analyses are necessary to assess possible sperm storage in females and complete spermatogenesis cycle in males (Almeida-Santos et al., 2014).

The diet of *M. altirostris* in Argentina is based on elongated reptiles. The same was reported of populations in Brazil and Uruguay (Carreira-Vidal, 2002; Silva, 1995; Silva & Aird, 2001). We reported 4 new genera in the diet of *M. altirostris*: *Amerotyphlops*, *Liodyphlops*, *Tomodon*, and *Bothrops*. Roze (1996) reported an egg in the stomach of *M. nigrocinctus zunilensis*. He asserted that no further content was found in the stomach and thus the egg-eating behavior arises as an open question. Our records of reptile eggs in 2 specimens with empty stomachs allow us to confirm for the first time the consumption of reptile eggs in the genus *Micrurus*, as suggested by Roze (1996). Lizards were not registered in the diet of this coral snake in Argentina, but this prey item was reported in populations from Uruguay (Carreira-Vidal, 2002). Probably the differences in diet between our data and populations from Brazil and Uruguay are influenced by the small sample analyzed in those studies and by differences in prey availability.

For *M. altirostris*, direction of prey ingestion is head-first, as was mentioned previously for snakes in general (Bellini et al., 2013; Greene, 1976; Pinto & Lema, 2002; Prieto et al., 2012; Rodriguez-Robles, 2002). This form of ingestion reduces time and deglutition effort and minimizes the possibility of prey escape (Albarelli & Santos-Costa, 2010; Andrade & Silvano, 1996; Pinto & Lema, 2002). Additionally, in the case of ophiophagous snakes, this behavior allows them to avoid the attack of potentially dangerous prey and reduce resistance of ventral scales (Greene, 1976; Pinto & Lema, 2002).

The assessment of the conservation status of neotropical snakes is hampered by the lack of basic natural history data (Giraudo et al., 2012; Leão et al., 2014). In the present study, new data were registered about reproduction (male-male combat, female and male reproductive cycle) and diet of *M. altirostris*. This work provides the first natural history data for a threatened population of coral snake, including relevant information for designing strategic conservation plans.

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