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MATING AND REPRODUCTIVE CYCLE IN THE NEOTROPICAL COLUBRID SNAKE *CHIRONIUS BICARINATUS*

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Abstract. *Chironius bicarinatus* is a common colubrid snake, widely distributed throughout the Atlantic Forest. Field observations of copulation and combat, combined with data on preserved and captive snakes, as well as on specimens brought to Instituto Butantan provided a better characterization of the reproductive cycle of this species. *Chironius bicarinatus* has a seasonal reproductive cycle with extended vitellogenesis and ovulation, and oviposition occurring at the onset of the rainy season (austral spring). Recruitment of newborns occurred mainly at the end of the rainy season. Clutch size ranged from five to 14 and relative clutch size ranged from 0.55 to 0.62. Copulation was observed four times, always in April (austral autumn) at the onset of vitellogenesis. These records correspond to the activity peak of males in the field. Thus, mating may occur prior to ovulation (in austral spring) indicating a dissociated reproductive pattern. We suggest that combat in November (in austral spring), recorded in a previous study, may be related to the presence of androgens in snakes during the non-mating season.

Keywords. reproduction, mating, copulation, combat, *Chironius bicarinatus,* Atlantic Forest.

Introduction

Recent studies have produced a lot of information on follicle or testicular cycles in several Neotropical species of snakes (Pizzatto and Marques 2002; Bizerra, Marques and Sazima, 2005; Almeida-Santos *et al.,* 2006; Marques *et al.,* 2006). Such data can be obtained easily from museum specimens (*e.g.,* Pizzatto and Marques 2002; Almeida-Santos *et al.,* 2006). However, important information on other parameters of the reproductive cycle, such as mating or combat, is scarce. These data are recorded mainly in nature, but Neotropical snakes are hardly ever found in the field (*e.g.,* Panger and Greene, 1998; Almeida-Santos and Marques, 2002).

Reproductive cycles in southeast Brazilian Atlantic Forest species are relatively well known and most species in this region seem to have a seasonal reproductive cycle (Marques, 1998; Marques and Sazima, 2004). Snakes of the colubrid genus *Chironius* are among the most abundant in the Atlantic Forest (Dixon *et al.,* 1993; Marques and Sazima, 2004). Five species inhabit the Atlantic Forest, being the semi-arboreal *C. bicarinatus* the most abundant in several localities (Argôlo, 2004; Marques *et al.,* 2004). Data on activity and reproductive cycle of *C. bicarinatus* are meager, but vitellogenic follicles and oviductal eggs have at least been found in the rainy season (in spring and summer), indicating a seasonal reproduction in females (Marques, 1998; Marques and Sazima, 2004). Although combat has been reported in *C. bicarinatus* (Almeida-Santos and Marques, 2002), information on copulation is absent for this species. Here we record the occurrence of copulation in nature, establishing a probable relationship among mating period, vitellogenesis, ovulation, and activity of *C. bicarinatus* from southeastern Brazil. Additional reproductive data on oviposition and hatching are also provided.

Materials and Methods

We obtained information on copulation in nature and additional data was obtained from the herpetological collection of Instituto Butantan and from captive individuals. Information about the female reproductive cycle was based on the dissection of 35 preserved specimens from the herpetological collection. All specimens were collected along the coast of São Paulo State, southeastern Brazil. The following data were gathered from each specimen: (1) snout-vent length (SVL; mm); (2) tail length (mm); (3) female reproductive maturity (considered mature if they had either oviductal eggs or ovarian vitellogenic follicles > 5 mm; see Shine, 1977a, b); (4) diameter of the largest ovarian follicles or oviductal eggs (0.1 mm).

Two captive snakes maintained in terraria of the Laboratório de Ecologia e Evolução of the Instituto Butantan, laid eggs. The females, eggs and newborns were weighed and measured. Relative clutch mass (RCM, ratio of total clutch mass and mother body mass after egg-laying) was calculated. The eggs were

incubated in a plastic container with moistened vermiculite, at room temperature, varying from 20 to 32°C. Data on combat was obtained from a previous publication (cf. Almeida-Santos and Marques, 2002).

Data on the seasonal activity pattern was inferred from the number of specimens brought each month to Laboratório de Ecologia e Evolução, Instituto Butantan, São Paulo, Brazil, mostly by laymen (see Marques *et al.,* 2001; Marques *et al.,* 2006). Records from 2000 to 2005 were used in the study and differences in abundance in the wet and dry season were compared using a Chi-square test (Zar, 1996).

Results

On 8 April 2003, a female was found about 2.5 m above ground on a tree at São Lourenço da Serra, State of São Paulo, (23°52'S, 46°55'W, approximately 800 m elevation), southeastern Brazil. One of us (RC) observed the snake throughout the week. The snake remained resting, coiled on a branch about 2.5 m above ground at night and about 1.5 m above ground during the day when it was apparently basking. On 11 April 2003 around 13:30 h, two snakes (the same female and a male) were found together about 1.5 m above ground (Table 1 and Figure 1) on the same tree. At about 15:00 h the snakes were observed closer together. A detailed inspection revealed their paired tails and cloacae clearly connected by the hemipenis (Figure 2). The female was opaque in color with loose skin, which it was clearly shedding (Figure 2). At 17:45 h the snakes had separated and moved away in different directions. The female did not return to the branch where it had been resting throughout the week.

On 10 April 2008 around 9:20 h one of us (RC) found two other snakes already copulating at the same locality cited above (Table 1). These snakes were found on the roof of a house about 3.0 meters above ground. Mating continued for at least 120 minutes. The female had loose skin, which it was also clearly shedding. Photographs and a complete videotaped sequence of this copulation have been stored on a DVD housed at the Laboratório de Ecologia e Evolução of the Instituto Butantan.

Information obtained from the Herpetological Collection of Instituto Butantan (voucher specimens IB 55747, IB 69068 and IB 69069) provided two additional data of copulation in the field (Table 1). Dissection of the preserved female (IB 69069) showed its follicles in vitellogenesis (diameter ranged from 6.7 to 6.9 mm). Previous data indicate that ritual combat occurs in spring, since two engaged males were observed in November. During this combat the bodies of males remain entwined and the anterior portion of their trunks upright. Each male attempts to obtain a higher position than that of its opponent, frequently pushing down the trunk of the opponent (see Almeida-Santos and Marques, 2002, for details).

Examination of ovarian follicles in dissected specimens revealed that females of *C. bicarinatus* have an extended seasonal reproductive cycle, with vitellogenesis starting at the end of rainy season (austral autumn), and oviductal eggs occurring mainly at the onset of the rainy season (September to December; Figure 3). Only one female with oviductal eggs was collected at the onset of the dry season (in May, see Figure 3).

Clutch size of five preserved females ranged from 5 to 14 eggs, with a mean of 8.4 ± 3.4. One captive female (785 mm SVL) laid seven eggs on 24 October 2005. The eggs averaged 42.5 mm in length (range: 40.3-44.4 mm) and weighed 9.3 g (range: 8.8-10.0 g). Relative clutch mass was 0.55. Another female (IB 71923, 940 mm SVL) collected on 3 November 2003 laid 14 eggs on the same day. Eggs averaged 30.9 mm (range: 29.2-36.0 mm) in length and weighed together 97 g. Relative clutch mass was 0.62. Thirteen neonates were born between 10 and 11 February 2004. Newborns averaged 264.1 ± 9.2 mm SVL (range: 242-283 mm) and weighed 5 to 6 g.

Adult males and females were collected throughout the year (Figure 4), but not as often in the dry season (May-August) as in the rainy season (September-April). This seasonal difference was significant ($\chi^2 = 38.22$, df = 1, n = 85, $p < 0.01$). The frequency of capture of adult males peaked in April (Figure 4).

Table 1. Records of copulation in the field. The data include body size in males and females (in mm), period, hour, minimum time of duration, and height of substrate (in meters) where copulation occurred. SVL: snout-vent length and TL: total length. Asterisks indicate females shedding their skin.

Male	Female	Mating period	Hour	Duration of copulation	Height of substrate
947 SVL	—	18 April 1995	—	—	—
ca 1500 TL	ca 1500 TL*	11 April 2003	13:30 h	180 min	1.5
1030 SVL	903 SVL	29 April 2003	14:00 h	—	2.0
ca 1500 TL	ca 1500 TL*	10 April 2008	9:20 h	120 min	3.0

FIGURE 1. Male and female *Chironius bicarinatus* on the tree ca 2.5 m above the ground.

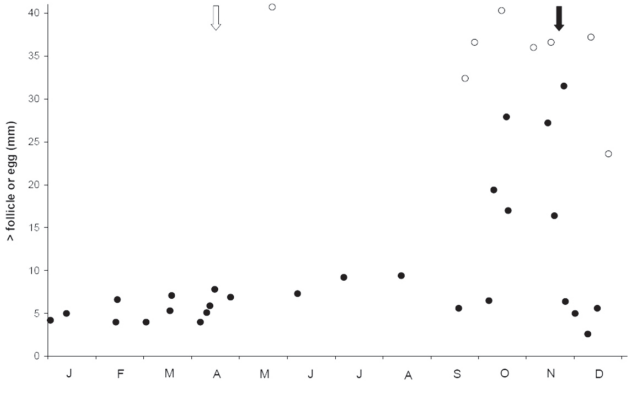

FIGURE 3. Seasonal variation in the diameter of the largest follicles in adult females of *Chironius bicarinatus* from southeastern Brazil. Open circles represent oviductal eggs. White arrow indicates copulation and black arrow indicates combat.

DISCUSSION

Similar to other Neotropical arboreal snakes, mating in *C. bicarinatus* can also occur above ground (*e.g.,* Cechin and Hartmann, 2001; Argôlo, 2004; Santos-Costa and Prudente, 2005). Mating observed

FIGURE 2. Detail of posterior portion of body of female (upper) and male (lower) *Chironius bicarinatus* showing the cloacae connected by hemipenis. Note the opaque loose skin of female, evidencing shedding of skin.

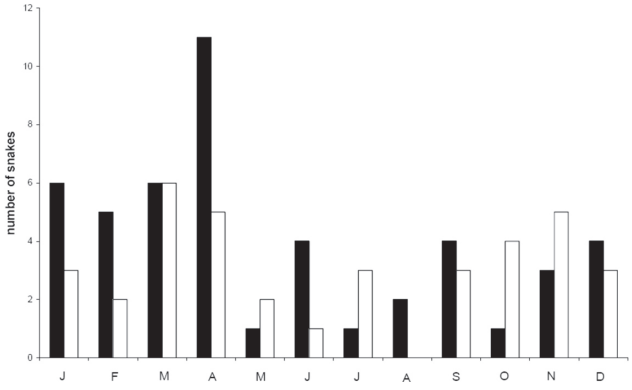

FIGURE 4. Seasonal activity of male (black bars) and female (white bars) *Chironius bicarinatus* from southeastern Brazil.

here at the end of the rainy season (in April, during austral autumn) was recorded during the start of vitellogenesis and before ovulation (in austral spring) indicating a dissociated reproductive pattern in *C. bicarinatus* (see Crews, 1984). However, ritual combat in this snake was recorded in nature during austral spring (Almeida-Santos and Marques, 2002). Ritual combat has been associated to the dispute for females (see Gillinghan, 1987). Nevertheless, such behavior may also be the result of the presence of androgens

in snakes during the non-mating season (Fox, 1977) when spermatogenesis is probably occurring. This hypothesis may be more plausible for *C. bicarinatus* because few males are found in nature during the onset of the rainy season, indicating that there is no increase in male activity to search for females in this period.

The function of male-male combat has been a matter of debate, but the main hypothesis is that the males are engaging in competition for females (Barker *et al.,* 1979; Gillingham *et al.,* 1983). Territorial defense (Lowe, 1948) and establishment of dominance (Carpenter and Gillingham, 1977; Carpenter, 1979, 1984) have also been suggested as probable functions. More data on the timing of mating are necessary before we can clearly evaluate the relevance of combat for reproductive competition in *C. bicarinatus.* However, there is no reason to expect that participation in fighting serves a single function; engaging in combat might also be an effective means of competing for certain resources or territories in which to court females (Carpenter, 1979, 1984).

The female *C. bicarinatus,* observed copulating in the field, was shedding its skin. Several authors have suggested that shedding is part of the estrus signal (Aldridge and Duvall, 2002). The proposed mechanism is that reproductive pheromones, released at the time of shedding, stimulate male courtship behavior (Klauber, 1972; Mason, 1992). This mechanism would explain the fact that females of *C. bicarinatus* were observed copulating while shedding their skin, as described herein, as well as for other Neotropical snakes (cf. Panger and Greene, 1998).

Encounter rate of male *C. bicarinatus* in nature is highest at the end of the rainy season (in austral autumn). This pattern occurs in all species of *Chironius* from the Atlantic Forest (cf. Marques *et al.,* 2001). This peak could be explained by an increase in activity of males searching for mates, as recorded here. Thus, mating at the end of the rainy season (in austral autumn) may be characteristic of the genus *Chironius* from the Atlantic Forest, when female ovarian follicles are still small at the onset of vitellogenesis (Marques, 1998; Marques and Sazima, 2004; this study). Apparently, females of *C. bicarinatus* may become sexually attractive and mate after the onset of vitellogenesis, similar to what has been described for other species of snakes (Macartney and Gregory, 1988). After this, the follicles attain a larger size at the onset of the rainy season when ovulation begins. Thus, females may be storing sperm in the oviduct until ovulation.

Chironius bicarinatus has an extended seasonal reproductive cycle. However, we observed oviductal eggs in a female collected in May. *Chironius flavolineatus* and *C. quadricarinatus,* studied in open areas at lower latitudes have continuous cycles (Pinto, 2006). Therefore, we cannot discard the possibility that *C. bicarinatus* and other Atlantic Forest species of this genus also have continuous cycles. Moreover, courtship in *C. flavolineatus* was recorded during the ovulation period (see Feio, 1999 and Pinto, 2006). Thus, the possibility of combat and mating in *C. bicarinatus* at the onset of the rainy season during the ovulation period can also not be discarded. A study characterizing male and female reproductive cycles of all species of *Chironius* would help to elucidate the extension of cycles and to verify if mating in this genus is restricted to one or two periods of the year.

Resumo

Chironius bicarinatus pertence à família Colubridae, está distribuída amplamente na Floresta Atlântica e é comum na natureza. Observações de cópula e combate na natureza, aliados a dados de indivíduos cativos, exemplares fixados e indivíduos trazidos à coleção do Instituto Butantan permitiram caracterizar melhor o ciclo reprodutivo dessa espécie. *Chironius bicarinatus* tem ciclo reprodutivo sazonal, com extenso período de vitelogênese e a ovulação e a oviposição ocorrendo no início da estação chuvosa. O tamanho da ninhada variou de 5 a 14 e o tamanho relativo da ninhada variou de 0,55 a 0,62. A cópula foi observada em quatro eventos, sempre no mês de abril (outono austral). Tais registros correspondem ao pico de atividade dos machos no campo. Assim, o acasalamento pode ocorrer antes da época da ovulação (que ocorre na primavera austral), indicando padrão reprodutivo dissociado. Sugerimos que o combate no mês de novembro (primavera austral), registrado em estudo anterior possa estar relacionado á presença de andrógeno nestas serpentes, fora do período de acasalamento.

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