

Reproductive biology of two sympatric colubrid snakes, *Chironius flavolineatus* and *Chironius quadricarinatus*, from the Brazilian Cerrado domain

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Abstract. The reproductive biology of *Chironius flavolineatus* and *C. quadricarinatus* from the Brazilian Cerrado domain is described, including sexual maturity, female fecundity, and reproductive cycles of males and females. Egg-laying is recorded here for the first time for *C. quadricarinatus*. Males of both species attain sexual maturity with a smaller snout-vent length than females. Females of *C. flavolineatus* have an extended reproductive cycle with egg production during the wet season, whereas males exhibit year-round sperm production. *Chironius quadricarinatus* has continuous reproductive cycles in both sexes. Differences in reproductive pattern between these two sympatric species are probably related to phylogenetic constraints on intrageneric lineages of *Chironius*.

Keywords: *Chironius*, fecundity, reproduction, Serpentes, South America.

Introduction

Neotropical snakes show greater diversity of reproductive patterns in comparison to snakes from temperate regions (Seigel and Ford, 1987; Greene, 1997). Snakes from temperate areas reproduce seasonally according to the warmer seasons of the year whereas in tropical areas reproductive cycles vary widely from seasonal to continuous (Seigel and Ford, 1987; Shine, 2003). However, there is a paucity of studies about the reproductive biology of Neotropical snakes. Analyses of gonads from preserved specimens may reveal several different patterns of reproductive biology in snakes, such as seasonal variation and fecundity (e.g., Pizzatto and Marques, 2002; Alves et al., 2005), but only females have been studied. Data characterizing male reproductive cycles of Neotropical snake species are scarce (cf. Almeida-Santos et al., 2004). Studies including both sexes could indicate variable reproductive features, such as

sperm storage or asynchronous reproduction (e.g., Seigel and Ford, 1987; Almeida-Santos et al., 2004).

The genus *Chironius* Fitzinger, 1826 is composed of diurnal, semi-arboreal and frog-eating snakes (Dixon et al., 1993; Marques and Sazima, 2004; Pinto et al., 2008), comprising 20 species occurring from Nicaragua to southern Brazil, northern Argentina and Uruguay (Dixon et al., 1993; Hollis, 2006). *Chironius flavolineatus* (Jan, 1863) and *C. quadricarinatus* (Boie, 1827) are sympatric in most of their distribution (Dixon et al., 1993) and occur mainly in the Brazilian Cerrado (central Brazilian savannas, see Eiten, 1978; Ab'Saber, 2003). Although sympatric and congeneric, these two species belong to different infrageneric clades of *Chironius* (Hollis, 2006). *Chironius flavolineatus* is closely related to species found in forested regions, such as Amazonian Forest (*C. scurrius*), Atlantic Rainforest (*C. fuscus* and *C. laevicollis*) and Montane Forest (*C. leucometapus*). *Chironius quadricarinatus* forms a clade with a Chacoan species, *C. maculiventris*. This species pair is sister to the entire remainder of the genus (see Hollis, 2006).

Reproductive data on the genus *Chironius* is available mainly to Atlantic Forest popula-

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tion (Marques and Sazima, 2004). Dixon et al. (1993) described information regarding the natural history of all *Chironius* species; however, the number of analyzed mature specimens was insufficient to reveal a reproductive pattern. Sporadic information on the morphology and reproduction of *C. flavolineatus* and *C. quadricarinatus* is also available (Dixon et al., 1993; Feio et al., 1999; Argolo, 2004), whereas Pinto et al. (2008) present data regarding the morphology and diet of these species. However, no detailed study has been published on the reproductive biology of these two Cerrado species. Herein, we present information on the reproductive biology of *Chironius flavolineatus* and *C. quadricarinatus*, including sexual maturity, reproductive cycles in males and females, and fecundity.

Materials and methods

We examined 256 specimens from Cerrado domain (fig. 1; *Chironius flavolineatus* $N = 108$; *C. quadricarinatus* $N = 148$) housed in the following herpetological collections: Museu Nacional, Universidade Federal do Rio de Janeiro (MNRJ), Rio de Janeiro, RJ; Instituto Butantan (IBSP), São Paulo, SP; Universidade Estadual de Campinas (ZUEC), Campinas, SP; Museu de Ciências e Tecnologia, PUCRS (MCP), Porto Alegre, RS; Coleção Herpetológica da Universidade de Brasília (CHUNB), Brasília, DF; Museu de Zoologia da Universidade Federal de Viçosa (MZUFV), Viçosa, MG; Museu de Ciências Naturais da PUC Minas (MCN-R), Belo Horizonte, MG. All examined specimens and localities are listed in the Appendix.

The following data were taken from each specimen: (1) sex; (2) snout-vent length (SVL); (3) reproductive maturity. The specific data were taken from males: (1) diameter of deferens ductus; (2) largest and smallest diameter of left and right testis to calculate total volume (e.g., Saint-Girons, 1982) throughout the year (Volsøe, 1944); and from females: (1) number of eggs; (2) largest diameter of the largest egg or secondary vitellogenesis follicles; (3) presence of multiple clutches.

Males were considered mature if they had enlarged testes and opaque deferens ductus (Shine, 1982), and females

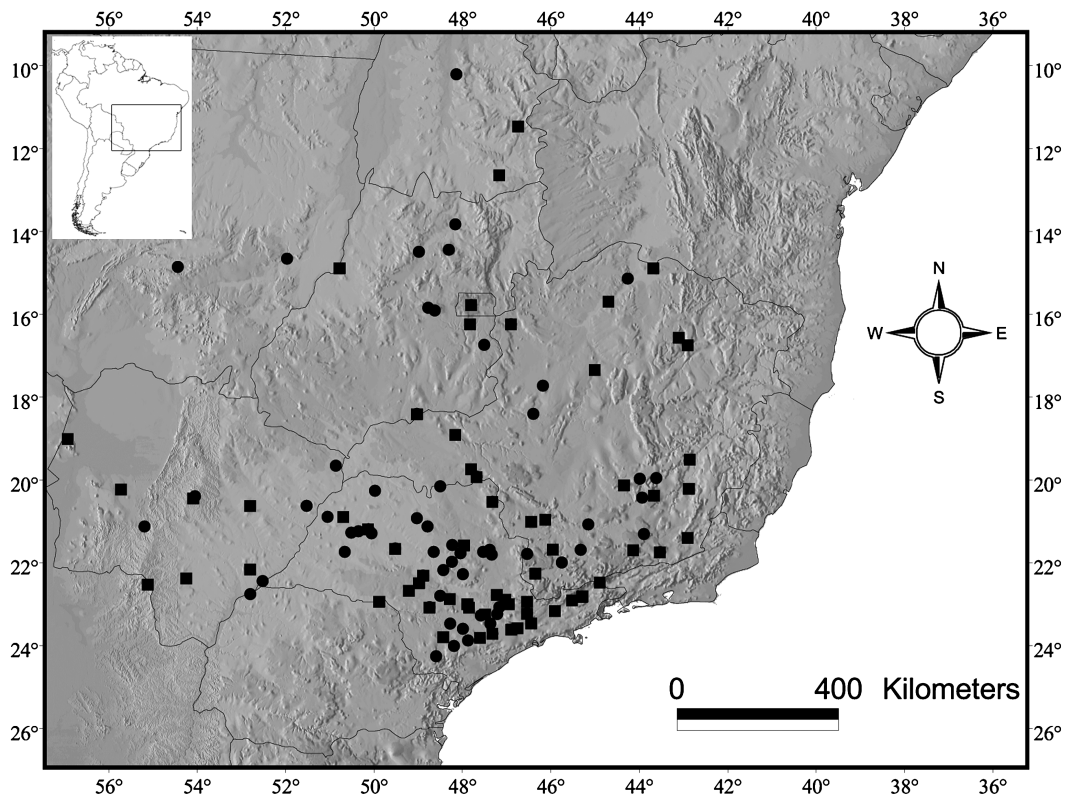


Figure 1. Geographic distribution of examined specimens of *Chironius flavolineatus* (circle) and *C. quadricarinatus* (square) in Cerrado Domain.

were considered mature if they had either ovarian follicles in secondary vitellogenesis (>5 mm) or/and oviduct eggs (e.g., Shine, 1980). The presence of multiples clutches was characterized by females that presented mature vitellogenic follicles and eggs simultaneously (see Shine, 1977a; Fitch, 1982).

Ductus deferens are recognized as the site of sperm storage (Jones, 1998; Sever et al., 2002), showing increased diameter when spermatozoa are present (Shine, 1977b; Pizzatto and Marques, 2002; Almeida-Santos et al., 2004), so the diameter of deferens ductus was measured near the cloacal plate (third ventral scale before cloacae). Volume of testes was calculated using the ellipsoid formula (e.g., James and Shine, 1985).

Additional data were obtained from two captive gravid females of *C. quadricarinatus*, which had laid eggs in a terrarium at the Laboratório de Ecologia e Evolução do Instituto Butantan. These two clutches were incubated in a plastic container with moistened vermiculite at room temperature varying from 19°C to 32°C. The following data were taken from each hatching: (1) sex; (2) SVL; (3) tail length (TAL); (4) total length (TL); (5) mass (M). The masses of these gravid female were also recorded.

All linear measures were taken with a dial calliper to the nearest 0.01 mm, except for snout-vent and tail lengths, which were taken with a flexible ruler to the nearest millimetre (mm). Female body and hatchling mass were recorded to the nearest 0.01 g.

Interspecific variation in size (SVL) at maturation was evaluated under MANOVA (see Pinto et al., 2008). Reproductive pattern was analyzed with graphical analysis of seasonality of the diameter of the largest follicles and eggs for females. Residuals of linear regressions from volume of testes and diameter of deferens ductus with SVL were used to justify the graphical analysis of seasonality in both species. Analysis of covariance (ANCOVA with SVL as covariate) was used to verify interspecific differences in all comparable variables.

Fecundity was estimated by linear regressions of female size (SVL) against number of eggs and egg size. The independence of number of eggs and egg size in each species were ensured by linear regressions (Stewart, 1979). Due to results in linear regression of female size and number of eggs and egg size, an analyse of covariance (ANCOVA with SVL as covariate) was used to verify interspecific differences in egg size, and an analyse of variance (ANOVA) was used to verify interspecific differences in number of eggs. Student *t*-tests for independent groups were used to test for seasonal occurrence of mature males and females among the dry and rainy season, thus testing for homogenous sampling for both species. Along the study region, the rainy and dry seasons are roughly homogeneous. The rainy season starts in October and extends up to March. The remaining of the year consists in the dry season.

All samples included only mature specimens and statistical analyses were made with STATISTICA for Windows version 6.0 (Statsoft, 2001). All continuous variables were transformed to their natural log prior to analysis. We used a significance index value of $P \leq 0.05$ for all analyses.

Results

Sexual maturity

Mature male *C. flavolineatus* (fig. 2a) averaged 671.0 ± 77.2 mm SVL (507-808 mm; $N = 40$), and mature females averaged 714.3 ± 64.6 mm SVL (606-894 mm; $N = 68$). Mature male *C. quadricarinatus* (fig. 2b) averaged 621.6 ± 69.1 mm SVL (504-771 mm; $N = 57$), and mature females averaged 645.7 ± 57.3 mm SVL (514-812 mm; $N = 91$). In both species, males mature with a smaller SVL than females. Females are also significantly larger than males in both species ($F_{1,252} = 13.5$; $P < 0.001$). For inter-specific differences see Pinto et al. (2008).

Female reproductive cycle

No seasonality in number of adults was found among the rainy and dry seasons, although data suggest that adults may be less common during dry season in *C. flavolineatus* (6.0 ± 2.7 vs 12 ± 7.7 , $t = -1.85$; $df = 10$; $P = 0.09$) and in *C. quadricarinatus* (9.3 ± 5.6 vs 14.5 ± 9.8 , $t = -1.12$; $df = 10$; $P = 0.28$).

Chironius flavolineatus exhibits a seasonal reproductive cycle with vitellogenic follicles and oviduct eggs occurring mainly during rainy season (fig. 3a), and *C. quadricarinatus* shows an extended reproductive cycle with oviduct eggs more abundant at the onset of the rainy season (fig. 3b). A female of *C. flavolineatus* with eggs in the oviduct and vitellogenic follicles in the same ovary was collected in October ($N = 1$) (fig. 3a). In *C. quadricarinatus* this condition was observed in July ($N = 1$) and September ($N = 1$) (fig. 3b).

Fecundity

Mean egg size in *C. quadricarinatus* (30.4 ± 5.5 ; 23.6-41.6 mm; $N = 20$) was significantly smaller than in *C. flavolineatus* (34.4 ± 6.4 ; 25.1-51.3 mm; $N = 21$) ($F_{1,39} = 5.0$; $P < 0.05$). However the mean number of eggs in the oviduct of *C. quadricarinatus* (5.1 ± 1.6 ; 3-8; $N = 21$) and *C. flavolineatus* (5.9 ± 1.9 ; 3-

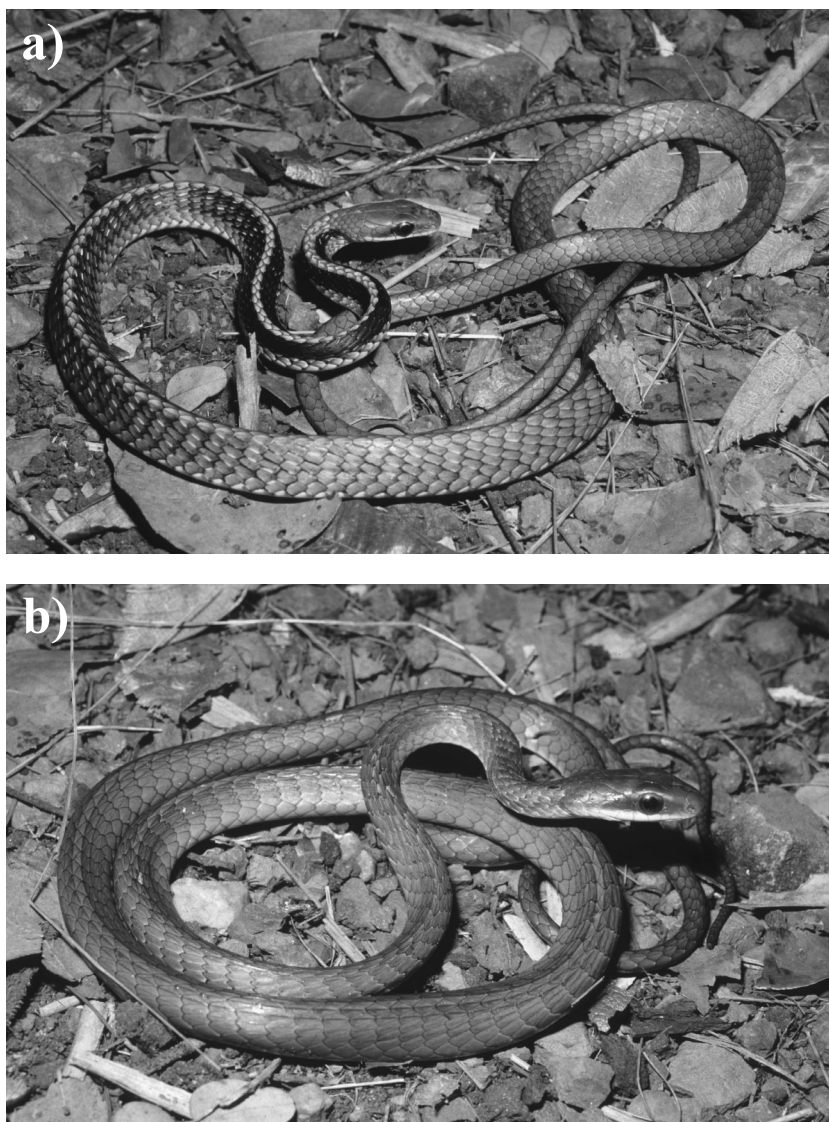


Figure 2. Male adult of *Chironius flavolineatus* (a; IBSP 57350) and male adult of *C. quadricarinatus* (b; IBSP 57351) from UHE Miranda, Uberlândia, MG, Brazil.

11; $N = 21$) was not significantly different ($F_{1,36} = 0.2$; $P = 0.63$). There was a significant positive correlation between clutch size and female snout-vent length in both species (*C. flavolineatus*, $F_{2,19} = 4.2$; $P = 0.05$; $R^2 = 0.2$; and *C. quadricarinatus*, $F_{2,17} = 26.4$; $P < 0.001$; $R^2 = 0.5$; fig. 4a, b). No significant difference was found in female size and egg size in both species (*C. flavolineatus*, $F_{2,19} = 0.005$; $P = 0.9$; and *C. quadricarinatus*, $F_{2,18} = 1.7$;

$P = 0.2$). An negative correlation was found between clutch size and egg size in *C. flavolineatus* ($F_{2,19} = 4.6$; $P < 0.05$; $R^2 = 0.2$; fig. 4c), whereas no correlation was observed in *C. quadricarinatus* ($F_{2,18} = 0.6$; $P = 0.5$; $R^2 = 0.3$; fig. 4d).

Captivity egg-laying records

Two oviposition events were recorded for *C. quadricarinatus* in captivity. A gravid female

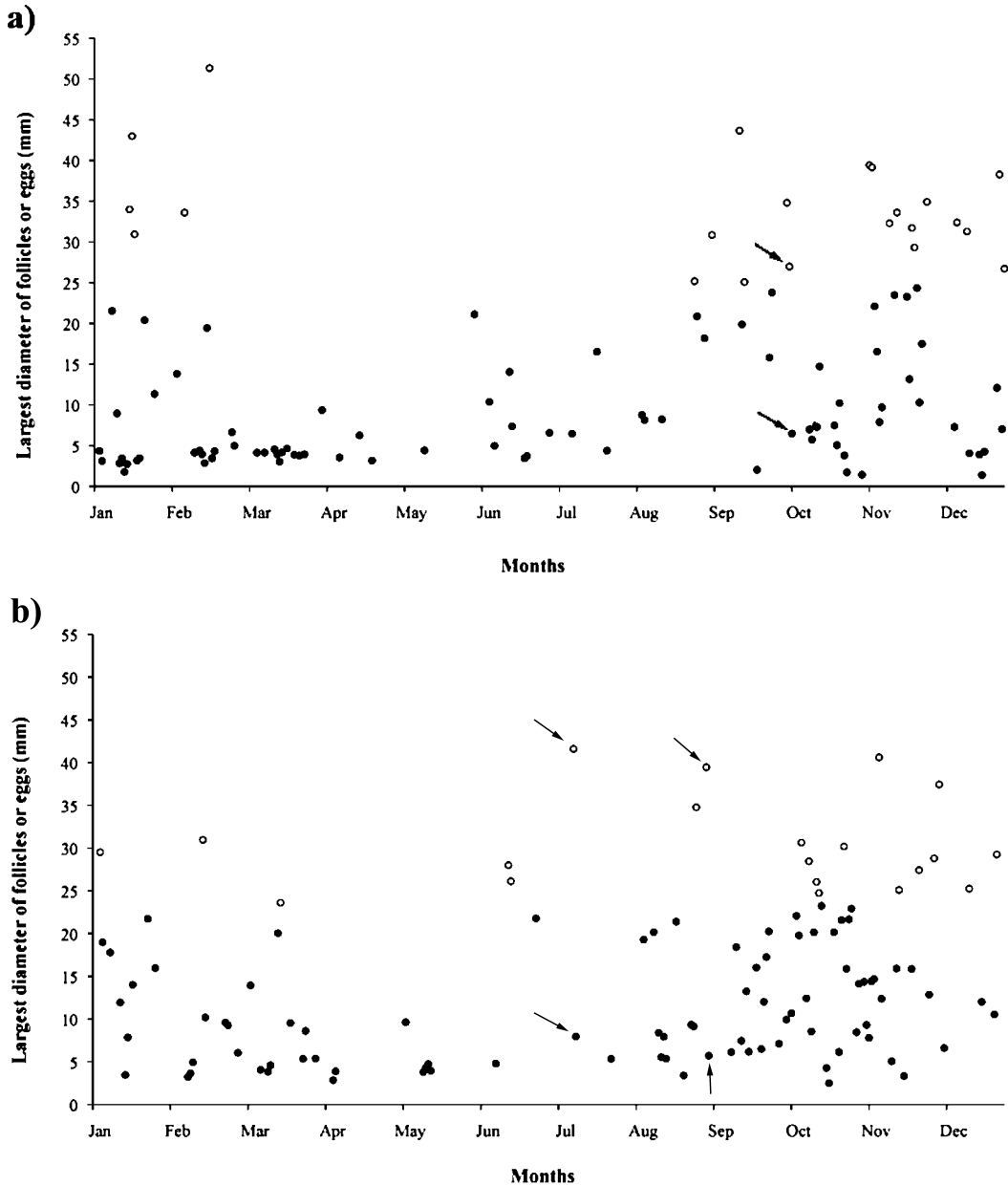


Figure 3. Seasonal variation in the largest diameter of the largest vitellogenic follicle (●) or egg oviductal (○) in *Chironius flavolineatus* (a) and *C. quadricarinatus* (b). Multiple clutches were represented by an arrow.

(IBSP 72091, SVL = 690 mm, TAL = 365 mm, M = 75 g), collected on November 19th, 2004, in Paulínia municipality, São Paulo state, laid six eggs on November 25th, 2004. Three males and three females hatched from these eggs on March 16th, 2005 (SVL = 211.8 ± 8.4 ; 200-221 mm; TAL = 115.5 ± 5.0 ; 110-122

mm; TL = 327.3 ± 10.9 ; 315-341 mm; M = 4.2 ± 0.4 ; 3.8-4.9 mm).

Another gravid female (IBSP 73817, SVL = 625 mm, TAL = 345 mm, M = 42 g) collected in December 2nd, 2005, in Pindamonhangaba municipality, São Paulo state, laid seven eggs in same day (Relative clutch mass = 0.43), none

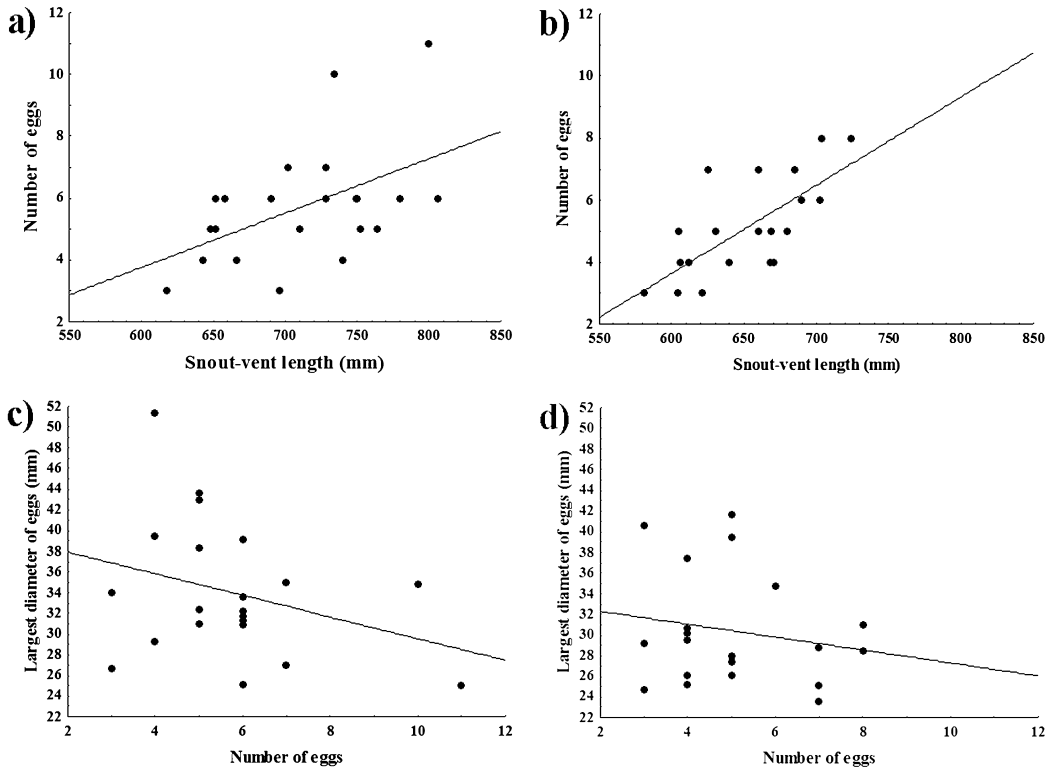


Figure 4. Relationship between female snout-vent length and number of eggs in *Chironius flavolineatus* (a; $R^2 = 0.2$) and *C. quadricarinatus* (b; $R^2 = 0.5$); and relationship between number of eggs and largest diameter of largest egg in *C. flavolineatus* (c; $R^2 = 0.2$) and *C. quadricarinatus* (d; $R^2 = 0.3$).

of which hatched. The largest diameter of eggs from this female was reported (26.2 ± 1.6 ; 24.0–28.1 mm) in reproductive cycle (fig. 3b).

Male reproductive cycle

Analyses of the seasonal variation of the deferens ductus (fig. 5) and volume of the testes (fig. 6) indicate that males of both species have continuous sperm production (in the testes) and storage (in the deferens ductus). Although the testes from *C. flavolineatus* were larger, there was no significant difference between the species ($F_{1,89} = 0.4$; $P = 0.5$).

Discussion

Dixon et al. (1993) suggested that *Chironius quadricarinatus* shows seasonal reproduction based on four specimens. The present study

shows a large number of gravid females at the end of year (wet season). However, the presence of gravid females in the dry season suggests an extended seasonal reproduction where most oviposition may occur during the middle of rainy season. In both species egg-laying is concentrated in warmer and wetter months as have been recorded in other Neotropical colubrid snakes (e.g., Pizzatto and Marques, 2002; Marques and Muriel, 2007). Thus most eggs will be incubated in suitable condition for embryonic development (see Vinegar, 1977).

Both *Chironius quadricarinatus* and *C. flavolineatus* present females simultaneously bearing mature vitellogenic follicles and eggs. This condition is often interpreted as multiple clutches, which may indicate aseasonal reproduction (e.g., Pinto and Fernandes, 2004; Marques and Muriel, 2007). However, *Chironius flavolineatus* presented a seasonal repro-

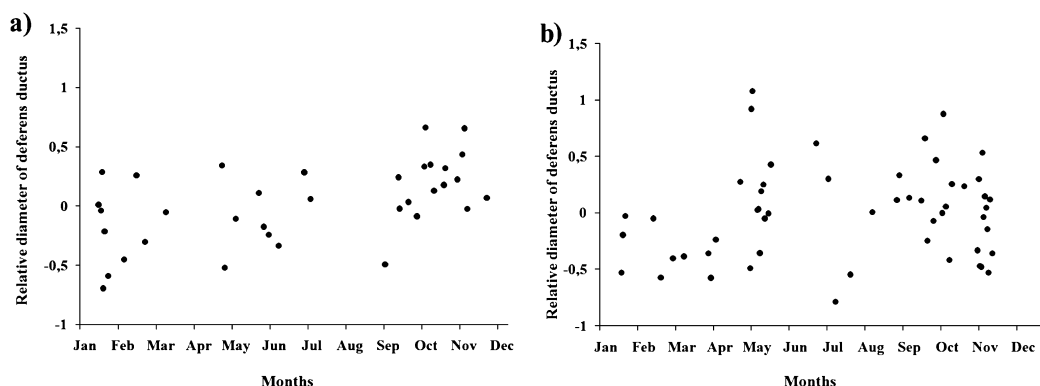


Figure 5. Seasonal variation in diameter of deferens ductus in *Chironius flavolineatus* (a) and *C. quadricarinatus* (b).

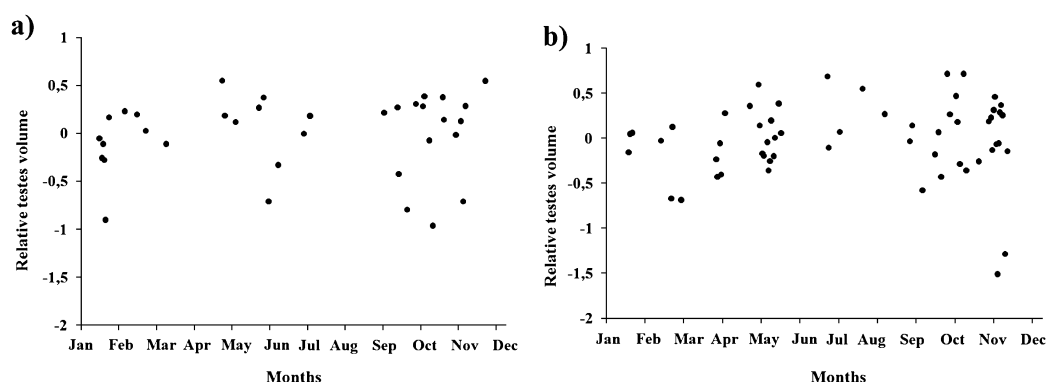


Figure 6. Seasonal variation in volume of testes in *Chironius flavolineatus* (a) and *C. quadricarinatus* (b).

ductive pattern with multiple clutches probably occurring only during the wet season. Multiple clutches may be common among tropical colubrid species. Apparently this strategy is related to high food availability and good environmental conditions through all the year (Brown and Shine, 2002).

Female snakes are often under stronger selective pressure to have larger size (SVL) than males (Shine, 1994) due to high correlation between clutch size and body size (Parker and Plummer, 1987). Also, female size has positive or negative effects in offspring and litter size (e.g., Stewart, 1979, 1989; Shine, 1981; King, 1993). *Chironius flavolineatus* and *C. quadricarinatus* females are larger than males (Pinto et al., 2008) and size had a significant positive correlation with clutch size (quantitative investment [Shine, 2003]), as reported to other

Neotropical colubrids (e.g., Zug et al., 1979; Censky and McCoy, 1988; Shine, 2003).

Females of *Chironius bicarinatus* from Atlantic forest have seasonal reproductive cycle and mating occurs at the onset of the dry season prior to a period of ovulation (at rainy season) indicating a dissociated reproductive pattern (Marques et al., 2009). However, male-male combat behaviour in this species was observed during rainy season (Almeida-Santos and Marques, 2002) suggesting that mating can occur during two periods of the year (Marques et al., 2009). *Chironius flavolineatus* females also present an extended seasonal reproduction with ovulation during the rainy season. Courtship behaviour was recorded in October during the rainy season (Feio et al., 1999). These data corroborate the idea that mating in *Chironius* occurs at least in two periods of the

year (Marques et al., 2009). Moreover the diameter of deferens ductus and volume of testes does not vary seasonally in both species. In species that mate only one period of the year the diameter and the volume do vary (e.g., Almeida-Santos et al., 2006).

Tropical snakes seem to exhibit a higher diversity of reproductive patterns when compared to species in temperate areas (e.g., Seigel and Ford, 1987; Greene, 1997), and some of this variation is related to phylogenetic constraints. Most *Xendodontini* have a consistent continuous reproductive cycle (Pinto and Fernandes, 2004; Pizzatto et al., 2008), although Pizzatto et al. (2008) identified some variation due to climate. Furthermore, differences in reproductive patterns may arise from differences in resource levels, since related sympatric species that occur in similar areas show different patterns of resource use (Shine, 2003; Ji and Wang, 2005; Santos et al., 2005; Pizzatto et al., 2008). *Chironius flavolineatus* and *C. quadricarinatus* are sympatric in most of their distribution and occur mainly in the same climatic conditions. Additionally they eat similar small size abundant anuran species (see Pinto et al., 2008). However they do not share the same reproductive pattern, providing evidence that proximal factors (climate, general habitat types) alone are not sufficient to explain variation in snake reproductive modes.

According to the most recent published phylogeny (Hollis, 2006), *Chironius flavolineatus* is closely related to species showing seasonal reproduction, such as *C. fuscus* and *C. laevicollis* (Marques and Sazima, 2004). Apparently, female reproductive cycle in *Chironius* genus is a conserved character among related species or species groups, but not in the entire genus. The relationship between *Chironius* and other colubrids perhaps explain these differences. *Chironius quadricarinatus* is nested within a clade, which is sister to all remaining *Chironius*. This early divergence may explain the retention of similar traits as those found in other related Colubrid snakes (e.g., Mastigodryas, Marques and

Muriel, 2007) that also show continuous reproductive cycle.

The central position of the Cerrado, its ancient age (see Silva and Bates, 2002) and its wide habitat heterogeneity all seem to favour the coexistence of sympatric lineages showing different eco-evolutionary traits (see Werneck and Colli, 2002; Costa et al., 2007; França et al., 2008; Nogueira et al., 2009; Werneck et al., 2009), such as those observed in *Chironius*. Moreover, the two species present slight differences in habitat use patterns, with *C. flavolineatus* being more often associated to forested sites than *C. quadricarinatus* (França et al., 2008). These differences may well be explained by the same historic-biogeographical factors that generate differences in reproductive traits although further research is still necessary to uncover the relationships between biogeography, habitat use and reproductive strategies in *Chironius*.

Acknowledgements. We are thankful to I. Sazima (ZUEC), S.M. Almeida-Santos and R. Sawaya (IBSP), C. Nogueira (CI), and especially W.B. Jennings (HSU) for helpful insights on early versions of the manuscript; F.L. Franco and V.J. Germano (IBSP), G.R. Colli and M.G. Zatz (CHUNB), I. Sazima (ZUEC), M. Di-Bernardo (*in memoriam*; MCP), L.B. Nascimento (MCN-R) and R.N. Feio (MZUFV) for the access and loan of the specimens. We are thankful to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ), and Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) for financial support.

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- 55525); Três Corações (IBSP 34154); Uberlândia (MCNR 1290). Minas Gerais/São Paulo: UHE Igarapava (Rifaina, Igarapava-SP; Conquista, Uberaba and Sacramento-MG) (MCNR 791-93). São Paulo: Agudos (IBSP 31520, 31555, 32066, 34314, 46183); Andradina (IBSP 19553); Angatuba (IBSP 32774, 45714, 46124); Américo Brasiliense (ZUEC 2470); Araçatuba (IBSP 25297, 25362, 31801, 32555); Araçariguara (IBSP 56960); Araraquara (IBSP 16580, 16588); Avaré (IBSP 58747); Bauru (IBSP 34476, 59585); Bento de Abreu (IBSP 23288); Birigui (IBSP 28828); Boa Esperança do Sul (IBSP 41106); Boituva (IBSP 18134, 32168); Brotas (IBSP 67874; ZUEC 1021); Buri (IBSP 20939); Capão Bonito (IBSP 33577, 43210); Catanduva (IBSP 23186); Catinguá (IBSP 16375); Cesário Longe (IBSP 43069); Colômbia (IBSP 32250); Descalvado (IBSP 23456, 43653, 64916); Espradia (IBSP 16586); Fernandópolis (IBSP 32666); Franca (IBSP 1183, 1815, 27878); Glicério (IBSP 22922); Guararapes (IBSP 32676); Ibitinga (IBSP 23367); Indaiatuba (IBSP 57466); Inúbia Paulista (IBSP 33472); Itapetininga (IBSP 45756, 55645); Itapeva (IBSP 28487); Itú (IBSP 31155, 46970, 59006, 68527, 70687); Ituverava (IBSP 32100); Jaboticabal (IBSP 11820, 50599); Jaú (IBSP 27775); Lins (IBSP 15449, 43624); Matão (IBSP 32580, 32626, 43040); Piedade (IBSP 33609, 58761, 71558); Pilar do Sul (IBSP 59643); Pirassununga (IBSP 27508, 33525, 69494, 69994); Pratânia (ZUEC 994); Ribeirão Branco (ZUEC 1585); Ribeirão Preto (IBSP 21811); Rosana (Porto Primavera) (IBSP 43640-41, 43643, 43645); Santa Rita do Passa Quatro (IBSP 31754); São José do Rio Preto (IBSP 16594, 30343, 30813); São Miguel Arcanjo (IBSP 40412, 52221); Sorocaba (IBSP 23021, 24568, 25326, 24586); Uchôa (IBSP 28082, 28132); Votuporanga (MCP 7297). Paraná: Porto Rico (IBSP 31244). Mato Grosso do Sul: Anaurilândia (IBSP 63043); Campo Grande (IBSP 42295, 42395, 42968-69); Jupiá (IBSP 21653, 21814, 22599, 22607, 21902, 29369); Nioaque (IBSP 40423); Paranaíba (IBSP 46116).

Received: February 1, 2010. Accepted: April 21, 2010.

Appendix: Specimens examined

Chironius flavolineatus – BRAZIL: Mato Grosso: Barra do Tapirapé (MNRJ 588-89); Xavantina (MNRJ 6697, 7778-79, 9273-76); Tocantins: Palmas (CHUNB 21959-60). BAHIA: Corumbá de Goiás (IBSP 49828); Goiás: Cristalina (IBSP 45195); Formosa (CHUNB 19698); Itumbiara (IBSP 42851); UHE Serra da Mesa, Minaçu (MCP 8192-96, 8219, 8227-28, 8426-27, 8568); UHE Serra da Mesa, Niquelândia (MCP 4286); Pirenópolis (CHUNB 3632); Uruaçu (MNRJ 7495); Distrito Federal: Brasília (CHUNB 3633; MNRJ 3242). Goiás/Minas Gerais: UHE Queimados (Luziânia-GO; Unaí-MG) (MNRJ 10929). MINAS GERAIS: Antonio Carlos (IBSP 18838, 19633, 22683, 24056); Barra Feliz (IBSP 17304); Belo Horizonte (MCNR 440); Conceição do Mato Dentro (MCNR 534); Cruzília (IBSP 30978); Jaíba (MZUFV 943-44, 946); João Pinheiro (MNRJ 12900-01); Juiz de Fora (IBSP 32266, 33044, 33180); Lima Duarte (MNRJ 6698); Matias Cardoso (IBSP 67911); Miguel Burnier (IBSP 16587); Nova Lima (MCNR 1466, 1474); Nova Ponte (MNRJ 4696); Perdões (MCNR 509); Poço de Caldas (IBSP 42435; ZUEC 2013); Presidente Olegário (MNRJ 6702); Santa Bárbara (IBSP 30000,

Chironius quadricarinatus – BRAZIL, Mato Grosso: Barra do Garças (IBSP 19957). Tocantins: Natividade (IBSP 32586); Santa Isabel (IBSP 12056). Goiás: Alto Paraíso de Goiás (CHUNB 20327); Aruanã (IBSP 42177); Itumbiara (IBSP 33588, 33590); Luziânia (CHUNB 20328, 20331). Distrito Federal: Brasília (CHUNB 20323). Goiás/Minas Gerais: UHE Queimados (Luziânia-GO and Unaí-MG) (MNRJ 10926-28; MCNR 1018). MINAS GERAIS: Brumadinho (MCNR 642); Carmo do Rio Claro (MNRJ 1841); Cataguases (MCNR 907); Coronel Fabriciano (IBSP 22996, 23124); Frutal (IBSP 37268); Grão Mogol (IBSP 56504); Jacuí (IBSP 64890); Juiz de Fora (IBSP 27832-34, 27950, 29144-45, 31341, 33358, 34201, 40749, 40752-53, 45665); Lagoa Santa (MNRJ 1324); Lima Duarte (IBSP 15323); Machado (IBSP 14362); Ouro Fino (IBSP 15270, 15486, 57303; ZUEC 72); Ouro Preto (MCNR 080); Pirapora (MNRJ 4019); Rio Casca (MNRJ 1967); Rio Pandeiro (IBSP 9168); São Vicente de Minas (IBSP 70580); Sossego (IBSP 28531); Uberaba (IBSP 10948, 31550, 31553); Uberlândia (IBSP 15680); UHE Miranda, Uberlândia (IBSP 57351); UHE Irapé (Botumirim) (MCNR 1598). Minas Gerais/São Paulo: UHE Igarapava (Rifaina, Igarapava-SP; Conquista, Uberaba and Sacramento-MG)

(MCNR 152). São Paulo: Agudos (IBSP 31487, 44193); Andradina (IBSP 27785, 28928); Aparecida (IBSP 19497, 30314, 30323); Araçatuba (IBSP 27424, 31735, 31746, 32616); Atibaia (IBSP 17511, 19400-01, 58565, 61203); Avaré (IBSP 13165, 23099, 23110); Bauru (IBSP 17035); Bofete (IBSP 70070); Botucatu (IBSP 22481, 59520); Bragança Paulista (IBSP 30573); Buri (IBSP 18267-68, 18298, 18651); Caçapava (IBSP 29108); Campinas (IBSP 17895, 18461, 58515; ZUEC 70-71, 164, 182, 503, 509, 887, 1020); Campo Alegre (IBSP 846); Campo Largo (IBSP 16583); Capivari (IBSP 70531); Carapicuíba (IBSP 27082, 55386); Cesário Longe (IBSP 66423); Coimbra (IBSP 16571); Conchas (IBSP 790); Cotia (IBSP 5194); Espírito Santo do Turvo (IBSP 66532); Franca (IBSP 315); Guaratinguetá (IBSP 28813); Guarulhos (IBSP 21519, 26904); Jundiaí (IBSP 29491, 62002, 66735, 68137);

Lins (IBSP 32496); Nova Odessa (ZUEC 2681); Paulínia (IBSP 72091); Piedade (IBSP 33610); Pilar do Sul (IBSP 50271, 58291; MCP 7299); Pindamonhangaba (IBSP 18824, 22107-09, 27414, 55637, 60674, 67527, 67875, 73817); Rincão (IBSP 28958); São José dos Campos (IBSP 18485, 23716, 26936, 28088, 28098, 28406-07, 29244, 67794, 68601). Mato Grosso do Sul: Água Boa (IBSP 56803-04); Campo Grande (IBSP 15242, 27784); Corumbá (IBSP 17003, 17045, 17211, 17284-85, 18822, 40932); Dourados (IBSP 24672); Jupiá (IBSP 22030, 22035, 21901, 22208, 22643, 22970, 27642, 29301, 29357, 29550); Miranda (IBSP 55126); Nova Andradina (IBSP 19142, 27413); Paranaíba (IBSP 46121); Ponta Porã (IBSP 17023, 18296, 23735, 26787, 42398).