Body size, reproductive biology and abundance of the rare pseudoboini snakes genera *Clelia* and *Boiruna* (Serpentes, Colubridae) in Brazil

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Abstract

Body size, reproductive biology and abundance of the rare pseudoboini snakes genera *Clelia* and *Boiruna* (Serpentes, Colubridae) in Brazil. Pseudoboini snakes of the genera *Clelia* and *Boiruna* are apparently rare in nature and certainly rare in collections. This work presents data on body size, reproduction and abundance of five Brazilian species of these genera, in the largest collection of snakes in Latin America, the Instituto Butantan. Despite scarcity of data, follicular cycle seems to be continuous in most species, except *Clelia rustica*, which occurs in highlands. Females are larger than males in all species, and fecundity is low when compared to other pseudoboini. Abundance is very low for all species even considering 100 years of collecting, and it is decreasing in recent decades when compared to other snakes (*Bothrops jararaca*, *Oxyrhopus guibei*, *O. clathratus*, *Philodryas patagoniensis*, *Sibynomorphus mikanii*, and *Spilotes pullatus*). The studied species present at least five traits of commonly threatened species and require more attention in researches and conservation policies.

Keywords: Serpentes, Colubridae, *Clelia*, *Boiruna*, abundance, body size, conservation, reproduction.

Introduction

Diversity of snake species in the Neotropics is enormous. Gathering detailed ecological data based on field observations of habitat use, behavior, and temporal variation in population sizes for each species in highly diverse communities is a daunting task, unlikely to be completed soon (Reed and Shine 2002). Populational studies are virtually absent for any species and despite availability of some studies on communities for some areas (Duellman 1978, Vitt and Vangilder 1983, Dixon and Soini 1986, Strussmann 1992, Martins 1994, Marques 1998, Sawaya 2003), the populational status and abundance of most species are largely unknown. This lack of general and specific knowledge places us far from realistic conservation policies. In sharp contrast to field work with snakes, studies on ecological attributes based on data from preserved museum specimens offer a good opportunity to quantify many life history traits to a degree not yet possible for most reptiles (Reed and Shine 2002).

Reproductive cycles of snakes can be seasonal even in tropical areas (Shine 2003). Studies

Colubridae is the most studied family of snakes in Neotropical areas. However, it is the largest family and constitutes an unnatural group, containing many unrelated species (Heise et al. 1995). Many monophyletic clades in this family, like the pseudoboini tribe, are unstudied, even documenting basic information such as body size and reproduction.


Snakes of genera Boiruna and Clelia are rarely found in the field, in spite of their medium to large size. Thus, they are not abundant in collections, even in large ones such as that of the Instituto Butantan, in São Paulo State. These snakes frequently show ontogenetic shifts in color pattern in which juveniles have a coral snake pattern (generally reddish with white collar and black dorsal stripes) and adults have a dark color pattern (for a detailed description see Zaher 1996 and Franco et al. 1997). In terms of reproduction, the only pseudoboini species studied is the false coral snake Oxyrhopus guibei, which reproduces continuously (Pizzatto and Marques 2002). Considering that this tribe is monophyletic (Zaher 1994) and that there are some phylogenetic patterns in reproduction (Vitt 1987, Shine 1989, Marques 1996a, b, 1998), it was logical to expect that species of Clelia and Boiruna could reproduce throughout the year. However, this is doubtful since these snakes are rare and it could be, at least in part, due to reproduction. A more obvious expectation is that at least largest species must present a high fecundity.

This work presents data on body size, sexual dimorphism and reproduction in Boiruna maculata, Clelia clelia, C. plumbea, C. rustica, C. quimi and C. montana, detecting similarities and differences among species and comparing them to Oxyrhopus guibei. I also present data on seasonal reception of these snakes in the Instituto Butantan collection compared to other species. I make inferences regarding their population status, relating life history traits with their apparent rarity.

Material and Methods

The examined specimens of Boiruna maculata, C. plumbea, C. quimi and C. montana were collected mainly in southeastern Brazil, São Paulo State (19.7 - 25.3° S, 44.2 - 53.2° W), where climate is seasonal, with a hot and rainy spring-summer (October to March) and a dry autumn-winter (April to September). However, C. montana occurs in highland areas where autumn-winter is more rigorous and spring-summer is milder. Clelia rustica specimens were collected in south Brazil (from 22.5 - 33.7° S, 48.2 - 57.6° W), where climate is also seasonal but with a mild and dry spring-summer (October to March) and cold and rainy autumn-winter (April to September). Clelia clelia were collected mostly in the Amazon domain (4.4 - 21.2° S, 45.7 - 73.6° W), where temperatures are high throughout the year but a dry season occurs in the winter (July to September).

I examined 107 individuals of Boiruna maculata (17 females, 34 males and 56 juveniles – juveniles are non-mature snakes, see below), 68 individuals of Clelia plumbea (23 females, 10 males and 35 juveniles), 21 indivi-
duals of *Clelia clelia* (4 females, 8 males and 9 juveniles), 28 individuals of *Clelia rustica* (16 females, 8 males and 4 juveniles), 30 individuals of *Clelia quimi* (10 females, 14 males and 6 juveniles) and seven individuals (3 males and 4 juveniles) of *Clelia montana* from the collections of Museu de História Natural do Capão da Imbuia (MHNCI), Museu de História Natural da Universidade Estadual de Campinas (ZUEC) and Instituto Butantan (IB). Each specimen was measured for snout-vent length (SVL in mm, with a ruler), sexed, and dissected by a mid ventral incision. With a vernier caliper I measured: (1) diameter of the largest follicle or oviductal egg (when eggs were present all of them were measured in length and width), (2) length, width and thickness of both testes and (3) diameter of deferent duct near the cloaca. Females were considered mature if they had vitellogenic follicles larger than 10 mm, oviductal eggs or folded oviducts (Shine 1978). Males were mature if they had turgid testes or opaque and convoluted deferent ducts (Shine 1980a, Slip and Shine 1988). Testes volume, which reflects testicular activity (Volsøe 1944, pers. obs.), were calculated using the ellipsoid formula \(\frac{4}{3}\pi abc\), where \(a = \) length/2, \(b = \) width/2 and \(c = \) thickness/2 (Pleguezuelos and Feriche 1999). Oviductal eggs and vitellogenic follicles were counted to estimate fecundity.

I analyzed snout-vent length differences among species and between sexes using factorial ANOVA with SVL as the dependent variable, sex and species as factors and a Post-hoc Tukey test (Zar 1999).

Testes volume and deferent duct diameter were significantly related to SVL. Thus, variation in testicular volume and diameter of deferent duct were compared among seasons by ANCOVA, using testes volume and deferent duct diameter as dependent variable, season as factor and SVL as continuous variable (Zar 1999).

Clutch size was compared among the studied species and *Oxyrhopus guihe* using linear regression of clutch size by female SVL for all species together and calculating its residuals.

Then, residuals for each species were compared by Kruskal-Wallis test (Zar 1999). Data for *O. guihe* were obtained from Pizzatto and Marques (2002). Reproductive frequency was estimated by the percentage of reproductive females (with ovarian follicles > 10 mm or oviductal eggs) in each sample (Seigel and Ford 1987).

I inferred species activity by the number of preserved snakes in collections by month. I compared the number of snakes for each species among four seasons (Summer, Autumn, Winter and Spring) using Chi-squared test (Zar 1999). I also recorded the number of individuals of the studied species that arrived at Instituto Butantan during each decade between 1917 and 2004. The number of snakes (abundance) by decade was compared using Chi-squared test (Zar 1999).

**Results**

**Body Size**

There were only three mature males of *Clelia montana* (SVL 762, 661 and 635 mm, respectively) in the sample and it was not included in body size analyses.

Females attained larger SVL than males in all species (factorial ANOVA, \(F_{(sex)} = 28.4, p < 0.01\), except *C. clelia* (Table 1). There were significant differences in body size among species (factorial ANOVA, \(F_{(species)} = 51.9, p < 0.01; F_{(interaction)} = 3.56, p < 0.01\). *Clelia quimi* was the smallest species and *C. plumbea* the largest (Table 1). *Clelia plumbea* differed significantly from *Boiruna maculata* (females: \(p < 0.01\), males: \(p < 0.01\), *C. quimi* (both females and males: \(p < 0.01\)) and *C. rustica* (females: \(p < 0.01\), males: \(p < 0.01\)). *Clelia quimi* differed also from *B. maculata* (females: \(p < 0.01\), males: \(p < 0.01\)) and *C. clelia* (females: \(p < 0.01\), males: \(p < 0.01\)). *Clelia rustica* also differed from *C. clelia* (females: \(p < 0.01\), males: \(p < 0.01\)) (Table 1).

Sexual size dimorphism was greatest in *C. plumbea* and it was similar in the other four species.
species (Table 1). Females of most species attain sexual maturity at 80-88% of mean adult body size, except to \textit{C. plumbea} and \textit{C. quimi}, which mature with smaller sizes (50% and 74%). In general, males attain sexual maturity with smaller body sizes than females but the ratio is more variable, from 48% in \textit{C. clelia} to 90% in \textit{C. plumbea} (see minimum sizes in Table 1).

Reproductive Cycles and Recruitment

\textit{Boiruna maculata:} vitellogenic follicles were found in July, August, October and February, while eggs were present in May, August, September and December (Figure 1A). Small snakes (SVL < 400 mm) were found from December to June, with a peak from March to June (Figure 2A). Testicular volume did not differ among seasons (ANCOVA: $F_{(slopes)} = 1.27$, $gl = 3$, $p > 0.05$, $F_{(intercept)} = 0.61$, $gl = 3$, $p > 0.05$) as well as residual diameter of deferent duct (ANCOVA: $F_{(slopes)} = 2.87$, $gl = 3$, $p > 0.05$, $F_{(intercept)} = 0.70$, $gl = 3$, $p > 0.05$).

\textit{Clelia plumbea:} vitellogenic follicles were found throughout the year (Figure 1B) as well as small snakes (SVL < 500 mm). A larger number of small snakes was collected in May, July and October (Figure 2B).

\textit{Clelia clelia:} none of sampled females had vitellogenic follicles or eggs and the smallest snake measured 466 mm (September), 480 mm (no data) and 493 mm (December) in SVL.

\textit{Clelia rustica:} vitellogenic follicles were found in January, March, November and December. One egg occurred in December (Figure 1C) and only one young (probably newborn, SVL = 267 mm) was sampled in March.

\textit{Clelia quimi:} vitellogenic follicles were found in July and eggs in September and October (Figure 1D). Two very small snakes (SVL = 215 and 226 mm,) were found in February, one in March (SVL = 238 mm), one in April (SVL = 260 mm) and one in May (SVL = 333 mm).

\textit{Clelia montana:} only three young (probably newborn) were collected in April (SVL = 276 mm), May (SVL = 275 mm) and December (SVL = 265 mm).

Fecundity

When all species were considered together, clutch size increased with female SVL ($R^2 = 0.44$, $\beta = 0.008$, $p < 0.01$, Figure 3). Clutch size differed among species of \textit{Boiruna} and \textit{Clelia} ($H_{(3,22)} = 13.89$, $p < 0.01$) and also with \textit{O. guibei} ($H_{(4,61)} = 17.04$, $p < 0.01$, Figure 3). \textit{Clelia quimi} (and also \textit{O. guibei}) had the largest clutch size and \textit{C. plumbea} the smallest (Figure 3, Table 2). Egg size was smaller in \textit{C. quimi} than in \textit{B. maculata} (Table 2) and it is probably due to differences in body size of the species. Reproductive frequency was 53% in \textit{C. clelia}, 56% in \textit{C. rustica}, 50% in \textit{C. plumbea} and 40% in \textit{C. quimi} (i.e. only half of adult females are reproducing each year).

\begin{table}[h]
\centering
\caption{Body size in five adult Pseudoboinae snakes genera \textit{Clelia} and \textit{Boiruna}. All measures in mm, SD = standard deviation. Significant results (\(p < 0.05\)) are in bold letters.}
\begin{tabular}{lcccccc}
\hline
& \textit{Boiruna maculata} & \textit{Clelia rustica} & \textit{Clelia plumbea} & \textit{Clelia quimi} & \textit{Clelia clelia} \\
\hline
\text{Mean SVL} & 1221.6 & 1079.1 & 1139 & 952.4 & 2004.5 & 1146.2 & 888.6 & 725 & 1632.2 & 1348.6 \\
\text{SD} & 143.4 & 178.2 & 97.97 & 147.9 & 450.6 & 322.6 & 148.1 & 95.4 & 295.8 & 347.9 \\
\text{Range} & 990-1467 & 745-1465 & 1000-1850 & 750-1195 & 1194-2790 & 1035-2240 & 660-1078 & 573-884 & 1372-2028 & 650-1763 \\
\text{N} & 17 & 32 & 16 & 8 & 25 & 10 & 10 & 14 & 4 & 8 \\
\text{t-test} & t = 2.84, p < 0.01 & t = 2.48, p < 0.05 & t = -3.66, p < 0.01 & t = 3.26, p < 0.01 & t = 1.39, p > 0.05 \\
\text{SSD} & 0.13 & 0.17 & 0.75 & 0.22 & 0.21 \\
\hline
\end{tabular}
\end{table}
Body size, reproductive biology and abundance of the rare pseudoboini snakes genera Clelia and Boiruna

Figure 1 - Seasonal distribution of vitellogenic follicles (dark circles) and eggs (white circles) in pseudoboinae snakes genera Boiruna (A) and Clelia (B-D) from Brazil.

Figure 2 - Number of young in Pseudoboinae snakes genera Boiruna (A) and Clelia (B) from Brazil.

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number of females ($\chi^2 = 20.00, df = 3, p > 0.05$) among seasons. Male samples were too small ($N = 8$).

*Clelia rustica*: although small sample size, females were collected more during November to December ($\chi^2 = 23.74, df = 1, p < 0.01$) and male samples were too small (Figure 4B).

*Clelia quimi*: there were no differences in number of females ($\chi^2 = 3.60, df = 3, p > 0.05$) or males ($\chi^2 = 1.43, df = 3, p > 0.05$, Figure 4C) among seasons.

**Number of Snakes Received at Instituto Butantan**

Snakes of genera *Boiruna* and *Clelia* were rarely collected before 1960 when a large increase was recorded (Figure 5A). After the 1960s, the number of snakes decreased and dropped to 39 from 1994 to 2004. *Boiruna maculata* is the most abundant species, followed by *C. plumbea* and *C. quimi*. *Clelia montana* is the rarest species with only six specimens recorded in total. Number of collected snakes is greatest from 1961 to 1971 and least from 1994 to 2004 for *B. maculata* ($\chi^2 = 27.9, df = 3, p < 0.01$), *Clelia clelia* ($\chi^2 = 26.0, df = 3, p = 0.028$), *C. plumbea* ($\chi^2 = 39.7, df = 3, p < 0.01$), *C. bicolor* ($\chi^2 = 8.4, df = 3, p = 0.038$) and *C. quimi* ($\chi^2 = 27.2, df = 3, p < 0.01$) (Figure 5A). Number of collected snakes of *C. rustica* did not vary among these periods ($\chi^2 = 5.2, df = 3, p > 0.05$). The decrease in number of snakes

**Activity**

Sample sizes for male and female *Clelia clelia* and *C. montana* were too small; data on activity for these species are not presented.

*Boiruna maculata*: number of females did not vary among seasons ($\chi^2 = 3.94, df = 3, p > 0.05$, Figure 4A). Although number of males also did not vary ($\chi^2 = 6.29, df = 3, p > 0.05$), they were more abundant in April and May compared to other months ($\chi^2 = 14.06, df = 1, p > 0.05$, Figure 4A).

*Clelia plumbea*: there were no differences in number of females ($\chi^2 = 20.00, df = 3, p > 0.05$) among seasons. Male samples were too small ($N = 8$).

*Clelia rustica*: although small sample size, females were collected more during November to December ($\chi^2 = 23.74, df = 1, p < 0.01$) and male samples were too small (Figure 4B).

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**Table 2** - Fecundity parameters of four pseudoboini snakes genera *Boiruna* and *Clelia*. Clutch size: mean ± standard deviation. Significant results are in bold letters.

<table>
<thead>
<tr>
<th></th>
<th>Boiruna maculata</th>
<th>Clelia plumbea</th>
<th>Clelia rustica</th>
<th>Clelia quimi</th>
</tr>
</thead>
<tbody>
<tr>
<td>R² (β)</td>
<td>0.59 (0.06)</td>
<td>0.21 (0.01)</td>
<td>0.21 (0.006)</td>
<td>0.83 (0.061)</td>
</tr>
<tr>
<td>Significant p</td>
<td>&lt; 0.05</td>
<td>p &gt; 0.05</td>
<td>p &gt; 0.05</td>
<td>p &gt; 0.05</td>
</tr>
<tr>
<td>Egg length (mm)</td>
<td>45.8 ± 8.1 [35]</td>
<td>-</td>
<td>-</td>
<td>31.7 ± 4.7 [23]</td>
</tr>
<tr>
<td>Egg width (mm)</td>
<td>19.7 ± 3.9 [35]</td>
<td>-</td>
<td>-</td>
<td>17.1 ± 2.9 [23]</td>
</tr>
</tbody>
</table>
collected after 1972 is not seen in other species, including two pseudoboini of the genus Oxyrhopus (Figure 5B).

**Discussion**

Female reproductive cycles are continuous in Boiruna maculata and Clelia plumbea, similar to Oxyrhopus guibei (Pizzatto and Marques 2002). In the other species, the small sample size makes this affirmation uncertain. There are many phylogenetic trends in reproductive patterns of snakes, with most species in the same monophyletic clade exhibiting the same reproductive timing. This trend was observed in Boinae snakes (pers. obs.), Xenodontini (Jordão 1996, Marques 1996a, Pizzatto 2003, Pinto and Fernandes 2004), coral snakes (genus Micrurus; Marques et al. 2003) and in pitvipers (Bothrops; Almeida-Santos and Salomão 2002, Nogueira et al. 2003, Hartmann et al. 2004). Probably, the genus Clelia is not monophyletic and many species in this genus seem to be more closely related to Pseudoboa (Zaher 1994). Moreover, C. quimi and C. rustica may belong to a clade unrelated with Clelia and Boiruna. On the other hand, climate can also affect reproduction (Seigel and Ford 1987) and the same species can present continuous cycle in warmer areas but seasonal pattern in colder areas (see Jordão 1996 for Waglerophis merremii, Maciel 2002 and Pizzatto 2003 for Liophis miliaris, Pinto and Fernandes 2004 for Liophis poecilogyrus). Considering climatic influence, it is more probable that species such as Clelia rustica and Clelia montana, which occur in cold areas - i.e., southwards and highlands areas, respectively - present a seasonal pattern. In this case, egg-laying should occurs in the spring and hatchlings in late summer to early autumn. Thus, eggs can incubate in higher temperatures (in spring-summer) avoiding low temperatures and possible abnormal embryologic development (Vinegar 1977).

Male cycle is more difficult to access. Although some tropical snakes have seasonal testicular cycles (Seigel and Ford 1987, Almeida-Santos and Salomão 2002), sperm production is commonly cheaper than egg production and continuous cycles are also expected in tropical areas (Fitch 1982, Saint-Girons 1982, Vitt 1983, Seigel and Ford 1987). Boiruna maculata, as well as Oxyrhopus guibei (Pizzatto and Marques 2002), have continuous
sperm production and it may occur in other pseudoboini such as *Clelia* spp.

Reproductive events may influence snake activity (Shine 1979, Marques et al. 2001). Despite the possibility of some bias in the searching effort throughout the year that can occur in collections, the number of snakes per season can be a good indicative of their activity (Shine 1980a, Marques et al. 2001). Frequently, males are more exposed to collection during the breeding season when they are more active looking for females (Duvall et al. 1992). More males *Boiruna maculata* are collected in April and May compared to other months; thus, it is possible that this species mates in autumn. More females *Clelia rustica* are collected in November-December when they have oviductal eggs.

Gravid snakes tend to increase thermoregulation time, are more active searching for sites for oviposition, and are slower than non-gravid (Shine 1979, 1980b, Seigel et al. 1987) being more susceptible for collection.

This study showed that members of the pseudoboini genera *Boiruna* and *Clelia* have the potential to reproduce continuously. Snakes from these genera seem to be uncommon and rare in nature (Marques et al. 1998) even when compared to other pseudoboini such as *Oxyrhopus guibei* (Pizzatto and Marques 2002). Despite the generalist diet (Amaral 1978, Vitt and Vangilder 1983, Pinto and Lema 2002, Teixeira and Vrcibradic 2003) and continuous reproduction, most species (at least those occurring in warmer areas) exhibit a low fecundity, in terms of relative clutch size and reproductive frequency, when compared to *Oxyrhopus guibei*. Low clutch size is unexpected in large species such as *Boiruna maculata*, *Clelia plumbea* and *Clelia clelia*. Rarity is largely affected by reproductive traits (McArthur and Wilson 1967, Kunin and Gaston 1993) and so, the low fecundity and late maturity of adults (large SVL) can be a factor contributing to low abundance of the studied species.

The oldest specimen analyzed in this study was a *Boiruna maculata* collected in 1917. Even considering a sample of 90 years of collecting and receiving of snakes, all species together totaled 436 individuals, and most species have a very small sample size. Considering reception of these pseudoboini snakes through the whole existence of the Institute Butantan, the maximum numbers recorded from 1961 to 1971 can be due a high incentive campaign for snake collection those years and can be also seen in other snakes (Figure 5B). However, in the

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**Figure 5** - Seasonal abundance of (A) Pseudoboinae snakes genera *Boiruna* and *Clelia* and (B) other snakes from 1917 to 2004. (A) Black bars: *Clelia clelia*, white: *C. plumbea*, diagonal stripes: *Boiruna maculata*, dotted: *C. rustica*, horizontal stripes: *C. bicolor*, grey: *C. quimi* and vertical stripes: *C. montana*. (B) Black bars: *Oxyrhopus clathratus*, white: *Bothrops jararaca*, diagonal stripes: *Philodryas patagoniensis*, dotted: *Sibynomorphus mikanii*, horizontal stripes: *Spilotes pullatus*, grey: *Oxyrhopus guibei*. **Phylomedusa** - 4(2), December 2005
following ten years, the reception of *Clelia* and *Boiruna* species decreased considerably and reached a minimum from 1994 to 2004, even with snakes rescued from a hydroelectric flooding occurred at this decade. During this rescue, only one *Clelia quimi* was collected. This decrease is not recorded for species such as *Bothrops jararaca*, *Oxyrhopus guibei*, *O. cla thratus*, *Philodryas patagoniensis*, *Sibynomorphus mikanii* or *Spilotes pullatus* (Figure 5B). Thus, numbers of these species are decreasing through the years, possibly due to populational decrease in nature. Reception of *Clelia montana* has been very low since the founding of the Institute. This low abundance probably is related to their restricted distribution in highlands (Franco et al. 1997).

None of the studied species are in the IUCN red list or local red lists due to absence of information for evaluation. However, there are several traits that increase risk of extinction, such as low abundance (Pimm et al. 1988, 1993, Mace and Kershaw 1997), small range size (Gaston 1994, Hanks et al. 1996, Manne et al. 1999), habitat specialization (Brown 1995), proximity to locations of high human density, large body size (McKinney 1997, Owens and Bennett 2000), geographic location (Ariño and Pimm 1995, Manne et al. 1999), higher trophic level (Primack 1993, Gaston and Kunin 1997, Manne and Pimm 2001) and “slow” life histories (small litters, slow growth, late maturation, long inter birth intervals and high survival rates) (MacArthur and Wilson 1967, Pimm 1991, Purvis et al. 2000, Webb et al. 2002, Pilgrim et al. 2004). Larger size of females in relation to males, absence of combat, ambush feeding behavior (Reed and Shine 2002) and “slow” life histories (Webb et al. 2002) may make snakes more vulnerable to extinction. Species of *Clelia* and *Boiruna* apparently have at least five (low abundance, “slow” life-history – low fecundity and possibly late maturation due to the large size of maturation – larger body size, higher trophic level, females larger than males and absence of combat) of these traits, which suggest that they deserve more attention in research and conservation policies.

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