REPRODUCTIVE STRATEGIES OF NEW WORLD CORAL SNAKES, GENUS MICRURUS

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ABSTRACT: New World Coral Snakes (genus Micrurus) occur from North to South America in a wide range of climates and habitats. Using both original and published data, we show that reproductive patterns diverge in the two phylogenetic lineages of Micrurus within the subtropical regions. Species with black rings arranged in triads are characterized by males larger than or equal to females, male combat behavior, and a broader season of vitellogenesis and oviposition. In these species, mating in autumn is synchronous with both spermatogenesis and vitellogenesis. Thus, females need to store sperm until ovulation in spring. In species with black rings arranged in monads, females are generally larger than males, there is no male–male combat, and seasonal vitellogenesis occurs in spring synchronous with mating. Egg laying occurs from late spring to summer, and hatchlings emerge from late summer to autumn. Spermatogenesis peaks during autumn, and males store sperm in the deferent duct over winter, until the mating season. Despite these phylogenetic trends, climatic influence on the extension of reproductive cycles was evident, with equatorial species exhibiting more continuous cycles and species from cold areas exhibiting more seasonal cycles. These two disparate reproductive strategies may be considered another differential trait between these two clades of Micrurus showing the high divergence between them.

Key words: Coral Snake; Micrurus; Phylogenetic lineage; Reproduction; Subtropical area

The diversity of reproductive tactics among snakes has always attracted considerable attention (Shine, 2003). The amplitude of such variation, especially of the reproductive cycles, is even more pronounced in tropical areas, making the recognition of patterns much harder (Seigel and Ford, 1987; Greene, 1997). Recent studies have expanded our knowledge of the reproductive cycles of tropical snakes (Mathies, 2011). However, with such a high species richness, lineage diversity, and climatic and ecological complexity, there is still an absence of data regarding basic reproductive aspects in many species, making generalizations about reproductive strategies of tropical snakes premature.

The semifossorial Coral Snakes are the only representatives of the family Elapidae in the New World, comprising at least 70 species in the monotypic genus Micruroides, and the speciose genus Micrurus (including Leptomicrurus; Slowinski, 1995, Campbell and Lamar, 2004). Micrurus species occur in a wide range of climates and habitats from the southeastern United States to South America (Campbell and Lamar, 2004). The genus includes two distinct phylogenetic lineages: one lineage includes approximately 40 species that possess black rings arranged in monads (BRM—one black ring in between two white/yellow rings and these rings in between red rings), and the other lineage with approximately 20 species possessing black rings in triads (BRT—three black rings separated by white/yellow rings, and this set of rings in between red rings; Slowinski, 1995, Campbell and Lamar, 2004). The two clades of Micrurus are similar ecologically, and the species from different lineage can be sympatric in several regions where they usually seem to live in syntopy and use the same type of food resource (Martins and Oliveira, 1998; Argôlo, 2004; Marques et al., 2004).

The reproductive biology within Micrurus is still not well known, but data are available for some species. Among the BRM species, data on sexual dimorphism, male and female
reproductive cycles, and seasonal activity are available for *M. fulvius* and *M. tener* from North America (Quinn, 1979; Jackson and Franz, 1981) and for *M. corallinus* from South America (Marques, 1996; Almeida-Santos et al., 2006). Information on reproductive biology is also available for *M. nigrocinctus* from Central America (Solórzano and Cerda, 1988; Goldberg, 2004). Data on BRT species are more scarce, with fragmentary information on the reproduction of *M. altirostris*, *M. decoratus*, and *M. pyrrocopterus* (Almeida-Santos et al., 1998; Marques, 2002; Marques et al., 2006; Avila et al., 2010).

Males of most species of the BRT group reach a larger size than females (Roze, 1996; Marques, 2002), whereas in BRM group the females usually attain larger body size (Jackson and Franz, 1981; Marques, 1996; Roze, 1996). In addition, the species of the two lineages from subtropical regions differ in seasonal surface activity, a difference that may be related to reproduction events, including mating (Marques et al., 2006). These data and record of combat only in the Coral Snakes of the BRT group (Almeida-Santos et al., 1998) indicate that differential reproductive strategies may occur between these two distinct phylogenetic lineages of *Micrurus*. The purpose of this study is to assess this hypothesis by using original and published data on reproductive cycles, reproductive behavior, and body sizes of *Micrurus* species from both groups.

**Materials and Methods**

Our original data are based on the analyses of four species of Coral Snakes with BRT pattern, from seasonal subtropical areas (between 20 and 30°S): 148 *M. altirostris* (43 females, 81 males, 24 immature; this species was included in *M. frontalis* in previous taxonomic classification), 60 *M. decoratus* (52 mature males, 8 immature), 126 *M. frontalis* (41 females, 58 males, 27 immature), and 149 *M. lemniscatus* (49 females, 45 males, 55 immature). Our sample was restricted to preserved snakes that were killed at the time of (or soon after) collection. All specimens were collected in southern and southeastern Brazil and belong to the Instituto Butantan (IB) and Museu de História Natural da Universidade Estadual de Campinas (ZUEC) collections. For each specimen, we measured the snout–vent length (SVL, in millimeters), and after dissection we recorded sex, diameter of the largest ovarian follicle or oviducal egg, length of the right testis, and diameter of the distal portion of deferent ducts (cf. Almeida-Santos et al., 2006). Females were considered sexually mature if they had enlarged follicles (> 5 mm) and oviducal eggs or folded oviducts, and males were considered mature if they had opaque and convoluted deferent ducts (Shine 1977a, 1980; Marques, 1996). Immature individuals < 300 mm in SVL were considered to be newborns (or at least a couple of months old, based on our own experience with these species), and data on dates of collection were used to infer recruitment periods.

Mean body sizes of mature males and females were compared using t-tests, and we calculated the index of sexual size dimorphism (SSD; [mean size of the larger sex/mean size of the smaller sex] – 1; this index is expressed as negative if males are the larger than females, Shine 1994). We described the female reproductive cycles by plotting the size of the largest follicle or egg by the date of collection, and we compared the length of the right testis and diameter of deferent ducts among the seasons by using ANOVA. Because both testis size and deferent duct diameter were related to SVL in all species, we used the residuals of the regression of these variables by SVL in all analyses, except for the deferent duct of *M. lemniscatus* in which there was no such correlation.

We compared our original data with published data for BRM Coral Snakes also from subtropical areas (approximately between 20 and 30°S and 25 and 30°N): *M. corallinus* from the Southern Hemisphere in eastern Brazil (Marques, 1996; Almeida-Santos et al., 2006) as well as *M. fulvius* and *M. tener* from the Northern Hemisphere in the southern United States (Quinn, 1979; Jackson and Franz, 1981). For comparison on sexual dimorphism, we also used data on adult body sizes of *M. nigrocinctus* from tropical regions in Northern Hemisphere (Solórzano and Cerda, 1988; Goldberg, 2004). Information on male–male combat and courtship for
Table 1.—Mean snout-vent length (SVL, in mm) ± SD, range (in parentheses) and degree of sexual size dimorphism (SSD) in Coral Snakes from the New World. BRM = black rings arranged in monads, and BRT = black rings in triads.

<table>
<thead>
<tr>
<th>Species</th>
<th>Female SVL</th>
<th>Male SVL</th>
<th>SSD</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BRM</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Micrurus corallinus</td>
<td>672 ± 123</td>
<td>563 ± 73</td>
<td>0.19</td>
<td>Marques, 1996</td>
</tr>
<tr>
<td></td>
<td>(425-650, n = 194)</td>
<td>(440-740, n = 125)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. fulvius</td>
<td>727 ± 80</td>
<td>547 ± 72</td>
<td>0.33</td>
<td>Jackson and Franz, 1981</td>
</tr>
<tr>
<td></td>
<td>(250-970, n = 52)</td>
<td>(260-700, n = 73)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. tener</td>
<td>630 ± 80†</td>
<td>539 ± 72</td>
<td>0.15</td>
<td>Quinn, 1979</td>
</tr>
<tr>
<td></td>
<td>(494-971, n = 71)</td>
<td>(400-635, n = 64)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. nigrocinctus</td>
<td>606 ± 86</td>
<td>530 ± 74</td>
<td>0.14</td>
<td>Goldberg, 2002</td>
</tr>
<tr>
<td></td>
<td>(490-783, n = 19)</td>
<td>(450-640, n = 15)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. n. nigrocinctus</td>
<td>588 ± 145</td>
<td>530 ± 83</td>
<td>0.11</td>
<td>Solórzano and Cerda, 1988</td>
</tr>
<tr>
<td></td>
<td>(327-1000, n = ??)</td>
<td>(331-692, n = ??)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. n. mosquitensis</td>
<td>696 ± 115</td>
<td>510 ± 97</td>
<td>0.36</td>
<td>Solórzano and Cerda, 1988</td>
</tr>
<tr>
<td></td>
<td>(382-887, n = ??)</td>
<td>(363-474, n = ??)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>BRT</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. altirostris</td>
<td>606 ± 98</td>
<td>704 ± 128</td>
<td>-0.16</td>
<td>Present work</td>
</tr>
<tr>
<td></td>
<td>(499-977, n = 45)</td>
<td>(459-1036, n = 93)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. decoratus</td>
<td>516 ± 41</td>
<td>510 ± 92</td>
<td>0.01</td>
<td>Marques, 2002, present work</td>
</tr>
<tr>
<td></td>
<td>(465-570, n = 6)</td>
<td>(360-802, n = 51)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. frontalis</td>
<td>757 ± 115</td>
<td>953 ± 201</td>
<td>-0.26</td>
<td>Present work</td>
</tr>
<tr>
<td></td>
<td>(500-957, n = 41)</td>
<td>(641-1425, n = 58)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. lemniscatus</td>
<td>806 ± 188</td>
<td>824 ± 196</td>
<td>-0.02</td>
<td>Present work</td>
</tr>
<tr>
<td></td>
<td>(500-1172, n = 36)</td>
<td>(458-1237, n = 45)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

† Single question mark (?) indicates information missing from reference.
Double question mark (??) indicates studies used 120 specimens in total of Micrurus n. nigrocinctus and 119 specimens of M. n. mosquitensis, but they did not specify the number of females and males.

Snakes from both lineages was summarized from observations by fellow researchers, our own, and published literature.

Results

BRT Coral Snakes

Body sizes and sexual dimorphism.—Male SVL was larger than female SVL in M. altirostris (t = 4.55, df = 136, P < 0.0001) and M. frontalis (t = 5.64, df = 97, P < 0.0001), but no significant difference was found for M. lemniscatus (t = 0.41, df = 80, P = 0.681) and M. decoratus (t = 0.16, df = 55, P = 0.875; Table 1). SSD was negative or close to zero for all BRT species (Table 1).

Mating and combat behavior.—In addition to previous records of combat behavior in captive males M. altirostris (cf. Almeida-Santos et al., 1998), three additional fighting males were observed in nature during the same season of the year (April and May; A. Tozzetti and J. L. Ucha, personal communication; Fig. 1). Copulation in this species also was recorded in April (S. Cechin, personal communication; Table 2).

Female reproductive cycles.—Records of enlarged follicles (> 5 mm) started during autumn and progressed through spring when ovulation occurred for M. altirostris, M. frontalis, and M. lemniscatus. The female reproductive cycle was strictly seasonal in M. altirostris, but M. frontalis and probably M. lemniscatus (based on the broad dispersion of enlarged follicles) seem able to produce eggs throughout the year.

Fig. 1.—Record of ritual combat in the field in Micrurus Coral Snakes with black rings arranged in triads (BRT). M. altirostris. Photograph by João L. Ucha; used with permission.
Table 2.—Time of the different events of the reproductive cycle in Coral Snakes from subtropical areas. BRM = black rings arranged in monads, and BRT = black rings in triads. Data from 1Almeida-Santos et al., 1998; 2Jackson and Franz, 1981; 3Marques, 1996; 4Marques, 2002; 5Marques et al., 2006; 6Quinn, 1979; and 7present work.

<table>
<thead>
<tr>
<th>Species/predominant season</th>
<th>Latitude</th>
<th>Follicles &gt; 5 mm</th>
<th>Ovulation/oviposition</th>
<th>Hatching</th>
<th>Mating</th>
<th>Peak of sperm production</th>
<th>Spawns in the deferent duct</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Micruroides corallinus 1, 2</td>
<td>20–30°S</td>
<td>September–December</td>
<td>November–January</td>
<td>February–May</td>
<td>October–November</td>
<td>April–June 1, 2, 5</td>
<td>July–December 1, 2, 5</td>
</tr>
<tr>
<td>Season</td>
<td></td>
<td></td>
<td>Late spring</td>
<td>Autumn</td>
<td></td>
<td>Spring</td>
<td>Winter–spring</td>
</tr>
<tr>
<td>BRT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. altirostris 4, 5</td>
<td>25–30°S</td>
<td>June–January</td>
<td>November</td>
<td>March–April</td>
<td>April</td>
<td>January–March 4, 5</td>
<td>January–December 4, 5</td>
</tr>
<tr>
<td>M. decoratus 4, 5</td>
<td>22–27°S</td>
<td>October–November</td>
<td>November</td>
<td>May</td>
<td>—</td>
<td>January–December 4, 5</td>
<td>January–December 4, 5</td>
</tr>
<tr>
<td>M. frontalis 7</td>
<td>20–24°S</td>
<td>February–September</td>
<td>August, January</td>
<td>November–March</td>
<td>—</td>
<td>January–December 7</td>
<td>January–December 7</td>
</tr>
<tr>
<td>M. lemniscatus 7</td>
<td>22–22°S</td>
<td>May–October</td>
<td>—</td>
<td>January–June</td>
<td>—</td>
<td>Extended or summer</td>
<td>Any</td>
</tr>
<tr>
<td>Season</td>
<td></td>
<td></td>
<td>Spring or autumn–spring</td>
<td>Autumn</td>
<td></td>
<td>Extended or summer</td>
<td></td>
</tr>
</tbody>
</table>

1 Behavioral observations.
2 Infers by maximum testis size or mass and deferent duct diameter.
3 Evidence by histology.
4 Single question mark (?) indicates uncertain data.

Summary of data—Females were larger and attained larger maximum body size than males, although there is no record of interactions between these snakes in nature. In our samples, the largest males and females were recorded from M. decoratus and M. lemniscatus, respectively. Newborn M. altirostris and M. decoratus were recorded to be 293 and 278 mm long, respectively.

BRM Coral Snakes

Males and females were recorded from late spring to early autumn, and M. lemniscatus from summer to autumn (Fig. 4).

For a longer period (Fig. 2). Because published data show mating occurs in summer and autumn, females may store sperm in the deferent duct (Figs. 3A and 3B). No seasonal variation in the size of the testis was detected in the M. decoratus (F3, 45 = 0.66, P = 0.57), M. altirostris (F3, 45 = 0.06, P = 0.978), or M. lemniscatus (F3, 46 = 1.13, P = 0.392).
ring from late spring to early summer, and hatchlings emerging from late summer to early autumn. Mating was recorded only in the spring (Table 2). Spermatogenesis peaked during autumn, and males have to store sperm in the deferent duct over winter until the mating season. For this reason, an increase in the diameter of the deferent duct was observed from winter until spring (Table 2).

**Discussion**

The data presented here indicate that reproductive strategies differ greatly between the two *Micrurus* lineages. The reproductive differences include sexual dimorphism, as is related to the presence or absence of combat, a behavioral trait widespread among snakes (Shine, 1994). Male–male combat was never recorded for any BRM species, consistent with the female-biased SSD (Shine, 1978, 1994). In contrast, male–male combat behavior has been observed in *M. altirostris* (Almeida-Santos et al., 1998; Marques et al., 2006), and the low SSD in the other BRT species suggests that this behavior may occur in the group (cf. Shine, 1978, 1994). Phylogenetic analyses based on morphological and molecular characters support a close relationship between northern Asian Coral Snakes (*Sinomicrurus*) and American Coral Snakes (*Micrurus* and *Micruroides*; Slowinski et al., 2001). This study suggests that snakes of the genera *Micrurus* and *Micruroides* are derived from an ancestor that dispersed from Asia into the Americas. Thus, the genera *Sinomicrurus*, *Micruroides*, and *Micrurus* form a monophyletic group (Slowinski et al., 2001; Castoe et al., 2007). Male–male combat is recorded in at least one Asian Coral Snake, *Sinomicrurus japonicus* (Ota and Iwanaga, 1996). Combat was never observed for *Micruroides*, but the low SSD (−0.01; data from Goldberg, 1997) suggests the existence of this behavior in this genus. The monophyletic group of Coral Snakes formed by *Sinomicrurus*, *Micruroides*, and *Micrurus* share a common ancestor with Afro–Asian cobras in the genera *Naja* and *Ophiophagus* (Heise et al., 1995; Keogh 1998; Slowinski et al., 2001; Fry et al., 2003) in which combat also has been documented (Shine, 1978, 1994). Phylogenetic distribution of male–male combat suggests that this trait
has evolved or has been lost many times within snake phylogeny (Shine, 1994). However, because behavior is often hard to observe in snakes, it is probable that combat is even more widespread than our current records indicate. The hypothesis of evolutionary relationships among elapids (e.g., Keogh, 1998; Fry et al., 2003) and reports of combat in many species (Shine, 1994) also suggest a similar scenario within elapid phylogeny, and it is possible the BRM Coral Snakes of the New World have lost this behavior.

In addition to differences in sexual dimorphism and combat behavior, the reproductive cycles in both males and females differ between the two *Micrurus* lineages. These differential reproductive cycles can explain differences in surface activity patterns between the two phylogenetic lineages of *Micrurus* in subtropical regions. The BRM Coral Snakes have a longer period of vitellogenesis, and the surface activity of these snakes is usually distributed more evenly throughout the year (see Marques et al., 2006). In addition, mating and ritual combat, behaviors that are related to the dispute between males for a single female (see Gillingham, 1987; Shine, 1994), in BRM Coral Snakes also have been recorded in autumn (Marques et al., 2006). Therefore, the surface...
activity peak in autumn verified for BRT Coral Snakes seems to be related to an increase of activity of males in this period (Marques et al., 2006). In contrast, vitellogenesis in the BRM Coral Snakes is very short and takes place in spring, simultaneously with mating. Thus, males searching for females in this period probably accounts for the male surface activity peak in spring recorded for BRM Coral Snakes (cf. Jackson and Franz, 1981; Marques, 1990).

Reproductive cycles in elapid snakes are highly variable (see Shine, 1977a,b), but detailed information is absent for various elapid clades, including other Coral Snakes such as the Asian Sinomicurus. In addition, the species belonging to the two clades of Micrurus are ecologically similar and often coexist within a region. Thus, it is not easy to trace how these two strategies have evolved within the genus Micrurus as well as which factors may lead to the differences between the two lineages. The climate has a great influence on the reproductive cycles of snakes (Saint-Girons, 1982). The Earth’s climate has changed continually since the middle Miocene (Hay et al., 2002) when the Micrurus genus probably evolved (Rage, 1987). There are many possible climate zones where each lineage of Micrurus may have emerged that may have been determinants of this differentiation.

Phylogenetic constraints are observed in a wide range of life-history traits, including several aspects of reproduction such as reproductive mode, reproductive seasonality, and clutch sizes (e.g., Pizzatto and Marques, 2007; Pizzatto et al., 2008a,b). Thus, phylogeny has an important role in reproductive cycles, but climate also can influence tropical and subtropical snakes (Pizzatto et al., 2008a,b). Our work shows that general reproductive strategy differs between the two Micrurus lineages and may be conservative within each lineage, but reproductive periods can be variable within a lineage. Climatic influence is obvious among the studied BRT species, because the species from higher latitudes (M. altirostris) exhibited more restricted cycles than species from lower latitudes (M. frontalis and M. lemniscatus). The BRM Coral Snakes M. corallinus, M. fulvius, and M. tener exhibit very similar timing of reproductive cycles. However, the areas where the populations were studied (southern and southeastern Brazil; Florida and Texas, USA) are similar in terms of climate (roughly between latitudes 20 and 30°). In contrast, data on the BRM M. nigrocinctus from a lower latitude region (Costa Rica, ±10° N: see Solórzano and Cerdas, 1988; Goldberg, 2004) suggests an aseasonal cycle, different from that of the other BRM species studied. However, sample sizes for this species were limited, and more
studies dealing with tropical and equatorial Coral Snake species are essential to better understand climatic influences on the reproductive cycles of Micrurus.

The two lineages of Micrurus differ in color pattern and other morphological characteristics, such as hemipenial shape and size of tail (Slovinski, 1995; Campbell and Lamar, 2004). The distinction between these two groups is supported by biochemical characters as well (Slovinski, 1995). The two reproductive strategies described here may be considered additional characteristics that differentiate these two clades of Micrurus, and they must have been fixed over time when the two lineages diverged.

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