

CAROLINA COELHO AUGUSTO SILVA

HISTÓRIA NATURAL E ANÁLISE CITOGENÉTICA DE
MICRURUS FRONTALIS (DUMÉRIL, BIBRON & DUMÉRIL, 1854)
(SERPENTES: ELAPIDAE)

Dissertação apresentada à Universidade Federal de Viçosa como parte das exigências do Programa de Pós Graduação em Biologia Animal, para obtenção do título de *Magister Scientiae*.

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Aprovada em 28 de Março de 2014

Dr. Diego José Santana Silva

Dr. Jorge A. Dergam dos Santos

Dr. Renato Neves Feio (Orientador)

À minha família, eterna incentivadora dos meus sonhos.

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RESUMO

SILVA, Carolina Coelho Augusto, M. Sc., Universidade Federal de Viçosa, Março de 2014. **História Natural e Análise Citogenética de *Micrurus frontalis* (Duméril, Bibron & Duméril, 1854) (Serpentes: Elapidae)**. Orientador: Renato Neves Feio. Coorientador: Jorge Abdala Dergam dos Santos.

Micrurus frontalis (Duméril, Bibron & Duméril, 1854) distribui-se ao longo do Cerrado do Brasil central, Paraguai, e na Mata Atlântica do sudeste brasileiro, atingindo a região costeira apenas no estado do Espírito Santo. Informações sobre sua história natural (principalmente dieta e reprodução) são escassas, assim como da maioria das cobras-corais tropicais. Quatorze espécies do gênero *Micrurus* têm o cariótipo descrito, e dessas, apenas quatro atingem o Brasil ao longo de sua distribuição. Visando aprimorar o conhecimento existente sobre história natural, padrões de variação morfológica e para melhor compreensão da evolução do genoma, o presente trabalho fornece dados sobre a dieta, atividade sazonal, ciclo reprodutivo e variação morfológica de espécimes tombados no Museu de Zoologia João Moojen, e pela primeira vez a descreve e caracteriza o cariotípica de *M. frontalis* procedente da região de Viçosa (20°45' S, 42°52' W), Minas Gerais, Brasil, utilizando as técnicas de AgNOR, Banda C, DAPI, CMA3 e FISH. Quatorze das 118 serpentes dissecadas (11,86%) apresentaram conteúdo estomacal. Com exceção das serpentes que não puderam ser identificadas, as demais presas são espécies com hábitos fossoriais (anfíbios e anfisbenídeos e serpentes) ou criptozóicos (lagartos). *Micrurus frontalis* possui o período de vitelogênese longo, com fêmeas com folículo vitelogênico encontradas em todas as estações do ano, com exceção da primavera. A espécie foi mais encontrada na estação chuvosa, período em que mais adultos apresentaram conteúdo estomacal e de provável início do ciclo reprodutivo das fêmeas. *Micrurus frontalis* possui número diploide de cromossomos $2N = 42$,

numero fundamental $NF = 24$ e fórmula cariotípica para fêmeas $42(1sm + 1st + 20t + 20mc)$, e para machos $42(2sm + 20t + 20mc)$. A marcação ag-NOR foi encontrada no primeiro par de cromossomos telocentricos. O cromossomo W possui o braço longo quase inteiramente heterocromático, e rico em DNA satélite. O quarto par de cromossomos telocêntrico apresentou padrões homozigóticos e heterozigóticos em relação à Banda-C DNA satélite.

ABSTRACT

SILVA, Carolina Coelho Augusto, M. Sc., Universidade Federal de Viçosa, March, 2014. **Natural History and Cytogenetic Analysis of *Micrurus frontalis* (Duméril, Bibron & Duméril, 1854) (Serpentes: Elapidae)**. Adviser: Renato Neves Feio. Co-adviser: Jorge Abdala Dergam dos Santos.

Micrurus frontalis (Duméril, Duméril & Bibron, 1854) is distributed along the Cerrado of central Brazil, Paraguay, and in the Atlantic Forest of southeastern Brazil, reaching the coastal region only in Espírito Santo. Information about its natural history (especially diet and reproduction) are scarce, as for most tropical coral snakes. Fourteen species of the genus *Micrurus* have the karyotype described and only four reach Brazil throughout its distribution. Aiming to enhance existing knowledge of natural history, patterns of morphological variation and to better understand the genome evolution, this work provides data on the diet, seasonal activity, reproduction, morphological variation and for the first time the karyotype description and characterization of *M. frontalis* from Viçosa (20 ° 45 ' S , 42 ° 52' W), Minas Gerais, southeastern Brazil, using the techniques of AgNOR, C Band, DAPI, CMA3 and fluorescence in situ hybridization (FISH). The diet of *M. frontalis* consists of serpentine reptiles, mainly fossorial or cryptozoic species and it has a long period of vitellogenesis, whereas females with vitellogenic follicles were found in all seasons, except in spring. The peak of activity happens in the rainy season, during which most adults had stomach contents. *Micrurus frontalis* has a diploid number of chromosome $2N = 42$, fundamental number $FN = 24$ and karyotype formula for females $42 (1sm + 1st + 20t + 20mc)$ and for males $42(2sm + 20t + 20mc)$ The W chromosome has the long arm almost entirely heterochromatic and rich in satellite DNA. The fourth pair of telocentric chromosomes showed homozygous and heterozygous patterns in relation to C-Band and satellite DNA.

1. INTRODUÇÃO GERAL

Os répteis foram os primeiros vertebrados a se adaptarem à vida predominantemente terrestre devido a algumas características como o surgimento do ovo amniótico e tegumento coberto por escamas, que propicia a retenção da umidade do corpo e facilita a locomoção em superfície irregular. O que chamamos comumente de “répteis” representa um grupo parafilético, pois exclui as Aves, portanto, os grupos pertencentes a Reptilia (Crocodylia, Sphenodontia, Squamata, Testudines e Dinosauria) não apresentam um ancestral comum exclusivo (Pough et al., 2008).

No mundo são conhecidas quase 10.000 espécies de répteis, sendo que aproximadamente 3.500 são serpentes (Uetz et al., 2014). O Brasil possui a segunda maior riqueza de répteis, com 788 espécies reconhecidas. Dessas, quase metade, 386, são serpentes (Uetz et al. 2014; Bérnils, 2010). Os ofídios compõem Squamata juntamente com os lagartos e anfisbenídeos, distribuindo-se ao longo de quase todo o mundo, com exceção dos pólos e algumas ilhas, devido à dependência da temperatura na termorregulação (Pough et al., 2008). Ocupam grande variedade de habitats, incluindo ambientes terrestres, subterrâneos, arbóreos, águas continentais e oceânicas, sofrendo grande variação adaptativa, mas mantendo um padrão morfológico homogêneo. A origem das Serpentes é relativamente recente, uma vez que o grupo surgiu provavelmente no Cretáceo, derivadas de lagartos fossoriais que tiveram alongamento do corpo e redução das patas (Cardoso et al., 2003). Estão distribuídas em 10 famílias no Brasil: Anomalepididae (sete espécies), Leptotyphlopidae (14 espécies), Tropidophiidae (uma espécie), Typhlopidae (uma espécie), Aniliidae (uma espécie), Boidae (12 espécies), Colubridae (34 espécies),

Dipsadidae (241 espécies), Elapidae (27 espécies) e Viperidae (28 espécies) (Bérnils, 2010).

Elapidae ocupa toda faixa intertropical do planeta (Slowinski et al., 1995) e é conhecida por abrigar as serpentes mais peçonhentas do mundo (Campbell & Lamar, 2004). Todos os seus membros possuem glândulas de veneno e dentição proteróglifa, além de outros aparatos defensivos. Espécies da África e Ásia, por exemplo, são capazes de levantar a cabeça alongando o pescoço dorsoventralmente, enquanto serpentes do novo mundo levantam a cauda em sinal de advertência ou para mimetizar a cabeça confundindo o predador (Campbell & Lamar, 2004). O veneno é conhecido pela alta atividade neurotóxica, miotóxica, hemorrágica além de efeitos cardiovasculares (Silva & Bucarechi, 2003). Estudos recentes sobre relacionamentos filogenéticos entre as famílias mostram que alguns grupos (Atractaspididae e Pseudoxyrhopiinae), antes tidos como Colubridae, estão intimamente relacionados a Elapidae, formando seu grupo irmão (Cardoso et al., 2003).

Tradicionalmente, duas subfamílias são reconhecidas em Elapidae: Hidrophiinae e Elapinae (Campbell & Lamar, 2004). A primeira é representada pelas cobras marinhas, contendo cerca de setenta espécies. Os hidrofines possuem uma série de características anatômicas, fisiológicas e comportamentais que auxiliam a vida nesse ambiente. Todos possuem glândulas de sal ao redor da língua que ajuda na regulação osmótica, corpo comprimido lateralmente e cauda longa além de válvulas de fechamento das narinas. São vivíparas e os filhotes nascem dentro da água. Ocorrem em águas tropicais quentes de parte do Oceano Pacífico e Índia (Campbell & Lamar, 2004).

A subfamília Elapinae tem como representantes as najas (*Naja*), as mambas (*Dendroaspis*), cobras-reis (*Ophiophagus*) e kraits (*Bungarus*), todas do Velho Mundo, além das corais-verdadeiras do novo mundo, distribuídas em três gêneros: *Micruroides*, *Leptomicrurus* e *Micrurus*, sendo que o último abriga a maior parte das espécies. A maioria dos Elapinae é ovípara, mas existem representantes vivíparos (Campbell & Lamar, 2004). *Micruroides* é encontrado no nordeste do México e no sudoeste dos Estados Unidos, enquanto *Leptomicrurus* habita florestas úmidas amazônicas e regiões baixas tropicais (Slowinsk, 1995; Campbell & Lamar, 2004).

O gênero *Micrurus* é o maior representante das serpentes-corais do Novo Mundo em diversidade e abundância, com mais de 70 espécies válidas. Ocorre do sudeste dos Estados Unidos à Argentina, e habita desde desertos a florestas de altitude (Campbell & Lamar, 2004). O gênero inclui dois grupos filogenéticos distintos, que podem ser diferenciados principalmente pelo padrão dos anéis pretos, dispostos em tríades ou em mônades. Essas duas linhagens também se diferem morfológica e bioquimicamente, além de apresentarem estratégias e padrões reprodutivos diferentes (Slowinski, 1995; Campbell & Lammar, 2004; Marques et al., 2013). As corais-verdadeiras estão presentes ao longo de todo o território do Brasil, representadas por 27 espécies atualmente reconhecidas (Bérnils, 2010). A grande maioria das espécies do gênero foi descrita e reconhecida com base em cores e padrões exclusivos de anéis e disposição das escamas. Entretanto, essas serpentes têm sido taxonomicamente problemáticas devido à variação morfológica, que é altamente conservadora, e à extrema variação de padrão de cores (Silva & Sites, 1999).

As cobras-corais possuem alimentação especializada, denominada estenofágica e ofiófaga. A maioria das espécies se alimenta de presas específicas, principalmente animais vermiformes como outras serpentes, anfisbenídeos, lagartos e cecílias

(Campbell & Lamar, 2004). O colorido vivo de padrão aposemático alerta o predador para o risco de envenenamento. Diversas espécies de colubrídeos e dipsadídeos não venenosos (falsas-corais) mimetizam o padrão de cores das corais verdadeiras garantindo proteção contra predadores que evitam esse tipo de coloração (Marques & Sazima, 1997; Campbell & Lamar, 2004). As cobras corais são animais de hábitos fossoriais, habitando galerias no solo ou sob a serapilheira. Devido à vida fossorial e dieta composta por animais de corpo alongado, essas serpentes possuem uma limitação na cinética craniana, e por isso, os acidentes ofídicos envolvendo as cobras corais não são tão expressivos quanto os acidentes envolvendo viperídeos (Cardoso et al., 2003).

Espécimes de *Micrurus* possuem corpo cilíndrico, cabeça pequena e cauda curta. Com base principalmente em características morfológicas como a forma do hemipênis e o padrão de distribuição dos anéis pretos (mônades e tríades), essas cobras podem ser divididas em dois grandes grupos facilmente distinguíveis (Cardoso et al., 2003). Os dois grupos estão presentes na Amazônia, porém em regiões mais povoadas do Brasil prevalece o grupo de tríades à exceção de *M. corallinus* (Cardoso et al., 2003).

Antes da revisão por Silva & Sites (1999), *Micrurus frontalis* era reconhecido como um complexo de espécies. Com base no padrão de coloração da cabeça, caracteres merísticos, padrão de tríades, osteologia cranial e morfologia do hemipênis, Silva e Sites revalidaram sete espécies: *Micrurus frontalis*, *M. altirostris*, *M. baliocoryphus*, *M. brasiliensis*, *M. diana*, *M. pyrrhocryptus* e *M. tricolor*. Posteriormente *Micrurus silviae* foi descrita para o Rio Grande do Sul (Di-Bernardo et al., 2007).

Micrurus frontalis se distribui desde o Paraguai ao Brasil central e sudeste, ocorrendo nos estados Mato Grosso, Goiás, Minas Gerais, Espírito Santo e Bahia e ocupando os domínios do Cerrado e Mata Atlântica (Silva e Sites, 1999). Dados sobre sua história natural, principalmente dieta e reprodução, assim como da maioria das cobras corais no Brasil são escassos.

Estudos das relações evolutivas envolvendo as cobras corais do novo mundo abordam aspectos morfológicos, imunológicos, paleontológicos, enzimáticos e moleculares (Roze e Bernal-Carlo, 1987; Slowinski, 1995; Silva e Sites, 2001). Silva e Sites (2001) utilizando dados principalmente enzimáticos e moleculares sugerem que o complexo *Micrurus frontalis* representa um grupo parafilético, entretanto a hipótese de monofiletismo não foi totalmente rejeitada. Já na filogenia proposta por Slowinski, 1995, o grupo “*M. frontalis*” é monofilético e grupo irmão de *Micrurus ibiboboca*. Ao mesmo tempo em que as serpentes do gênero *Micrurus* são morfológica e ecologicamente conservadas (Cadle & Sarich 1981), apresentam diferenças significativas em relação à citogenética (Gutiérrez et al. 1988). Em *Micrurus* o número diplóide de cromossomos (2n) varia de 26, em *Micrurus ruatanus*, a 42, em *M. lemniscatus* e *M. ibiboboca* (Luykx et al., 1992; Serafim et al. 2007; Becak e Becak 1969). Gutiérrez & Bolaños (1981) ressaltam a necessidade de estudos citogenéticos em maior número de espécies de corais, sendo imprescindível o uso de técnicas de bandeamento cromossômico e de caracterização de DNA. Estes estudos são importantes, pois permitem detectar pequenas variações nas estruturas cromossômicas, auxiliando o entendimento do papel dos rearranjos cromossômicos no processo evolutivo, logo, esses dados podem enfim ter um papel crucial em análises filogenéticas. (Serafin et al., 2007).

Visando aprimorar o conhecimento existente sobre esta espécie, o presente trabalho fornece dados sobre a dieta, reprodução, atividade sazonal e variação no padrão de coloração de espécimes de *M. frontalis* de Minas Gerais, sudeste do Brasil. Além de informações sobre história natural, esse trabalho descreve o cariótipo de *Micrurus frontalis*. As técnicas de Banda C, AgNOR, FISH, DAPI e CMA3 são apresentadas pela primeira vez para o gênero. Deste modo, os resultados citogenéticos poderão contribuir para melhor compreensão da evolução cariotípica dentro de Elapidae.

Este trabalho está dividido em três capítulos: O primeiro aborda história natural de *Micrurus frontalis*, o segundo as análises citogenética e o terceiro variação no padrão de coloração na espécie.

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1. OBJETIVOS

Objetivo geral:

Aumentar o conhecimento existente sobre a espécie *Micrurus frontalis* procedentes de Viçosa, MG.

Objetivos específicos:

- I. Apresentar dados sobre a dieta da cobra-coral *M. frontalis*;
- II. Descrever o padrão de atividade de *M. frontalis*, verificando a influência de fatores climáticos na atividade sazonal das serpentes;
- III. Caracterizar o ciclo reprodutivo de *M. frontalis*;
- IV. Descrever o cariótipo da espécie e fornecer dados sobre o padrão de bandeamento cromossômico, utilizando as técnicas de Banda C, AgNOR, FISH, DAPI e CMA3;
- V. Descrever uma variação no padrão de coloração de *Micrurus frontalis*.

2. ARTIGOS CIENTÍFICOS

I - Coelho-Augusto, C.; Costa, H.C.; Feio, R. N. Diet, Reproduction and Activity Patterns of the Coral Snake *Micrurus frontalis* (Serpentes: Elapidae)

II - Coelho-Augusto, C.; Peixoto, M. A.; Dergam, J.A.; Feio, R.N. Chromosomal Polimorphism in *Micrurus frontalis* (Duméril, Duméril & Bibron, 1854) (Serpentes: Elapidae)

III - Coelho-Augusto & Feio, R. N. Variation in the color pattern of *Micrurus frontalis*

2.1 Coelho-Augusto, C.; Costa, H.C.; Feio, R. N. Diet, Reproduction and Activity
Patterns of the Coral Snake *Micrurus frontalis* (Serpentes: Elapidae)

Diet, Reproduction and Activity Patterns of the Coral Snake *Micrurus frontalis*

(Serpentes: Elapidae)

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Resumo

Micrurus frontalis (Duméril, Bibron & Duméril, 1854) distribui-se ao longo do Cerrado do Brasil central ao Paraguai e na Mata Atlântica do sudeste brasileiro, atingindo a região costeira no estado do Espírito Santo, provavelmente devido à barreira imposta ao sul pela Serra do Mar. Dados da história natural (principalmente dieta e reprodução) dessa cobra são escassos, assim como a da maioria das cobras-corais tropicais, exceto *Micrurus corallinus*. O presente trabalho fornece dados sobre a dieta, reprodução e padrão de atividade de espécimes *M. frontalis* da região de Viçosa (20°45' S, 42°52' W) no domínio da Mata Atlântica, estado de Minas Gerais. Quatorze das 118 serpentes dissecadas (11,86%) apresentaram conteúdo estomacal. Com exceção das serpentes que não puderam ser identificadas, as demais presas são espécies com hábitos fossoriais (anfíbios e serpentes) ou criptozóicos (lagartos). Sete presas foram ingeridas a partir da cabeça, provavelmente durante atividade de alimentação na superfície, como ocorre com *M. corallinus*. Vinte e seis espécimes adultos de *M. frontalis* tiveram suas gônadas analisadas (sete fêmeas e dezenove machos). *Micrurus frontalis* possui o período de vitelogênese longo, com

fêmeas com folículo vitelogênico encontradas em todas as estações do ano, com exceção da primavera. A espécie foi mais encontrada na estação chuvosa, período em que mais adultos apresentaram conteúdo estomacal e de provável início do ciclo reprodutivo das fêmeas. Os machos com maiores testículos, que indica a produção de esperma, foram encontrados principalmente durante a estação chuvosa, indicando um ciclo reprodutivo provavelmente pré-nupcial, em que a espermatogênese antecede ou coincide com o acasalamento. Os resultados corroboram registros de que a dieta de *M. frontalis* constitui-se de répteis serpentiformes, principalmente espécies fossoriais ou criptozoicas, e anfisbenídeos como suas presas mais comuns. Assim como a maioria das serpentes tropicais, *M. frontalis* apresenta seu pico de atividade durante os meses mais quentes e chuvosos.

Abstract

Micrurus frontalis (Duméril, Duméril & Bibron, 1854) is distributed along the Cerrado of central Brazil and Paraguay, and in the Atlantic Forest of southeastern Brazil, reaching the coastal region only in Espírito Santo. Information about its natural history (especially diet and reproduction) are scarce, as for most tropical coral snakes, except for *M. corallinus*. This study provides data on the diet, reproduction and activity patterns of *M. frontalis* from the region of Viçosa (20 ° 45 ' S, 42 ° 52' W) in the Atlantic Forest of Minas Gerais. Fourteen of the 118 dissected snakes (11.86 %) had stomach contents. Except for the snakes that could not be identified, other prey species have fossorial (amphisbaenians and snakes) or cryptozoic (lizards) habits. Seven prey were eaten from the head, probably during feeding activity on the surface, as with *M. corallinus*. Twenty-six adult specimens of *M. frontalis* had their

gonads examined (seven females and nineteen males). *Micrurus frontalis* has a long period of vitellogenesis, whereas females with vitellogenic follicles were found in all seasons, except in spring. The peak of activity happens in the rainy season, during which most adults had stomach contents. Males with larger testes, which indicates the production of sperm, were found mainly during the rainy season, probably indicating a prenuptial reproductive cycle. The results corroborate reports that the diet of *M. frontalis* consists of serpentine reptiles, mainly fossorial or cryptozoic species, and amphisbaenians as their most common prey. Like most tropical snakes, *M. frontalis* presents their peak of activity during warm and rainy months.

Introduction

Micrurus frontalis (Duméril, Duméril & Bibron, 1854) is distributed along Paraguay, Cerrado of central Brazil and southeastern Brazilian Atlantic Forest, reaching the coastal region only in Espírito Santo, probably due to the barrier imposed in the south by Serra do Mar (Silva & Sites, 1999). Although it is a fairly common snake, information on its natural history (especially diet and reproduction) are still scarce, as for most tropical coral snakes, except for *M. corallinus* (Marques Sazima, 1997; Campbell and Lamar 2004). The genus *Micrurus* includes two different phylogenetic groups that can be distinguished mainly based on the arrangement of black rings in triads or monads. As well as color pattern, the distinction between these two lineages is supported by morphological, biochemical and reproductive characters (Slowinski, 1995; Campbell & Lamar, 2004; Marques et al., 2013). *Micrurus frontalis* has fossorial habits, living in holes or galleries in the soil or under dry leaves (Amaral, 1977, Campbell and Lamar, 2004) and both diurnal and nocturnal habits, mainly active from the end of the rainy season to early dry season (Sazima & Abe 1991; Marques et al, 2006).

According to Amaral (1977), the feeding habits of *M. frontalis* are similar to their counterparts, preying on lizards, snakes and anfisbenians, especially the genus *Atractus*. Azevedo (1961) and Roze (1966) reported *Leposternon microcephalum*, *Amphisbaenia darwini*, *A. mertensi*, *A. roberti*, *A. steindachneri*, *Leptothyphlops munoai*, *Erythrolampus* sp., *Sibynomorphus mikanii* and *Ophiodes* sp. as prey of *M. frontalis* complex. Lema et al. (1983) also indicate that this species feeds on lizards and snakes, and describe an exemplary set swallowing an adult of *Sibynomorphus turgidus*. Sazima & Abe (1991) found specimens of amphisbaenids *Amphisbaenia dubia*, *A. mertensi* and *A. steindachneri* as food items of *M. frontalis*. Recently, Maffei et al. (2009) recorded *M. frontalis* fettering the lizard *Ameiva ameiva*.

The activity pattern of snakes can be influenced by several factors, including the weather, prey availability and reproduction. In temperate regions this pattern is directly influenced by temperature, however, in tropical countries this factor is more homogeneous throughout the year, making the recognition of patterns much harder (Seigel and Ford, 1987; Greene, 1997). Rainfall is concentrated during the summer in these countries, and may directly influence the pattern of activities of neotropical coral snakes (Marques et al, 2006).

Like most coral snakes, *M. frontalis* is an oviparous animal, however very little is known about their activity patterns and reproductive cycle (Campbell & Lamar, 2004). Recently Marques et al. (2013) have enlarged our Knowloge of reproductive cycle of new world coral snakes. Almeida-Santos et al., 1998 depicts a combat between males of the species in April. *Micrurus frontalis* has a long period of vitellogenesis, which starts after the rainy season going forward through spring, when ovulation occurs (Marques et al., 2006; Marques et al 2013). This paper presents data on diet, reproductive cycle and reports possible influences of climate,

prey availability and reproduction in activity patterns of *M. frontalis* in the region of Viçosa, MG.

Material and Methods

Study Area

The municipalities of Viçosa, Teixeiras and Ponte Nova, located in Minas Gerais, southeastern Brazil, were considered in this work as the region of Viçosa. This zone is inserted into the morphoclimatic area of Atlantic Forest, one of the 34 ecoregions considered as hotspots of biodiversity, which means that in addition to having high endemism and high diversity of species, this region is subject to accelerated destruction caused by human activities (Mittermeier et al. 2005). Viçosa presents an original vegetation formed by semideciduous submontane and montane forest (IBGE 2007), currently reduced from 23.6% to 12% (IEF 2007), humid mesothermal climate with hot summers and dry winters (Cwa the Köppen system) (Vianello & Alves 1991), and altitude varying between 550 and 750m. (Ribon et al. 2003).

Diet Analysis

All specimens of *Micrurus frontalis* deposited in the herpetological collection of Museu de Zoologia João Moojen (MZUFV), Universidade Federal de Viçosa, Minas Gerais, coming from the municipalities of Viçosa, Teixeiras and Ponte Nova were dissected. For the analysis of diet, the contents were removed from all digestive tract (stomach, intestines, and rectum). Food items were identified to the lowest possible taxonomic level based on morphological characteristics and literature (Costa et al., 2009; Costa et al., 2010) . All prey had their total length (TL) measured with

the aid of line and ruler. When possible, we also recorded the position in which the prey were ingested.

Reproduction

A total of 118 specimens of *M. frontalis* deposited in the herpetological collection MZUFV belonging to the region of Viçosa had their gonads examined. We measured for each specimen the caudal and rostral-vent length and recorded the month of entry in the collection. Sexual maturity was determined in accordance with the opacity and/ or convoluted deferente ducts for males and ovary greater than 5 mm for females (Shine 1977; Shine 1980; Marques, 1996). The eggs of adult females were differentiated into primary and secondary vitellogenesis. The right adult males testicle were also measured.

Activity Pattern

MZUFV often receives snakes collected by population and fire department in the region of Viçosa, which are subsequently incorporated into the herpetological collection (Costa et al., 2010). The pattern of seasonal activity of *Micrurus frontalis* in the study area was inferred from the date of entry in the collection to each specimen analyzed, excluding juveniles, to avoid interference caused by the recruitment period (Marques, 2000; Marques et al. 2001). Temperature and rainfall data collected at the Meteorological Station of the Universidade Federal de Viçosa were used to correlate the climate factors with the activity pattern of *M. frontalis*. We calculated the monthly average temperature and rainfall throughout the sample period, and then the average for each month. Statistical analyzes were performed using R software, version 2.12.2 (25/02/2001). Data on diet presented in this paper were used to discuss possible interference of prey availability. Females with

secondary vitellogenesis in follicles were differentiated to identify possible influences on activity patterns. Adult males had the right testicle measured.

Results

Diet

Fourteen of the 118 dissected snakes (11.86%) had identifiable prey in their digestive tract (Table 1), seven were adults. All prey have fossorial (amphisbaenians and snakes) or cryptozoic habits (lizards). The majority (42.86%, n = 6) of the prey are amphisbaenians. 28.57% (n = 4) were represented by lizards. The rest of the content found (28,57%, n = 4) were snakes, which just one could be identified to species level and the other only to family level. Six prey were ingested from the head. The only prey ingested from the tail was a *Leposternon microcephalum*. The unidentified Gymnophthalmidae was recognized from a tail found in the stomach of one of the specimens examined, with its base toward the head of a specimen of *M. frontalis*.

TABLE 1. Items found in the digestive tract of *M. frontalis*.

MAJOR ITEN	INTEN	N	%
Amphisbaenians			
Amphisbenidae	<i>Amphisbaena alba</i>	1	
	<i>Leposternon microcephalum</i>	4	42,86
	Unidentified	1	
Lizards			
Gymnophthalmidae	<i>Ecleopus gaudichaudi</i>	2	
	Unidentified	1	28,57
	Unidentified	1	
Snakes			
Dpsadidae	Unidentified	1	
	<i>Elapomorphus quinquelineatus</i>	2	28,57
	Unidentified	1	

Reproduction

Twenty-six adult specimens of *Micrurus frontalis* had their gonads examined (seven females and 19 males) (Figure 1). We found three females in primary vitellogenesis, two in March and one in December, while four had their follicle in secondary vitellogenesis, two registered in March, one in May and another in August. Males were recorded during all year. Newborns were found from July to February. Data on testes size and total length are in Table 2.

No size variation between males and females were detected (SVL $p=0,143435$), but the tail in males were larger than in females (TL $p= 0,000606$) (Figure 2).

TABLE 2. Measures of ovarian follicle and testis of *Micrurus frontalis* specimens from Viçosa, Minas Gerais, southeastern Brazil. LOF (Largest Ovarian Follicle); RTS (Right Testicle Size), TL (Total Length)(mm)

	LOF	TL		RTS	TL
				0,10	72,2
				0,10	52,5
				0,12	68,8
				0,12	70,9
	0,06	54,9		0,13	86,4
	0,05	57,6		0,14	96
	0,20	58,7		0,15	100
FEMALE	0,05	66,6	MALE	0,15	111,5
	0,14	97		0,16	91,5
	0,22	88		0,16	105,5
	0,17	99,9		0,17	98
				0,17	48,5
				0,17	139
				0,18	122,5
				0,18	52,5
				0,20	107,3
				0,23	118,8
				0,24	125
				0,25	105,4

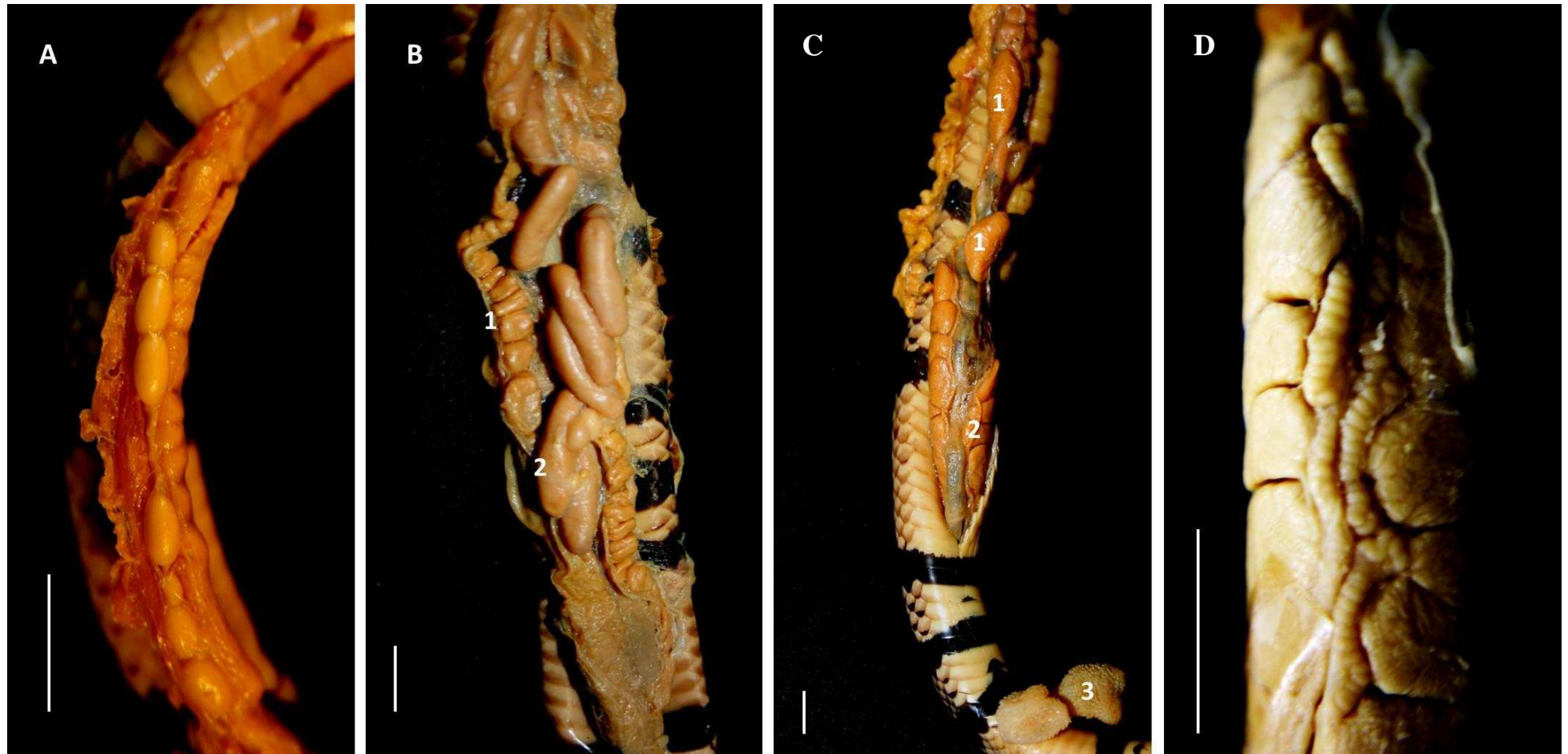


FIGURE 1. Female and male reproductive systems of *Micrurus frontalis* from the region of Viçosa, Minas Gerais, southeastern Brazil - A) Female in primary vitellogenesis showing the ovarian follicle. B) female in secondary vitellogenesis showing: (1)oviduct (2) ovarian follicle. C) Adult male showing: (1) testes; (2) deferens duct; and (3) everted hemipenes. D) Deferens ducts coiled. Bar Scale: 1cm

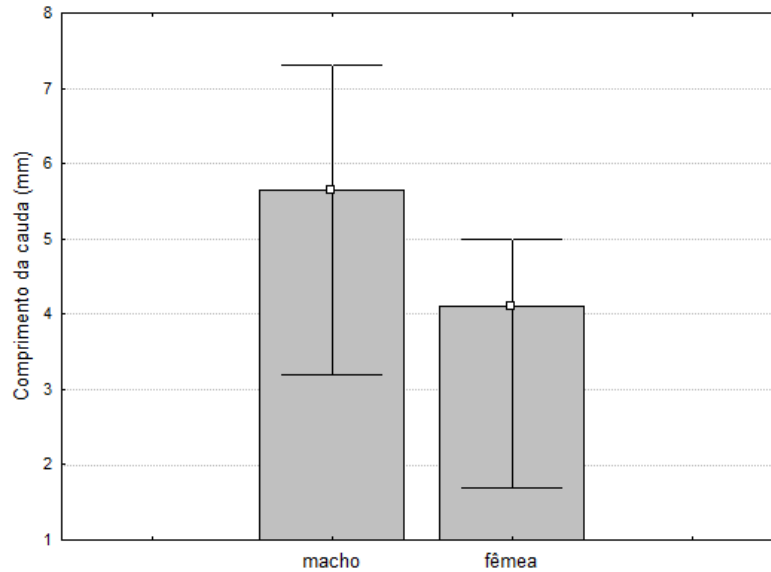


FIGURE 2. Sexual dimorphism in tail length of *Micrurus frontalis* from the region of Viçosa, MG.

Activity Pattern

The sample period extends for 29 years and two months (350 months), from December 1983 to January 2014. *Micrurus frontalis* was found during all year, with the highest incidence in the rainy season, when we registered 18 catches (69.23 %), while 8 catches (30.77%) were recorded in the dry season . However, we did not find any correlation between the temperature and rainfall ($p > 0.05$) and the activity pattern of these snakes (Figure 3).

March had the highest number of snakes with prey (3) and females with secondary vitellogenic follicles (3), corresponding to the peak activity of *M. frontalis*, during which nine specimens were recorded. Five of the six adults with gastrointestinal contents were recorded in the rainy season. A fourth female with follicles in secondary vitellogenesis was recorded in August.

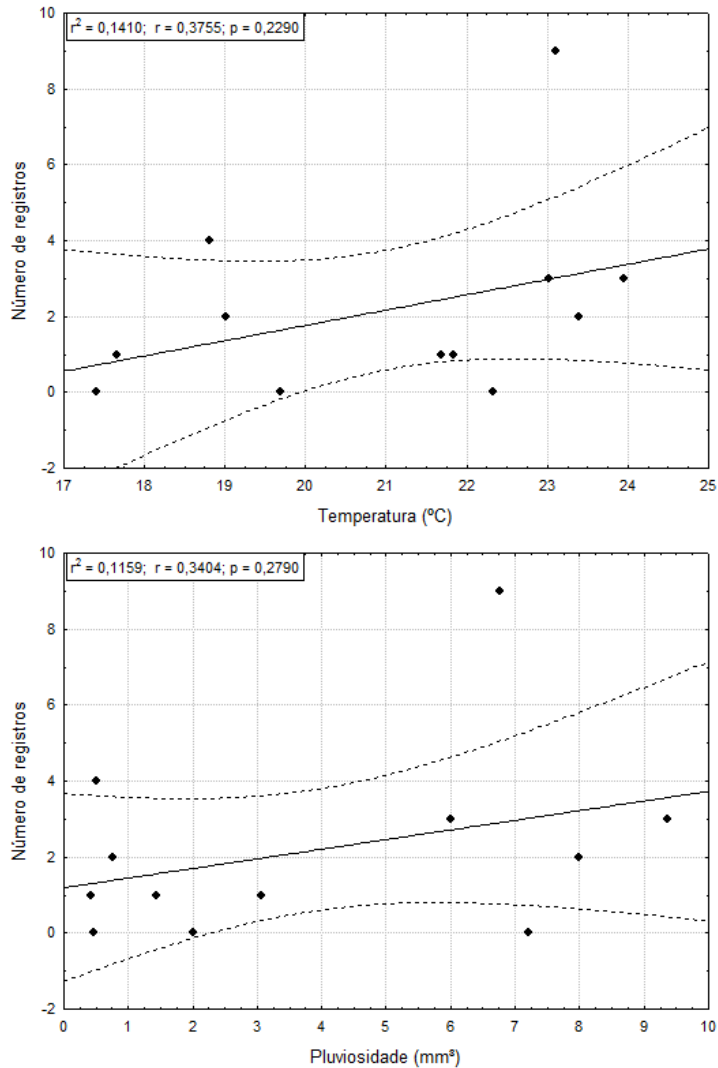


FIGURE 3. Influence of temperature and rainfall on activity pattern in *Micrurus frontalis* from Viçosa, Minas Gerais, southeastern Brazil.

Discussion

Diet

All prey found for *Micrurus frontalis* have fossorial (amphisbaenians and snakes) or cryptozoic (lizards) habits (Table 3). Most prey were amphisbaenians, as well as for most species of the genus *Micrurus* (Sazima and Marques, 1997). Marques (2001) and Terribile & Silva (2005) mention anfisbaenias and caecilians as the main prey of *M. ducoratus*, and Marques and Sazima (1997) report the same to *M. coralinus*. *Leposternon microcephalum* was the most frequent among preys found, possibly because it is the most abundant amphisbaenian in the Atlantic Forest,

as Marques Sazima (1997) recorded to *M. corallinus*. Among the lizards found, the one which could be identified to the level of species, *Ecleopus gaudichaudi*, occupies the same environments that *M. frontalis* in Viçosa: Secondary forest and anthropogenic environments (Costa et al , 2009; Costa et al 2010). As found to *M. frontalis*, snakes are very common prey for all coral snakes (Campbell & Lamar, 2004). *Elapomorphus quinquelineatus* is a fossorial snake and it has nocturnal and diurnal habit (Cardoso et al. 2001; Pontes & Rocha 2008) and according to Costa et al. 2010 as *Micrurus frontalis*, *E. quinquelineatus* is found in disturbed habitats.

TABLE 3. Taxa recorded as preys of *Micrurus frontalis*

TAXON	SOURCE
SQUAMATA	
AMPHISBAENIANS	
<i>Amphisbaena alba</i> (Linnaeus, 1758)	This study
<i>Amphisbaena dubia</i> L. (Müller, 1924)	Sazima & Abe (1991)
<i>Amphisbaena mertensii</i> (Strauch, 1881)	Sazima & Abe (1991)
<i>Amphisbaena steindachneri</i> (Strauch, 1881)	Sazima & Abe (1991)
<i>Leposternon microcephalum</i> (Wagler, 1824)	This study
Unidentified Amphisbaenians	Roze et al. (1996); This study
LIZARDS	
<i>Ameiva Ameiva</i> (Linnaeus, 1758)	Maffei et al. (2009)
<i>Ecleopus gaudichaudi</i> (Duméril & Bibron, 1839)	This study
Unidentified Gymnophthalmidae	This study
SNAKES	
<i>Elapomorphus quinquelineatus</i> (Raddi, 1820)	This study
<i>Sibynomorphus turgidus</i> (Cope, 1868)	Lema et al. (1983)
Unidentified Dipsadidae	This study

Six prey were ingested from the head, probably during feeding activity on the surface, like in *M. corallinus*. The only prey ingested from the tail was a *Leposternon microcephalum*, which may be related to underground feeding or to the fact that these animals do not have overlapping ventral scales, like snakes and lizards,

facilitating the intake by the tail (Marques Sazima 1997). The unidentified Gymnophthalmidae was recognized from a tail found in the stomach of one of the specimens examined, with its base toward the head of the serpent. It seems that the snake bit and swallowed only the lizard's tail, which may be an autotomized tail.

Reproduction

Females of *Micrurus frontalis* were found with enlarged follicles during all year, and as observed by Marques (1996) and Marques et al. (2013), this snake has a long period of vitellogenesis. Whereas the production process of secondary vitellogenesis seems to be restricted to the dry season (autumn and winter), as cited by Pizzato et al. (2007), species of *Micrurus* seems not having a continuous reproductive cycle. Marques et al. (2006) mention that vitellogenesis begins soon after the rainy season, however, females with primary vitellogenic follicles were found here in the middle and end of the rainy season (December and March) and with secondary vitellogenic follicles in the end of the rainy season. Therefore, vitellogenesis begins in the end of spring (middle of rainy season), but females are able to reproduce during the dry season (autumn and winter).

Males were found during all year, but those which had larger testes, indicating the production of sperm (Pizzato et al . , 2007), were found during the rainy season in the months of December, January , February and March. Thus the reproductive cycle of males in *Micrurus frontalis* is probably prenuptial, when spermatogenesis precedes or coincides with mating, unlike *M. corallinus*, in which spermatogenesis occurs in autumn and males stocking sperm until spring, when it occurs copulation (post-nuptial cycle) (Pizzato et al . , 2007) .

Smaller juveniles (less than 30 mm), probably newborn (Marques et. al 2013) , were mostly found at the beginning of the rainy season, more specifically in the spring (72 % , n = 22). These data indicate that births starts in winter and extends during the rainy season. Other species of *Micrurus* as *M. lemniscatus* and *M. altirostris*, show a similar reproductive cycle, with females able to reproduce during the dry season and the birth occuring in the rainy season (Azevedo, 1960; Martins & Oliveira, 1998; Campbell and Lamar, 2004). However, according to Marques et al. (2013) newborns were recorded from late the middle to the end of rainy season.

Our work shows tha the period of reproduction of *Micrurus frontalis* extends from the end of the rainy season (early autumn) to the dry season (winter). Diffeenrent from recoreded by Marques et al. (2013) we did not find any female able to reproduce in spring, therefore the reproductive cycle of males and females may coincid (Figure 4). The reproductive peak happens at the end of the rainy season (early autumn) once there are more records of males and females in gametogenesis and vitellogenesis respectively at this time. Almeida-Santos et al. (1998) described a fight between males of *Micrurus frontalis* in early April and Marques et al. (2006) and Marques et. al (2013) reported male combats in the *M. frontalis* lineage in autumn supporting the data find here. Thus, as mating occurs in autumn (Marques et. al., 2013) and males have a peak of sperm production in the summer, mating probably synchronizes with spermatogenesis.

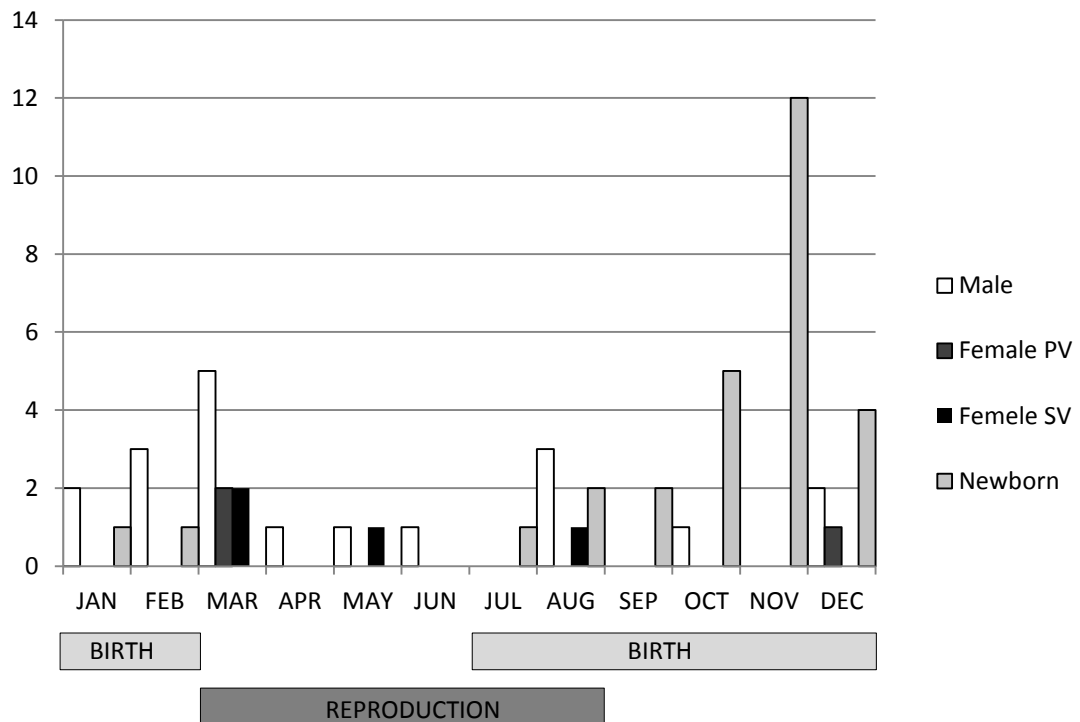


Figure 4. Reproductive cycle of *Micrurus frontalis* from the region of Viçosa, Minas Gerais, southeastern Brazil, showing males, females in primary vitellogenesis (PV), females in secondary vitellogenesis (SV) and newborn recorded for each month.

Activity Pattern

Most tropical snakes have their peak in warm and rainy months, whereas temperature and humidity directly influence the metabolism of reptiles (Marques et al . 2000). *Micrurus frontalis* was more common in the rainy season corroborating previous data (Marques et al . , 2006).

Another factor that can influence the behavior of snakes is the availability of prey (Marques et al., 2000). Five of six adult snakes with stomach contents were recorded in the rainy season, when there is a greater activity of anfibenias and lizards, as they feed on invertebrates, which are also more abundant in spring and summer (Marques et al . , 2006).

The reproductive cycle can also interfere in the seasonal pattern of snakes since some females during vitellogenesis are most active (Marques, 1996; Marques et al,

2000) . The first peak found here (March) is similar to that reported by Marques et al. (2006), that report that the majority of specimens of *Micrurus frontalis* appear at the end of the rainy season, but they also mention that the second peak occurs early in the dry season, while here, the second peak happens in the middle of this season. As Marques et al., 2006 describe, there is an absence of capture in the spring (early rainy season), and here there was no recorded snakes in September and November, while in October there was only one individual registered. The period of vitellogenesis in *M. frontalis* is larger than in other species of the same genus as *M. corallinus*, being its most casual encounter throughout the year (Marques 1996, Marques et al., 2006; Marques et al., 2013).

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2.2 Coelho-Augusto, C.; Peixoto, M. A.; Dergam, J. A.; Feio, R. N. Chromosomal Polimorphism in *Micrurus frontalis* (Duméril, Bibron & Duméril, 1854) (Serpentes: Elapidae)

Chromosomal Polimorphism in *Micrurus frontalis* (Duméril, Bibron & Duméril, 1854) (Serpentes: Elapidae)

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Resumo

O gênero *Micrurus* possui mais de 70 espécies distribuídas na América Neotropical. Quatorze apresentam o cariótipo descrito, mas apenas quatro atingem o Brasil. *Micrurus frontalis* possui número diploide de cromossomos $2N = 42$, número fundamental $NF = 24$ e fórmula cariotípica para fêmeas $42(1sm + 1st + 20t + 20mc)$, e para machos $42(2sm + 20t + 20mc)$, exibindo o padrão ZZ-ZW, sendo o W maior que o Z. A marcação ag-NOR foi encontrada no primeiro par de cromossomos telocentricos. O cromossomo W possui o braço longo quase inteiramente heterocromático, e rico em DNA satélite. As sequências de DNA repetitivo encontradas nesse cromossomo foram: $(C)_{30}$, $(GA)_{20}$, $(GAT)_{10}$ and $(CAT)_{10}$. O quarto par de cromossomos telocêntrico apresentou padrões homocigóticos e heterocigóticos em relação à Banda-C DNA satélite. O polimorfismo cromossômico não mostrou-se limitado pelo sexo.

Abstract

The *Micrurus* genus has more than 70 species distributed in Neotropical America. Fourteen have the karyotype described, but only four reach Brazil. *Micrurus frontalis* has a diploid chromosome number $2N = 42$, fundamental number $NF = 24$ and karyotype formula for females $42(1sm + 1st + 20t + 20mc)$ and 42 for

males (2sm + 20t + 20mc) displaying a ZZ-ZW pattern, and W being greater than the Z. Ag-NOR staining was found in the first pair of telocentric chromosomes. The W chromosome has the long arm almost entirely heterochromatic and rich in satellite DNA. The following DNA repetitive sequences in this chromosome were found: (C)₃₀, (GA)₂₀, (GAT)₁₀ and (CAT)₁₀. The fourth pair of telocentric chromosomes showed homozygous and heterozygous patterns in relation to C-Band and satellite DNA. The chromosomal polymorphism pattern is not sex-limited.

Introduction

The genus *Micrurus* includes 72 species being distributed throughout the Neotropical America, which 24 occur in Brazil (Campbell & Lamar, 2004). Fourteen species have their karyotype described, but only *Micrurus corallinus*, *M. lemniscatus*, *M. surinamensis* and *M. ibiboboca* reach Brazil throughout its distribution (Serafim et al., 2007). Based primarily on morphological characteristics such as the shape of the hemipenes and distribution pattern of black rings (monads and triads), these snakes can be divided into two different phylogenetic groups easily distinguishable (Cardoso et al., 2003). Both groups are found in the Amazon, but in more populated regions as southern Brazil, the group of triads, except for *M. corallinus*, prevails (Cardoso et al., 2003).

Studies of evolutionary relationships involving the new world coral snakes include morphological, immunological, paleontological, enzymatic and molecular aspects (Roze and Bernal - Carlo, 1987; Slowinski, 1995; Silva and Sites, 2001). While the snakes of the genus *Micrurus* are morphologically and ecologically preserved (Cadle & Sarich 1981), they show significant differences in cytogenetic data (Gutiérrez et al. 1988).

In *Micrurus* the diploid number of chromosomes ($2n$) varies from 26 in *Micrurus ruatanus* to 42, in *M. lemniscatus* and *M. ibiboboca* (Luykx et al., 1992; Serafim et al., 2007; Beçak and Beçak 1969). As most of elapids, the species of new world coral snakes have sex chromosomes that show a ZZ-ZW pattern, once males are the homomorphic sex (ZZ) and females the heteromorphic sex (ZW) (Serafim et al., 2007, Campbell & Lamar, 2004). Besides the variability among species of the genus *Micrurus*, Gutiérrez & Bolaños, 1981 showed differences in number and morphology of chromosomes for *Micrurus nigrocinctus* complex. However, the validity of some subspecies is questionable (Villa, 1984; Vences et al., 1998) and as *Micrurus nigrocinctus mosquitensis*, they will probably be elevated to full species status (Campbell & Lamar, 2004).

Whereby the number of snakes analyzed in most of papers are very low ($N = 1$ to 4), except for *Micrurus nigrocinctus* and *M. mosquitensis*, cytogenetics description for *Micrurus* is almost based only in male specimens (eg. Gutiérrez & Bolaños 1979, Gutiérrez et al 1988; Serafim et al 2007). Moreover, chromosome banding were never used to characterize any species of New World Coral snakes, thus knowledge of the cytogenetic patterns in *Micrurus* are still very poorly known.

Gutiérrez & Bolaños (1981) emphasized the need of cytogenetic studies on a larger number of coral snakes species, and reported the importance of using chromosome banding techniques and characterization of DNA. These studies are important since they permit detecting small variations in chromosome structure, aiding the understanding of the function of chromosomal rearrangements in evolution, therefore it can have an important role in phylogenetic analyzes (Serafim et al., 2007).

Aiming to enhance existing knowledge of this species and the genus, this paper describes the karyotype of *Micrurus frontalis*, using the techniques of C-Band, AgNOR, FISH, DAPI and CMA3. Thus, the cytogenetic results contribute to better understanding karyotypic evolution and irradiation within Elapidae.

Material and Methods

The Museu de Zoologia João Moojen, Universidade Federal de Viçosa (UFV), often receives snakes collected by population and fire department, which are returned to nature, sent for research and/or incorporated into the herpetological collection (Costa et al., 2010). For description and chromosome characterization, twelve specimens (seven males and five females) of *Micrurus frontalis* from Viçosa (20°45' S, 42°52' W), Minas Gerais, southeastern Brazil, were analysed.

Mitotic chromosomes were obtained from intestinal epithelial cells (Schmid, 1978). Colchicine solution 0.1%, in dosage 0.01 mL/mg was intraperitoneally injected in each specimen, six hours before death. For euthanasia we administered intraperitoneally 0.01 mL/mg of Hypnol to induce anesthesia and 60 mg/kg of pentobarbital (lethal dose) as recommended by Brazilian Federal Board of Veterinary Medicine (CFMV), Federal Board of Biology (CFBio) and CONCEUA.

After the procedures, we examined for each specimen about 20 metaphases (when possible) under a light microscope for determination of the modal value (2n) and the fundamental number (NF). Best metaphases were photographed at 10 digital system photomicroscope equipped with epifluorescence Olympus BX53 with the 100x immersion objective to 1000x magnification. Homologous chromosomes were paired according to the position of the centromere, emordem decreasing size. The pairing of chromosomes were based on visual observation, aided by direct

measurements with Image Pro Plus software (IPP Version 15 4.5). The chromosomes were classified according to their centromeric index (Levan et. al.) in metacentric (m), submetacentric (sm), subtelocentric (st) and telocentric (t) and microchromosomes (mc).

The material was processed to obtain C-band pattern (constitutive heterochromatin) according to Boyce, 1989 with some modification (figure 1); and Ag - NOR banding , aiming to highlight the nucleolus organizer regions by silver nitrate impregnation (Howell 1980) .

The protocol described by Schweizer (1976) was used to DAPI (4'-6-diamidino-2-phenylindole) and CMA₃ (chromomycin A₃).

Fluorescence in situ hybridization (FISH) was performed according to Poltronieri *et al.*, with some modifications, using the following sequences of repetitive DNA: (A)₃₀, (C)₃₀, (GA)₂₀ (CA)₂₀ (TA)₂₀ (GC)₂₀ (TAT)₁₀ (GAT)₁₀ (CGG)₁₀ (CAT)₁₀

Results

Specimens of *Micrurus frontalis* showed the diploid number of chromosomes $2N = 42$, fundamental number $FN = 24$ and karyotype formula for females $42 (1sm + 1st + 20t + 20mc)$ (Figure 1) $42 (2sm + 20t + 20mc)$ for males (Figure 2). The pair of sex chromosomes of males was represented by two submetacentric chromosomes, while in females the equivalent pair was represented by a submetacentric chromosome and a bigger W subtelocentric chromosome.

All specimens showed NORs in the telomeric region of the bigger arm of the first pair of telocentric chromosomes (Figures 3 and 4). Females presented a heterochromatic block on the long arm of W chromosome (Figure 3) and eleven

specimens showed a heterochromatic band in one chromosome of the fourth pair of telocentric (Figure 5). Only one individual showed the same heterochromatic band on both chromosomes of the fourth pair of telocentric (Figure 6). DAPI pattern coincides with C-band pattern for all samples (Figure 7). GC-rich regions were observed only in females in the upper region of the long arm, entire short arm and centromeric region of chromosome W (Figure 8).

Of all the repetitive DNA probes tested, *Micrurus frontalis* presented different pattern of distribution of the following repetitive sequences: C30 , GA , GAT and CAT . All centromeric and telomeric regions, and much of the microchromosomes proved rich in cytosine. In females, the short arm and more than half of the long arm of W chromosome were stained (Figure 9). The centromeric and telomeric regions of the sex chromosomes, besides the long arm of the W, showed rich in GA repetitive sequences (Figure 10). The same regions on sex chromosomes, with the exception of centromeric, are also rich in GAT (Figure 11), in addition to the telomeric regions of autosomes (Figure 12). Only one specimen showed heterozygosity for the fourth pair of telocentric chromosome. CAT distribution revealed the same pattern of C-banding distribution (figure 13 and 14)

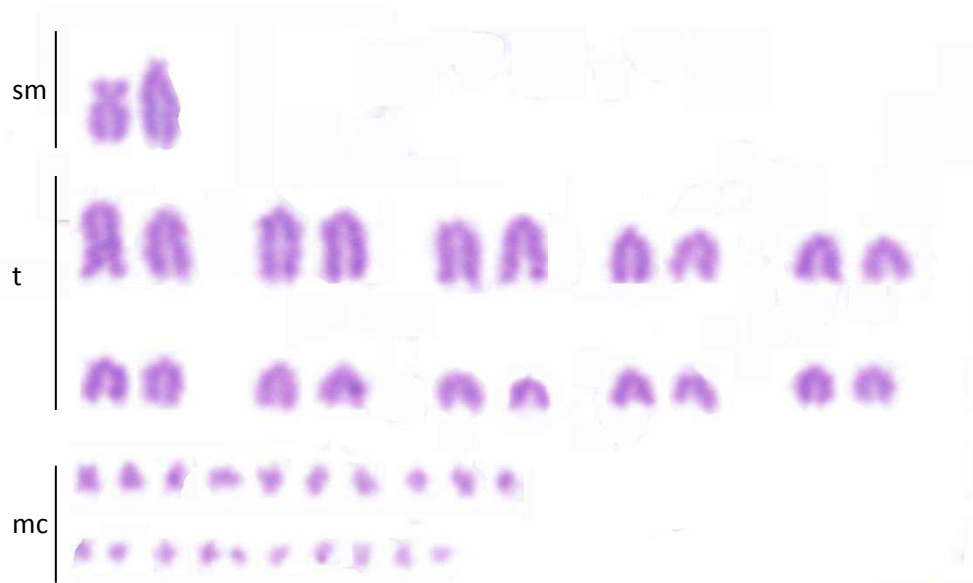


Figure 1. *Micrurus Frontalis* karyotype. Female

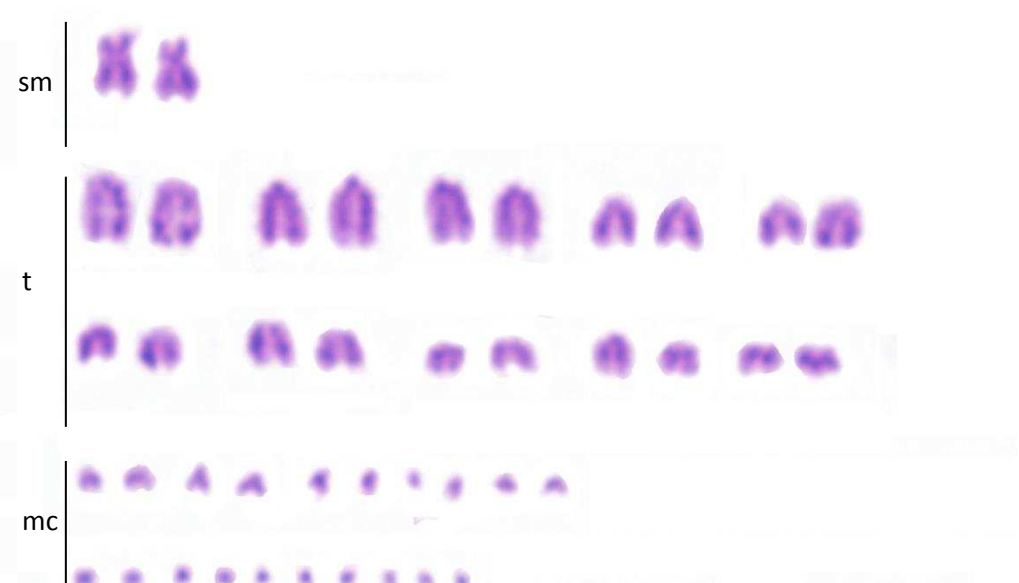


Figure 2. *Micrurus Frontalis* karyotype. Female



Figure 3. Silver nitrate staining showing the Nucleolus Organizer Regions (NOR) in *Micrurus frontalis*. Female



Figure 4. Silver nitrate staining showing the Nucleolus Organizer Regions (NOR) in *Micrurus frontalis*. Male

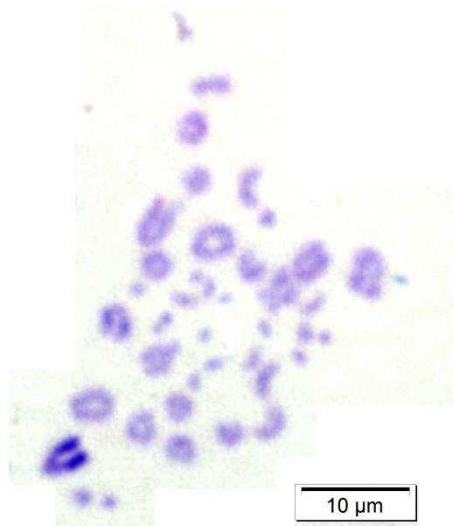


Figure 5. C-band distribution pattern in males of *Micrurus frontalis*.

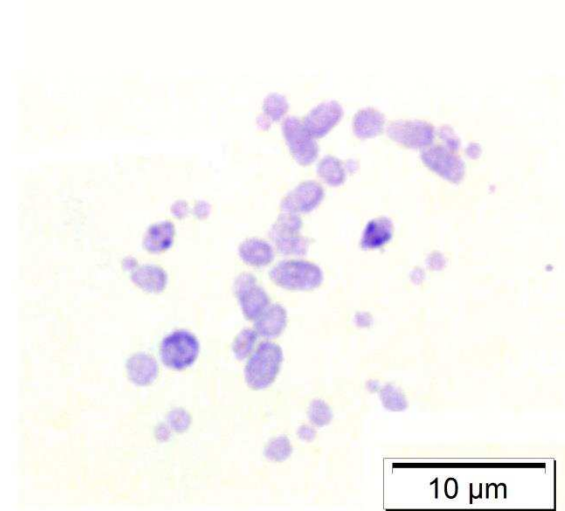


Figure 6. Polymorphism in the distribution pattern of C-band in *Micrurus frontalis*.



Figure 7. DAPI distribution pattern in males of *Micrurus frontalis*.

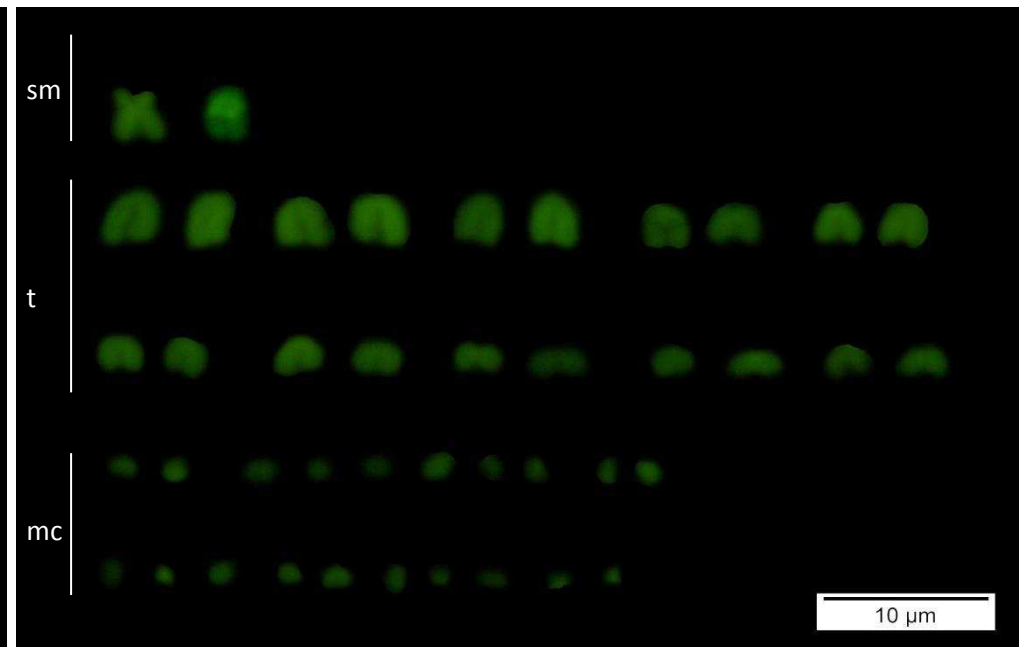


Figure 8. CMA3 distribution pattern in males of *Micrurus frontalis*.

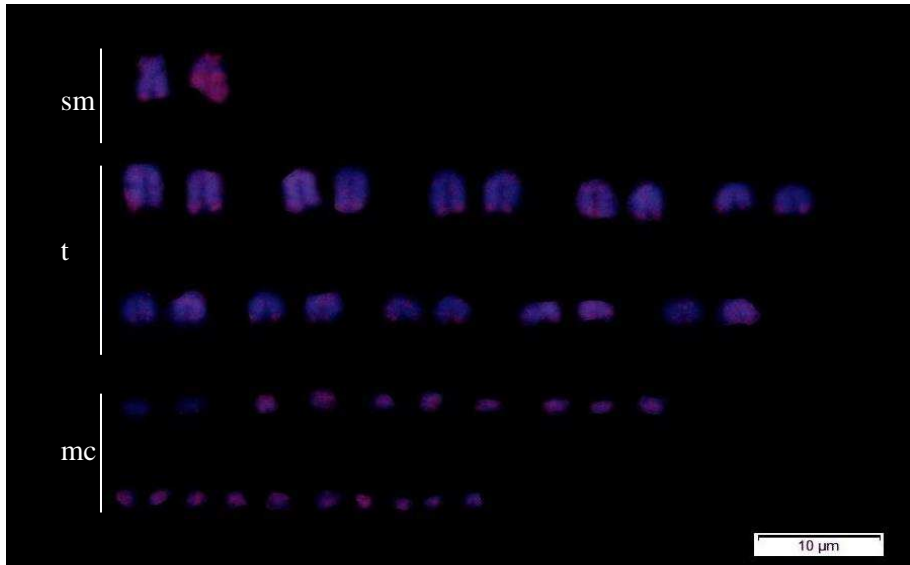


Figure 9. Distribution pattern of repetitive DNA sequence (C30) by Fluorecence In Situ Hybridization (FISH) in males of *Micrurus frontalis*.



Figure 10. Distribution pattern of repetitive DNA sequence (GA) by Fluorecence In Situ Hybridization (FISH) in males of *Micrurus frontalis*.

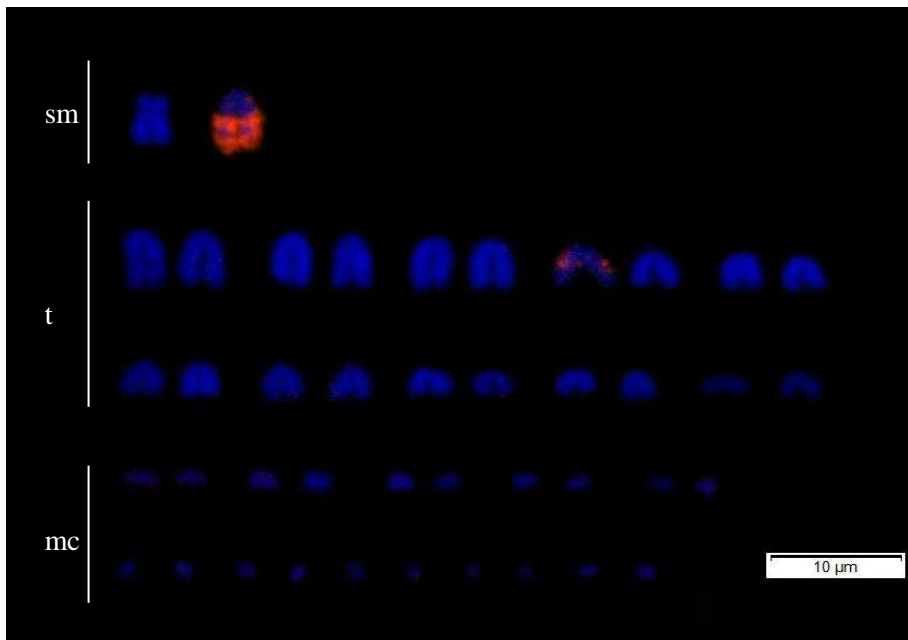


Figure 11. Distribution pattern of repetitive DNA sequence (GAT) by Fluorecence In Situ Hybridization (FISH) in female of *Micrurus frontalis*.

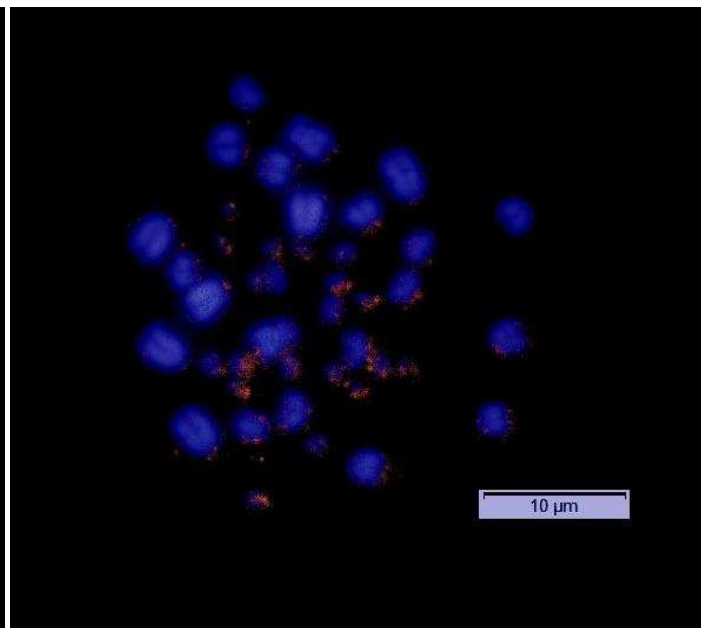


Figure 12. Metaphase showing the distribution pattern of repetitive DNA sequence (GAT) by Fluorecence In Situ Hybridization (FISH) in males of *Micrurus frontalis*.

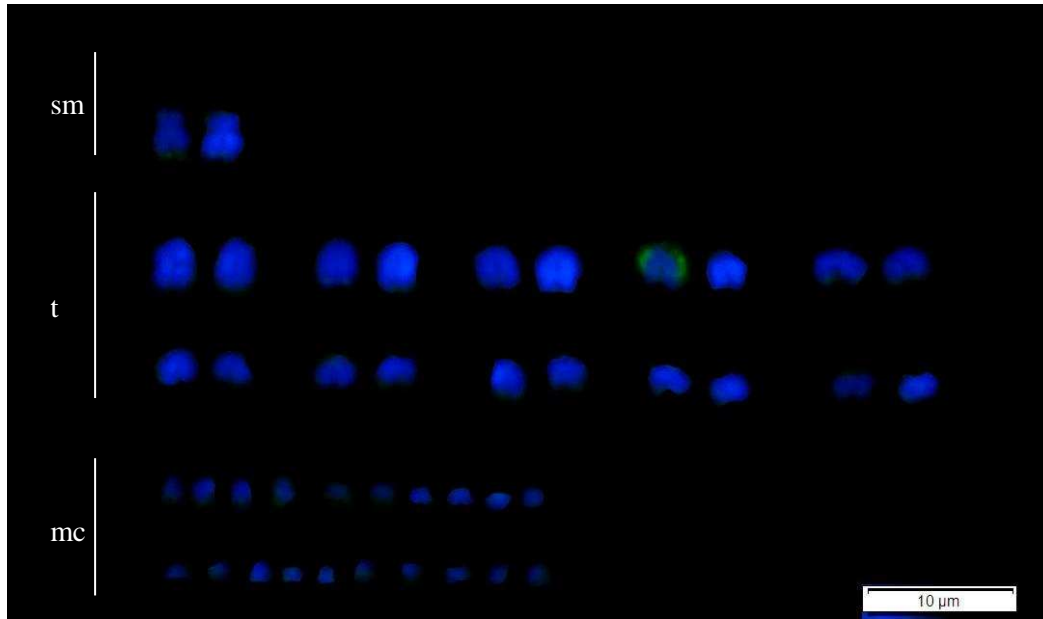


Figure 13. Distribution pattern of repetitive DNA sequence (CAT) by Fluorecence In Situ Hybridization (FISH) in males of *Micrurus frontalis*.

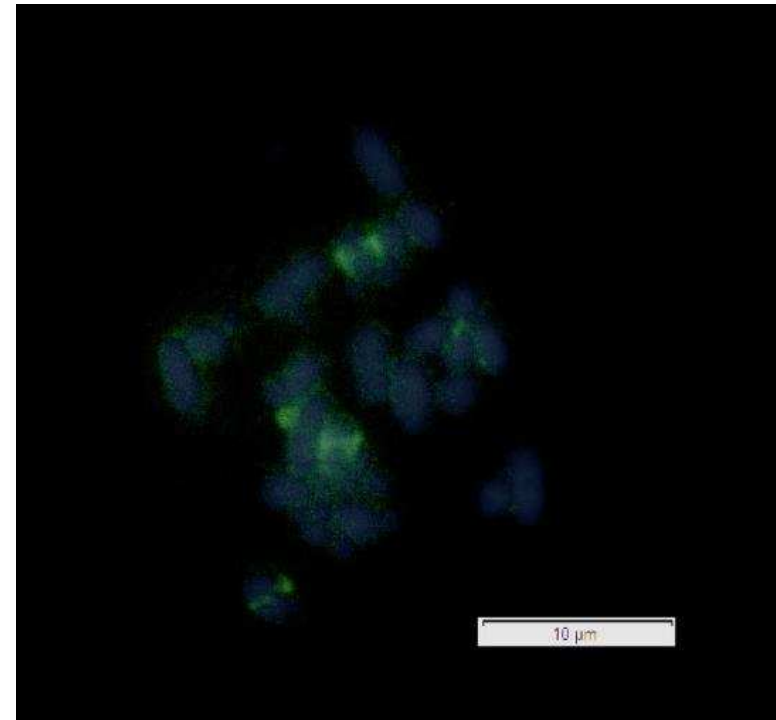


Figure 14. Metaphase showing the distribution pattern of repetitive DNA sequence (CAT) by Fluorecence In Situ Hybridization (FISH) in female of *Micrurus frontalis*.

Discussion

The karyotype analyzes show numerical and structural similarities between the chromosomes of South American coral snakes *Micrurus frontalis*, *M. corallinus*, *M. ibiboboca*, *M. lemniscatus* and *M. surinamensis* (Table 1). These snakes present 20 microchromosomes, higher diploid chromosomes number among New World coral snakes, ranging from 38 to 42 and the fundamental number of chromosome arms $FN= 24$. Gutierrez & Bolaños (1988) and Serafim et al. (2007) noted that cytogenetically, South American coral snakes are similar and present characters that differ them from Central America species. Serafim et al. (2007) also suggest that South American coral snakes tend to increase diploid number of chromosomes, while Central American coral snakes tend to reduce this number, considering $2n=36$ chromosomes as ancestral.

Micrurus frontalis, *M. lemniscatus* and *M. ibiboboca* show the same number of chromosomes ($2N = 42$), nine pairs of telocentric chromosomes (probably autosomes) and secondary constriction in the first pair of macrochromosomes. Among five snakes that have the karyotype described in South America, *Micrurus corallinus* present more differences in the morphology of chromosomes. While this snake exhibit a high number of subtelocentric chromosomes, and low number of telocentric chromosomes, other South American coral snakes have a high number of telocentric chromosomes and few subtelocentric chromosomes (probably only Z chromosome).

According to the phylogeny proposed by Silva & Sites (2001), based on molecular characters for South American triad coral snakes, *Micrurus surinamensis* is the most basal species among those which have the karyotype described.

TABLE 1. Karyotype of the genus *Micrurus*. Macrochromosomes were based in males.

Species	Karyotype	Macrochromosomes	Microchromosomes	Sex	NF	Source
CENTRAL AMERICAN SPECIES						
<i>Micrurus aleni</i>	34	12m + 2sm + 6st	14		26	Gutierrez & Bolaños, 1979
<i>Micrurus browni</i>	26	4m + 6sm + 2st + 4t	10		26	Gutierrez et al. 1988
<i>Micrurus diastema apiatus</i>	30	4m + 8sm + 2 st	16	ZZ/ZW: sm + sm/sm + sm	26	Gutierrez et al. 1988
<i>Micrurus diastema sapperi</i>	30	4m + 8sm + 2 st	16	ZZ/ZW: sm + sm/sm + sm	26	Gutierrez et al. 1988
<i>Micrurus elegans</i>	30	6m + 4sm + 4st + 2t	14		26	Gutierrez et al. 1988
<i>Micrurus fulvius</i>	32	4m + 8sm + 4st	16	ZZ/ZW: sm + sm/sm + st	26	Graham, 1977
<i>Micrurus hippocrepis</i>	30	6m + 6sm + 2st	16		26	Gutierrez et al. 1988
<i>Micrurus mosquitensis</i>	34	4m + 8sm + 4st	14	ZZ/ZW: sm + sm/sm + st	26	Gutierrez & Bolaños, 1979
<i>Micrurus multifaciatu</i>	30	8m + 4sm + 2st	20		26	Gutierrez & Bolaños, 1979
<i>Micrurus nigrocinctus</i>	26	4m + 6sm + 6st	10	ZZ/ZW: sm + sm/sm + st	26	Gutierrez & Bolaños, 1979
<i>Micrurus ruatanus</i>	26	4m + 6sm + 6st	10	ZZ/ZW: sm + sm/sm + st	26	Luykx et al 1992
SOUTH AMERICAN SPECIES						
<i>Micrurus corallinus</i>	40	4sm + 12st + 4t	20		24	Serafim et al., 2007
<i>Micrurus frontalis</i>	42	1sm + 18t	20	ZZ/ZW: sm + sm/sm + st	24	Present Data
<i>Micrurus ibiboboca</i>	42	2sm + 18t	20		24	Serafim et al., 2007
<i>Micrurus lemniscatus</i>	42	2sm + 18t	20		24	Beçak & Beçak, 1969
<i>Micrurus surinamensis</i>	38	4m + 2sm + 12t	20		24	Gutierrez et al. 1988

Cytogenetic data support this hypothesis, since *Micrurus surinamensis* present lower number of macrochromosomes (18) and four metacentric chromosomes. The second pair of metacentric chromosomes of *Micrurus surinamensis* exhibit a secondary constriction and is possibly homologous to the first pair of telocentric chromosomes of other species from South America. Probably two centromeric fissions occurred in metacentric chromosomes, only morphological characteristics which differ *Micrurus surinamensis* from *M. frontalis*, *M. ibiboboca* and *M. lemniscatus*.

According to Silva e Sites (2001), *Micrurus frontalis* represents a paraphyletic group, though they do not rule out the hypothesis of monophyly. More karyotypic studies can help in comprehension of South American coral snakes phylogeny. Figure 15 was adapted from Silva & Sites (2001) and karyotypic data of South American *Micrurus* was added.

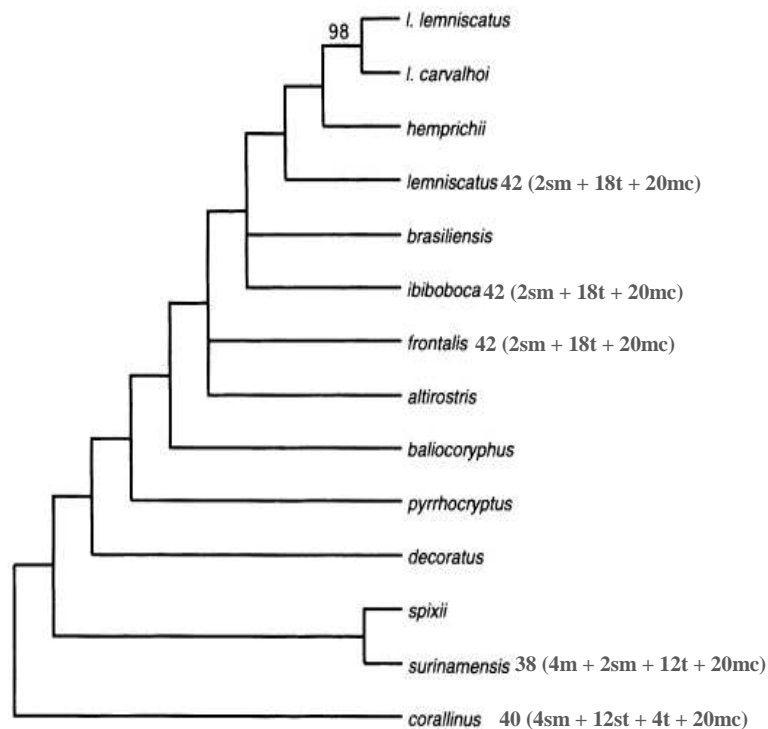


FIGURE 15. Relationships for all South American triad species of *Micrurus* based on molecular data (Silva & Sites, 2001), plus karyotypic data.

On the other hand, the phylogeny proposed by Piron et al 2013 does not group North American and South American snakes. However considering just the snakes that have their karyotype described, the discussion proposed here does not change. More species must be studied to better understand the evolution of the karyotype in *Micrurus*.

Sex Chromosomes

According to Mengden & Stock (1980), snakes sex chromosomes are the most variable element in serpents cytogenetics, representing many states of differentiation (Singh, 2011). The first step in differentiation of sex chromosomes is the heterochromatinization of one of the sex homologous (Ray-Chaudhuri et al., 1971). However, Singh et al. 1976 suggest that the distribution of a specific satellite DNA precedes this step. W chromosome of *Micrurus frontalis*, like all snakes, except for some Boidae species, present a big region of heterochromatin tightly colored by C-banding (Ray-Chaudhuri and Singh, 1975). Besides, *M. frontalis* shows a high accumulation of satellite DNA in almost the same region of the heterochromatin, except for the distribution pattern of the repetitive sequence (C)₃₀. This sequence occurs in the short arm of the W chromosome, where we did not observe any pattern of heterochromatinization. This fact may support the hypothesis that the origin of satellite DNA is the initial process of differentiation of sex chromosomes (Singh, 1976; Singh 1980; Matsubara et al., 2006; Singh 2011).

Micrurus frontalis, as well as other New World coral snakes, shows a ZZ / ZW pattern of sex chromosomes. However, different from these snakes, the W chromosome in *M. frontalis* is bigger than Z chromosome. According to Singh (2011), accumulation of heterochromatin and mutation occur before structural rearrangements, and highly differentiated W chromosome is much smaller than the

Z. Despite other *Micrurus* exhibit the W smaller than Z, this genus probably show a intermediate state of differentiation of sex chromosomes, being an important tool for understanding the evolution of sex chromosomes, since species from the genus are in different stages of differentiation.

Polymorphism

The only chromosomal polymorphism of *Micrurus* was recorded by Gutierrez & Bolaños (1979) to two subspecies of *Micrurus nigrocinctus*. However, *M. n. mosquitensis* (*M. nigrocinctus* Schmidt 1933), become a full specie (Campbell & Lammar, 2004).

All chromosomal polymorphism in *Micrurus frontalis* is related to the fourth pair of telocentric chromosomes. Satellite DNA is usually correlated to sex Chromosomes (Singh et al. 1994; Matsubata et al. 2006). Except for one female that showed a homozygous positive pattern of heterochromatin and CAT in this pair of homologous, all other specimens (males and females) presented heterozygous pattern to both characters. An other female presented an heterozygous region of GAT in the equivalent pair of chromosomes, while remaining specimens presented a homozygous positive pattern of this repetitive sequences.

The review of *Micrurus frontalis* complex, based mainly in morphological characters, was done by Silva & Sites (1999), that described seven species: *Micrurus frontalis*, *M. altirostris*, *M. baliocoryphus*, *M. brasiliensis*, *M. Diana*, *M. pyrrocryptus* and *M. tricolor*. Later *Micrurus silviae* was described to Rio Grande do Sul (Di-Bernardo et al., 2007). The polymorphism found here is not sex-limited and studies on more individuals from different regions are needed to identify possible different patterns between population.

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2.3. Coelho-Augusto, C. &Feio, R. N. Variation in the color pattern of
Micrurus frontalis

Variation in the color pattern of *Micrurus frontalis*

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MICRURUS FRONTALIS (Coral Snake). VARIATION IN THE COLOR PATTERN.

The genus *Micrurus* is the largest representative of new world coral snakes in diversity and abundance, with more than 70 valid species occurring from southeastern United States to Argentina (Campbell and Lammar 2004. *Venomous Reptiles Vol I*). The genus includes two different phylogenetic groups, which can be distinguished mainly by the pattern of black rings arranged in triads or monads. These two lineages also differ morphologically and biochemically, besides having different reproductive pattern and strategies (Slowinsk, J. B. 1995. *Journal of Herpetology*, 29:325-338; Campbell and Lammar 2004. *Venomous Reptiles Vol I*, 2004; Marques, Otavio A. V. , Pizzatto, L., Almeida Santos, S. M. 2013. *Herpetologica*, 69(1):58-66).

In addition of being a taxonomic and phylogenetic character, the color pattern of coral snakes has an environmental and evolutionary importance, since the bright red and black rings seems to have an aposematic and cryptic function (Campbell and Lammar 2004. *Venomous Reptiles Vol I*). Several harmless species of colubrid and dipsadid coral snakes (false coral) mimic the color pattern of coral snakes ensuring protection from predators that avoid this type of color (Marques and Sazima, 1997. *Herpetol. Nat. Hist.* 5: 88-93; Campbell and Lammar 2004. *Venomous Reptiles Vol I*). These pattern are important for understanding coral sanake mimicry systems (e.g.

Greene and McDiarmid 1981. *Science* 213:1207–1212; Kikuchi and Pfennig 2010. *The American Naturalist* 176: 830-834).

While we were analyzing specimens of *Micrurus frontalis* from the municipalities of Viçosa (20°45' S, 42°52' W), Ponte Nova (20°24' S, 42° 53' W) and Teixeiras (20° 39' S 42° 51' W), Minas Gerais, Brasil, deposit in the herpetological collection of Museu de Zoologia João Moojen, UFV, we observed that some specimens showed a tendency for the concentration of black pigment in the center of the red rings between two triads (Figure 1). Silva, N. and Sites, J. *Herpetological Monographs*, 13:142-194, in the redescription of the holotype of *Micrurus frontalis* reported that all red and white rings of *Micrurus frontalis* are black-tipped, but any tendency of accumulation were observed.. One hundred and eighteen individual were analyzed (including alive specimens) and eight (6,78%) presented this variation in the color pattern.

In 1962, Azevedo (Azevedo, 1962. *Séries Científicas do Museu Rio-Grandense de Ciências Naturais*. 26: 1-7) presented this black tipping on red dorsal scale as an anomalie for *Micrurus multicinctus* and *M. ibiboboca* from Rio Grando do Sul, Brasil. He described as large elongated and oval spots for *M. multicinctus* as the total obscuration of scales constituting false black ring for *M. ibiboboca*. This kind of variation seems to be common between coral snakes and it is possible that the amount of black tipping vary between population of *Micrurus frontalis*, as recorded to *M. diastema* from Mexico by Fraser 1973 (Fraser, 1973. *Copeia* 1:1-17) and may be an important character to distinguish two subspecies.

Stuart, 1963 (Stuart, 1963. *Miscellaneous Publications. Museum of Zoology, University of Michigan* 122: 1–150) and Fraser 1973 (Fraser, 1973. *Copeia* 1:1-17) reported clinal trends in some color pattern, as the amount of black tipping in dorsal scales and number of black rings for *Micrurus diastema* from Guatemala and Mexico

and considered these characteristics as important in the delimitation of certain subspecies of *Micrurus diastema*.

Further studies involving variation in the color pattern of *Micrurus frontalis* should be done to identify possible differences between populations, and to better understand the mechanisms involved in the evolution and speciation of *M. frontalis*.

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FIGURE 1. Variation in the color pattern of *Micrurus frontalis*, showing black spots on red rings.
Photo: Costa, H. C.

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CONCLUSÕES GERAIS

Micrurus frontalis se alimenta principalmente de lagartos, anfisbenídeos e serpentes, sendo *Leposternon microcephalum* sua principal presa. Os machos estão mais ativos no período chuvoso, enquanto as fêmeas, na época seca, provavelmente devido ao ciclo de espermatogênese e vitelogênese, respectivamente. O acasalamento ocorre no final da estação chuvosa e o nascimento dos filhotes acontece principalmente na primavera.

Em relação ao padrão do cariótipo, as corais verdadeiras do Novo Mundo pertencentes ao gênero *Micrurus* podem ser divididas em dois grandes grupos: Serpentes Centro Americanas e Serpentes Sul Americanas. *Micrurus frontalis* mostrou-se mais semelhante às corais da mesma linhagem evolutiva (*M. lemniscatus*, *M. ibiboboca* e *M. carvalhoi*), que por sua vez apresentaram mais semelhanças com *Micrurus coralinus*, única coral Sul Americana pertencente a outra linhagem evolutiva e possui o cariótipo descrito. O cromossomo sexual W de *Micrurus frontalis* encontra-se num estágio evolutivo intermediário, e pode ajudar a elucidar os mecanismos moleculares envolvidos na origem e diferenciação dos cromossomos sexuais.

Os Polimorfismos encontrados podem significar diferenças cromossômicas entre as populações de *Micrurus frontalis*, sendo um ponto de partida para determinar alterações envolvidas na evolução e especiação.

ANEXOS

1. Material examinado em análises de História Natural. MZUFV corresponde à Coleção Herpetológica do Museu de Zoologia João Moojen, onde os espécimes encontram-se depositados.

MZUFV 220, 221, 222, 227, 228, 229, 230, 231, 232, 233A, 233B, 242, 245, 248, 249, 250, 251, 287, 314, 316, 347, 355, 359, 360, 361, 430, 431, 438, 439, 441, 453, 454, 461, 470, 472, 475, 480, 474, 507, 509, 510, 518, 519, 520, 521, 522, 523, 537, 575, 632, 633, 634, 672, 673, 674, 675, 721, 728, 729, 730, 731, 736, 809, 818, 828, 966, 968, 986, 1015, 1044, 1084, 1099, 1136, 1156, 1177, 1196, 1241, 1242, 1245, 1248, 1252, 1269, 1296, 1297, 1372, 1448, 1468, 1469, 1472, 1478, 1481, 1516, 1521, 1536, 1550, 1571, 1582, 1656, 1666, 1701, 1769, 1853, 1932, 1943.

2. Material examinado em análises citogenéticas.

	SEXO	LOCALIDADE	DATA
CT2443	M	Viçosa, MG	mai/11
CT2862	M	Viçosa, MG	mar/12
CT2863	M	Viçosa, MG	mar/12
CT2938	M	Viçosa, MG	mai/12
CT2939	F	Viçosa, MG	mai/12
CT3335	F	Viçosa, MG	fev/13
CT3336	M	Viçosa, MG	fev/13
CT3394	F	Viçosa, MG	mai/13
CT3466	F	Viçosa, MG	jul/13
CT3586	F	Viçosa, MG	dez/13
CT3587	M	Viçosa, MG	dez/13
CT3609	M	Viçosa, MG	jan/13