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DÉBORA FABIANE NEVES DA SILVA

“APTIDÃO REPRODUTIVA DE QUATRO ESPÉCIES DE LAGARTOS DO GÊNERO *TROPIDURUS* DO GRUPO *TORQUATUS* (TROPIDURIDAE) NO BRASIL”

“REPRODUCTIVE APTITUDE OF FOUR SPECIES LIZARDS OF THE GENUS *TROPIDURUS* FROM *TORQUATUS* GROUP (TROPIDURIDAE) IN BRAZIL”

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Tese apresentada ao Instituto de Biologia da Universidade Estadual de Campinas para a obtenção do título de Doutora em Biologia Celular e Estrutural, na Área de Biologia Celular.

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RESUMO

O gênero *Tropidurus* compreende atualmente 30 espécies de lagartos ovíparos que ocorrem na América do Sul cis-Andina, apresentando variações no ciclo reprodutivo categorizados como contínuo, sazonal e descontínuo. Considerando essas variações reprodutivas, o objetivo foi avaliar a aptidão reprodutiva de quatro espécies de *Tropidurus* do grupo *torquatus* com ampla distribuição geográfica e elevada representatividade nas coleções zoológicas do Brasil. Um total de 276 indivíduos machos divididos em 65 populações e 201 fêmeas com 43 populações das espécies *Tropidurus torquatus*, *T. hispidus*, *T. itambere* e *T. oreadicus* foram analisadas. Amostras de testículo, ducto epididimal e ovário foram incluídas em resina plástica e preparadas para análise em microscopia de luz. Os parâmetros da aptidão reprodutiva incluíram o comprimento na maturidade sexual, tamanho de ninhada, presença ou ausência de espermatozoides, ovos e folículos. Nos macho*s*, as células germinativas foram morfologicamente semelhantes, porém a produção de espermatozoides variou ao longo do tempo com maior continuidade para as espécies *T. torquatus* e *T. hispidus*, e sazonal para *T. itambere* e *T. oreadicus*. O ducto epididimal apresentou hipertrofia do epitélio e atividade secretora relacionadas com a espermiação e regressão nos túbulos seminíferos. O comprimento na maturidade sexual variou no seu tamanho mínimo entre as populações e biomas. Nas fêmeas, o ovário apresentou apenas uma cama germinativa com oogônias e oócitos primários associados ao estroma. O desenvolvimento folicular foi bastante conservado no aspecto morfoestrutural, com duas fases: uma pré-vitelogênica e outra vitelogênica. Durante a maturação folicular foi observado maior duração da conecção entre a ponte intercelular e o oocito na vitelogênese, além de uma organização distinta do alvéolo cortical nas quatros espécies de *Tropidurus*. A atresia, folículos pós-ovulatórios e corpo lúteo, foram detalhadas também pela primeira vez para as espécies *Tropidurus torquatus*, *T. itambere* e *T. oreadicus*. A aptidão reprodutiva foi sazonal para *T. hispidus* e *T. itambere*, porém mais extensa para *T. torquatus* e *T. oreadicus*, com a duração de oito meses. O número de cama germinativa, ocorrência de corpo lúteo e atresia sugerem controle intríseco de cada uma dessas espécies no tamanho de ninhada e folículos viáveis para reprodução.

Palavras-chave: Tropiduridae, Lagartos Ovíparos, Aptidão Reprodutiva, Espermatogênese, Corpo Lúteo

ABSTRACT

The *Tropidurus* genus currently comprises 30 oviparous lizards that occupy South American cis-Andina continent, presenting variations in the reproductive cycle characterized as continuous, seasonal and non-continuos. Considering the reproductive variations, the aim was to evaluate the reproductive aptitude of four *Tropidurus* species of *torquatus* group, with wide distribution and high representative specimens in the zoological collections in Brazil. A total of 276 male individuals in 65 populations and 201 females in 43 populations of the species: *Tropidurus torquatus*, *T. hispidus*, *T. itambere* and *T. oreadicus* were evaluated. Samples of testicular, epididymal duct and ovary were included in plastic resin and prepared for analysis under light microscopy. Reproductive aptitude parameters included the minimum size at sexual maturity, clutch size, presence or absence of free spermatozoa, eggs and mature follicles. Males presented conserved germinative cells in morphology, however the spermatozoa production varied in time with continuity for *T. torquatus* and *T. hispidus* and seasonal for *T. itambere* and *T. oreadicus*. The epididymal duct showed epithelial hypertrophy and secretory activity related to the spermiation and regression in the seminiferous tubules. Sexual maturity varied in the minumum size among populations and biomes. Females presented the ovary with one germinative bed with oogonia and primary oocytes associated with stroma. Follicular development was conserved in morphostructural aspects, with two phases: previtellogenic and vitellogenic phases. During follicular maturation was observed longer duration of the connection between the intercellular bridge and the oocytes in vitellogenesis, besides a distinct organization of the cortical alveoli in four *Tropidurus* species. The atresia, post-ovulatory follicles and corpus luteum, were also detailed for the first time for *Tropidurus torquatus*, *T. itambere* and *T. oreadicus*. The reproductive aptitude was seasonal for *T. hispidus* and *T. itambere*, but extended for *T. torquatus* and *T. oreadicus*, with a duration of eighteen months. Germinal bed number, occurrence of corpus luteum and atresia suggest intrisic control of each of these species in clutch size and viable follicles for reproduction.

Keywords: Tropiduridae, Oviparous lizards, Reproductive aptitude, Spermatogenesis, Corpus Luteum

SUMÁRIO

1. INTRODUÇÃO

Lepidosauria compreende o maior grupo dentro de Reptilia, sendo representado pelos tuataras (Sphenodontidae), lagartos e serpentes (Squamata), os quais possuem como característica o tegumento coberto por escamas e uma fenda cloacal tranversal (Pough, 2008). Em lagartos, a história evolutiva e a filogenia os têm separado em dois grupos: Iguania e Scleroglossa (Pianka e Vitt, 2003). Iguania é reconhecido como um dos clados mais diversificado (Evans, 2003), inicialmente distribuídos em 11 subfamílias [Iguaninae, Crotaphytinae, Phrynosomatinae, Hoplocercinae, Liolaeminae, Polychrotinae, Coritophaninae, Leiocephalinae, Tropidurinae, Leisaurinae e Oplurinae] (Pianka e Vitt, 2003). Ao longo do tempo, diversas espécies pertencentes a essas subfamílias têm sido alvo de vários estudos e, principalmente, modelos para estudos reprodutivos (e.g. Tinkle *et al.*, 1970; Licht, 1971; Vitt e Goldberg, 1983; Vitt, 1991; Ibargüengoytía e Cussac, 1998; Van Sluys, 1993; Lovern, 2004; Ortiz *et al.*, 2014).

1.1 Modos de reprodução e os órgãos reprodutores em lagartos

Reprodução é um importante aspecto da história de vida relacionado a manutenção e permanência das espécies em um local ao longo do tempo (Pianka e Vitt, 2003). Em lagartos, de forma geral, podemos identificar dois modos de reprodução: oviparidade e viviparidade. Oviparidade é caracterizada pelo desenvolvimento embrionário fora do corpo sustentado pelo vitelo e protegido dentro do ovo, enquanto a viviparidade é definida pelo desenvolvimento no interior do útero, e dependente da transferência de nutrientes da mãe para o embrião (Pough, 2008). A oviparidade é considerada uma condição ancestral dentro de Squamata, e a viviparidade constitui um modo de reprodução que evoluiu várias vezes, de forma independente, sendo que nos lagartos ocorreu pelo menos 45 vezes (Pough, 2008).

O órgão reprodutor nos lagartos é posicionado na região dorsal do abdômen, ocorrendo aos pares. Nos machos são observados dois testículos, cada um deles associado a um ducto epididimal (Fig. 1), o qual se diferencia e se estende até atingir o hemipênis (Pianka e Vitt, 2003). De acordo com Jones (2002), o ducto epididimal em animais com fertilização externa e interna representa múltiplas funções, destacando o transporte, a maturação e a estocagem de espermatozoides. Morfologicamente, esse ducto é revestido por um epitélio pseudo-estratificado cilíndrico ciliado com as células basais ou tronco (Jones, 2002).

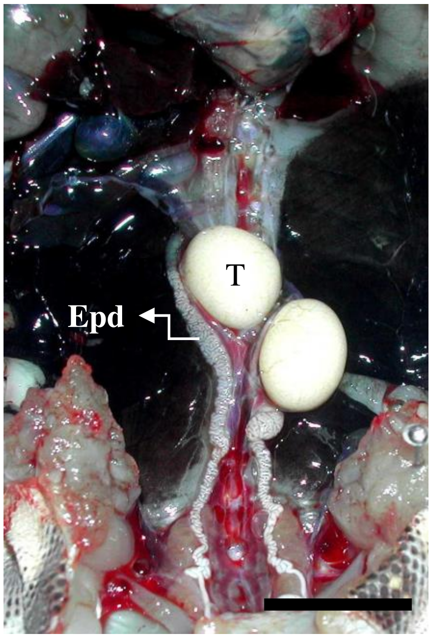

Figura 1: Órgão reprodutor masculino do lagarto *Tropidurus torquatus* (Wied, 1820) (Tropiduridae). Legenda: T = Testículo e Epd = Epidídimo. Aproximação de 1.5X, resolução de 7.2 MP. Barra preta = 20 mm. Foto: Tirada pela autora Débora F. N. da Silva na disciplina de Anatomia Animal Comparada na turma de Ciências Biológicas 2005/1 da Universidade Federal de Mato Grosso.

Nas fêmeas, os ovários são estruturas saculiformes aderidas por fino tecido conjuntivo à parede dorsal do abdômen (Fig. 2), contendo na sua superfície a cama germinativa e o estroma associado (Uribe *et al*., 1995). A cama germinativa é constituída por oogônias em divisão, oócitos isolados e folículos primordiais (Guraya, 1989). O número de camas germinativas, apesar de ser uma característica gênero-específica, pode variar em quantidade para espécies de lagartos de diferentes famílias e com variados modos de reprodução, podendo ser encontradas uma ou várias camas germinativas por ovário (Jones *et al*., 1982). À medida em que os folículos crescem, modificações estruturais do epitélio e dos componentes do ooplasma levam esses folículos pré-vitelogênicos à etapa de incorporação progressiva de vitelo, sendo esse um processo essencial ao desenvolvimento do embrião (Guraya, 1989).

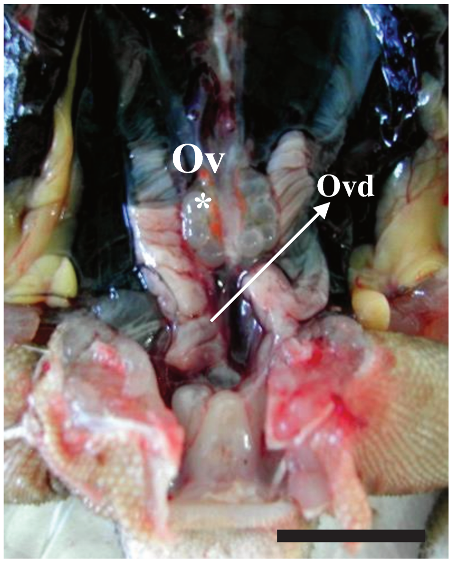

Figura 2: Órgão reprodutor feminino do lagarto *Tropidurus torquatus* (Wied, 1820) (Tropiduridae). Legenda: Ov* = Ovário, Ovd = Oviduto. Aproximação de 1.0X, resolução de 7.2 MP. Barra preta = 15 mm. Foto: Tirada pela autora Débora F. N. da Silva na disciplina de Anatomia Animal Comparada na turma de Ciências Biológicas 2005/1 da Universidade Federal de Mato Grosso.

Para cada tipo de gônada ocorrem processos de diferenciação das células germinativas, resultando em células altamente especializadas, sendo estas, nos machos, os espermatozoides, e nas fêmeas, o oócito maduro. Tanto a espermatogênese como a oogênese são processos regulados por hormônio, com participação de células acessórias. Nos machos, o hormônio luteinizante (LH) atua sobre as células de Leydig, induzindo a síntese de testosterona, a qual induz a célula de Sertoli, que sob a ação do hormônio folículo estimulante (FSH), estimula a diferenciação das células germinativas (Hess, 2008). Nas fêmeas, o ciclo ovariano também é regulado pelos hormônios FSH e LH, além do estrogênio e da progesterona, sendo o pico de estrogênio associado ao desenvolvimento folicular, e o pico de progesterona associado à manutenção do corpo lúteo e da gestação (Al-Amri *et al*., 2012).

1.2 Túbulos seminíferos e espermatogênese

Podem ser identificados pelo menos dois tipos de organização e desenvolvimento das células germinativas em vertebrados. A primeira é encontrada em vertebrados anamniotas (ex: peixes e anfíbios), os quais possuem testículos em forma de lóbulos com cistos de células germinativas se desenvolvendo de forma sincronizada. A segunda forma é observada em amniotas (ex: répteis, aves e mamíferos), que apresentam o testículo com túbulos seminíferos (Fig. 3) contendo células em vários estágios de desenvolvimento (Gribbins e Gist, 2003). Nos lagartos, os túbulos seminíferos são delimitados externamente pelo mesotélio e pela túnica albugínea, enquanto no seu interior, apresentam o arranjo em diferentes direções, envolvidos por tecido insterstiticial, células de Sertoli e células mioides (Ferreira *et al*., 2002). Espermatogênese envolve mudanças nas células germinativas, da base do túbulo em direção ao lumen: espermatogônias proliferam por mitose e, por meiose se diferenciam em espermatócitos e espermátides e, por fim, passam por espermiogênese até se diferenciar em espermatozoide (Gribbins e Gist, 2003). Durante a espermiogênese, as principais etapas estão relacionadas com o alongamento nuclear, a formação dos complexos acrossomal e axonemal e a eliminação do citoplasma residual (Ferreira e Dolder, 2003a).

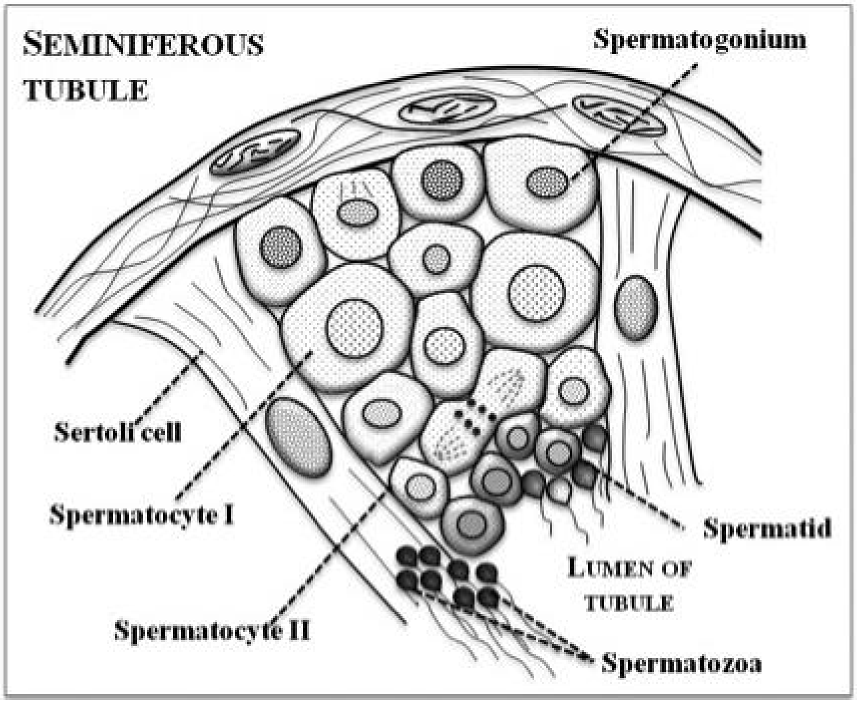

Figura 3: Esquema geral da espermatogênese em lagartos. Legenda: Spermatogonium = Espermatogônia; Sertoli cell = Célula de Sertoli; Spermatocyte I = Espermatócito primário; Spermatocyte II = Espermatócito secundário; Spermatid = Espermátide; Lumen of tubule = Lume do túbulo; Spermatozoa = Espermatozoide. Fonte: Mendéz de la Cruz *et al*., 2014.

Múltiplas abordagens são utilizadas nos estudos reprodutivos de lagartos, podendo o enfoque ser direcionado para as diferentes fases da espermatogênese com a predominância das células germinativas na fase de proliferação, espermiação e regressão (Estrada-Flores *et al*., 1990), ou para o período completo da formação de uma coorte de células germinativas (Gribbins, 2011). Nos machos de lagartos, as gônadas apresentam ciclo com duas fases: uma caracterizada pela atividade das células germinativas que se desenvolvem e se diferenciam dentro do túbulo durante a espermatogênese. A outra fase que é caracterizada pela inatividade das células germinativas ou quiescência, sendo observadas apenas associações entre espermatogônias e células de Sertoli (Licht, 1971). Mayhew e Wright (1970) descreveram oito estágios do ciclo espermatogênico para lagartos do gênero *Uma* sp. (Phrynosomatidae), sendo as principais mudanças relacionadas à presença de células em divisão, abertura do lúmem, espermatozoides na luz do túbulo e ausência de células em divisão. Já para o lagarto vivíparo *Sceloporus mucronatus* (Cope, 1885) (Phrynosomatidae) foram descritas quatro fases da espermatogênese (quiescência – recrudescência – atividade testicular máxima – regressão), sendo todas relacionadas ao tipo celular presente e à morfometria do epitélio seminífero e do epidídimo (Estrada-Flores *et al.*, 1990). Essas fases são bem estabelecidas para lagartos que habitam zona temperada, ou em localidades com as quatro estações bem definidas, onde mudanças estruturais do epitélio germinativo apresentam relação com as mudanças ambientais pontuais (Licht, 1971). Entretanto, para lagartos que habitam ambientes tropicais, onde as diferenças estacionais não predominam, as mudanças no epitélio germinativo geralmente estão associadas a outros fatores ambientais (Van Sluys, 1993). Para algumas espécies do gênero *Tropidurus* (Tropiduridae) no Brasil, observam-se variações no epitélio germinativo ao longo do ano, sendo a pluviosidade e a temperatura os possíveis fatores ambientais relacionados a sua reprodução sazonal (Wiederhecker *et al.*, 2002; Van Sluys *et al.*, 2010; Ferreira *et al.*, 2011).

1.3 Ovário e o desenvolvimento folicular

Os ovários dos lagartos estão localizados na região dorsal, no interior da cavidade abdominal, onde são sustentados pelo mesovário e revestidos por um epitélio simples pavimentoso (Uribe *et al.*, 1996). O estroma é constituído por tecido conjuntivo, vasos sanguíneos e linfáticos, e apresenta folículos em diferentes estágios de desenvolvimento, folículos atrésicos e o corpo lúteo (Uribe *et al.*, 1996). O desenvolvimento folicular nos répteis (Fig. 4) resulta de um complexo processo que envolve modificações do núcleo ou vesícula germinal, dos componentes do ooplasma, síntese e incorporação do vitelo, e do epitélio folicular durante sua maturação (Guraya, 1989).

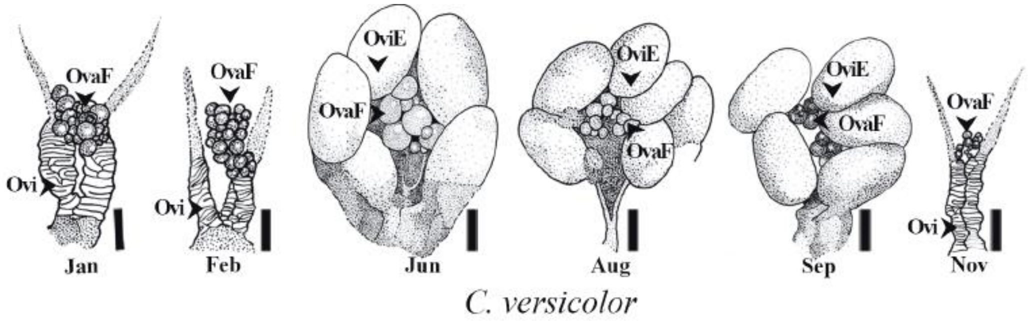

Figura 4: Esquema do ovário e do crescimento folicular do lagarto ovíparo *Calotes versicolor* (Daudin, 1802) (Agamidae) em diferentes meses do ano. Legenda: Folículo ovariano = OvaF, Ovo no oviduto = OviE, Oviduto = Ovi. Fonte: Meesook *et al*., 2016.

Nos estudos de Uribe *et al.* (1995; 1996) para as espécies de lagartos vivíparo *Sceloporus torquatus* (Wiegmann, 1828) e ovíparo *Ctenossaura pectinata* (Wiegmann, 1834) (Iguanidae), foram descritos nove estágios do desenvolvimento folicular dividido em uma fase pré-vitelogênica e outra vitelogênica. Na espécie ovípara, a pré-vitelogênese foi dividida em quatro estágios (I – IV) e a vitelogênese em cinco estágios (V – IX). Na fase da pré-vitelogênese, o estágio I foi caracterizado por oócitos localizados na cama germinativa com o núcleo apresentando cromossomos fibrilares na prófase da meiose I. No estágio II, o oócito migra em direção ao estroma ovariano e se torna um folículo primordial. No estágio III o oócito aumenta de tamanho e o núcleo se torna excêntrico com cromossomos plumosos. O estágio IV foi o último estágio do folículo pré-vitelogênico, sendo caracterizado por um núcleo com cromossomos plumosos e vários nucléolos evidentes. Ainda nesse estágio, a zona pelúcida é homogênea e de fácil visualização e, a camada granulosa apresenta-se polimórfica com três tipos celulares constituintes: as células pequenas, intermediárias e piriformes. Na vitelogênese, o estágio V foi caracterizado por oócito maior com vários grânulos que se depositaram no ooplasma. No estágio VI, os oócitos apresentam aumento no depósito de vitelo, através da fusão dessas vesículas. No estágio VII aumenta a deposição de vitelo e a camada granulosa regride, ficando com apenas uma camada de células cúbicas e achatadas, enquanto a camada da teca aumenta em quantidade de vasos sanguíneos. No estágio VIII, o oócito possui os pólos animal e vegetal distintos e, a granulosa com apenas uma camada de células achatadas. Já a teca torna-se altamente vascularizada. No último estágio da vitelogênese, estágio IX, o núcleo localiza-se no pólo animal e vesículas com vitelo permanecem irregularmente distribuídas. Após completar a vitelogênese, o folículo maduro é liberado do epitélio folicular durante a ovulação, e a estrutura restante forma o corpo lúteo. Guraya (1989) define corpo lúteo como células foliculares que após a ovulação hipertrofiam para formar uma massa de células com função luteal, envolvidas por camadas de células da teca. Já a atresia, definida como um processo relacionado à degeneração folicular (Guraya, 1989), apresenta ocorrência em ambos tipos de folículos, pré-vitelogênicos e vitelogênicos, porém com maior frequencia nos pré-vitelogênicos (Uribe *et al*., 1996). De acordo com Guraya (1973), a atresia caracteriza-se pelo aumento das células da granulosa, formando uma invaginação em direção ao interior do oócito, o que indica fagocitose e a consequente dobra da zona pelúcida até a sua ruptura. Esses processos de formação do corpo lúteo e atresia são essenciais para determinar a quantidade de folículos viáveis para fecundação, podendo estar relacionado com a quantidade de cama germinativa de cada espécie, influenciando sua fecundidade durante a estação reprodutiva (Jones *et al*., 1982).

1.4 Reprodução em lagartos tropicais e a aptidão reprodutiva

Estratégia reprodutiva é o conjunto de adaptações para promover a forma mais eficiente de uma população sobreviver e ter sucesso reprodutivo sob as condições particulares de um determinado ambiente (Tinkle *et al*., 1970; Ballinger, 1977). As adaptações envolvidas na estratégia reprodutiva em lagartos incluem a diferenciação morfológica e funcional dos órgãos relacionados à produção e condução de gametas, repercutindo nos padrões estabelecidos de comportamento, na anatomia e fisiologia das espécies (Ferreira *et al*., 2011). Diferentes estratégias reprodutivas foram reportadas para os lagartos. Inicialmente destacaram-se as formas conhecidas como maturadores-precoces, caracterizados por indivíduos com baixa expectativa de vida e elevada fecundidade com ninhadas múltiplas, e maturadores-tardios, com alta expectativa de vida e baixa fecundidade com ninhadas únicas (Tinkle *et al*., 1970). Posteriormente, Dunham *et al*., (1988) reconheceram como sendo três as estratégias reprodutivas básicas em lagartos: ovíparos com ninhadas múltiplas, ovíparos com ninhadas únicas e vivíparos com ninhadas múltiplas. As demais características reprodutivas como maturação precoce ou tardia e ninhadas grandes ou pequenas, por sua vez, se encaixam dentro dessas estratégias de acordo com a história de vida de cada espécie (Dunham *et al*., 1988).

Outro aspecto importante da estratégia reprodutiva em lagartos envolve a duração do ciclo reprodutivo (Guillette Jr. e Casa-Andreu, 1987). Alguns estudos têm abordado a relação entre ciclos reprodutivos de lagartos vivendo em ambientes temperados e tropicais (Hahn, 1964; James e Shine, 1985; Guillete Jr. Casas-Andreu, 1987). No estudo de Angelini *et al*. (1976) foi observado que em lagartos de ambientes temperados, a reprodução é um processo continuamente regulado por fatores endógenos, ou genético, e por fatores ambientais. No estudo de Licht (1971) em ambiente subtropical foi observado que em *Anolis carolinensis* (Voigt, 1832) (Dactyloidae) a atividade testicular apresentou variações relacionadas à sazonalidade climática, sendo fotoperíodo e temperatura fatores proximais na regulação da espermatogênese. Entretanto, nas espécies tropicais, outros fatores podem estar relacionados com a sazonalidade reprodutiva como a precipitação e a umidade para o desenvolvimento dos embriões (James e Shine, 1985; Wiederhecker *et al*., 2002). Modificações estruturais do epitélio germinativo durante o ciclo testicular em relação as condições ambientais (e.g. Mendonça e Litch, 1986; Cree *et al*., 1992), a disponibilidade de alimento ou reserva lipídica (e.g. Diaz *et al*., 1994), ou ainda associadas as variações na atividade das células intersticiais (e.g. Grimalt *et al*., 1995; Van Wyk, 1995; Colli e

Pinho, 1997). Incluem-se também as adaptações ecomorfológicas com elevada especialização de habitat e modo de forrageio como fatores que influenciam no investimento reprodutivo de diferentes espécies de lagartos tropicais (e.g. Vitt, 1981; Vitt, 1991; Colli *et al*., 1991). Assim, com o objetivo de padronizar a reprodução dos lagartos tropicais, Sherbrook (1975) agrupou os ciclos reprodutivos em três tipos, considerando a aitividade reprodutiva anual: 1) Ciclo reprodutivo contínuo, caracterizado pela baixa variação dos parâmetros reprodutivos; 2) Ciclo contínuo com variação dos parâmetros reprodutivos, e 3) Ciclo reprodutivo não-contínuo (descontínuo), caracterizado por um período de inatividade reprodutiva.

A reprodução também pode ser avaliada através do estudo utilizando espécimes depositados em acervos de coleções zoológicas, contribuindo igualmente com o conhecimento dos padrões de reprodução nos lagartos tropicais. Nesse contexto, a aptidão reprodutiva representa uma nova abordagem de estudo com a proposta de avaliar o status reprodutivo de uma ou mais espécie coletada em diferentes épocas e localidades. Essa abordagem traz algumas vantagens inerentes como acesso a espécimens sob diferentes condições temporais e pressões ambientais (e.g. Cassel *et al*., 2012), contribuindo com possíveis medidas de conservação dessas espécies no Brasil. Outra vantagem está relacionada a dispensa de coletas de novos indivíduos, pois as espécies com maior abundância são também as mais representativas nos acervos zoológicos, representando modelo ideal de estudo. Metodologicamente, a determinação da aptidão reprodutiva pressupõe a caracterização do estágio gametogênico, evidenciando a presença de espermatozoides maduros e de folículos e ovos, inferirindo assim a condição reprodutiva de forma pontual. De fato, essa abordagem favorece a construção de um panorama comparável entre diferentes condições ao longo do tempo em uma determinada população de espécie ou entre populações, fornecendo dados inéditos para a compreensão da estratégia reprodutiva de um determinado grupo de espécies.

1.5 Características do Gênero *Tropidurus*

O gênero *Tropidurus* (Wied-Neuwied, 1825), *sensu estrictu*, compreende os lagartos Sul-Americanos cis-Andinos atualmente classificados em 30 espécies alocadas em quatro grupos: grupo *bogerti*, grupo *semitaeniatus*, grupo *spinulosus* e grupo *torquatus* (*sensu* Frost *et al.*, 2001). No estudo de Rodrigues (1987) foi realizada uma revisão sistemática do grupo *torquatus*, sendo listadas 11 espécies [*T. etheridgei* (Cei, 1982), *T. catalanensis* (Gudynas e Skuk, 1983), *T. hispidus* (Spix, 1825), *T. hygomi* (Reinhardt e Lütken, 1861), *T. torquatus* (Wied, 1820)], mais setes descritas (*T. cocorobensis*, *T. erythrocephalus*, *T. insulanus*, *T. itambere*, *T. montanus*, *T. mucujensis* e *T. oreadicus*). Posteriormente, Frost *et al.*, (2001) realizaram um estudo filogenético do gênero *Tropidurus*, baseado em dados morfológicos e moleculares, o qual incluiu as espécies *T. chromatops* (Harvey e Gutberlet, 1988) e *T. psammonastes* (Rodrigues *et al.*, 1988), totalizando 14 espécies no grupo *torquatus*. Atualmente, esse grupo apresenta o total de 16 espécies com as duas últimas descritas recentemente: *T. imbituba* (Kunz e Borges-Martins, 2013) e *T. sertanejo* (Carvalho *et al.*, 2016).

Segundo Rodrigues (1987) todas as espécies do grupo *torquatus* mostram distribuições geográficas que só podem ser compreendidas com base na atuação de ciclos climáticos recentes, baseando-se na teoria dos refúgios florestais. No entanto, Carvalho *et al.*, (2013) estabeleceu a biogeografia para o gênero, relatando que a distribuição atual de *Tropidurus* pode ser explicada pelo surgimento de diversos eventos vicariantes, sendo pouco provável que as alterações de ciclos climáticos no período Quaternário tenham influenciado o padrão de distribuição geográfica dessas espécies. Porém, o autor não descarta que talvez essas alterações tenham influenciado o rearranjo das distribuições dessas espécies.

O grupo *torquatus* apresenta espécies de lagartos que habitam formações abertas da América do Sul cis-Andina, da Venezuela à Argentina, sendo espécies de porte relativamente pequeno, caracterizadas pela presença de escamas dorsais carenadas e imbricadas e pela ausência da crista médio-dorsal (Rodrigues, 1987; Carvalho *et al.*, 2013). Uma parte das espécies apresenta ampla distribuição geográfica e a maioria explora diferentes tipos de hábitats com sobreposição na distribuição de algumas espécies, as quais podem ocorrer tanto em sintopia quanto em simpatria, ou ainda de forma mais restrita ou endêmica (Rodrigues, 1987; Carvalho *et al.*, 2013). Devido a esse padrão de distribuição, o grupo *torquatus* pode ser considerado como modelo ideal para estudos de adaptações reprodutivas em lagartos tropicais, fornecendo evidências sobre a estratégia reprodutiva utilizada pelas espécies do gênero.

Dados relacionados à análise dos ciclos reprodutivos para a família Tropiduridae foram publicados em diversos estudos [e.g. *Microlophus albermalensis* (Baur, 1890) (= *Tropidurus albermalensis*) Stebbins *et al.*, (1967), *T. hispidus* Prieto *et al.*, (1976), Ribeiro *et al.*, (2012), *T. semitaeniatus* (Ribeiro *et al.*, 2012), *M. delanonis* (Baur, 1890) (= *Tropidurus delanonis*) Werner, (1978), *M. quadrivittatus* (Tschudi, 1845) (= *Tropidurus quadrivittatus*) e *M. theresioides* (Donoso-Barros, 1966) (= *Tropidurus theresioides*) Goldberg e Rodrigues, (1986), *T. torquatus* (Cruz-Landim e Cruz-Höfling, 1977; Cruz-Höfling e Cruz-Landim, 1978, Vieira *et al.*, 2001, Wiederhecker *et al.*, 2002, Van Slyus *et al.*, 2010), *T. itambere* (Van Sluys, 1993, Ferreira e Dolder, 2003a, 2003b, Ferreira *et al.*, 2006, Ferreira e Dolder, 2007, Ferreira *et al.*, 2009, Ferreira *et al.*, 2011), *T. etheridgei* (Ferreira *et al.*, 2011), *T. oreadicus* (Meira *et al.*, 2007, Ferreira *et al.*, 2011), *T. spinulosus* e *T. gaurani* (Ferreira *et al.*, 2011)].

2 OBJETIVO GERAL

Determinar as variações da aptidão reprodutiva de quatro espécies de lagartos do gênero *Tropidurus* do grupo *torquatus*, estabelecendo um panorama comparável da condição espacial e temporal relacionados a estratégia reprodutiva dessas espécies no Brasil.

2.1 Objetivos Específicos

- Estabelecer o tamanho corpóreo médio em machos e fêmeas das espécies *Tropidurus hispidus*, *T. torquatus*, *T. oreadicus* e *T. itambere* do grupo *torquatus* proveniente de diferentes localidades no Brasil.

- Identificar as células germinativas e estabelecer pontualmente em qual fase da espermatogênese se encontram as diferentes populações de lagartos das espécies *Tropidurus hispidus*, *T. oreadicus, T. itambere* e *T. torquatus* do grupo *torquatus*.

- Avaliar, através da ausência ou da presença de espermatozoides nos túbulos seminíferos e no ducto epididimal, o tamanho corpóreo na maturidade sexual e a aptidão reprodutiva dos machos das espécies *Tropidurus hispidus*, *T. oreadicus, T. itambere* e *T. torquatus* do grupo *torquatus*.

- Quantificar os ovos e/ou folículos vitelogênicos nas fêmeas das espécies de *Tropidurus hispidus*, *T. oreadicus, T. itambere* e *T. torquatus* do grupo *torquatus* e estabelecer o tamanho corpóreo na maturidade sexual e o tamanho médio da ninhada.

- Identificar o ovário e suas estruturas, e estabelecer o desenvolvimento folicular e as suas modificações estruturais até a formação do folículo maduro.

- Identificar o corpo lúteo, a atresia folicular e estabelecer o período no qual ocorrem.

- Determinar a aptidão reprodutiva de lagartos fêmeas das espécies *Tropidurus hispidus*, *T. oreadicus, T. itambere* e *T. torquatus* do grupo *torquatus*, através da presença de ovos, folículos maduros e corpo lúteo.

3 MATERIAL E MÉTODOS

3.1 Espécimes e Coleções

Foram analizados o total de 476 indivíduos distribuídos em quatro espécies do gênero *Tropidurus* do grupo *torquatus*: *Tropidurus hispidus*, *T. torquatus*, *T. oreadicus* e *T. itambere*. Os espécimens foram dividos em 276 amostras de indivíduos machos e 200 de indivíduos fêmeas. Todos os indivíduos foram agrupados em populações para cada localidade, considerando a região e o bioma correspondente, sendo sumarizados nas Tabelas 1 e 2.

Para a coleta dos dados macroscópicos, os indivíduos foram medidos, sendo a distância mensurada a partir da escama rostral até a cloaca, chamado de comprimento rostro-cloacal (CRC). Essas medidas foram estimadas em suas médias e desvios padrão para cada população de cada espécie de diferentes localidades (Tabela 2 do artigo 2 e Tabela 3 do artigo 3). Posteriormente, foi feita uma incisão longitudinal no abdômen de todos os indivíduos, onde nas fêmeas quando presente, foram contabilizados folículos vitelogênicos ≥ 3mm e a presença de ovos nos ovidutos de acordo com Van Sluys (1993).

A estimativa do tamanho da ninhada (Tabela 3 do artigo 3) foi obtida através da média entre folículos vitelogenciso e ovos por fêmea reprodutiva, de acordo com Van Sluys *et al*., (2010). Para o comprimento mínimo na maturidade sexual (Tabela 2 – artigo 2 e Tabela 3 – artigo 3) foi relacionado o comprimento corpóreo (CRC) com a presença de gametas maduros nos indivíduos, de acordo com (Van Sluys, 1993).

Para estabelecer a razão gonadossomática, foram regitrados o peso de apenas um dos testículos e o peso do lagarto, sendo o resultado dessa razão multiplicado por 100. Amostras de ovário e folículos com até 5 mm de espessura foram retiradas para a análise em microscopia de luz, assim como nos machos, onde foram retiradas as amostras de testículo e ducto epididimal.

Tabela 1: Relação das populações de lagartos machos do gênero *Tropidurus* do grupo *torquatus* avaliadas no estudo. Tamanho amostral (N) e números tombo dos espécimes. Acrônimos: **CHUNB** (Coleção Herpetológica da Universidade de Brasília), **INPA-HERPETO** (Coleção de Anfíbios e Répteis do Instituto Nacional de Pesquisas da Amazônia), **MPEG** (Museu Paraense Emílio Goeldi), **UFMT-R** Coleção Zoológica da Universidade Federal de Mato Grosso Setor Reptilia), **ZUEC-REP** (Museu de Zoologia da Universidade Estadual de Campinas "Adão José Cardoso"), **ZUFG** (Coleção Zoológica da Universidade Federal de Goiás).

Espécie	**Bioma**	**Região**	**Localidade**	**N**	**Data de Coleta (mês/ano)**	**Tombo**
Tropidurus torquatus	Cerrado	Centro-oeste	Brasília - DF	5	12/2000, 12/2001, 01/2003	CHUNB 43271, 48039, 44770, 49660, 49661
Tropidurus torquatus	Cerrado	Centro-oeste	Cuiabá - MT	82	01,11-12/2005, 01-07/2006, 10-12/2006, 01,03,05-07/2007, 06-12/2012, 01-05/2013	UFMT-R 2770, 2882, 2883, 3038, 3039, 3190, 3419, 3420, 3543, 3544, 3606, 3607, 3767, 3961, 3992, 4186, 4255, 4256, 4565, 4566, 4882, 4883, 4889, 4904, 5467, 5725, 5828, 5829, 5909, 5910, 11590, 11591, 11593, 11594, 11596, 11603, 11605, 11606, 11609-11611, 11614-11616, 11618, 11620, 11624-11627, 11630-11632, 11635-11637, 11640-11644, 11649, 11653, 11655, 11657, 11659, 11663, 11666, 11668, 11669, 11674-11681, 11685-11687, 11689
Tropidurus torquatus	Cerrado	Centro-oeste	Itarumã - GO	1	09/2008	ZUFG 124
Tropidurus torquatus	Cerrado	Centro-oeste	Santa Terezinha - MT	1	03/1995	CHUNB 10912
Tropidurus torquatus	Cerrado	Centro-oeste	São Domingos - GO	1	03/2004	CHUNB 37622
Tropidurus torquatus	Cerrado	Centro-oeste	Silvânia - GO	1	07/2008	ZUFG 115
Tropidurus torquatus	Cerrado	Centro-oeste	Sítio D' Abadia - GO	1	01/2008	ZUFG 249
Tropidurus torquatus	Cerrado	Nordeste	Bahia	1	01/1995	ZUEC-REP 1873
Tropidurus torquatus	Cerrado	Norte	Lajeado - TO	2		ZUFG 509, 511
Tropidurus torquatus	Cerrado	Norte	Macaúba - TO	1	05/1962	MPEG 1235
Tropidurus torquatus	Cerrado	Sudeste	Unaí - MG	5	10/1993, 03/1994, 02/1999	CHUNB 01720-01722, 05431, 36286
Tropidurus torquatus	Amazônia	Norte	Manaus - AM	3	04/2006, 06/2007	INPA-H 016528, 019892, 019894
Tropidurus torquatus	Mata Atlântica	Nordeste	Itacaré - BA	1	07/1997	CHUNB 25227
Tropidurus torquatus	Mata Atlântica	Sudeste	Alto Caparaó - MG	1	03/2002	CHUNB 46225
Tropidurus torquatus	Mata Atlântica	Sudeste	Vale do Rio Doce - MG	1		ZUEC-REP 234
Tropidurus torquatus	Mata atlântica	Sudeste	Linhares - ES	1	07/1995	ZUEC-REP 2863

Tropidurus torquatus	Mata Atlântica	Sudeste	Maricá - RJ	3	12/1979	CHUNB 03971, 03973, 04019
Tropidurus torquatus	Mata Atlântica	Sudeste	Presidente Kennedy - ES	1	09/1997	CHUNB 24986
Tropidurus hispidus	Caatinga	Nordeste	Petrolina - PE	1	08/1991	CHUNB 05830
Tropidurus hispidus	Cerrado	Nordeste	Estreito - MA	1	08/1991	CHUNB 05193
Tropidurus hispidus	Amazônia	Nordeste	Arari - MA	3	02/1978	MPEG 11593, 11613, 11614
Tropidurus hispidus	Amazônia	Nordeste	Santa Luzia do Paruá - MA	4	06/1975, 06/1976	MPEG 10141, 10147, 10167, 11110
Tropidurus hispidus	Amazônia	Nordeste	São Raimundo - MA	1	11/1975	MPEG 9392
Tropidurus hispidus	Amazônia	Norte	Boa Vista - RR	1	06/1970	MPEG 3964
Tropidurus hispidus	Amazônia	Norte	Caracaraí-RR	8	07/1993	MPEG 17181-17183, 17224, 17232, 17236, 17249, 17258
Tropidurus hispidus	Amazônia	Norte	Carajás - PA	2	05/1983	MPEG 12935, 12937
Tropidurus hispidus	Amazônia	Norte	Marabá - PA	4	05/1969	MPEG 2880, 2881, 2884, 2887
Tropidurus hispidus	Amazônia	Norte	São José do Ribamar - MA	32	04-05/2007	ZUEC-REP 3193, 3194, 3196, 3198, 3204, 3207, 3209-3211, 3214, 3222, 3223, 3230-3232, 3235, 3237, 3243, 3245, 3250, 3251, 3256, 3257, 3259, 3274, 3276, 3278, 3281, 3284, 3287, 3290, 3297
Tropidurus itambere	Cerrado	Centro-oeste	Brasília - DF	2	11/2005	CHUNB 44132, 44134
Tropidurus itambere	Cerrado	Centro-oeste	Minaçu - GO	5	06/2001, 01,05/2008	CHUNB 48416, 48417, 52775, 53173, 53174
Tropidurus itambere	Cerrado	Centro-oeste	Pirenópolis - GO	6	06,09/2000	CHUNB 31678, 31679, 31682, 31690, 31707, 31708
Tropidurus itambere	Cerrado	Nordeste	Bahia	1	07/1995	ZUEC-REP 1873
Tropidurus itambere	Cerrado	Sudeste	Paracatu - MG	1	10/2001	CHUNB 26900
Tropidurus itambere	Mata Atlântlica	Sudeste	Campinas - SP	1	10/1993	ZUEC-REP 1814
Tropidurus itambere	Mata Atlântica	Sudeste	Itatiba - SP	3	04/1988	ZUEC-REP 617-619
Tropidurus itambere	Mata Atlântica	Sudeste	Pedro Canário - ES	1	07/1974	ZUEC-REP 326
Tropidurus itambere	Mata Atlântica	Sudeste	Poços de Caldas - MG	1	08/1998	ZUEC-REP 2165
Tropidurus itambere	Mata Atlântica	Sudeste	Valinhos - SP	1	03/1987	ZUEC-REP 533
Tropidurus oreadicus	Cerrado	Centro-oeste	Alto Paraíso de Goiás - GO	1	09/2005	CHUNB 43642
Tropidurus oreadicus	Cerrado	Centro-oeste	Alvorada do Norte - GO	2	08/2003	CHUNB 33311, 33316
Tropidurus oreadicus	Cerrado	Centro-oeste	Barro Alto - GO	1	11/2007	ZUFG 80
Tropidurus oreadicus	Cerrado	Centro-oeste	Cocalzinho de Goiás - GO	3	01/2003	CHUNB 34655-34657
Tropidurus oreadicus	Cerrado	Centro-oeste	Minaçu - GO	3	06/2001, 12/2002, 05/2008	CHUNB 29641, 48409, 53176

Tropidurus oreadicus	Cerrado	Centro-oeste	Niquelândia - GO	3	04,05/2006	ZUFG 09, 10, 50
Tropidurus oreadicus	Cerrado	Centro-oeste	Pirenópolis - GO	8	12/1990, 05-07,09/2000	CHUNB 06294, 06295, 31681, 31688, 31689, 31701, 31720, 31732
Tropidurus oreadicus	Cerrado	Centro-oeste	São Domingos - GO	7	03/2001, 09,11/2003, 03,11/2004, 04/2007, 01/2008	CHUNB 25325, 33330, 35359, 37622, 43833; ZUFG 186, 200
Tropidurus oreadicus	Cerrado	Nordeste	Balsas- MA	2	12/2002	CHUNB 43773, 43774
Tropidurus oreadicus	Cerrado	Nordeste	Carolina - MA	1	11/2007	CHUNB 52034
Tropidurus oreadicus	Cerrado	Nordeste	Cocos - BA	4	12/2000, 11/2007, 04/2008	CHUNB 50202, 51239, 51490, 52378
Tropidurus oreadicus	Cerrado	Nordeste	Correntina - BA	1	10/1993	CHUNB 06159
Tropidurus oreadicus	Cerrado	Norte	Almas - TO	1	02/2004	CHUNB 42334
Tropidurus oreadicus	Cerrado	Norte	Caseara - TO	1	09/2005	CHUNB 45197
Tropidurus oreadicus	Cerrado	Norte	Dianópolis - TO	1	09/2003	CHUNB 33303
Tropidurus oreadicus	Cerrado	Norte	Lajeado - TO	3		ZUFG 406, 408, 411
Tropidurus oreadicus	Cerrado	Norte	Mateiros - TO	5	12/1999, 02/2002, 11/2004	CHUNB 24302, 28585, 40506, 40523, 40524
Tropidurus oreadicus	Cerrado	Norte	Palmas - TO	4	12/1999, 12/2000	CHUNB 11301, 13863, 13864, 13865
Tropidurus oreadicus	Cerrado	Norte	Paranã - TO	2	09/2003	CHUNB 33302, 33305
Tropidurus oreadicus	Cerrado	Norte	Porto Nacional - TO	2	07/2005	CHUNB 47767, 47769
Tropidurus oreadicus	Amazônia	Norte	Amazonas	1	03/2013	INPA-H 033020
Tropidurus oreadicus	Amazônia	Norte	Belém - PA	6	09,10/2004, 03,05/2006, 06/2007	MPEG 22219, 22220, 22229, 24803, 24805, 24806
Tropidurus oreadicus	Amazônia	Norte	Macapá - AP	2	09/1991	CHUNB 06458, 06461
Tropidurus oreadicus	Amazônia	Norte	Marabá - PA	5	07/1984, 06/1985	MPEG 13689, 13691, 13692, 14105, 14235
Tropidurus oreadicus	Amazônia	Norte	Parauapebas - PA	2	07/1986	CHUNB 05704, 05705
Tropidurus oreadicus	Amazônia	Norte	Porto Velho - RO	13	03,07/2004, 11/2010, 05,11/2011	INPA-H 14980, 14981, 14988, 32944, 32946, 32949, 32950, 33014, 33016, 33017, 33024, 33025, 33027
Tropidurus oreadicus	Amazônia	Norte	Vigia - PA	1	10/1973	MPEG 7091

Tabela 2: Relação das populações de lagartos fêmeas do gênero *Tropidurus* do grupo *torquatus* avaliadas no estudo. Tamanho amostral (N) e números tombo dos espécimes. Acrônimos: **CHUNB** (Coleção Herpetológica da Universidade de Brasília), **INPA-HERPETO** (Coleção de Anfíbios e Répteis do Instituto Nacional de Pesquisas da Amazônia), **MPEG** (Museu Paraense Emílio Goeldi), **UFMT-R** Coleção Zoológica da Universidade Federal de Mato Grosso Setor Reptilia), **ZUEC-REP** (Museu de Zoologia da Universidade Estadual de Campinas "Adão José Cardoso"), **ZUFG** (Coleção Zoológica da Universidade Federal de Goiás).

Espécie	**Bioma**	**Região**	**Localidade**	**N**	**Data de Coleta (mês/ano)**	**Tombo**
Tropidurus torquatus	Cerrado	Centro-oeste	Brasília - DF	1	12/2001	CHUNB 48038
Tropidurus torquatus	Cerrado	Centro-oeste	Cuiabá - MT	74	11-12/2005, 01,03,06,10-11/2006, 01-07/2007, 06-12/2012, 01-05/2013	UFMT-R 2880, 2881, 3040, 3188, 3189, 3551, 3963, 4185, 4257, 4567, 4881, 4890, 4905, 5334, 5468, 5469, 5723, 5724, 5827, 5830, 5911, 11589, 11592, 11595, 11597-11602, 11604, 11607, 11608, 11612, 11613, 11617, 11619, 11621-11623,11628, 11629, 11633, 11634, 11638, 11639, 11645-11648, 11651, 11652, 11654, 11656, 11658, 11660-11662, 11664, 11665, 11667, 11670-11673, 11682-11684, 11688, 11690-11693
Tropidurus torquatus	Cerrado	Centro-oeste	Sítio D' Abadia - GO	2	01/2008	ZUFG 250, 251
Tropidurus torquatus	Cerrado	Norte	Lajeado - TO	1		ZUFG 510
Tropidurus torquatus	Cerrado	Sudeste	Buritizeiro - MG	1	12/2000	CHUNB 44501
Tropidurus torquatus	Cerrado	Sudeste	Unaí - MG	2	09/1997, 04/1999	CHUNB 24706, 32876
Tropidurus torquatus	Amazônia	Norte	Iracema - RR	1	11/2011	INPA-H 31617
Tropidurus torquatus	Amazônia	Norte	Manaus - AM	2	06/2007	INPA-H 19893, 19895
Tropidurus torquatus	Mata Atlântica	Sudeste	Presidente Kennedy - ES	2	09/1997	CHUNB 24985, 24987
Tropidurus hispidus	Caatinga	Nordeste	São José do Ribamar - MA	35	04-05/2007	ZUEC-REP 3195, 3199, 3202, 3203, 3205, 3206, 3213, 3215-3217, 3219, 3221, 3225-3228, 3236, 3241, 3252-3255, 3270-3273, 3275, 3279-3283, 3285, 3286, 3288, 3291
Tropidurus hispidus	Caatinga	Nordeste	Exu - PE	1	07/1993	CHUNB 13465
Tropidurus hispidus	Caatinga	Nordeste	Petrolina - PE	1	09/1991	CHUNB 05832
Tropidurus hispidus	Cerrado e Amazônia	Nordeste	Arari - MA	2	02/1978	MPEG 11540, 11558
Tropidurus hispidus	Amazônia	Nordeste	Santa Luzia do Paruá - MA	4	06/1975, 06/1976	MPEG 10148, 11098, 11128, 11130
Tropidurus hispidus	Amazônia	Norte	Caracaraí - RR	5	07/1993	MPEG 17180, 17206, 17207, 17240, 17251
Tropidurus hispidus	Amazônia	Norte	Manaus - AM	1	07/2008	INPA-H 21223
Tropidurus hispidus	Amazônia	Norte	Marabá - PA	3	05/1969	MPEG 2883, 2885, 2886
Tropidurus itambere	Cerrado	Centro-oeste	Aparecida do Rio Doce - GO	1	12/2006	ZUFG 69

Tropidurus itambere	Cerrado	Centro-oeste	Minaçu - GO	1	05/2008	CHUNB 53175
Tropidurus itambere	Cerrado	Centro-oeste	Pirenópolis - GO	5	07/2000	CHUNB 31676, 31677, 31696, 31731, 31744
Tropidurus itambere	Cerrado	Sudeste	Paracatu - MG	2	10/2001	CHUNB 26898, 26899
Tropidurus itambere	Mata Atlântica	Sudeste	Campinas - SP	5	05/1988, 09/1994, 05,07,10/1995	ZUEC-REP 633, 1795, 1848, 2735, 2736
Tropidurus itambere	Mata Atlântica	Sudeste	Itatiba - SP	2	04/1988	ZUEC-REP 615, 616
Tropidurus itambere	Mata Atlântica	Sudeste	Ourinhos - SP	1	03/2002	ZUEC-REP 2660
Tropidurus itambere	Mata Atlântica	Sudeste	Poços de Caldas - MG	1	08/1998	ZUEC-REP 2163
Tropidurus itambere	Mata Atlântica	Sudeste	Pinheiros - ES	1	07/1974	ZUEC-REP 327
Tropidurus oreadicus	Cerrado	Centro-oeste	Alvorada do Norte - GO	1	03/2004	CHUNB 37605
Tropidurus oreadicus	Cerrado	Centro-oeste	Minaçu - GO	3	06/2001, 12/2002	CHUNB 29640, 48407, 48408
Tropidurus oreadicus	Cerrado	Centro-oeste	Niquelândia - GO	2	01,05/2006	ZUFG 11, 51
Tropidurus oreadicus	Cerrado	Centro-oeste	Pirenópolis - GO	1	07/2000	CHUNB 31702
Tropidurus oreadicus	Cerrado	Centro-oeste	Sítio D' Abadia - GO	1	01/2007	ZUFG 213
Tropidurus oreadicus	Cerrado	Nordeste	Balsas - MA	1	12/2002	CHUNB 43775
Tropidurus oreadicus	Cerrado	Nordeste	Carolina - MA	2	11/2007	CHUNB 52033, 52035
Tropidurus oreadicus	Cerrado	Nordeste	Cocos - BA	5	12/2000, 10/2003, 11/2006, 11/2007	CHUNB 49160, 49163, 50201, 51240, 51489
Tropidurus oreadicus	Cerrado	Norte	Dianópolis - TO	1	09/2003	CHUNB 33306
Tropidurus oreadicus	Cerrado	Norte	Lajeado - TO	6		ZUFG 270, 405, 407, 409, 410, 412
Tropidurus oreadicus	Cerrado	Norte	Mateiros - TO	2	12/1999	CHUNB 24299, 24301
Tropidurus oreadicus	Cerrado	Norte	Palmas - TO	4	12/1999, 07/2001	CHUNB 11302, 24626-24628
Tropidurus oreadicus	Cerrado	Norte	Porto Nacional - TO	1	07/2005	CHUNB 47768
Tropidurus oreadicus	Amazônia	Norte	Macapá - AP	1	09/2001	CHUNB 06467
Tropidurus oreadicus	Amazônia	Norte	Marabá - PA	1	07/1984	MPEG 13690
Tropidurus oreadicus	Amazônia	Norte	Parauapebas - PA	3	07/1986, 07/1992	CHUNB 05706, 06356, 06366
Tropidurus oreadicus	Amazônia	Norte	Porto Velho - RO	9	05,11/2011, 03/2013	INPA-H 32943, 32945, 32947, 32948, 32951, 33015, 33018, 33019, 33023

3.2 Dados macroscópicos

3.3 Protocolo histológico e microscopia de luz

Todas as amostras foram desidratas em solução alcoolíca na concentração de 92% pelo período de 4 horas e, posteriormente infiltradas e incluídas em resina plástica do tipo metacrilato glicol (Historesin® – Leica) de acordo com a metodologia de Ferreira *et al*., (2011). Cortes do tipo semi-seriados na espessura de 3µm com a distância mínima de 30 µm entre cortes foram corados pelos corantes básicos azul de toluidina a 1%, azul de metileno a 5% e pela combinação ácido periódico-Schiff (PAS)+ metanil yellow+ hematoxilina férica de acordo com Quintero-Hunter *et al*., (1991). A coloração básica foi utilizada para ressaltar as estruturas com radicais carregados encontrados em estruturas como o DNA (ácido desoxirribonucleico), proteínas e carboidratos. Devido aos problemas com a coloração de algumas amostras com o azul de toluidina, possivelmente relacionados a forma de fixação, optou-se por utilizar o azul de metileno que permitiu melhor coloração dessas amostras. A cobinação de PAS+Metanil yellow+Hematoxilina férrica foi utilizada especificamente para material fixado em aldeído e incluído em resina plástica. Essa coloração evidencia componentes da membrana basal, fibrina e coloide em magenta, radical amino de proteínas, núcleo em marrom com contra-coloração em amarelo das outras estruturas. Posteriormente, os cortes foram fotografados e medidos nas suas diferentes estruturas, utilizando o software Leica Application Suite versão 3.7 (LAS V3.7).

3.4 Descrição morfológica e aptidão reprodutiva

A espermatogênese foi dividida em quatro fases: Quiescência – Proliferação – Espermiação – Regressão, baseado na classificação proposta por Licht (1967), e Mayhew e Wright (1970). O ducto epididimal foi caracterizado de acordo com o seu aspecto morfológico relacionado as fases da espermatogênese, categorizados em quatro tipos: a) ducto epididimal com epitélio atrofiado e com o lumen vazio; b) ducto epididimal com epitélio hipertrofiado e sem espermatozoide no lumen; c) ducto epididimal com epitélio hipertrofiado, com espermatozoides e secreção no lumen; d) ducto epididimal com epitélio em transição entre hipertrofia e atrofia sem espermatozoide e secreção no lumen. Para a descrição do desenvolvimento folicular e suas células associadas foi utilizado como base os trabalhos de Uribe et al., (1995; 1996). A descrição da morfologia do corpo lúteo e folículos atrésicos foi baseada nos estudos de Guraya (1973, 1989).

A maturidade sexual foi estabelecida através da análise histológica do testículo e do ducto epididimal nos machos, enquanto nas fêmeas foi avaliado através da presença de folículos vitelogênicos ≥ 3 mm e/ou ovos nos ovidutos. O individuo com menor tamanho corporal que apresentou espermatozoides no lumen do túbulo seminífero ou no ducto epididimal foi considerado maduro sexualmente, e na fêmea com o menor tamanho com folículos vitelogênicos e/ou ovos nos ovidutos, conforme Van Sluys *et al.*, (2010). Assim, a aptidão reprodutiva foi estabelecida através de critério qualitativo, sendo considerado apenas os indivíduos que atingiram a maturidade sexual. Nos machos a aptidão foi relacionada com a espermatogênese, considerando aptos os indivíduos que apresentassem espermatozoides no lumen do túbulo seminífero e no ducto epididimal. Nas fêmeas a aptidão foi relacionada ao desenvolvimento folicular, sendo apta a reprodução os indivíduos que apresentassem folículos maduros, corpo lúteo, folículos vitelogênicos ≥ 3 mm e ovos nos ovidutos, conforme Van Sluys *et al.*, (2010).

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5. LISTA DE ARTIGOS

5.1 GERMINATIVE CELLS AND SPERMATOGENESIS OF THE LIZARD TROPIDURUS TORQUATUS (TROPIDURIDAE) FROM A URBAN AREA IN THE CERRADO BIOME OF THE BRAZILIAN MIDWEST

Débora Silva[1]; Mamynne Rodrigues[2]; Mahmoud Mehanna[3]; Adelina Ferreira[4]; Heidi Dolder[5]
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5.2 SPERMATOGENESIS AND REPRODUCTIVE APTITUDE OF FOUR SPECIES OF LIZARD TROPIDURUS FROM TORQUATUS GROUP (TROPIDURIDAE) IN BRAZIL

Débora Silva[1]; Adelina Ferreira[2]; Heidi Dolder[3]; Mahmoud Mehanna[4]
À submeter: Zoologischer Anzeiger

5.3 FOLLICULAR DEVELOPMENT AND REPRODUCTIVE APTITUDE IN FOUR SPECIES OF BRAZILIAN *TROPIDURUS* LIZARDS

Débora Silva[1]; Mônica Cassel[2]; Mahmoud Mehanna[3]; Adelina Ferreira[4]; Heidi Dolder[5]
Submetido: Anatomical Record

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GERMINATIVE CELLS AND SPERMATOGENESIS OF THE LIZARD *Tropidurus torquatus* (Tropiduridae) FROM A URBAN AREA IN THE CERRADO BIOME OF THE BRAZILIAN MIDWEST

CÉLULAS GERMINATIVAS E ESPERMATOGÊNESE DO LAGARTO Tropidurus torquatus *(Tropiduridae) DE UMA ÁREA URBANA NO BIOMA CERRADO DO CENTRO-OESTE BRASILEIRO*

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ABSTRACT: *Tropidurus* comprises a Neotropical genus of lizard that currently has about 30 species widely distributed in the South American. Among these species, *Tropidurus torquatus*, which has the characteristic of great physiological plasticity, occupying a variety of habitats in open areas and urbanized environments. Considering this, the aim of the study was to investigate the germinative cells and spermatogenesis of a population of *T. torquatus* in an urban area under Cerrado Biome influences to understand how to establish the temporal development of germinative cells and spermatogenesis during a period of one year. Individuals were obtained in the Zoological Collection of Vertebrates at the Universidade Federal de Mato Grosso (UFMT), and the germinative cells and full spermatogenesis were described with light microscopy. *Tropidurus torquatus* presented germ cells with similar characteristics already documented for the other species of lizards and reptiles. Spermatogonia type A and B, primary and secondary spermatocytes, and spermatids were present in almost all months evaluated. The gonadosomatic ratio presented its highest value in October, moment in which spermatogenesis presented all the germinative cells and spermatozoa in the lumen, of the seminiferous tubules. In the seasonal climate of the Cerrado Biome, we observe discontinuous spermatogenesis in *T. torquatus* with the production of spermatozoa in almost every month of the year, however with sperm storage in the epididymis during the phase of testicular regression.

KEYWORDS: Reproduction. Male lizard. Savanna. Brazil

INTRODUCTION

The adjustments involved in gametogenesis include, among others, morphological and functional differentiation of the organs related to the production and conducing of the gametes, fertilization and embryo development. In lizards, the temporal development of the germ cells is reflected in the testicular architecture (GRIBBS et al., 2003), allowing the definition of three types of reproduction: continuous reproduction (WILHOFT, 1963; INGER; GREENBERG, 1966), continuous with variation in spermatogenic activity (LICHT; GORMAN, 1970; SEXTON et al., 1971), and discontinuous or seasonal reproduction (LICHT; GORMAN, 1970; MARION; SEXTON, 1971). According to Ferreira; Dolder (2002), reproduction, histologically described, is considered continuous if all germ cell classes are present in the seminiferous tubules at different stages of spermatogenesis during all periods of the year. However, discontinuous reproduction is characterized by its opposite with periods of the year in which only primordial cells of spermatogenesis are observed, while in other periods, mature cells of spermatogenesis fill the entire lumen of the tubule (FERREIRA; DOLDER, 2002).

Tropidurus (WIED-NEUWIED, 1825) is a Neotropical genus of lizard with one of the widest distributions in the South American continent (CARVALHO, 2013). It is a lizard considered an early maturing animal, characterized by a short life expectancy and high fertility (TINKLE et al., 1970). Considering the wide geographic distribution of *T. torquatus* and its versatility to reproduce in different types of habitats, including urban areas.

The objective of this study was to evaluated the germinative cells and spermatogenesis of *T. torquatus* belonging to a population in the Cerrado Biome from urban area of the city Cuiabá, Mato Grosso state, Brazil. Additionally, comparison of spermatogenesis for the same species and its

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congeners from the other localities in South America was established.

MATERIAL AND METHODS

Specimens and Climate Data

For this study, male specimens of *Tropidurus torquatus* deposited in the Zoological Collection of Vertebrates of the Universidade Federal de Mato Grosso were used. According to the records, these specimens were collected monthly in a period from june/2012 to may/2013, on the campus of this university in the urban area of the Cuiabá city, capital of Mato Grosso in the Brazilian Midwest (Fig. 1). This region in the Cerrado Biome is under the influence of tropical climate (Aw) following the KÖPPEN classification (1931), which is characterized by a seasonal precipitation, where rainfall is abundant for a period at the wet season and scarce in the dry season (SOUZA et al. 2013). To collect climate data, we considered the average monthly precipitation values, which were obtained by consulting the database of the Instituto Nacional de Pesquisas Espaciais (INPE) and the Centro de Previsão de Tempo e Estudos Climáticos (CPTEC).

Figure 1. Map of the municipally of Cuiabá in Mato Grosso state, Brazil within the Cerrado Biome.

Measurements and Light Microscopy

For evaluation of the specimens, we recorded the snout-vent length (SVL) with a digital caliper with an accuracy of 0.001 mm. The gonadosomatic ratio (GR) was obtained using the ratio of the weight of the right testicle and the lizard weight for each individual sampled. After this evaluation, samples of the right testis and epididymis were submitted to a histological protocol adapted by FERREIRA et al. (2011), for light microscopy. Subsequently, the samples were embedded in methacrylate plastic resin and sectioned with 3 µm thickness at intervals of 30 µm between sections. The sections were stained with 1% toluidine blue (pH 5 ~ 6), totaling three sections for each sample from each individual. For photo documentation and histological analysis, a capture system coupled to the optical microscope with the software Leica Application Suite (LAS V.3) was used. All histological material was deposited in the Laboratory of Morphology of the Universidade Federal de Mato Grosso.

A qualitative approach was used to determine the occurrence and duration of spermatogenesis through the identification of the all germ cells, their occurrence and duration throughout the months of a year. The observation of only early germ cells and absence of spermatozoa in the seminiferous tubules and epididymis were considered to indicate a period with no reproductive individuals.

RESULTS

Specimens and Germinative Cells

The total of 52 male lizards of *Tropidurus torquatus* were analyzed, having the average SVL of 102.03 mm (± 14.06). The testes were located in the dorsal region of the abdomen, occurring in pairs. The seminiferous tubules presented all types of germline cells (Figure 2A) during period of study. Spermatogonia type A was observed in the periphery of the seminiferous tubules, with an oval shape and poorly staining cytoplasm and the nucleus having an evident nucleolus (Figure 2A). Spermatogonia type A go into mitosis, resulting in spermatogonia type B. Spermatogonia type B were also observed on the basal lamina of the tubule, but with a rounded shape, more strongly stained cytoplasm and the nucleus which may present a conspicuous nucleolus or aggregates of heterochromatin (Figure 2A). Both spermatogonia were present in the seminiferous tubules throughout the study period, but were more evident in the proliferation phase in the months of May and June, decreasing progressively until the phase of regression between february to march (Figure 2B).

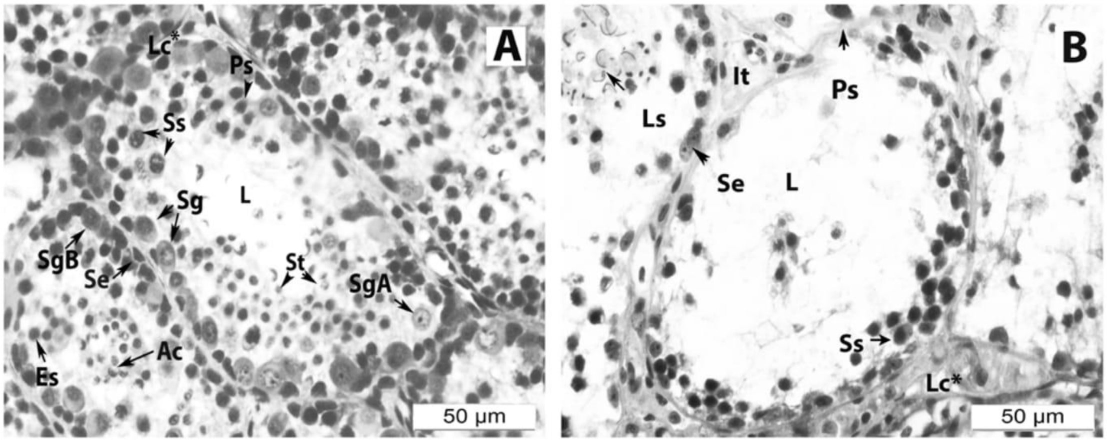

Figure 2. Light microscopy of the seminiferous tubules of the lizard *Tropidurus torquatus* during the study period in a locality of the urban area of the Cerrado Biome in the city of Cuiabá, Mato Grosso, Brazil. A – Seminiferous tubule with germinative cells and interstitial tissue during the testicular spermiation phase in July and B – during regression in march. Abbreviations: Sg = Spermatogonia; SgA = Spermatogonia A; SgB = Spermatogonia B; Ps = Primary spermatocytes; Ss = Secondary spermatocytes; St = Spermatids; Es = Early spermatids; Ls = Late spermatids; L = Lumen; Se = Sertoli cell; Lc* = Leydig cell, It = Interstitial tissue and Ac=Acrosome vesicle.

The primary spermatocytes correspond to the cells that go into meiosis, originating from Spermatogonia type B. In morphology, these cells showed different characteristics of the nucleus along meiosis I (Figure 3A – C). The spermatocyte during the leptotene phase presented a more strongly stained nucleus with filamentous chromatin. During the zygotene phase the nucleus showed dense chromatin with the formation of small lumps, occupying the entire nuclear area. In pachytene, cells were larger with heavily stained nuclei, with little gaps being observed in the nucleoplasm (Figure 3A). In metaphase of meiosis I the alignment of homologous chromosomes was observed in the central region of the cell. Primary spermatocytes were observed during all months of the study, especially in the phases of proliferation and spermiation of *T. torquatus* (Figure 3A – C). Secondary spermatocytes were a product of meiosis I, but they are difficult to visualize and differentiate from early spermatids. Secondary spermatocytes present the nucleus with more compacted chromatin, evident nucleolus and reduced cytoplasm in relation to primary spermatocytes (F Figure 3A, C). These spermatocytes were observed near the periphery of the seminiferous tubule when spermiation was reduced and close to the lumen when spermiation was more intense with release of mature spermatozoa (Figure 3B).

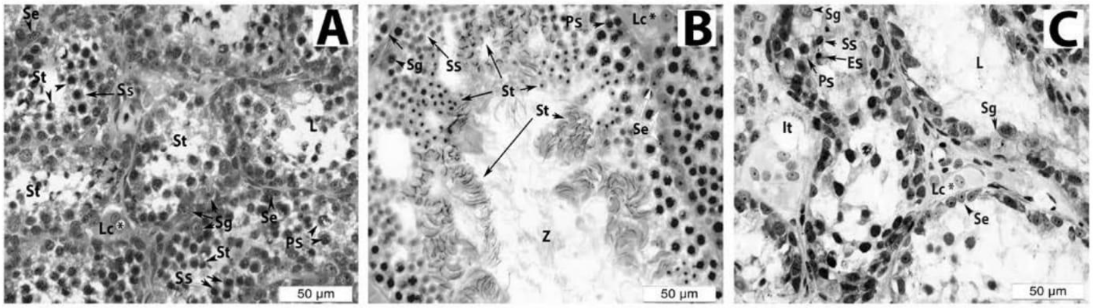

Figure 3. Light microscopy of the seminiferous tubules of the lizard *Tropidurus torquatus* during the study period in a locality of the urban area of the Cerrado Biome in the city of Cuiabá, Mato Grosso, Brazil. A – Seminiferous tubules in the spermiation phase during the spermatogenesis in June. B – Seminiferous tubules during the peak of the spermiation in October. C – Seminiferous tubules during testicular regression with no spermatozoa in the tubule lumen in May. Abbreviations: Sg = Spermatogonia; Ps = Primary spermatocytes; Ss = Secondary spermatocytes; St = Spermatids; Es = Early spermatids; L = Lumen; Se = Sertoli cell; Lc* = Leydig cell; It = Interstitial tissue and Z = Spermatozoa.

Spermatids were present during the stages of spermiation and regression of spermatogenesis (Figure 3A – C) and have different structural characteristics during their maturation. We observed the initial spermatids or round spermatids with a strongly stained nucleus and strongly condensed chromatin (Figure 2A, 3B). The acrosome was observed as the formation of a blank space over the nucleus of spermatids (Figure 2A). Later, we observed the elongated spermatids with a thick dense filamentous shape surrounded by a less strongly stained cytoplasm (Figure 2A). Spermiation occurred in almost every month of the study period, decreasing in intensity during the regression phase, and ceasing in the months of February to April (Figure 3A – C).

Spermatogenesis

We observed that spermatogenesis was divided into four phases: proliferation, spermiation, regression, and quiescence. The proliferation phase starts in May, where the seminiferous tubules had spermatogonia and primary spermatocytes, which subsequently produced the first spermatids (Figure 3A). During June to February the spermiation phase was characterized by a high germinal epithelium with many layers of cells, and in most of them secondary spermatocytes and spermatids can be found (Figure 3B). With increasing intensity of spermatogenesis, many spermatids changed their structure, completing maturation with the progressive release of spermatozoa in the lumen of the tubules (Figure 3B). The regression phase was observed during February to April, and is characterized by diminishing layers of germ cells, detachment of part of the germinal epithelium and emptying lumens (Figure 2B, 3C). In this phase, spermatozoa were rarely visualized in the lumen of the seminiferous tubules, being stored in the epididymis. The quiescent phase was brief, occurring in April and a few individuals possessed tubules with only two cell types, spermatogonia and Sertoli cells, that reorganized the testicular architecture for next proliferation phase.

The epididymis showed structural changes in the epithelium and in its production of secretion during spermatogenesis of *Tropidurus torquatus*. During spermiation phase, the epithelium showed hypertrophy with tall cells and many secretory vesicles observed in the cytoplasm during the months of June to January (Figure 4 A). Spermatozoa were observed in the epididymis lumen in the same period (Figure 4 A). In the months of February to April, during the phase of testicular regression, the epithelium of the epididymis decreased in height, with few vesicles observed in the cytoplasm of epithelial cells (Figure 4 B). The complete absence of spermatozoa in the lumen of the epididymis was observed only in April.

The gonadosomatic ratio showed variations over the months of study, reaching the highest value in October when spermatogenesis presented all the germinative cells, mostly spermatids and spermatozoa in the lumen (Figure 5). However, after December the gonadosomatic ratio decreased progressively, following the changes in the arrangements of germinative cells that decrease in quantity, leading to the regression phase of the spermatogenesis (F Figure 5). The lowest value of the index occurred in May, where the tubule remains empty without spermatozoa in the lumen (Figure 5).

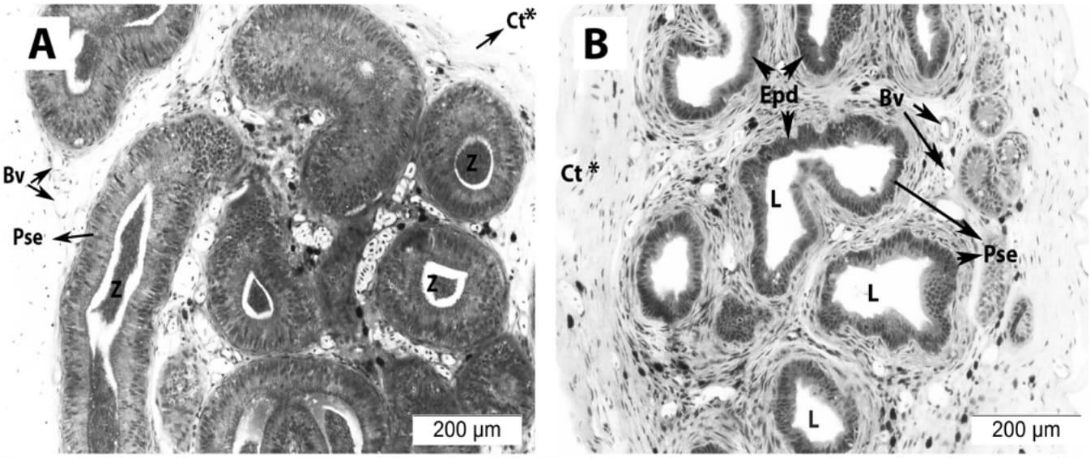

Figure 4. Light microscopy of the epididymis of the lizard *Tropidurus torquatus* from the urban area of the Cerrado Biome in the city of Cuiabá, Mato Grosso, Brazil. A – Period with reproduction demonstrated by the presence of free spermatozoa in the lumen of the epididymis from July to January. B – Period without reproduction shown by the absence of spermatozoa in the lumen of the epididymis in April. Abbreviations: Ct* = Conjunctive tissue; Epd = Epididymis; Pse = Pseudostratified columnar epithelium; Z = Spermatozoa; Bv = Blood vessels and L = Lumen.

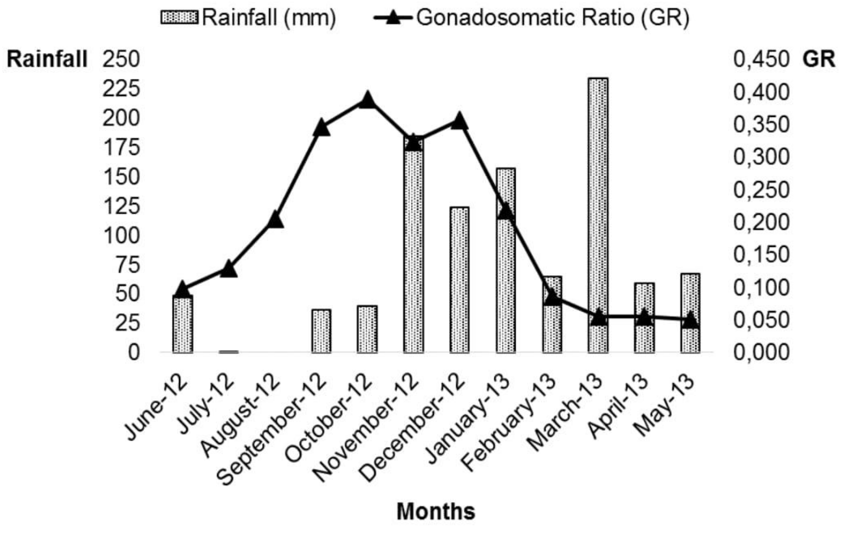

Figure 5. Distribution of values of the gonadosomatic ratio (GR) for the lizard *Tropidurus torquatus* from the Cerrado Biome in the urban area in the city of Cuiabá, Mato Grosso, Brazil. The values correspond the period of June/2012 to May/2013, which was calculated for all individuals and the average values distributed for each month.

DISCUSSION

We evaluated the development of germinative cells in the lizard *Tropidurus torquatus* during the period of one year. Significant morphological differences and changes in the testicular architecture were identified during spermatogenesis the spermatogonia observed were identified as type A when had an oval shape and evident nucleolus, while the type B were lower with rounded shape and had heterochromatin aggregates in the nucleus. The same types of spermatogonia were identified in the lizard *Podarcis muralis* (GRIBBINS; GIST, 2003), as the permanent cells of tubules during all spermatogenic phases. In the lizard *Mabuya brachypoda* the spermatogonia type A had the same oval shape, but with one or two prominent nucleoli (HERNÁNDEZ-FRANYUTTI; URIBE, 2012), while in *T. torquatus* we observed only one nucleolus. In addition, in *Sceloporus bicanthalis*, spermatogonia type A with a clear nucleolus or sometimes the absence of a nucleolus were found (GRIBBINS, 2011). Despite the diversity in the presence and quantity of nucleoli of spermatogonia, the other germinative cells observed in *T. torquatu*, such as primary spermatocytes, secondary spermatocytes and spermatids were similar to the characteristics described in other cell types of different species of lizards (GRIBBINS; GIST, 2003; GRIBBINS, 2011; HERNÁNDEZ-FRANYUTTI; URIBE, 2012).

The analysis of germinative cells showed that spermatogenesis in *Tropidurus torquatus* had a discontinuous pattern, however with presence of spermatozoa in the seminiferous tubules and epididymis occurred almost every month of the year except in April. Previous studies with males of the *T. torquatus* species showed continued spermatogenesis in different Biomes and phytophysiognomies like as Caatinga (VITT; GOLDBERG, 1983), Cerrado (WIEDERHECKER et al., 2002), Pampa (ARRUDA, 2009), Restinga (VAN SLUYS et al., 2010), Atlantic Forest (GOMIDES et al., 2013), and Chaco (ORTIZ et al., 2014). These studies showed continuous production of spermatozoa for *T. torquatus*, but clearly decreased reproductive activity in the months of January to April, period in which we observed testicular regression in our study. The only exception was observed in the population of the Atlantic forest, which maintained the reproductive activity without variations between seasons. This demonstrates that spermatogenesis in *T. torquatus* can occur regardless of seasonal variations, the rainy and dry season being the extremes of these variations in Brazil. According to WIEDERHECKER et al. (2002), the continuous production of spermatozoa does not require high energy expenditure, and may be a consequence of the high hormone levels needed for territorialism activities. All the locations cited above, are near urban areas or areas with some kind of anthropic activity, such as pastures, fishing or housing. Thus, the comparison of our results with those of other studies mentioned above occurs under similar conditions of human influence.

We compared variation in spermatogenesis for the congeners of *Tropidurus torquatus*. For example, *T. hispidus* was presented as having continuous spermiation in the Caatinga Biome (RIBEIRO et al., 2012), but the spermiation was restricted from March to August in the Amazon Forest (PRIETO et al., 1976). The species *T. itambere* was considered reproductive throughout the year in the Atlantic Forest (VAN SLUYS, 1993). However, the relationship between the types of germinative cells and the size of seminiferous tubules showed differences of spermatogenic activity, with all types of germinative cells present, but with regression during February to April (FERREIRA et al., 2009). For *T. oreadicus* from the Cerrado Biome, spermatogenesis was more intense during the months of June to September, during the dry season (MEIRA et al., 2007; FERREIRA et al., 2011). Another species of the Cerrado, *T. montanus*, presented reproductive males throughout the year, but in greater proportion from September to January (VAN SLUYS et al., 2002). Lizards of the genus *Tropidurus* have spermatogenesis that can be characterized as continuous, seasonal or discontinuous, depending on the aspect considered. Some species have continuous production of spermatozoa, despite the decrease in testicular parameters (e.g. *T. torquatus*, WIEDERHECKER et al., 2002; ORTIZ et al., 2014). Others have a seasonal reproduction, concentrating spermatogenesis in a specific period, such as *T. itambere* and *T. hispidus* (VAN SLUYS et al., 1993; RIBEIRO et al., 2012). Alternatively, they can be seasonal with interruption of spermatogenesis as observed in our study and in *T. torquatus* in the Restinga (VAN SLUYS et al., 2010).

Thus reproductive studies also should consider the development of germinative cells, which is the most appropriate way to explain during which period the species is able to reproduce and how it can change over time. The presence of spermatozoa in the lumen of the tubules and epididymis is a strong indicator of reproductive fitness, and the analysis of all types of germinative cells is also important to detect a regression stage, which can interrupt, or not, the progression of spermatogenesis.

CONCLUSIONS

This population of male lizards of *Tropidurus torquatus*, inhabiting an urban area of the city of Cuiabá under influences of the Cerrado Biome, presented all the types of germinative cells with morphologic and structural similarities to other species of lizards and other reptiles.

Spermatogenesis occurred in the almost all months in the year, but spermiation was discontinuous with a brief period for testicular regression.

The epididymis presented structural variation of the epithelium during spermiation and testicular regression, the storage of spermatozoa being observed even when the tubules had empty lumen. These results are, in part, similar to another population of *T. torquatus* from the Cerrado Biome. However, there is a limited number of descriptive studies of germinative cells and spermatogenesis for the other populations of this species in Brazil.

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RESUMO: O gênero de lagartos neotropicais *Tropidurus* tem cerca de 30 espécies amplamente distribuídas no continente sul-americano. Entre essas espécies, *Tropidurus torquatus* é a que apresenta grande plasticidade fisiológica, ocupando uma variedade de hábitats em áreas abertas e ambientes urbanizados. Considerando isso, o objetivo do estudo foi investigar as células germinativas e a espermatogênese de uma população de *T. torquatus* em uma área urbana sob a influência do Bioma Cerrado para entender como é estabelecido o desenvolvimento temporal das células germinativas e a espermatogênese durante o período de um ano. Indivíduos foram obtidos na Coleção Zoológica de Vertebrados da Universidade Federal de Mato Grosso (UFMT). A caracterização das células germinativas e da espermatogênese foi realizada através de microscopia de luz. *Tropidurus torquatus* apresentou células germinativas com características similares às já documentadas para outras espécies de lagartos e répteis. Espermatogônias do tipo A e B, espermatócitos primários e secundários e espermátides estiveram presentes praticamente em todos os meses. A razão gonadossomática apresentou seu valor máximo em outubro, momento em que a espermatogênese apresentou todas as células germinativas e espermatozoides no lúmen do túbulo seminífero. No clima sazonal do Bioma Cerrado observamos espermatogênese descontínua em *T. torquatus* com a produção de espermatozoides em quase todos os meses do ano, porém com estocagem nos epidídimos durante a fase de regressão testicular.

PALAVRAS-CHAVE: Reprodução. Lagarto macho. Savana. Brasil.

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5.2 SPERMATOGENESIS AND REPRODUCTIVE APTITUDE OF FOUR MALES SPECIES OF LIZARD TROPIDURUS FROM TORQUATUS GROUP (TROPIDURIDAE) IN BRAZIL

ABSTRACT

Reptiles represent an interesting experimental model for reproductive studies and, among them, lizards stand out because they present variations of reproductive cycles, which can be categorized as continuous, discontinuous or seasonal. The genus *Tropidurus* includes species inhabiting the cisandean South America. In Brazil, *Tropidurus* occurs in open areas habitats such as savanas, rock outcrops and sandy soils. Considering the reproductive variations already documented for the genus, our objective was to relate the spermatogenesis and reproductive aptitude of four *Tropidurus* species of the *torquatus* group (*Tropidurus hispidus*, *T. torquatus*, *T. itambere* and *T. oreadicus*) in Brazil. The study evaluated 276 specimens from six zoological collections of Brazil. Testicular and epididymal duct samples were removed, dehydrated and included in plastic resin (Historesin-Leica) for analysis with light microscopy. Spermatogenesis was divided into four phases (Quiescence – Proliferation – Spermiation – Regression) and the germ cells were structurally similar among the species studied. Spermatogonia A and B types were found mainly during the proliferative phase while spermatocytes and spermatids were more abundant in the spermiation phase. The epididymal duct presented different morphological aspects such as hypertrophy and secretory activity associated with spermiation phase and spermatozoa retention during testicular regression. Reproductive aptitude was extended in *T. torquatus* and *T. hispidus*, and seasonal for *T. oreadicus* and *T. itambere*. The four species presented spermatozoa in the epididymal duct with storage time related to the testicular regression of each one. The pattern of spermiation and spermatozoa retention by the epididymal duct was an important reproductive strategy for this group of lizard species in Brazil.

Keywords: Tropidurus lizards, Aptitude, Germinative cells, Epididymal morphology, Reproductive strategy.

INTRODUCTION

Reptiles, among the amniotes, are of particular interest in reproductive studies, since they have the seminiferous epithelium with the arrangement and spatial relationship between germ cells very similar to those of birds and mammals (Gribbins, 2011). Generally, studies of spermatogenesis use multiple approaches, and for most species the most used of them is the one that identifies the association between germ cells and the stages of spermatogenesis (Hess and França, 2008). In tropical lizard species, characteristics of spermatogenesis have been evaluated suggesting at least three reproductive cycles: 1) continuous whithout variation in reprodductive activity; 2) contonuous with a seasonal variation and 3) non-continuous with periods when all individuals are reproductively inactive (Sherbrook, 1975). This variety of reproductive cycles may be related to mechanisms that stimulate gonadal development and behavior, which would explain the reproduction of some lizard species independently of environmental factors (Clerke and Alford, 1993). The reproductive aptitude representes a new study approach with the proposal to evaluate the reproductive status of one or more species collected at different periods and localities, occasionally. The main advantage of this method is the absence of new collections, using only specimens of the zoological collections.

Lizards of the genus *Tropidurus* are widely distributed in cis-Andean South America (Rodrigues, 1987). In Brazil, these species occurs in open formations of Caatinga, Cerrado, Amazon and Atlantic Forest and Pampa biomes (Rodrigues, 1987, Carvalho et al., 2013). The reproduction of some *Tropidurus* species was evaluated and presented a continuous pattern of reproduction in certain populations (e.g. Vitt and Goldberg, 1983, Gomides et al. 2013, Ortiz et al. 2014) and a markedly seasonal reproduction (Meira et al. 2007, Ribeiro et al. 2012). According to Gribbins (2011), in seasonally reproducing reptilian species, spermiation was observed to occur in the summer or late fall, with a brief period of quiescence, in which spermatogenesis may be slower or cease altogether. However, in species of reptiles that reproduce continuously, new generations of germ cells enter spermiogenesis with increase or reduction of seminiferous epithelium occurring repeatedly (Gribbins, 2011).

Considering the variations in the spermatogenetic pattern reported for *Tropidurus* species, the aim was to establish the spermatogenetic pattern and to evaluate the reproductive aptitude of four species belonging to *torquatus* group in Brazil, namely *T. hipsidus*, *T. torquatus*, *T. itambere* and *T. oreadicus*. For this approach, we identified the presence and types of germ cells, the length and duration of spermatogenetic phases, the presence of spermatozoa in the seminiferous tubule, the morphology and spermatozoa storage in the epididymal duct.

MATERIAL AND METHODS

Specimens and Populations studied

A total of 276 male individuals belonging to *T. hispidus* (10 populations), *T. torquatus* (18 populations), *T. itambere* (10 populations) and *T. oreadicus* (27 populations) were acquired from six zoological collections from different regions of Brazil: Acronyms: **CHUNB** (Coleção Herpetológica da Universidade de Brasília), **INPA-HERPETO** (Coleção de Anfíbios e Répteis do Instituto Nacional de Pesquisas da Amazônia), **MPEG** (Museu Paraense Emílio Goeldi), **UFMT-R** Coleção Zoológica da Universidade Federal de Mato Grosso Setor Reptilia), **ZUEC-REP** (Museu de Zoologia da Universidade Estadual de Campinas “Adão José Cardoso”), **ZUFG** (Coleção Zoológica da Universidade Federal de Goiás), all summarized in Table 1 (Supplementary Data). The Arcgis software version 10.2 was utilized for mapping the localities these populations in Brazil (Fig. 1).

Histological Protocol

The samples of the right testis and epididymal duct were removed and submitted to the routine histological protocol according to the study of Ferreira et al. (2011) and later infiltrated and included in plastic resin (Historesin®-Leica). Semi-serial sections at a thickness of 3 µm with a minimum distance of 30 µm between cuts were stained with 1% toluidine blue at pH ≅ 7, or with 5% methylene blue at pH ≅ 7, and by the combination of periodic acid Schiff (PAS) + metanil yellow + ferric hematoxylin according to the protocol of Quintero-Hunter et al. (1991). The photodocumentation of the slides was performed under an optical microscope with the Leica Application Suite software (LAS V3.7).

Spermatogenesis and Morphology of Epididymal Duct

The stages of spermatogenesis were established in four distinct phases (Quiescence – Proliferation – Spermiation – Regression), based on the classification proposed by Licht (1967), Mayhew and Wright (1970). The epididymal duct was also characterized according to its morphological aspects during spermatogenesis. Thus, four different aspects were observed according to the morphology: a) Epididymal duct with atrophied epithelium and without spermatozoa or with an empty lumen; b) Epididymal duct with hypertrophied epithelium, but without spermatozoa in the lumen; c) Epididymal duct with hypertrophied epithelium having spermatozoa and secretion in the lumen and d) Epididymal duct in the transition from hypertrophy to atrophy without secretory activity in the lumen.

Sexual Maturity and Reproductive Aptitude

All individuals were measured with a digital calipter with precision of (0,001 mm) to obtain their snout-vent length (SVL). The sexual maturity was determined for each species based on the smallest SVL of na individual with free spermatozoa in the lumen of the seminiferous tubule or stored in the epididymal duct, according to the methodology used by Van Sluys et al. (2010). For the evaluation of reproductive aptitude, only the individuals who presented the sexual maturity were considered. The following qualitative criterion were utilized: the type of germinative cell present, presence or absence of lumen in the seminiferous tubules, presence or absence of free spermatozoa in the lumen, hypertrophy or atrophy of the epididymal duct epithelium, and the presence or absence of secretion and spermatozoa stored in the epididymal duct.

RESULTS

Spermatogenesis

The seminiferous tubules of the four *Tropidurus* species lizards presented the germinative epithelium with cells at different stages of spermatogenesis, in which four phases (quiescence, proliferation, spermiation and regression) could be recognized throughout the study period (Fig. 2A – 2I). The quiescence, or phase of inactivity, showed reduced size of seminiferous tubules, which were occluded (Fig. 2A), containing type A spermatogonia and Sertoli cells (Fig. 2B). Type A spermatogonia are large and oval, lightly stained cytoplasm, with a nucleus having loose chromatin and a very evident nucleolus (Fig. 2B). Subsequently, in the proliferation phase, the mitotic activity was intensified giving rise to the type B spermatogonia (Fig. 2B). Type B spermatogonia was also located in the tubular periphery with a rounded shape and more strongly stained cytoplasm, but the nucleus presented more compact chromatin (Fig. 2B). During the proliferation phase, the first spermatocytes appeared causing an increase in the seminiferous tubule

(Fig. 2C). The primary spermatocytes were the most abundant cells in the tubule, being very similar to the type B spermatogonia, but larger and generally not located at seminiferous tubule perifery (Fig. 2D). The secondary spermatocytes, which were smaller than the primary spermatocytes, and the nucleus reached a highest level of chromatin compaction (Fig. 2D, 2E). These cells became abundant before the spermiation phase began. The spermiation phase is characterized by the presence of large numbers of spermatids, which assumed different aspects being sequentially observed as undifferentiated round spermatids, then round with an acrosomal vesicle and finally elongated spermatids (Fig. 2F – 2H). Still during this phase the lumen of the seminiferous tubules becomes evident and gradually filled with spermatozoa released by spermiation (Fig. 2F). We observed that during spermiation there was a great change in the seminiferous tubule structure, which formed groups of germinative cells at various stages of maturation associated with Sertoli cells in a chalice structure (Fig. 2G, 2H). This germinative cells chalice indicates the most advanced stage of spermatogenesis, where small gaps were formed, isolating populations of germinative cells leading into a differentiation process from the periphery into the lumen of the seminiferous tubule (Fig. 2G, 2H).

As spermatogenesis progressed, there was a decrease in the number of germinative cells layers, which was a significant feature for the beginning of the testicular regression (Fig. 2I). The regression phase, subsequent to the reproductive maximum, was characterized by a low germinative epithelium, usually with two or three cell layers mostly composed of Sertoli cells at tubule periphery and few spermatocytes (Fig. 2I). At the end of the regression phase the lumen appeared wide and empty, with Sertoli cells and some spermatids (Fig. 2I). Sertoli cells presented pyramidal shape with weakly stained cytoplasm and a very evident nucleolus (Fig. 2G), and have a fundamental role in the reorganization of the seminiferous tubule at the end of the regression.

Morphology of the Epididymal Duct

Four different morphological aspects of the epididymal duct were identified, based on the hypertrophy and secretory activity of epithelial cells, besides the presence or absence of spermatozoa in the lumen (Fig. 3A – 3I). The epididymal duct was composed of basal stem cells and a cylindrical pseudostratified epithelium with stereocilia at the apical portion (Fig. 3A, 3B). During the proliferation phase in the seminiferous tubules the epididymis duct showed hypertrophy of the epitelial cells with the onset of numerous vesicles, which were located near the apical portion of the cells, indicating an increase in secretory activity (Fig. 3C, 3D). Later, during the maximum spermiation activity in the semminiferous tubules, the epididymal duct showed hypertrophied cells and the lumen filled with spermatozoa and secretion, which was released by vesicles located in the apical portion of the epithelial cells (Fig. 3D). When seminiferous tubules entered testicular regression, with an empty lumen or with few spermatozoa, the epididymal duct still remained hypertrophied in appearance with many spermatozoa in the lumen (Fig. 2I, 3E). Thus, the epithelium remained hypertrophied and with secretory activity, maintaining spermatozoa for some time during testicular regression (Fig. 2I, 3D – 3F). Subsequently, the epididymal duct decreased its secretory activity and the lumen becomes empty due to elimination of the spermatozoa (Fig. 3G, 3H). The last modification observed in the epididymal duct was the decrease in the epithelium height, which becames atrophied with no evidence of secretory activity in its cells (Fig. 3I). Thus, the lumen remained empty with the atrophied epithelium, only lining the epididymal duct (Fig. 3I).

Sexual Maturity and Reproductive Aptitude

Sexual maturity varied among *Tropidurus* species (Table 2 Supplementary Data). For *Tropidurus torquatus* of Cerrado biome the minimum size at sexual maturity was 51 mm in

Brasilia-DF (Fig. 4A). At the Amazon, a population registered in Manaus-AM with maturity 87,78 mm, while in Atlantic Forest the sexual maturity was 62 mm (Fig. 4A). For *T. hispidus* sexual maturity was 57 mm in Marabá-PA (Amazon biome) and in the Cerrado and Caatinga SVL was 81 mm and 90mm, respectively for Estreito-MA and Petrolina-PE (Fig. 4B). *Tropidurus itambere* reached sexual maturity with 47 mm for population in Pirenópolis-GO (Cerrado) and 64,72 mm for Poços de Caldas-MG in the Atlantic Forest (Fig. 4C). The smaller individual at reproductive maturity of *T. oreadicus* was 51 mm in Cocalzinho de Goiás-GO (Cerrado) and 56 mm in Marabá-PA (Amazon) (Fig. 4D).

Reproductive aptitude was established as the period in wich reproduction was viable due to the presence of spermatozoa in the lumen of the seminiferous tubules or in the epididymal duct. All the *Tropidurus* species of the present study had spermatozoa during most of the year in the seminiferous tubules (Fig. 2E, 2F e Tabela 2), or in the epididymal duct during a short period of seminiferous tubule regression (Fig. 3C – 3F). For the *T. torquatus* the reproductive aptitude was recorded in most months sampled, with Cerrado population apt to reproduce between the dry and wet season, with reproductive inaptitude in April (Fig. 2I and Tabela 2). Population from Amazon biome show reproductive aptitude in July whereas in the Atlantic forest the aptitude occurred in March, July, September and December (Table 2). For the species *T. hispidus* the reproductive aptitude occurred in the month of February to November in the Amazon biome, and in August in both Cerrado and Caatinga (Table 2). For the congener *T. itambere* the reproductive aptitude was strongly seasonal in both biomes Cerrado and Atlantic Forest, occurring from July to November (Table 2). For *T. oreadicus* the reproductive aptitude occurred from May to November in the Cerrado biome and in March to November for the Amazon biome (Table 2).

DISCUSSION

We described the germinative cells, such as types A and B spermatogonia in the periphery of the seminiferous tubules contacting the basal lamina for the *Tropidurus* species of the present study. Morphologically, these cells presented similar characteristics among the four species evaluated and typical characteristics described for other lizard species such as shape, chromatin condensation and nucleolus (e.g. Gribbins and Gist, 2003, Rheubert et al. 2009, Silva et al. 2016). Spermatocytes were the most numerous cells of the seminiferous tubule, especially in the proliferative phase of spermatogenesis. According to Gribbins (2011) this is the spermatogenesis phase which include the formation of primary and secondary spermatocytes, and is the shortest period of sperm development. For all species of this study spermiation was marked by the different types of spermatids presente in the seminiferous tubules epithelium, sequentially identified as undifferentiated round spermatids, round spermatids with acrossomoal vesicles and elongated spermatids. Similarly, other lizards have different morphological aspects of the spermatids, in relation to acrosome formation, nuclear elongation and flagellum formation (e.g. Ferreira and Dolder, 2003, Rheubert et al. 2009).

In previous studies, important aspects of spermatogenesis of the genus *Tropidurus* were registered, mainly related to the duration and differentiation of the germinative cells along the months (e.g. Wiederhecker et al. 2002, Van Sluys, 1993, Ferreira et al. 2009). In general, it is possible to identify two spermiation patterns for the genus: the first is related to the species that continuously produces spermatozoa (e.g. Vitt and Goldberg, 1983, Wiederhecker et al. 2002, Silva et al. 2016), and the second refers to those species that have spermiation occurring in periods of specific periods of the year (e.g. Van Sluys, 1993, Meira et al. 2007, Ferreira et al. 2009). At present study, spermatogenesis in *Tropidurus torquatus* and *T. hispidus* presented a prolonged spermiation pattern, occurring in all the months analised. These results corroborate previous studies focus in *T. torquatus*, which demonstrated a continuous spermiation (e.g. Vitt and Goldber, 1983, Wiederhecker et al. 2002, Arruda, 2009, Van Sluys et al. 2010, Gomides et al. 2013, Ortiz et al, 2014, Silva et al. 2016). According to Wiederhecker et al. (2002), the continuous production of spermatozoa does not require a high energy level, and can be influenced by the hormonal maintenance related to the territory defense for feeding and reproduction. For *T. hispidus*, spermiation was observed continuously (Ribeiro et al. 2012) as at this study, but also restricted to a few months of the year (Prieto et al. 1976). At the other hand, for *T. itambere* and *T. oreadicus* we recorded a seasonally markedly spermiation. *Tropidurus itambere* was reproductive from July to november in Cerrado biome, and the regression was observed from March to June, when it was not reproductive. Differently, in another population of *T. itambere* reproductive aptitude was resgitered throughout the year (Vans Sluys, 1993). However, the evaluation of germinative cells and morphometry of the seminiferous tubules showed diferences in the intensity of spermatogenic activity in the same species during the year (Ferreira et al. 2009). For *T. oreadicus*, reproductive aptitude occurred during eigteen months of the year (May to January) in Cerrado biome, with the period of inaptitude and testicular regression in May. Germinative cells proliferation occurred in various months of the year, indicating that spermiation is seasonal, occurring in sucessive waves beginning in May. The same was observed by Ferreira et al. (2011) with different populations of *T. oreadicus* which were reproductively apt between September and November. However, for another population, the reproductive aptitude occurred from April to June (Meira et al. 2007). Thus, the reproductive capacity of *T. oreadicus* had a seasonal characteristic with a variation of up to eight months and a short period of reproductive inaptitude of three months.

The epididymal duct for amniote species, among then lizards, exert multiple functions related to transport, maturation and storage of spermatozoa (Jones, 2002). Considering its importance for spermatozoa viability and fertilization, modifications of its morphology are necessary and conditioned to the different stages of spermatogenesis in lizards. According to Ferreira et al. (2009), major changes found in the epididymal duct of the lizard *T. itambere* were related to the secretory activity and height of their epithelial cells. Additionally, it was described for species *T. torquatus* that the type of secretion produced by cells of the renal sexual segment, in contact with the epididymal duct, was distinct between sexually mature and immature individuals (Mendes et al. 2009). Similarly, we identified different aspects in morphology of the epididymal duct of the four *Tropidurus* species studied, being related to hypertrophy of the epithelium and the presence of secretory vesicles. The cylindrical pseudostratified epithelium presented changes in its height, such as hypertrophy or atrophy of its cell and in the secretory activity, being related to different phases of spermatogenesis. The same variations were observed for *T. torquatus* (e.g. Wiederhecker et al. 2002) and for *T. itambere* (Ferreira et al. 2009). However, none of these studies documented the retention of spermatozoa in the epididymal duct, especially during testicular regression. In our study, all species presented spermatozoa in the epididymal duct during part of the regression phase in the semminiferous tubules. Many factors may explain this spermatozoa retention extending the period of reproductive aptitude. We can cite the timing of reproductive activity between males and females, considering humidity as an important regulatory factor for reproduction and embryo development in female of *T. torquatus* (Wiederhecker et al. 2002). Another important fator to consider is the spermatozoa maturation by retention in the seminal receptacle of the female lizards. In the study by Ferreira and Dolder (2007) for *T. itambere* spermatozoa maturation was found to be related to changes in carbohydrate composition in the seminal receptacle of females, making up an essential part of its maturation. In spite of increasing the viability of spermatozoa by storing them in the epididymal duct, we found that retention occurred for a quick period during the regression phase in the seminiferous tubules

CONCLUSION

The *Tropidurus* species from *torquatus* group (*Tropidurus toquatus*, *T. hispidus*, *T. itambere* and *T. oreadicus*) presented morphological similarities in germinative cells during spermatogenesis, but different patterns of spermiation between species. The reproductive aptitude was related to spermatozoa retetion in epididymal duct and to the regression phase in the seminiferous tubules. The extended spermiation occurred in *T. torquatus* and *T. hispidus*, and a seasonal pattern of spermiation in *T. oreadicus* and *T. itambere* species. The aspects of the spermatogenesis of these *Tropidurus* species demonstrated the pattern of spermiation and retention of spermatozoa in the epididymal duct as an important reproductive strategy of this group of lizards in Brazil.

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FIGURE LEGENDS

Figure 1: Distribution of four *Tropidurus torquatus* group in different localities of Brazil. *Tropidurus torquatus* (triangle), *Tropidurus hispidus* (star), *Tropidurus itambere* (hexagon) and *Tropidurus oreadicus* (cross).

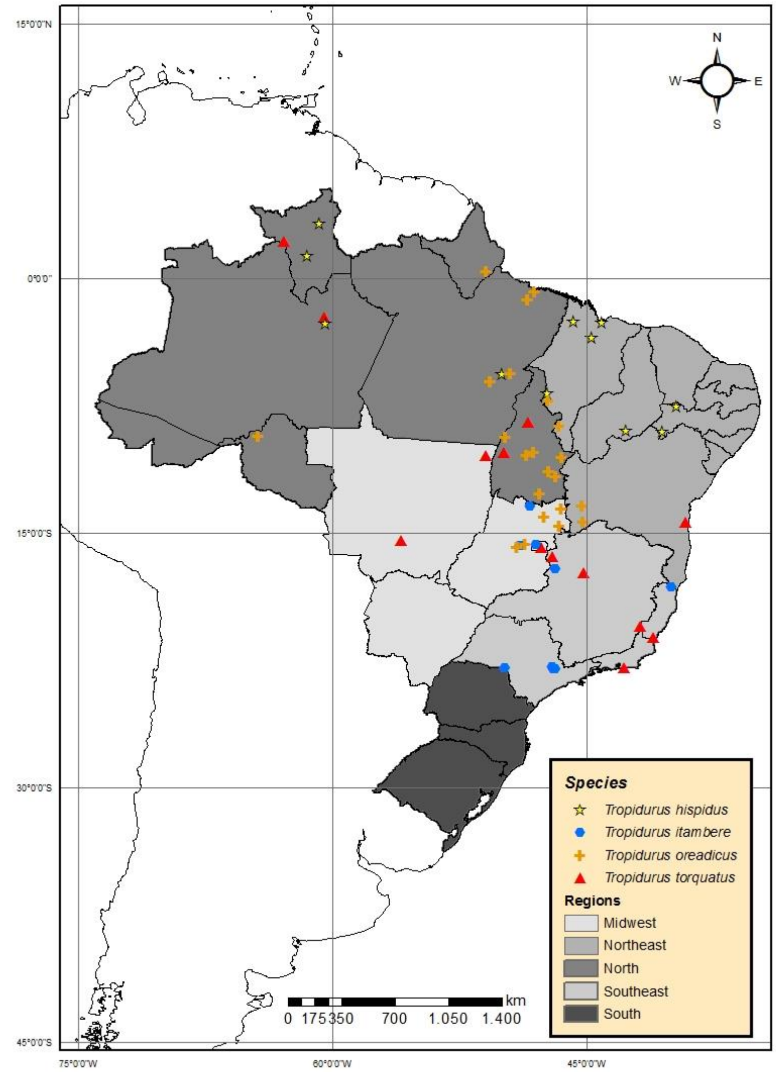
Figure 2 A – I: Light microscopy of the seminiferous tubules of *Tropidurus* species (*torquatus* group). The species *T. oreadicus* (CHUNB 24302) during quiescence phase **(A, B)**, *T. oreadicus* (INPA-H 33016) and *T. torquatus* (UFMT-R 11641) during the proliferation phase of the germiantive cells, generating many spermatocytes **(C, D).** *Tropidurus oreadicus* (CHUNB 43774, CHUNB 45197), *T. hispidus* (ZUEC-REP 3231), *T. oreadicus* (INPA-H 33016) during the spermiation phase, with many spermatids in various stages of differentiation and spermatozoa in the lumen of the seminiferous tubules **(E, F, G, H)**. Regression phase of the spermatogenesis presenting germinative epithelium with few layers of cells in the seminiferous tubules of *T. torquatus* (UFMT-R 11636) **(I)**. Legend: **Ac** = Acrosome, **Bv** = Blood vessel, **Gc** = Germinative chalice, **It** = Interstitial tissue, **L** = Lumen, **Lc** = Leydig cells, **Sc** = Primary spermatocyte, **Se** = Sertoli cells, **SgA** = Spermatogonia A, **SgB** = Spermatogonia B, **St** = Seminiferous tubule, **Std*** = Spermatid, Elongated spermatids (**Arrowheads**), **Ss** = Secondary spermatocyte, **Z** = Spermatozoa, **(+)** = Basal lamina. Coloration: Toluidine blue at 1% (pH ≅ 7), Methylene blue at 5% (pH ≅ 7), Periodic Acid Schiff (PAS) + Metanil Yellow + Ferric Hematoxylin.

Figure 3 A – I: Light microscopy of the epididymal duct of *Tropidurus* species (*torquatus* group) during the spermatogenesis. Epididymal duct with epithelial cells in hypertrophy and empty lumen of the species *Tropidurus torquatus* (UFMT-R 11680) **(A)** and *T. oreadicus* (CHUNB 35359) **(B)**.

Hypertrophied epithelial cells with spermatozoa filled lumen of the species *T. oreadicus* (CHUNB 35359) **(C)**, *T. hispidus* (ZUEC-REP 3196) **(D)** and *T. oreadicus* (CHUNB 50202, CHUNB 43774) **(E, F)**. Epididymal duct with atrophied cells with no spermatozoa, but with secretion in the lumen of the lizard species *T. itambere* (ZUEC-REP 533) **(H)**. Epididymal duct with atrophy in the epitelial cells at the period with reproductive inaptitude in the species *T. torquatus* (UFMT-R 11620) **(G)** and *T. oreadicus* (INPA-H 33027) **(I)**, respectively. Legend: **Bc** = Basal cells, **Bv** = Blood vessel, **Ct** = Connective tissue, **Epd** = Epididymal duct, **Gs** = Secretion granules, **L** = Lumen, **Pse** = Cylindrical pseudostratified epithelium, **S** = Secretion, **(Arrowhead)** = Stereocilia e **Z** = Spermatozoa. Coloration: Toluidine blue at 1% (pH ≅ 7), Methylene blue at 5% (pH ≅ 7), Periodic Acid Schiff (PAS) + Metanil Yellow + Ferric Hematoxylin.

Figure 4A – D: Distribution of the sexual maturity of *Tropidurus* species (*torquatus* group) at different populations in Brazil. Populations of *Tropidurus torquatus* in the biomes Cerrado, Amazon and Atlantic Forest (**A**), *T. hispidus* in Caatinga, Cerrado and Amazon (**B**), *T. itambere* in Cerrado and Atlantic Forest (**C**) and *T. oreadicus* in Cerrado and Amazon biomes (**D**)**.** Horizontal axis (X) with Brazilian biomes and the vertical axis (Y) with snout-vent length (SVL) in milliliters.

FIGURE 1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N
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Species
☆ Tropidurus hispidus
● Tropidurus itambere
✚ Tropidurus oreadicus
▲ Tropidurus torquatus
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FIGURE 2

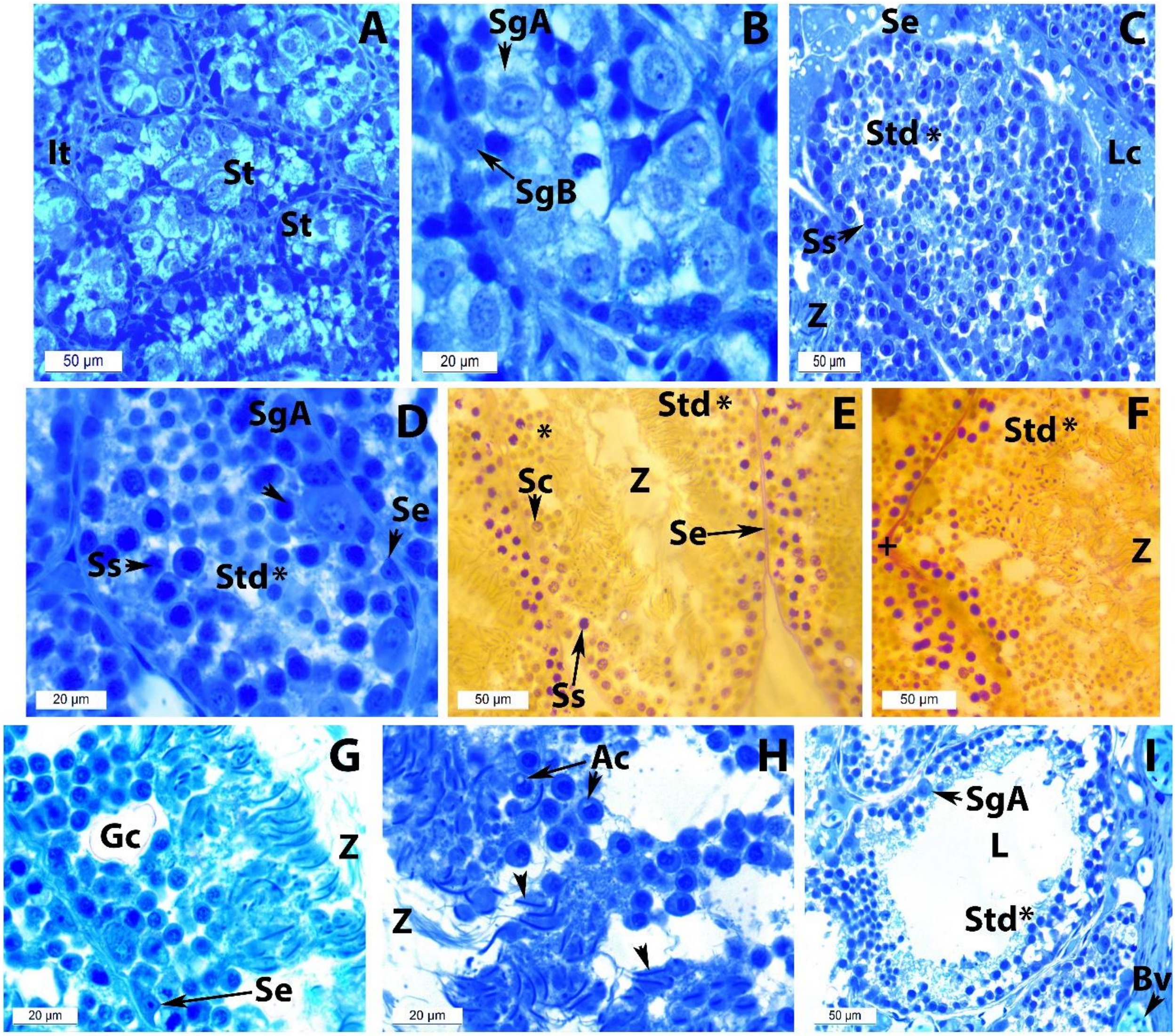
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FIGURE 3

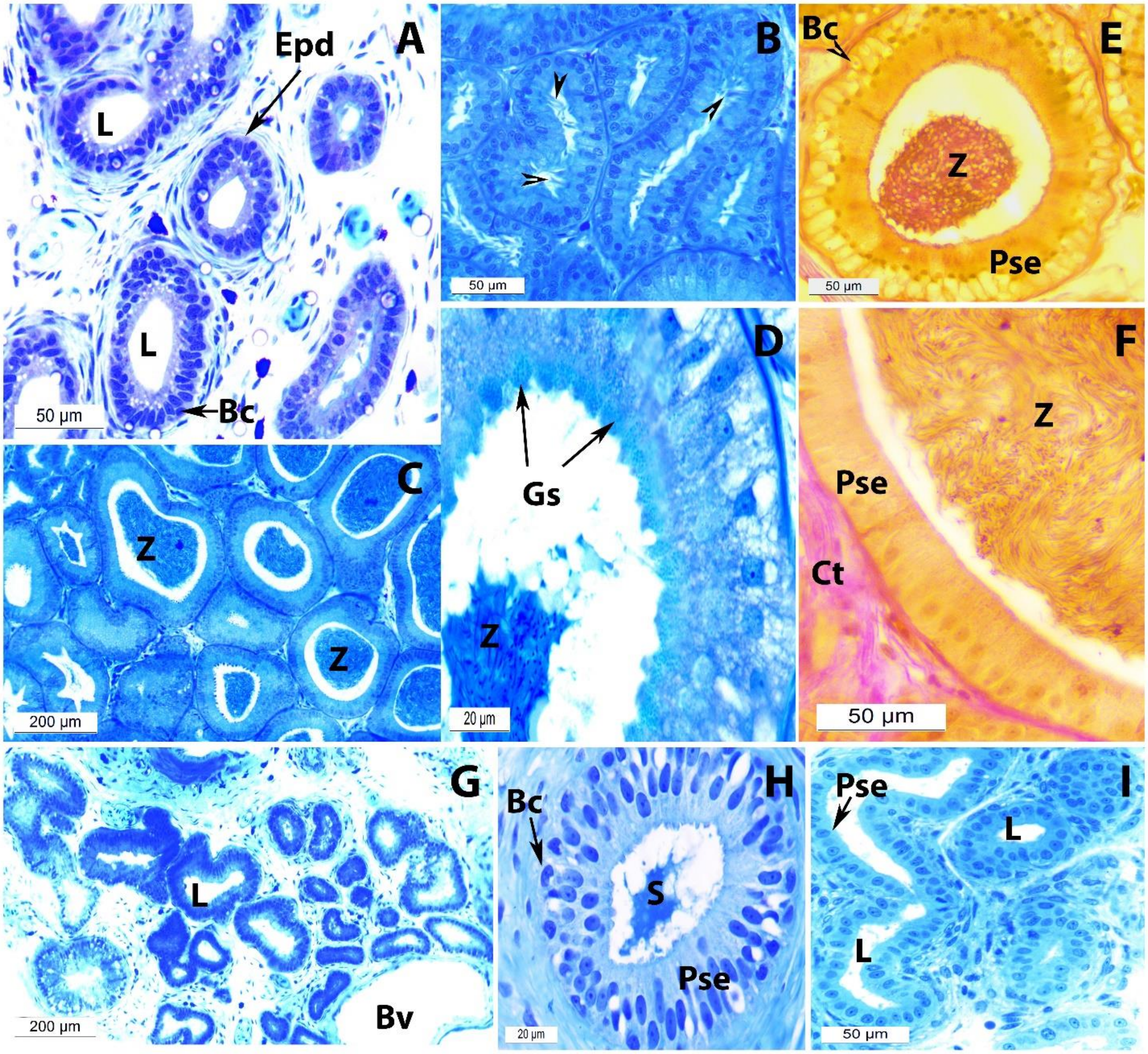
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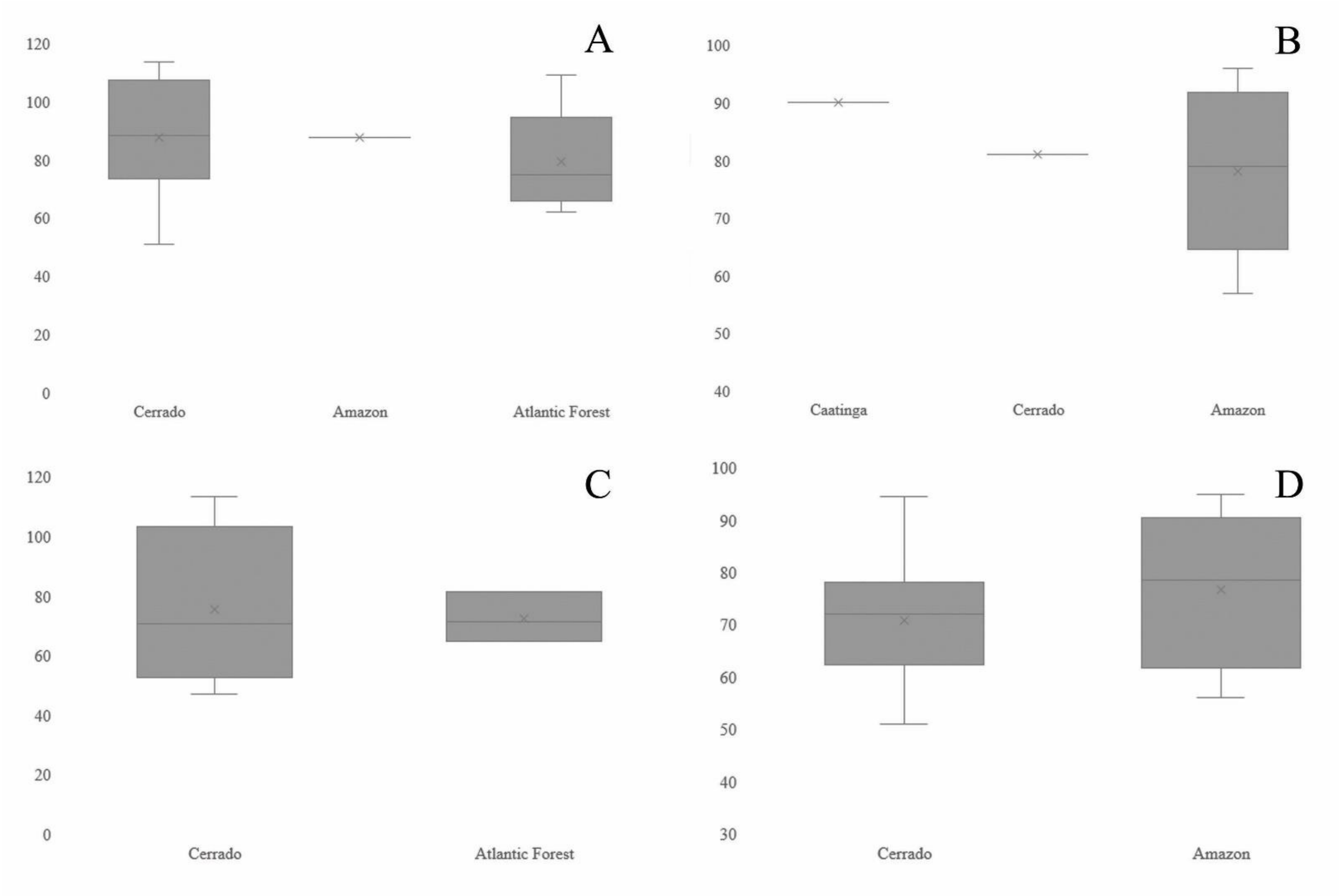
FIGURE 4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Supplementary Data

Table 1: Relation of *Tropidurus* species (*torquatus* group) evaluated in this study. Samples (N) and Voucher numbers. Acronims: **CHUNB** (Coleção Herpetológica da Universidade de Brasília), **INPA-HERPETO** (Coleção de Anfíbios e Répteis do Instituto Nacional de Pesquisas da Amazônia), **MPEG** (Museu Paraense Emílio Goeldi), **UFMT-R** Coleção Zoológica da Universidade Federal de Mato Grosso Setor Reptilia), **ZUEC-REP** (Museu de Zoologia da Universidade Estadual de Campinas "Adão José Cardoso"), **ZUFG** (Coleção Zoológica da Universidade Federal de Goiás).

Species	**Biome**	**Region**	**Locality**	**N**	**Colection date (month/year)**	**Voucher**
Tropidurus torquatus	Cerrado	Centro-oeste	Brasília - DF	5	12/2000, 12/2001, 01/2003	CHUNB 43271, 48039, 44770, 49660, 49661
Tropidurus torquatus	Cerrado	Centro-oeste	Cuiabá - MT	82	01,11-12/2005, 01-07/2006, 10-12/2006, 01,03,05-07/2007, 06-12/2012, 01-05/2013	UFMT-R 2770, 2882, 2883, 3038, 3039, 3190, 3419, 3420, 3543, 3544, 3606, 3607, 3767, 3961, 3992, 4186, 4255, 4256, 4565, 4566, 4882, 4883, 4889, 4904, 5467, 5725, 5828, 5829, 5909, 5910, 11590, 11591, 11593, 11594, 11596, 11603, 11605, 11606, 11609-11611, 11614-11616, 11618, 11620, 11624-11627, 11630-11632, 11635-11637, 11640-11644, 11649, 11653, 11655, 11657, 11659, 11663, 11666, 11668, 11669, 11674-11681, 11685-11687, 11689
Tropidurus torquatus	Cerrado	Centro-oeste	Itarumã - GO	1	09/2008	ZUFG 124
Tropidurus torquatus	Cerrado	Centro-oeste	Santa Terezinha - MT	1	03/1995	CHUNB 10912
Tropidurus torquatus	Cerrado	Centro-oeste	São Domingos - GO	1	03/2004	CHUNB 37622
Tropidurus torquatus	Cerrado	Centro-oeste	Silvânia - GO	1	07/2008	ZUFG 115
Tropidurus torquatus	Cerrado	Centro-oeste	Sítio D' Abadia - GO	1	01/2008	ZUFG 249
Tropidurus torquatus	Cerrado	Nordeste	Bahia	1	01/1995	ZUEC-REP 1873
Tropidurus torquatus	Cerrado	Norte	Lajeado - TO	2		ZUFG 509, 511
Tropidurus torquatus	Cerrado	Norte	Macaúba - TO	1	05/1962	MPEG 1235
Tropidurus torquatus	Cerrado	Sudeste	Unaí - MG	5	10/1993, 03/1994, 02/1999	CHUNB 01720-01722, 05431, 36286
Tropidurus torquatus	Amazônia	Norte	Manaus - AM	3	04/2006, 06/2007	INPA-H 016528, 019892, 019894
Tropidurus torquatus	Mata Atlântica	Nordeste	Itacaré - BA	1	07/1997	CHUNB 25227
Tropidurus torquatus	Mata Atlântica	Sudeste	Alto Caparaó - MG	1	03/2002	CHUNB 46225
Tropidurus torquatus	Mata Atlântica	Sudeste	Vale do Rio Doce - MG	1		ZUEC-REP 234
Tropidurus torquatus	Mata atlântica	Sudeste	Linhares - ES	1	07/1995	ZUEC-REP 2863
Tropidurus torquatus	Mata Atlântica	Sudeste	Maricá - RJ	3	12/1979	CHUNB 03971, 03973, 04019

Tropidurus torquatus	Mata Atlântica	Sudeste	Presidente Kennedy - ES	1	09/1997	CHUNB 24986
Tropidurus hispidus	Caatinga	Nordeste	Petrolina - PE	1	08/1991	CHUNB 05830
Tropidurus hispidus	Cerrado	Nordeste	Estreito - MA	1	08/1991	CHUNB 05193
Tropidurus hispidus	Amazônia	Nordeste	Arari - MA	3	02/1978	MPEG 11593, 11613, 11614
Tropidurus hispidus	Amazônia	Nordeste	Santa Luzia do Paruá - MA	4	06/1975, 06/1976	MPEG 10141, 10147, 10167, 11110
Tropidurus hispidus	Amazônia	Nordeste	São Raimundo - MA	1	11/1975	MPEG 9392
Tropidurus hispidus	Amazônia	Norte	Boa Vista - RR	1	06/1970	MPEG 3964
Tropidurus hispidus	Amazônia	Norte	Caracaraí-RR	8	07/1993	MPEG 17181-17183, 17224, 17232, 17236, 17249, 17258
Tropidurus hispidus	Amazônia	Norte	Carajás - PA	2	05/1983	MPEG 12935, 12937
Tropidurus hispidus	Amazônia	Norte	Marabá - PA	4	05/1969	MPEG 2880, 2881, 2884, 2887
Tropidurus hispidus	Amazônia	Norte	São José do Ribamar - MA	32	04-05/2007	ZUEC-REP 3193, 3194, 3196, 3198, 3204, 3207, 3209-3211, 3214, 3222, 3223, 3230-3232, 3235, 3237, 3243, 3245, 3250, 3251, 3256, 3257, 3259, 3274, 3276, 3278, 3281, 3284, 3287, 3290, 3297
Tropidurus itambere	Cerrado	Centro-oeste	Brasília - DF	2	11/2005	CHUNB 44132, 44134
Tropidurus itambere	Cerrado	Centro-oeste	Minaçu - GO	5	06/2001, 01,05/2008	CHUNB 48416, 48417, 52775, 53173, 53174
Tropidurus itambere	Cerrado	Centro-oeste	Pirenópolis - GO	6	06,09/2000	CHUNB 31678, 31679, 31682, 31690, 31707, 31708
Tropidurus itambere	Cerrado	Nordeste	Bahia	1	07/1995	ZUEC-REP 1873
Tropidurus itambere	Cerrado	Sudeste	Paracatu - MG	1	10/2001	CHUNB 26900
Tropidurus itambere	Mata Atlântlica	Sudeste	Campinas - SP	1	10/1993	ZUEC-REP 1814
Tropidurus itambere	Mata Atlântica	Sudeste	Itatiba - SP	3	04/1988	ZUEC-REP 617-619
Tropidurus itambere	Mata Atlântica	Sudeste	Pedro Canário - ES	1	07/1974	ZUEC-REP 326
Tropidurus itambere	Mata Atlântica	Sudeste	Poços de Caldas - MG	1	08/1998	ZUEC-REP 2165
Tropidurus itambere	Mata Atlântica	Sudeste	Valinhos - SP	1	03/1987	ZUEC-REP 533
Tropidurus oreadicus	Cerrado	Centro-oeste	Alto Paraíso de Goiás - GO	1	09/2005	CHUNB 43642
Tropidurus oreadicus	Cerrado	Centro-oeste	Alvorada do Norte - GO	2	08/2003	CHUNB 33311, 33316
Tropidurus oreadicus	Cerrado	Centro-oeste	Barro Alto - GO	1	11/2007	ZUFG 80
Tropidurus oreadicus	Cerrado	Centro-oeste	Cocalzinho de Goiás - GO	3	01/2003	CHUNB 34655-34657
Tropidurus oreadicus	Cerrado	Centro-oeste	Minaçu - GO	3	06/2001, 12/2002, 05/2008	CHUNB 29641, 48409, 53176
Tropidurus oreadicus	Cerrado	Centro-oeste	Niquelândia - GO	3	04,05/2006	ZUFG 09, 10, 50
Tropidurus oreadicus	Cerrado	Centro-oeste	Pirenópolis - GO	8	12/1990, 05-07,09/2000	CHUNB 06294, 06295, 31681, 31688, 31689, 31701, 31720, 31732

Tropidurus oreadicus	Cerrado	Centro-oeste	São Domingos - GO	7	03/2001, 09,11/2003, 03,11/2004, 04/2007, 01/2008	CHUNB 25325, 33330, 35359, 37622, 43833; ZUFG 186, 200
Tropidurus oreadicus	Cerrado	Nordeste	Balsas- MA	2	12/2002	CHUNB 43773, 43774
Tropidurus oreadicus	Cerrado	Nordeste	Carolina - MA	1	11/2007	CHUNB 52034
Tropidurus oreadicus	Cerrado	Nordeste	Cocos - BA	4	12/2000, 11/2007, 04/2008	CHUNB 50202, 51239, 51490, 52378
Tropidurus oreadicus	Cerrado	Nordeste	Correntina - BA	1	10/1993	CHUNB 06159
Tropidurus oreadicus	Cerrado	Norte	Almas - TO	1	02/2004	CHUNB 42334
Tropidurus oreadicus	Cerrado	Norte	Caseara - TO	1	09/2005	CHUNB 45197
Tropidurus oreadicus	Cerrado	Norte	Dianópolis - TO	1	09/2003	CHUNB 33303
Tropidurus oreadicus	Cerrado	Norte	Lajeado - TO	3		ZUFG 406, 408, 411
Tropidurus oreadicus	Cerrado	Norte	Mateiros - TO	5	12/1999, 02/2002, 11/2004	CHUNB 24302, 28585, 40506, 40523, 40524
Tropidurus oreadicus	Cerrado	Norte	Palmas - TO	4	12/1999, 12/2000	CHUNB 11301, 13863, 13864, 13865
Tropidurus oreadicus	Cerrado	Norte	Paranã - TO	2	09/2003	CHUNB 33302, 33305
Tropidurus oreadicus	Cerrado	Norte	Porto Nacional - TO	2	07/2005	CHUNB 47767, 47769
Tropidurus oreadicus	Amazônia	Norte	Amazonas	1	03/2013	INPA-H 033020
Tropidurus oreadicus	Amazônia	Norte	Belém - PA	6	09,10/2004, 03,05/2006, 06/2007	MPEG 22219, 22220, 22229, 24803, 24805, 24806
Tropidurus oreadicus	Amazônia	Norte	Macapá - AP	2	09/1991	CHUNB 06458, 06461
Tropidurus oreadicus	Amazônia	Norte	Marabá - PA	5	07/1984, 06/1985	MPEG 13689, 13691, 13692, 14105, 14235
Tropidurus oreadicus	Amazônia	Norte	Parauapebas - PA	2	07/1986	CHUNB 05704, 05705
Tropidurus oreadicus	Amazônia	Norte	Porto Velho - RO	13	03,07/2004, 11/2010, 05,11/2011	INPA-H 14980, 14981, 14988, 32944, 32946, 32949, 32950, 33014, 33016, 33017, 33024, 33025, 33027
Tropidurus oreadicus	Amazônia	Norte	Vigia - PA	1	10/1973	MPEG 7091

Table 2: Mean snout-vent lenght and range of (SVL), minimum size at sexual maturity and reproductive aptitude of males *Tropidurus* species (*torquatus* group) evaluated in this study. Samples (N), reproductive individuals (*N) and non-reproductive (nr) individuals.

Species	**Biome**	**Locality**	**N (N*)**	**Collecting date (month/year)**	**SVL**		**Sexual Maturity**	**Spermatogenic Stage**	**Reproductive Aptitude**
					Mean (SD)	**Range**			
Tropidurus torquatus	Cerrado	Brasília - DF	5 (2)	12/2000, 12/2001, 01/2003	60.8 (27.9)	31 - 103	51	Spermiation	December
Tropidurus torquatus	Cerrado	Cuiabá - MT	82 (62)	01,11-12/2005, 01-07/2006, 10-12/2006, 01,03,05-07/2007, 06-12/2012, 01-05/2013	102.75 (13.13)	68.54 - 123.57	72,8	Spermiation	May - February
Tropidurus torquatus	Cerrado	Itarumã - GO	1 (0)	09/2008	98.2 (0.0)	98,2	no data	no data	no data
Tropidurus torquatus	Cerrado	Santa Terezinha - MT	1 (1)	03/1995	109 (0.0)	109	109	Spermiation	March
Tropidurus torquatus	Cerrado	São Domingos - GO	1 (0)	03/2004	32 (0.0)	32	no data	no data	no data
Tropidurus torquatus	Cerrado	Silvânia - GO	1 (0)	07/2008	48.02 (0.0)	48,02	nr	Quiescence	no data
Tropidurus torquatus	Cerrado	Sítio D' Abadia - GO	1 (1)	01/2008	96.87 (0.0)	96,87	96,87	Spermiation	January
Tropidurus torquatus	Cerrado	Bahia	1 (1)	01/1995	113.47 (0.0)	113,47	113,47	Spermiation	January
Tropidurus torquatus	Cerrado	Lajeado - TO	2 (1)		97.15 (8.41)	91.21 - 103.1	103,1	Regression, Spermatozoa Storaged in Epididymal	no data
Tropidurus torquatus	Cerrado	Macaúba - TO	1 (1)	05/1962	80 (0.0)	80	80	Spermiation	May
Tropidurus torquatus	Cerrado	Unaí - MG	5 (4)	10/1993, 03/1994, 02/1999	99.8 (12.27)	75 - 111	75	Spermiation	February and October
Tropidurus torquatus	Amazônia	Manaus - AM	3 (1)	04/2006, 06/2007	64.34 (21.47)	45.63 - 87.78	87,78	Spermiation	June
Tropidurus torquatus	Mata Atlântica	Itacaré - BA	1 (1)	07/1997	75 (0.0)	75	75	Spermiation	July
Tropidurus torquatus	Mata Atlântica	Alto Caparaó - MG	1 (1)	03/2002	109 (0.0)	109	109	Spermiation	March
Tropidurus torquatus	Mata Atlântica	Vale do Rio Doce - MG	1 (0)		107.94 (0.0)	107,94	no data	no data	no data
Tropidurus torquatus	Mata atlântica	Linhares - ES	1 (1)	07/1995	70.04 (0.0)	70,04	70,04	Spermiation	July
Tropidurus torquatus	Mata Atlântica	Maricá - RJ	3 (3)	12/1979	81.33 (1.15)	80 - 82	80	Spermiation	December
Tropidurus torquatus	Mata Atlântica	Presidente Kennedy - ES	1 (1)	09/1997	62 (0.0)	62	62	Spermiation	September
Tropidurus hispidus	Caatinga	Petrolina - PE	1(1)	08/1991	90 (0.0)	90	90	Spermatozoa Storaged in Epididymal	August
Tropidurus hispidus	Cerrado	Estreito - MA	1 (1)	08/1991	81 (0.0)	81	81	Spemiation	August
Tropidurus hispidus	Amazônia	Arari - MA	3 (3)	02/1978	100 (3.6)	96 - 103	96	Spermiation	February
Tropidurus hispidus	Amazônia	Santa Luzia do Paruá - MA	4 (4)	06/1975, 06/1976	82 (6.88)	77 - 92	77	Spermiation	June
Tropidurus hispidus	Amazônia	São Raimundo - MA	1 (1)	11/1975	81 (0.0)	81	81	Spermiation	November
Tropidurus hispidus	Amazônia	Boa Vista - RR	1 (1)	06/1970	88 (0.0)	88	88	Spermiation	June
Tropidurus hispidus	Amazônia	Caracaraí-RR	8 (5)	07/1993	83.5 (20.4)	46 - 102	93	Spermiation	July
Tropidurus hispidus	Amazônia	Carajás - PA	2 (2)	05/1983	71 (11.31)	63 - 79	63	Spermiation	May

Tropidurus hispidus	Amazônia	Marabá - PA	4 (1)	05/1969	56.25 (5.68)	48 - 60	57	Spermiation	May
Tropidurus hispidus	Amazônia	São José do Ribamar - MA	32 (27)	04-05/2007	77.93 (7.11)	59.6 - 91.97	69,57	Spermiation	April–May
Tropidurus itambere	Cerrado	Brasília - DF	2 (1)	11/2005	67.5 (2.12)	66 - 69	69	Spermiation	November
Tropidurus itambere	Cerrado	Minaçu - GO	5 (0)	06/2001, 01,05/2008	48.2 (6.83)	37 - 55	nr	Regression	no data
Tropidurus itambere	Cerrado	Pirenópolis - GO	6 (6)	06,09/2000	56.83 (7.02)	47 - 66	47	Spermiation	June and September
Tropidurus itambere	Cerrado	Bahia	1 (1)	07/1995	113.47 (0.0)	113,47	113,47	Spermiation	July
Tropidurus itambere	Cerrado	Paracatu - MG	1 (1)	10/2001	72 (0.0)	72	72	Spermiation	October
Tropidurus itambere	Mata Atlântlica	Campinas - SP	1 (1)	10/1993	81.29 (0.0)	81,29	81,29	Spermiation	October
Tropidurus itambere	Mata Atlântica	Itatiba - SP	3 (0)	04/1988	65.18 (16.1)	54.21 - 83.65	nr	Quiescence	no data
Tropidurus itambere	Mata Atlântica	Pedro Canário - ES	1 (1)	07/1974	71.26 (0.0)	71,26	71,26	Spermiation	July
Tropidurus itambere	Mata Atlântica	Poços de Caldas - MG	1 (1)	08/1998	64.72 (0.0)	64,72	64,72	Spermiation	August
Tropidurus itambere	Mata Atlântica	Valinhos - SP	1 (0)	03/1987	87.05 (0.0)	87,05	nr	Regression	no data
Tropidurus oreadicus	Cerrado	Alto Paraíso de Goiás - GO	1 (1)	09/2005	63 (0.0)	63	63	Spermiation	September
Tropidurus oreadicus	Cerrado	Alvorada do Norte - GO	2 (2)	08/2003	78.5 (0.7)	78 - 79	78	Spermiation	August
Tropidurus oreadicus	Cerrado	Barro Alto - GO	1 (1)	11/2007	58.89 (0.0)	58,89	58,89	Spermiation	November
Tropidurus oreadicus	Cerrado	Cocalzinho de Goiás - GO	3 (1)	01/2003	43 (8.0)	35 - 51	51	Spermiation	January
Tropidurus oreadicus	Cerrado	Minaçu - GO	3 (0)	06/2001, 12/2002, 05/2008	41.33 (10.02)	30 - 49	nr	Quiescence and Proliferation	no data
Tropidurus oreadicus	Cerrado	Niquelândia - GO	3 (1)	04,05/2006	58.66 (21.88)	40.38 - 82.9	82,9	Spermiation	May
Tropidurus oreadicus	Cerrado	Pirenópolis - GO	8 (6)	12/1990, 05-07,09/2000	68.25 (15.9)	52 - 94	52	Spermiation	May–July, September, and December
Tropidurus oreadicus	Cerrado	São Domingos - GO	7 (3)	03/2001, 09,11/2003, 03,11/2004, 04/2007, 01/2008	59.4 (27.45)	30.42 - 97	71	Spermiation	September and November
Tropidurus oreadicus	Cerrado	Balsas- MA	2 (1)	12/2002	71.5 (2.12)	70 - 73	73	Spermiation	December
Tropidurus oreadicus	Cerrado	Carolina - MA	1 (1)	11/2007	60 (0.0)	60	60	Spermiation	November
Tropidurus oreadicus	Cerrado	Cocos - BA	4 (3)	12/2000, 11/2007, 04/2008	73 (21.77)	43 - 93	72	Spermiation	November–December
Tropidurus oreadicus	Cerrado	Correntina - BA	1 (1)	10/1993	75 (0.0)	75	75	Spermiation	October
Tropidurus oreadicus	Cerrado	Almas - TO	1 (0)	02/2004	34 (0.0)	34	no data	no data	no data
Tropidurus oreadicus	Cerrado	Caseara - TO	1 (1)	09/2005	75 (0.0)	75	75	Spermiation	September
Tropidurus oreadicus	Cerrado	Dianópolis - TO	1 (1)	09/2003	71 (0.0)	71	71	Spermiation	September
Tropidurus oreadicus	Cerrado	Lajeado - TO	3 (1)		94.16 (3.5)	90.5 - 97.45	94,54	Spermatozoa Storaged in Epididymal	no data
Tropidurus oreadicus	Cerrado	Mateiros - TO	5 (3)	12/1999, 02/2002, 11/2004	59.2 (21.09)	32 - 78	72	Spermiation	November
Tropidurus oreadicus	Cerrado	Palmas - TO	4 (3)	12/1999, 12/2000	81.5 (2.88)	78 - 85	78	Spermiation and Spermatozoa Storaged in Epididymal	December

Tropidurus oreadicus	Cerrado	Paranã - TO	2 (2)	09/2003	86 (7.07)	81 - 91	81	Spermiation	September
Tropidurus oreadicus	Cerrado	Porto Nacional - TO	2 (2)	07/2005	74.5 (9.19)	68 - 81	68	Spermiation	July
Tropidurus oreadicus	Amazônia	Amazonas	1 (0)	03/2013	41.58 (0.0)	41,58	nr	Quiescence	no data
Tropidurus oreadicus	Amazônia	Belém - PA	6 (1)	09,10/2004, 03,05/2006, 06/2007	57.33 (18.13)	39 - 89	89	Spermiation	May
Tropidurus oreadicus	Amazônia	Macapá - AP	2 (2)	09/1991	97 (2.83)	95 - 99	95	Spermiation	September
Tropidurus oreadicus	Amazônia	Marabá - PA	5 (4)	07/1984, 06/1985	65.6 (7.63)	56 - 76	56	Spermiation	June–July
Tropidurus oreadicus	Amazônia	Parauapebas - PA	2 (2)	07/1986	72.5 (6.36)	68 - 77	68	Spermiation	July
Tropidurus oreadicus	Amazônia	Porto Velho - RO	13 (9)	03,07/2004, 11/2010, 05,11/2011	78.44 (14.3)	48.41 - 95.26	63,48	Spermiation and Spermatozoa Storaged in Epididymal	March, May, July, and November
Tropidurus oreadicus	Amazônia	Vigia - PA	1 (1)	10/1973	89 (0.0)	89	89	Spermiation	October

5.3 FOLLICULAR DEVELOPMENT AND REPRODUCTIVE APTITUDE IN FOUR SPECIES OF BRAZILIAN *TROPIDURUS* LIZARDS

ABSTRACT

The follicular development and reproductive aptitude of four species of oviparous lizards of the genus *Tropidurus* were evaluated anatomically and histologically. Therefore, specimens were measured, the number of follicles and eggs were counted, and the right ovary of each individual was collected, processed according to histological routine, and photodocumented. For all species, ovaries are divided in a cortical germinal bed, where are located the oogonia and stage I oocytes, and a medullar stroma, where the remaining follicular development stages occur. There were no microscopic differences in the development of the ovarian follicles between the four species of the *T. torquatus* group. In addition, the only measure that presented significant variation throughout the follicular development was the thickness of the granulosa layer in the stage VII follicles. Regarding sexual maturity, close values were observed among species, with the smallest length recorded for *T. hispidus*. The clutch size were higher for *T. itambere* and *T. torquatus* species with a maximum of five and six eggs in the oviducts, respectively. *T. oreadicus* and *T. hispidus* had a maximum of six and five follicles, respectively, but neither of the species presented eggs in the oviducts. In addition, the period of reproductive aptitude varied among the four species of lizards of the *T. torquatus* group. Finally, besides the morphological aspects observed in four species of *Tropidurus*, this is the first study to relate data on the number of germinal bed and frequency of corpus luteum and atresia to reproductive aptitude.

Keywords: Tropiduridae; reproductive aptitude; oogenesis; germinal bed; follicle growth; corpus luteum; atresia; sexual maturity.

INTRODUCTION

Ovarian follicular growth in reptiles, as in other vertebrates, is essential in the female reproductive process (Lozano et al., 2014). Follicular development process begins in regions called germinal beds and is subsequently displaced into the stroma (Guraya, 1989). In reptiles this process occurs when the oogonia is individualized and starts to receive specific signals for differentiation and growth (Guraya, 1989). Although the general sequence of follicular development has been described in various species of reptiles, some variation among species have been reported (e.g. Guraya, 1978; Van Wyk, 1984; Uribe et al., 1996; Lozano et al., 2014). In particular, there is scarce information of ovarian development for Brazilian lizard species, which are distributed aong distinct biomes and present high diversity of reproductive strategies, such as species that reproduce continuously and those that reproduce seasonally (Vitt, 1992). Moreover, it is important to analyse the identification and definition of morphological changes in the oocyte follicle and its surrounding structures during ovarian maturation for understanding the development of oogenesis (Tokarz, 1978; Guraya, 1989), including in tropical oviparous lizards.

The reproductive aptitude determines the reproductive viability of a species through morphological inference of the gonads, reflecting the physiological and behavioral response during a specific moment of organism life. Additionally, this study approach allows establish a comparative panorama between the reproductive status o one more species over time.

The genus *Tropidurus* comprises species of cisandean South American oviparous lizards that occurs along a wide geographical extention in Brazil, distributed from southern Amazon River to Pampa biome in the south (Rodrigues, 1987, Carvalho et al., 2013). *Tropidurus* species included into four species group according to their morphological and molecular characteristics (*bogerti*, *semitaeniatus*, *spinulosus*, and *torquatus*, sensu Frost et al., 2001). The *torquatus* group comprises 16 species (Carvalho et al., 2016), of which four were studied here: *Tropidurus torquatus* (Wied, 1820), *T. hispidus* (Spix, 1825), *T. itambere* (Rodrigues, 1987) and *T. oreadicus* (Rodrigues, 1987). Studies on reproduction have demonstrated reproductive variations within the genus *Tropidurus* with species presenting continuous reproduction (e.g. Vitt and Goldberg, 1983) and others with seasonal reproduction (e.g. Wiederhecker et al., 2002; Ferreira et al., 2009, 2011). *Tropidurus* species present early maturity (Tinkle, 1969), multiple clutches per breeding season (Wiederhecker et al., 2002), and variation in clutch size among species (e.g. Vitt, 1981, Van Sluys, 1993, Van Slyus et al., 2010) and among populations of the same species (Kiefer et al., 2008).

Although several studies have documented the reproductive strategies for the genus *Tropidurus* (e.g. Wiedehecker et al., 2002, Van Sluys et al., 2010, Ferreira et al., 2011) an approach describing ovarian morphology, follicular development and its relation to reproductive aptitude is still missing, especially comparing species. Thus, we aimed to describe the structural modifications of the developing ovarian follicles and to relate them with the reproductive aptitude, sexual maturity and variations of the clutche size produced by females of four species of the *torquatus* group.

MATERIALS AND METHODS

Two hundred one female specimens of the *torquatus* group from 43 populations were acquired from six zoological collections in Brazil. Acronims: **CHUNB** (Coleção Herpetológica da Universidade de Brasília), **INPA-HERPETO** (Coleção de Anfíbios e Répteis do Instituto Nacional de Pesquisas da Amazônia), **MPEG** (Museu Paraense Emílio Goeldi), **UFMT-R** Coleção Zoológica da Universidade Federal de Mato Grosso Setor Reptilia), **ZUEC-REP** (Museu de Zoologia da Universidade Estadual de Campinas “Adão José Cardoso”), **ZUFG** (Coleção Zoológica da Universidade Federal de Goiás). Samples preserved in ethanol 70% were quantified *T. torquatus* (N= 86 from nine localities), *T. hispidus* (N= 52 from eighteen localities), *T. itambere* (N=19 from nine localities) and *T. oreadicus* (N= 44 from 17 localities). Invoice numbers, details of the specimens' origins, and dates of collection can be found in Supplementary data (Table 1).

For the histological analysis, the individuals were dissected and the right ovaries were removed, in its absence the left ovary was used instead. The ovaries were entirely removed and submitted to histological routine for analysis under light microscopy. Thus, samples were placed in glycol methacrylate resin (Historesin® Leica) and cut semi-serially into 3µm thick sections, with minimum of 30µ m of distance between each section. The sections were stained with 1% toluidine blue or 5% methylene blue both in (pH ≅ 7). A histochemical staining was also performed with the combination of Periodic acid – Schiff's reactive (PAS) + iron hematoxylin + metanil yellow (Quintero-Hunter et al., 1991). This histochemical technique shows: 1) basal membrane, fibrins, and colloids in magenta; 2) proteins rich in amine radicals, nucleus, and rough endoplasmic reticulum in brown; 3) counter-staining of the remaining structures in yellow or orange (Quintero-Hunter et al., 1991). Then, sections were analyzed and photodocumented under a Leica DM500 light microscope coupled with Leica DFC295 camera and a digital image capture device Leica Application Suite (LAS V3.7).

In addition to the histological description, the morphometry of the ovarian follicles was performed for each described stage and for each species. The characters considered for measurement were follicular diameter, thickness of the granulosa cell layer, thickness of the zona pellucida, and thickness of the theca cell layer. For each species, up to 10 follicles per stage of development were measured, and then mean value ± standard desviation were calculated, in micrometers. In order to verify if there was any difference in the morphometry of the ovarian follicles among the different species, the measurements obtained for each stage of development were compared using ANOVA with ($p \leq 0.05$).

For the analysis of reproductive aptitude, the snout-vent length (SVL, in mm) measurement has been taken for all individuals with a digital calipter (0.001 mm) and the number of eggs and follicles ≥ 3mm present in the oviducts were recorded, according to Van Sluys (1993). The sexual maturity corresponds to the smaller SVL of the lizard that is able to reproduce. For this study, it was considered the females with the smallest SVLpresenting follicles ≥ 3mm and / or eggs in the oviduct (Van Sluys, 1993). The clutch size was obtained through the mean ± standard deviation among the number of eggs or follicles ≥ 3mm in the oviducts. On the concomitante occurrence of eggs and follicles ≥ 3mm in the oviducts, only eggs or follicles were considered, not being counted both at the same time (Van Sluys, 1993).

RESULTS

The ovaries of *Tropidurus torquatus* group from the study are pair organs attached to the dorsal wall of the abdominal cavity by a connective tissue – the mesovarium (Fig. 1A). Histologically, the ovary was divided in two regions: cortical germinal bed and medullar stroma (Fig. 1A). Externally, a simple squamous epithelium cover the ovary (Fig. 1B) and the stroma region is composed by blood vessels and connective tissue surrounding ovarian follicles at different stages of development and structures in involution process (Fig. 1A-B).

For all species analyzed, it was observed one germinal bed situated on the dorsal surface of each ovary composed by loose connective tissue surrounding oogonia and early oocytes intermixed with epithelial cells (Fig. 1C). It was also observed a basal membrane clearly delimiting the germinal bed (Fig. 1C). Because there were no differences in the development of the ovarian follicles among species, the description of the microscopic characteristics are presented uniquely according to their development. This consideration also applies to illustrations.

The oogonia (Fig. 1C) are spherical or ovoid with small amounts of homogeneous ooplasm and spherical nuclei. The nuclei show loose chromatin and an evident nucleolus. The earliest stages of development from oogonia to the formation of the oocyte follicle are comprised in the previtellogenesis phase, which, in turn, is divided in five stages. In stage I there is the start of meiosis in oogonia, arresting in diplotene, and now called as young oocytes (Fig. 1C). These oocytes are spherical or ovoid and remain adjacent to the germinal bed. Its nucleus is central and spherical with loose chromatin and an evident nucleolus. The ooplasm stains lightly, and it is possible to observe a distinct Balbiani corpuscle as a basophilic mass near the nucleus (Fig. 1C). Few follicular cells surround these oocytes and they appear as squamous cells with elongated and dense nuclei (Fig. 1C).

Stage II oocytes (Fig. 1D) show a lightly basophilic ooplasm with large fibers aggregated into distinct bundles that are located at the center of the cell. Its nucleus has fibrillar chromatin and an evident nucleolus. In the follicular epithelium, the granulosa is monolayered and surrounds the oocyte. During this stage, intermediate cells begin to appear interspersed with many small granulosa cells. The larger granulosa cells are ovoid with a lightly stained cytoplasm, and a large, spherical nucleus. In this stage, there is also the association of the first cells of the theca layer surrounding the oocyte follicle.

In the next stage (III), the oocyte (Fig. 2A) shows nucleus with fibrillary chromatin surrounded by fine granular material and the ooplasm has fibers aggregated more homogeneously distributed than those observed in stage II oocytes. As in the previous stage, the granulosa consists of small cells and intermediate cells, but now the intermediate cells are greater in number and size. In stage IV, oocyte (Fig. 2B-C) has an eccentric nucleus with lampbrush chromosomes and numerous nucleoli. The ooplasm contains an abundance of fibrillary cytoplasmic clumps homogeneously distributed.

As folliculogenesis progresses during this stage, the ooplasm exhibits vacuoles, known as cortical alveoli, which increase in number toward the center of the oocyte. The zona pellucida is clearly distinct at this stage and appears as a single layer. Also in this stage, the granulosa becomes stratified and polymorphic showing three distinct cell types: small, intermediate, and pyriform cells (Fig. 2B, D). Small cells are spherical and have round nuclei; they are more abundant in the region closest to the theca and interspersed among the pyriform cells (Fig. 2D). Each intermediate cell is oval with a round nucleus, single nucleolus, and basophilic cytoplasm; these cells are prevalent in the region of contact with the oocyte (Fig. 2D). Pyriform cells exhibit a large, central nucleus containing clumps of heterochromatin and an evident nucleolus (Fig. 2D). Their cytoplasm contains vacuoles and there is considerable variation in the cytoplasmic staining characteristics of the pyriform cells in the granulosa layer at this stage of folliculogenesis. In addition, these cells show narrow but long cytoplasmic prolongations that apparently join the zona pellucida (Fig. 2D). The theca cell layer is more developed in at stage being divided in external and internal theca; external theca comprises cells with spherical nucleus and many blood vessels, and the internal theca has more squamous cells (Fig. 2B).

The oocyte in stage V (Fig. 2E-F) has a thin peripheral ooplasm immediately beneath the oolemma and lacks the fibrillary clumps but it contains fine basophilic granules, and the fibrillary cytoplasmic clumps are present more centrally in the oocyte. In addition, just below the zona pellucida it starts a new deposition of fibrillary material on the periphery of the ooplasm (Fig. 2F). The granulosa layer has become thicker with the three cell types present (Fig. 2E-F). Small cells are the most intensely stained cells and they are observed on both the apical and basal sides of the granulosa layer (Fig. 2F). The intermediate cells are well stained and still abundant in the region in contact with the zona pellucida (Fig. 2F). Moreover, the pyriform cells contain a large nucleus with one nucleolus and a lightly stained cytoplasm (Fig. 2F). This stage marks the end of previtellogenesis.

Next, there is the vitellogenic development of oocyte follicles. This phase has a subdivision in stages ranging from VI to IX (Fig. 3A-F). Oocyte in stage VI (Fig. 3A-B) is characterized by presenting largest cortical alveoli in the center of the follicle and small yolk vesicles near its periphery. The periphery of the ooplasm has a homogeneous appearance with deposition of fibrous components below the zona pellucida (Fig. 3B). The zona pellucida is evident (Fig. 3B) and it is still observed all cell types in the granulosa cell layer (Fig. 3A-B).

In stage VII, the follicle (Fig. 3C-D) is in active vitellogenesis and is characterized by showing large number of yolk vesicles in the ooplasm. The number, size and morphological diversity of yolk vesicles continue to grow, so the vesicles are smaller closest to the oocyte periphery than those which occupy its center (Fig. 3C). Still, it is interesting to note that there is the formation of a belt-like structure of yolk vesicles associated with cortical alveoli in the center of the oocyte (Fig. 3C). The zona pellucida is highly evident and bilayered (Fig. 3D). The granulosa layer is narrower with small and intermediate cells present (Fig. 3D), and only a layer of oval pyriform cells which still presents cytoplasmic prolongations towards the zona pellucida (Fig. 3D).

With the advancement of vitellogenesis, the oocyte follicle reaches stage VIII (Fig. 3E). The zona pellucida is tightly defined with two layers: the inner striated layer (zona radiata) and the outer more homogeneous, clearer, and PAS-positive layer (hyaline band). Yolk vesicles occur in almost all ooplasm and in a large variety of sizes. At this stage, the follicle has a thin granulosa layer with pyriform and intermediate cells both in a more cubic shape.

The last stage of vitellogenesis is stage IX (Fig. 3F). At this stage, it is observed the last structural changes of the follicle, as the granulosa layer that consists of squamous and strongly stained cells. The yolk vesicles fill the ooplasm of the oocyte to its limits with the zona pellucida, and the theca layer is highly vascularized. Oocyte follicles that develop to the last stage of vitellogenesis are also called pre-ovulatory oocytes, and the ovulation process is characterized by the release of mature oocyte, disruption of the cell layers surrounding the oocyte, and subsequent formation of the corpus luteum.

The corpus luteum can be morphologically distinguished in three stages (Fig. 4A-E). In stage I, or luteogenesis, is possible to see the opening from which the oocyte was ovulated. In addition, there is the formation of a temporary round shape endocrine gland with an outer coating of theca cells and a central, irregular cavity filled with the remaining follicular cells that progressively increase in volume becoming the granulosa-lutein cells (Fig. 4A). Granulosa-lutein cells have morphological characteristics of steroid-secreting cells: lightly stained cytoplasm with large amounts of secretory granules and vacuoles, and nuclei with condensed chromatin (Fig. 4B). Changes in the stage II, or luteal maturity, involve thickening of the theca layer and reduction in the number of granulosa-lutein cells, compaction of the central cavity, and formation of connective tissue septa that orientates the infiltration of blood vessels into the central cavity (Fig. 4C-D). In Stage III, or luteal regression, it was observed an increased vascularity throughout the structure of the corpus luteum, few theca cells, and fibers (Fig. 4E).

As for the follicles that are not ovulated, they can undergo involution and form atretic follicles (Fig. 5A-E), which were classified into seven stages. In the early stages (stages I, II, and III; Fig. 5A-B) there is a gradual disintegration of the zona pellucida to the point where it is not possible to distinguish it. There is also the proliferation and hypertrophy of the granulosa cells with phagocytic characteristics, and invagination of theca cells into the oocyte (Fig. 5A-B). In stage IV, the atretic follicles have fewer ooplasm, which was phagocytized by the granulosa cells (Fig. 5C). In addition, granulosa cells decrease in number, remaining attached to the theca cell layer, while there is an increase in the number of vacuoles (Fig. 5C). In subsequent stages (V and VI), the atretic follicle becomes smaller, being characterized by the total phagocytosis of the ooplasm and the remaining of few granulosa cells (Fig. 5D). In stage VII, it is observed a small, round structure; in it central portion are observed only vacuoles among connective tissue septa, and the theca cell layer becomes extremely thin and its outer and inner layers are indistinguishable (Fig. 5E).

Morphometry data are presented in Table 2 (Supplementary data). In this table, the measurements of each stage of follicular development of each of the four species of the *Tropidurus torquatus* group are shown. There was no significant difference for the majorities of measurements made for each stage of follicular development (Table 2) among the four species. The only measure that presented significant difference was the thickness of the granulosa layer in the stage VII vitellogenic follicle (Anova F = 3.996; $p \leq 0.05$ df= 3). The thickness of this layer differed significantly between *T. hispidus* and *T. itambere* ($t = -4.384$; $p \leq 0.01$ df = 7) and between *T. oreadicus* and *T. itambere* ($t = -3.423$; $p = 0.05$ df = 7) (Table 2).

Regarding sexual maturity (Fig. 6 A-D and Table 3), similar SVL values were registred among species, with the smallest length recorded for *Tropidurus torquatus* in Presidente Kennedy-ES (64 mm) in Atlantic Forest biome. For *T. hispidus* the SVL at sexual maturity was (67.59 mm) in Caatinga from São José do Ribamar-MA, for *T. itambere* it was (59 mm) from Paracatu-MG in Cerrado, and for *T. oreadicus* from Porto Nacional-TO the minimum SVL was (44 mm) in Cerrado biome. The clutch size for *T. torquatus* was (5.06 ±2.25; n= 44) in Cerrado. For *T. hispidus* from Caatinga was (3.5 ±1.73; n= 4), while for *T. itambere* in Atlantic Forest was (5.66 ±0.57; n= 3) and for *T. oreadicus* the clutch size was (4.14 ±1.34; n= 7) in Amazon. All data for sexual maturity and clutch size is summarized in Table 3 (Supplementary data). Considering the presence of mature follicles, corpus luteum and atretic follicles (Table 3), the reproductive aptitude for *Tropidurus torquatus* in Cerrado occurred from August to March. For

T. hispidus occurred in April and May in Caatinga and in July at Amazon. For Cerrado population of *T. itambere* the aptitude occurred in October and December, while in Atlantic Forest was from July to October. *Tropidurus oreadicus* was apt to reproduce from May to January at Cerrado and from March to November in Amazon biome.

DISCUSSION

Ovaries of four species of the *torquatus* group presented similar morphology to other species of lizards (e.g. Uribe et al., 1995, 1996; Hernández-Franyutti et al., 2005; Lozano et al., 2014). Nevertheless, currently there is no description of the amount of germinal beds for other *Tropidurus* species, except for *T. torquatus* (Jones et al., 1982). At the present study was observed only one germinal bed per ovary for *T. torquatus*, *T. hispidus*, *T. itambere* and *T. oreadicus*. According to Jones et al. (1982), species of tropical lizards presented mostly one germinal bed per ovary, which could be related to the small amount of eggs produced by these species.

In fact, the number of eggs produced by the four species of the *Tropidurus torquatus* group in this study showed mean of 5.06 eggs for *T. torquatus* (Cerrado), 3.5 eggs for *T. hispidus* (Caatinga), 5.66 eggs for *T. itambere* (Atlantic Forest) and 4.14 eggs for *T. oreadicus* (Amazon). These data are very similar to the mean of eggs already reported for the continental populations in Brazil, as to *T. torquatus* from Cerrado with a mean 6.1 eggs (Wiederhecker et al., 2002). However, for coastal population the mean was smaller (2.0 to 3.3) in the Restinga (Kiefer et al., 2008). These variantion among *T. torquatus* population in egg production resulted, in part from female body size, but ecological fators can be influencied that differences to (e.g. Vitt, 1981; Rodrigues, 1987; Kiefer et al., 2008). For *T. hispidus* mean of clutch size was 8.1 in Caatinga (e.g. Ribeiro et al., 2012), being higher than recorded in this study. For *T. itambere* from Atlantic Forest the mean was 3.5 (Van Sluys, 1993), while for the population evaluated in this study was 5.66. *Tropidurus. oreadicus* from Cerrado presented clutch size of 3.66 (Meira et al., 2007), but in Amazon was 4.2 (Vitt, 1993), very similar to the value found in this study (4.14). Moreover, these data corroborate the argument of Jones et al. (1982), in which tropical species that present one germinal bed per ovary produce smaller amount of eggs than those that have two or more germinal beds. However, it is worth mentioning that other factors may also regulate the amount of eggs produced by species of the genus *Tropidurus* (e.g. Vitt, 1981; Rodrigues, 1987; Kiefer et al., 2008; Brandt and Navas, 2011).

Lizard species with one germinal bed usually have follicles developing in a hierarchical arrangement with larger follicles at vitellogenesis (Jones et al., 1982). This characteristic was observed in the species studied, which presented oogonia and primary oocytes in the germline. In the germinal bed, oocytes were involved and individualized by follicular cells, which increased in number and cell type as the oocyte was directed towards the stroma. Small cells were the first ones to appear around the oocyte. These cells differentiate into intermediate cells in a second moment and the latter, in its turn, differentiate in pyriform cells.

A sequential formation of follicular epithelium in a polymorphic structure has already been described for Squamata reptiles (Andreucetti, 1992), being widely shared among various species of lizards, such as *Podarcis siculus* (Rafinesque-Schmaltz, 1810) (Lacertidae) (Motta et al., 1995; Raucci and Di Fiori, 2010), *Cyrtopodion kachhense* (Stoliczka, 1872) (Gekkonidae) (Hojati et al., 2013), and in the species *Tropidurus hispidus* and *T. semitaeniatus* (e.g. Santos et al., 2015). For the species of the present study, it was observed changes in the follicular epithelium similar to those already described for other lizards during vitellogenesis (e.g. Uribe et al., 1995, 1996; Hernández-Franyutti et al., 2005; Lozano et al.,2014). Many studies report the role of the follicular epithelium during the development of the follicle (e.g. Motta et al., 1995; Vieira et al., 2010), highlighting the role of pyriform cells in the transport of substances to the oocyte via intercellular bridge (Andreucetti, 1992). Specifically with regard to pyriform cells, we found that the communication of these cells via intercellular bridge with the zona pellucida was maintained until oocytes in stage VII of vitellogenesis. While, for *Ctenosaura pectinate* (Weigmann, 1834) (Iguanidae), it was observed that this communication was broken once settled vitellogenesis (Uribe et al., 1996).

Still on the vitellogenesis process, it was found that the organization of the cortical alveoli was different compared to other species. For the four species of the genus *Tropidurus*, the alveoli were randomly distributed in the ooplasm during previtellogenesis. However, during vitellogenesis these alveoli regrouped forming a concentric belt-like structure in the center of the oocyte, dividing the ooplasm into two compartments. Later they filled completely the central portion, increasing in quantity toward the periphery. In other species of lizards, however, the organization of alveoli occurs through its accumulation in the periphery toward the center of the vitellogenic oocyte (Uribe et al., 1995, 1996; Lozano et al., 2014).

Another characteristic that has variation between the previtellogenic and vitellogenic phases was the fiber arrangement in the ooplasm. Initially fibrillary material was homogeneously distributed in the ooplasm. Sequentially, still in previtellogenesis, a thin deposition of fibers was built up below the zona pellucida. Next, a thick layer of fibrillary material was formed below the zona pellucida and was visualized until the end of vitellogenesis. Few studies discuss these characteristics in species of lizards, although Uribe et al. (1996) recognize that the pattern of aggregation and dispersion of the fibers may have a functional role in follicular growth.

After ovulation, the corpus luteum formation consists of an essential process in the ovary of reptiles, mainly because it is associated with hormone regulation (Shanbhag, 2002). In lizards, it was reported the role of luteal cells of the granulosa and theca layer, mostly in the production of steroid hormones (e.g. Fox and Guillette Jr, 1987; Al-Amri et al., 2012). For the species *T. torquatus*, *T. hispidus*, *T. itambere* and *T. oreadicus*, it was identified three stages of the corpus luteum according to its formation, maturation and regression as described by Guraya (1989). These stages have been described for other lizards, as *Sceloporus torquatus* (Weigmann, 1828) (Phrynosomatidae) (Uribe et al., 1995) and *S. grammicus* (Weigmann, 1828) (Lozano et al., 2014), and it is common to most lacertids. However, not all species have the same definition of the involution stages (Fox and Guillette Jr, 1987), which, in turn, depend on the phase of the ovarian cycle.

The morphological evidence of the corpus luteum in the ovaries of the four species of the *Tropidurus torquatus* group, in our study, corroborates the data obtained in other reproductive studies with other populations of these species in Brazil. Thus, *T. torquatus* presented corpus luteum for a longer period, from July to April, months in which it is also able to reproduce (e.g. Wiederhecker et al., 2002; Van Sluys et al., 2010; Gomides et al., 2013). For *T. hispidus*, the presence of corpus luteum was restricted to April and May and corroborates that reported by Ribeiro et al. (2012), in which it is suggested that the reproduction of the females is strongly influenced by the rainfall, being able to reproduce from November to April. *Tropidurus itambere* and *T. oreadicus* presented corpus luteum predominantly from October to December, with reproductive aptitude seasonally and strongly influenced by the rainy season in Brazil (Van Sluys, 1993; Meira et al., 2007; Ferreira et al., 2009, 2011).

As for the atretic follicles, seven stages of involution were identified for the species, which were different in accordance with the modifications observed in the morphology of follicle regression. These modifications included, mainly, invagination of the theca layer toward the oocyte, increase of the granulosa cells, disruption of the zona pellucida, and phagocytosis of the ooplasm. Some studies have documented similar structural changes in the granulosa and theca layers, and in the ooplasm, during the folicular regression, but without any established classification (Uribe et al., 1995; Lozano et al., 2014). Atresia is considered a common process of degeneration in vertebrates ovary (Guraya, 1973), and it may occur in both previtellogenic and vitellogenic follicles (Lozano et al., 2014).

At the present study, atresia was observed in both types of follicles. In the viviparous species *Sceloporus torquatus* (Uribe et al., 1995) and *S. grammicus* (Lozano et al., 2014), it was observed a higher prevalence of atresia in previtellogenic follicles. However, for *T. hispidus* and *T. semitaeniatus,* atresia was only observed in previtellogenic follicles (Santos et al., 2015). According to its occurrence rate, it was observed that atretic oocytes were present in all months of the year for *T. torquatus*, from April to July for *T. hispidus*, from January to August for *T. oreadicus*, and from July to October for *T. itambere*. Taking into account the aspects already known reproduction of these species in Brazil (e.g. Van Sluys, 1993; Wiederhecker et al., 2002;

Meira et al., 2007; Ferreira et al., 2009, 2011; Van Sluys et al., 2010; Ribeiro et al., 2012; Gomides et al., 2013), our data suggest that atresia acts controlling the amount of follicles that would be viable for reproduction, and may occur seasonally for *T. hispidus*, *T. oreadicus* and *T. itambere*, or continuously as for *T. torquatus*.

Finally, this study evidenced that the morphological aspects of the follicular development were similar in the four lizard species of group *torquatus* in Brazil. We highlighted the description of unprecedented data in relation to the duration of the intercellular bridge of pyriform cells with the oocyte, the organization of cortical alveoli and the pattern of aggregation of the cytoplasmic fibers. Furthermore, for the first time, data on the number of germinal bed and frequency of corpus luteum and atresia were related to reproductive aptitude. This relation suggests an intrinsic control of each of these species in clutch size and viable follicles for reproduction.

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FIGURE LEGENDS

Fig. 1. Ovarian morphology and the earliest stages of oocyte follicle development in *Tropidurus torquatus*, *T. hispidus*, *T. itambere* and *T. oreadicus*. A) Histological ovarian organization: the ovary is attached to the dorsal wall of the abdominal cavity by the mesovarium (M) and it is divided in stroma (Str), composed by blood vessels (bv), connective tissue (CT), ovarian follicles (OF) at different stages of development and structures in involution process (Inv), and germinal bed (GB); B) Details on the simple squamous epithelium (SE) covering the ovary and the blood vessels (bv) and connective tissue (CT) of the medullar stroma (Str); C) Germinal bed delimited by a basal membrane (bm) and composed by loose connective tissue (CT), presenting spherical or ovoid oogonia (oo) with a spherical nucleus (n) and an evident nucleolus (nl) and spherical or ovoid stage I oocytes (sI) with a central and spherical nucleus (n), an evident nucleolus (nl), a distinct Balbiani corpuscle (Bc) in its ooplasm and surrounded by few squamous follicular cells (fc); D) Stage II oocytes: show a lightly basophilic ooplasm with large fibers aggregated (circle) at the center of the cell, its nucleus (n) has fibrillary chromatin and an evident nucleolus (nl), the granulosa (GR) is composed by intermediate cells (ic) interspersed with many small cells (sc), there is also the association of the first cells of the theca layer (TH). (A-B) Stained with 5% methylene blue; (C) Stained with PAS + iron hematoxylin + metanil yellow; (D) Stained with 1% toluidine blue.

Fig. 2. Advanced stages of the previtellogenesis phase of the oocyte follicles development in in *Tropidurus torquatus*, *T. hispidus*, *T. itambere* and *T. oreadicus*. A) Stage III oocytes: show nucleus (n) with fibrillary chromatin and the ooplasm has fibers aggregate (circle) more homogeneously distributed, the granulosa layer (GR) consists of small cells (sc), some of them in metaphase (sc-m), and intermediate cells (ic); B) Stage IV oocytes: it has an eccentric nucleus (n) with lampbrush chromosomes, the ooplasm contains fibrillary clumps (circle) and some cortical alveoli (ca),

and the zona pellucida (zp) appears as a single layer, also the granulosa layer (GR) becomes stratified and polymorphic showing three distinct cell types, and theca layer is more developed being divided in external (eTH) and internal theca (iTH); C) Detail of the lampbrush chromosomes (arrowhead) and the numerous nucleolus (nl); D) Emphasis on the zona pellucida (zp) and granulosa layer with three distinct cell types: small cells (sc), intermediate cells (ic), and pyriform cells (pc), the latter showing narrow but long cytoplasmic prolongations (arrow) that apparently join to the zona pellucida; E) Stage V oocytes: it has fibrillary clumps (circle) present more centrally in the oocyte and the thin peripheral ooplasm (po) containing fine basophilic granules, the granulosa layer (GR) has become thicker; F) Below the zona pellucida (zp) it starts a new deposition of fibrillary material (double arrow) and in the granulosa layer there are still small cells (sc), intermediate cells (ic), and pyriform cells (pc). (A) Stained with 1% toluidine blue; (B-E) Stained with 5% methylene blue; (F) Stained with PAS + iron hematoxylin + metanil yellow.

Fig. 3. Vitellogenic phase of the oocyte follicles development in *Tropidurus torquatus*, *T. hispidus*, *T. itambere* and *T. oreadicus*. A) Stage VI oocyte: present largest cortical alveoli (ca) in the center of the follicle and small yolk vesicles (yv) near its periphery; B) Details of the periphery of the oocyte: deposition of fibrous components (double arrow) below the zona pellucida (zp) and the granulosa layer (GR); C) Stage VII oocyte: have large number of yolk vesicles (yv) in the ooplasm, and there is the formation of a belt-like structure (dashed circle) of yolk vesicles associated with cortical alveoli (ca) in the center of the oocyte; D) The zona pellucida (zp) is highly evident and bilayered, and the granulosa layer (GR) is narrower, but pyriform cells (pc) still present cytoplasmic prolongations (arrow) towards the zona pellucida; E) Stage VIII oocyte: the zona pellucida (zp) is tightly defined in two layers, the zona radiata (zr) and the PAS-positive hyaline band (hb), yolk vesicles (yv) occur in almost all ooplasm, and the

granulosa layer (GR) is thinner with cells in a more cubic shape; F) Stage IX oocyte: the granulosa layer (GR) consists of squamous cells, the yolk vesicles (yv) fill all the ooplasm, and the theca layer (TH) is highly vascularized (*). (A-D, F) Stained with 5% methylene blue; (E) Stained with PAS + iron hematoxylin + metanil yellow.

Fig. 4. Involution process of the corpus luteum in *Tropidurus torquatus*, *T. hispidus*, *T. itambere* and *T. oreadicus*. A) In stage I is possible to see the opening (arrow) where the oocyte was ovulated, also there is the formation of a temporary round shape endocrine gland with an outer coating of theca cells (TH) and a central cavity filled with granulosa-lutein cells (GL); B) Details of the granulosa- lutein cells: lightly stained cytoplasm with large amounts of secretory granules (sg) and vacuoles (vc), and nuclei (n) with condensed chromatin; C-D) Stage II is characterized by the thickening of the theca layer (TH), compaction of the central cavity filled by a reduced number of granulosa-lutein cells (GL), and formation of connective tissue septa (arrowhead) that orientates the infiltration of blood vessels (bv) into the central cavity; E) Finally, in stage III, it was observed an increased vascularity (*) throughout the structure, few theca cells (TH), and fibers (fb). (A-C) Stained with 5% methylene blue; (D-E) Stained with PAS + iron hematoxylin + metanil yellow.

Fig. 5. Involution process of the follicular atresia in *Tropidurus torquatus*, *T. hispidus*, *T. itambere* and *T. oreadicus*. A-B) Early stages of atresia are characterized by a gradual disintegration of the zona pellucida (zp), proliferation and hypertrophy of the granulosa cells (GR), and invagination of theca cells towards the oocyte (arrow); B) Emphasis in the proliferation of cells in the granulosa layer (GR) and the disintegration of the zona pellucida

(zp); C) In stage IV, the atresia has fewer ooplasm (oo), granulosa cells (GR) decrease in number, remaining attached to the theca layer (TH), while there is an increase in the number of vacuoles (vc); D) In subsequent stages (V and VI) there is the total phagocytosis of the ooplasm (oo) and few granulosa cells (GR); E) In the final stage, there is a small, round structure with a vacuolated (vc) central portion among which are seen connective tissue septa (*), and the theca layer (TH) becomes extremely thin. (A-B, E) Stained with PAS + iron hematoxylin + metanil yellow; (C-D) Stained with 5% methylene blue.

Fig. 6. Distribution of the sexual maturity of *Tropidurus* species (*torquatus* group) at different populations in Brazil. Populations of *Tropidurus torquatus* in the biomes Cerrado, Amazon and Atlantic Forest (**A**), *Tropidurus hispidus* in Caatinga, Cerrado and Amazon (**B**), *Tropidurus itambere* in Cerrado and Atlantic Forest (**C**) and *Tropidurus oreadicus* in Cerrado and Amazon biomes (**D**)**.** Horizontal axis (X) with Brazilian biomes and the vertical axis (Y) with snout-vent length (SVL) in milliliters.

FIGURE 1

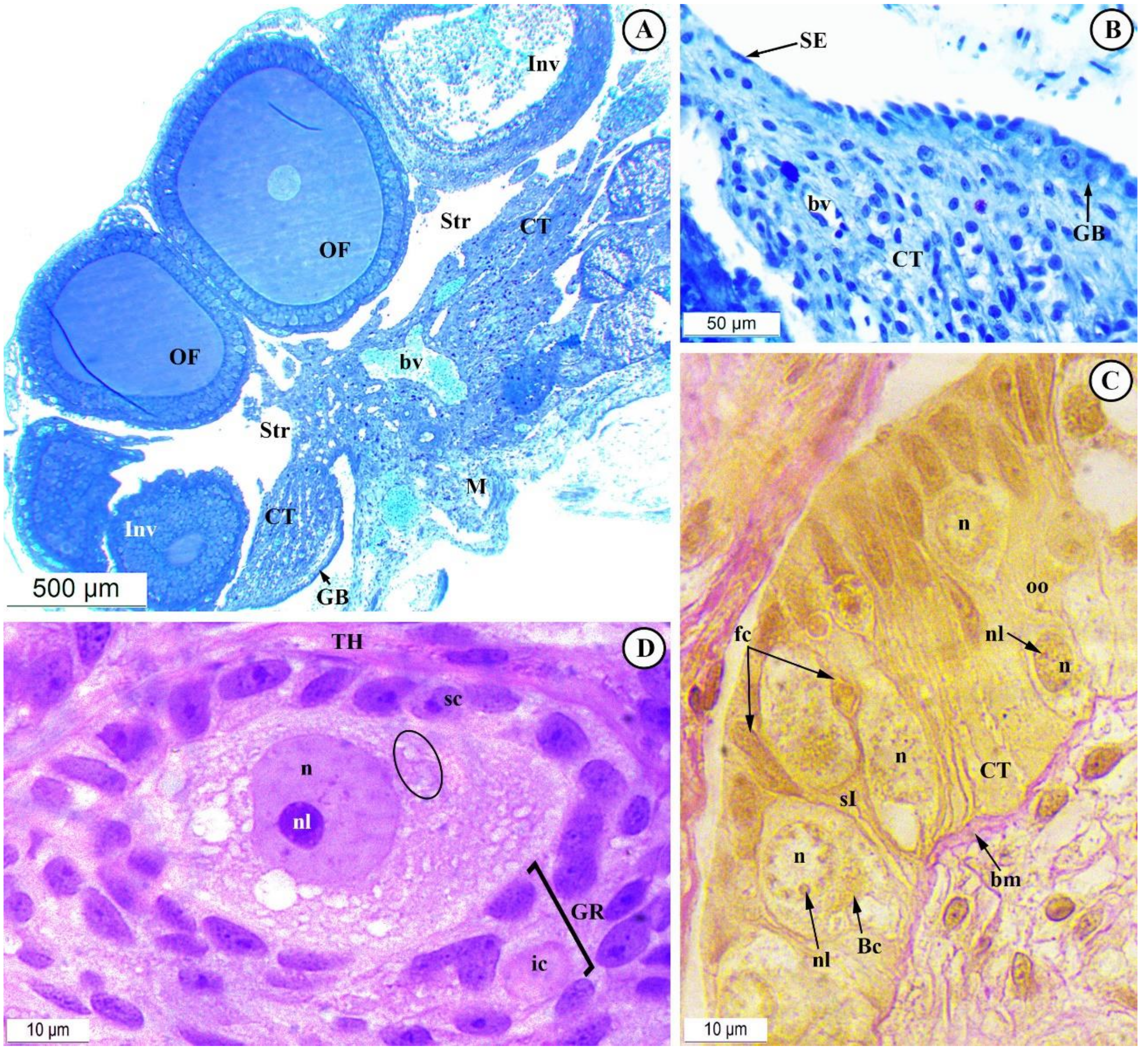
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FIGURE 2

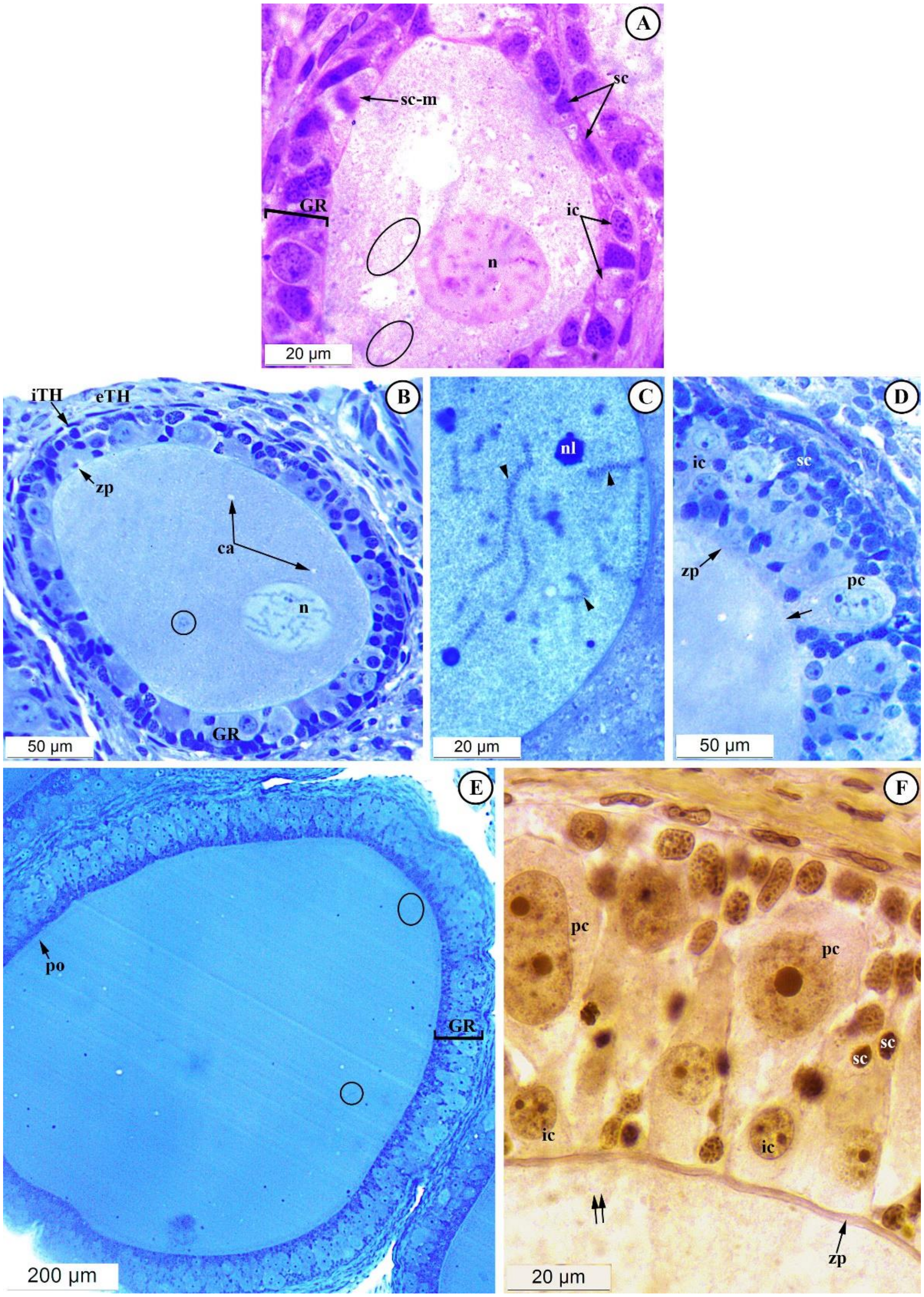
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FIGURE 3

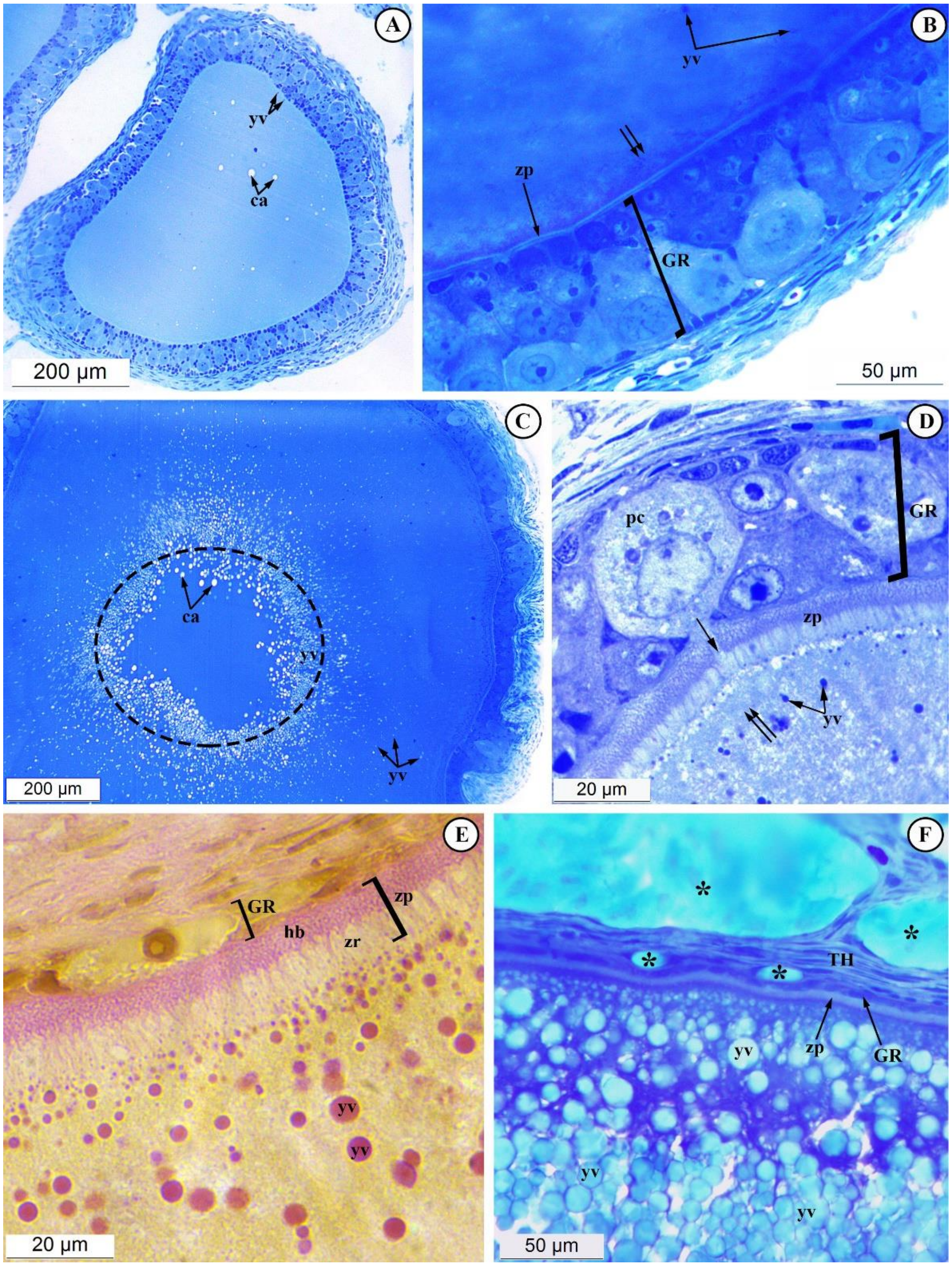
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FIGURE 4

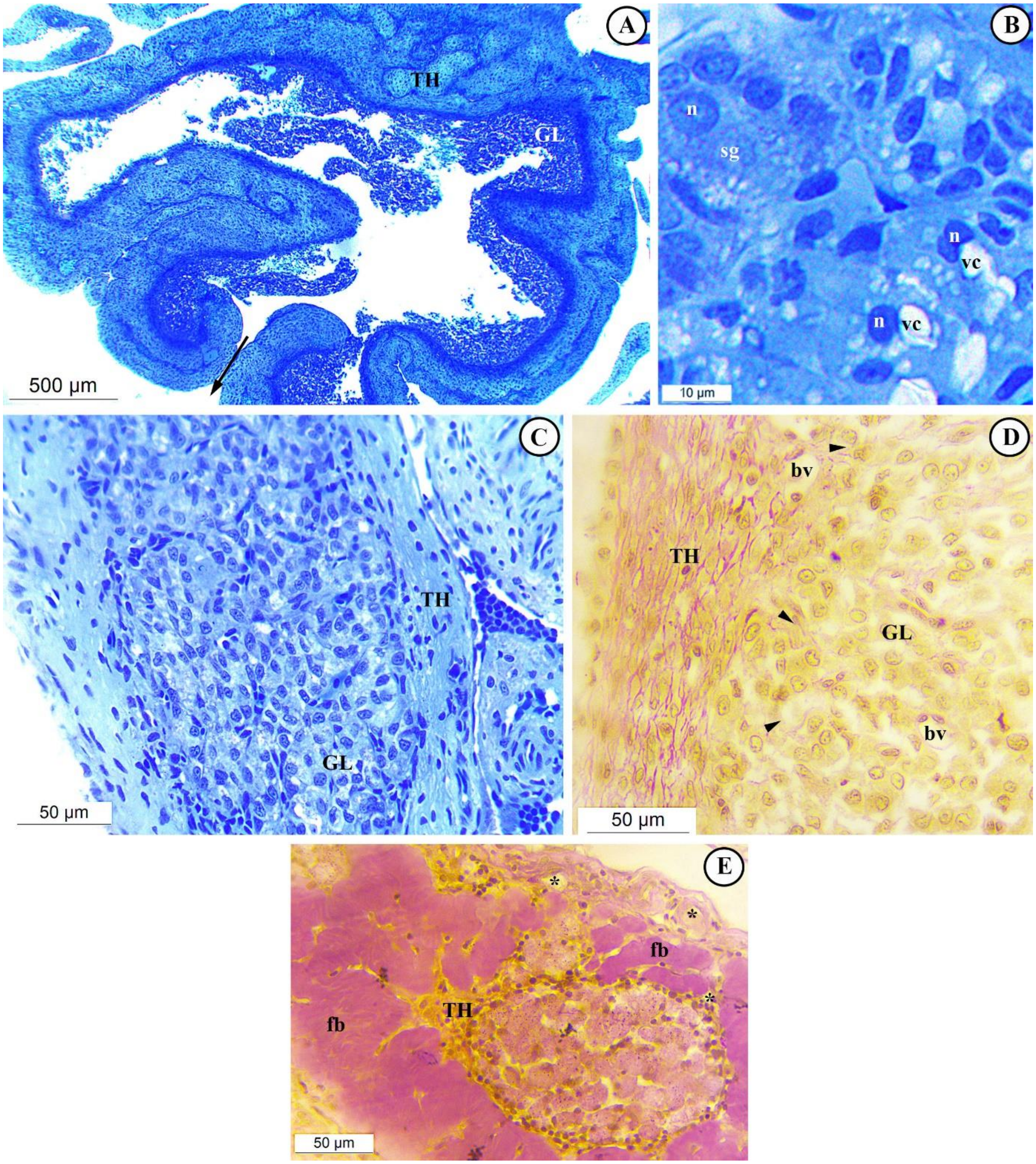
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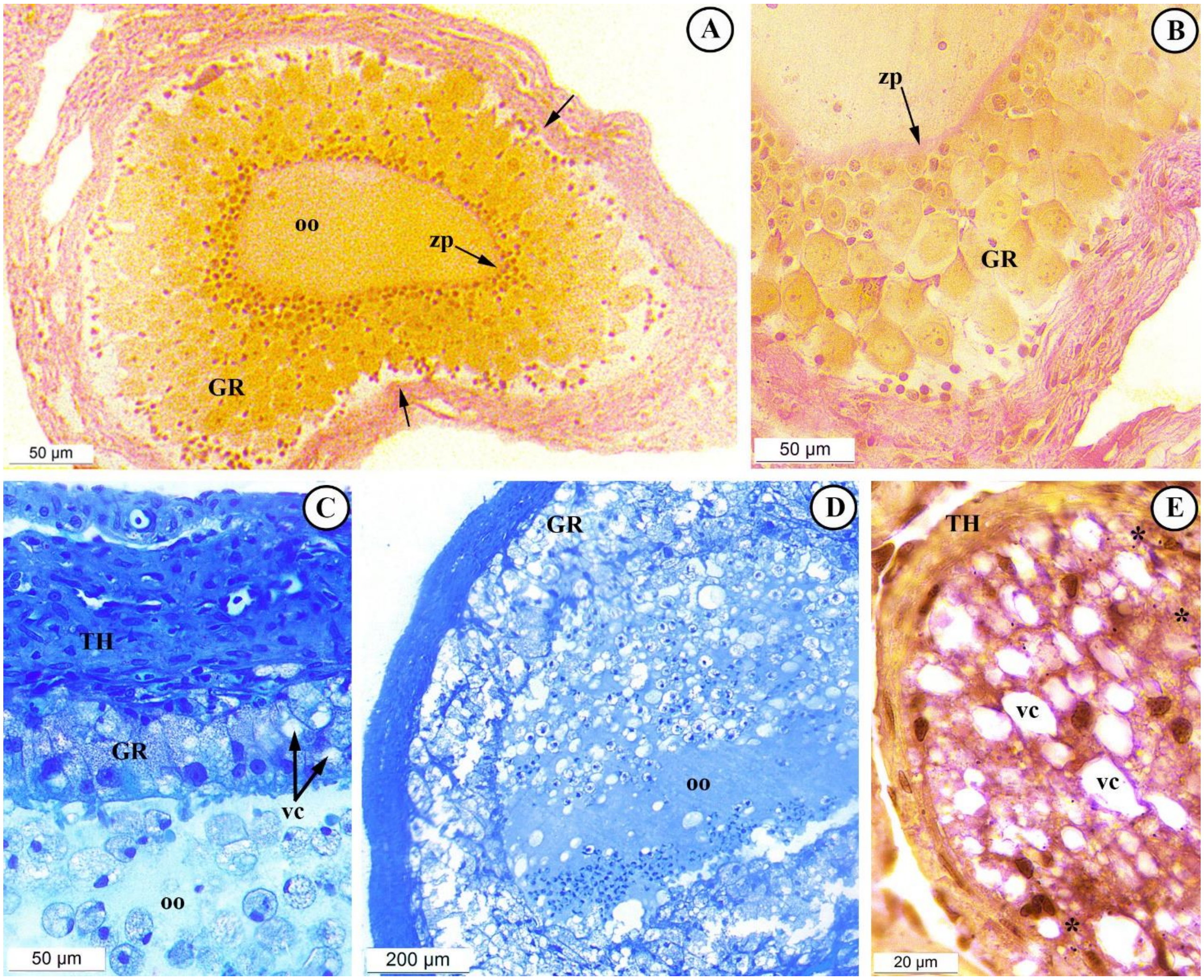
FIGURE 5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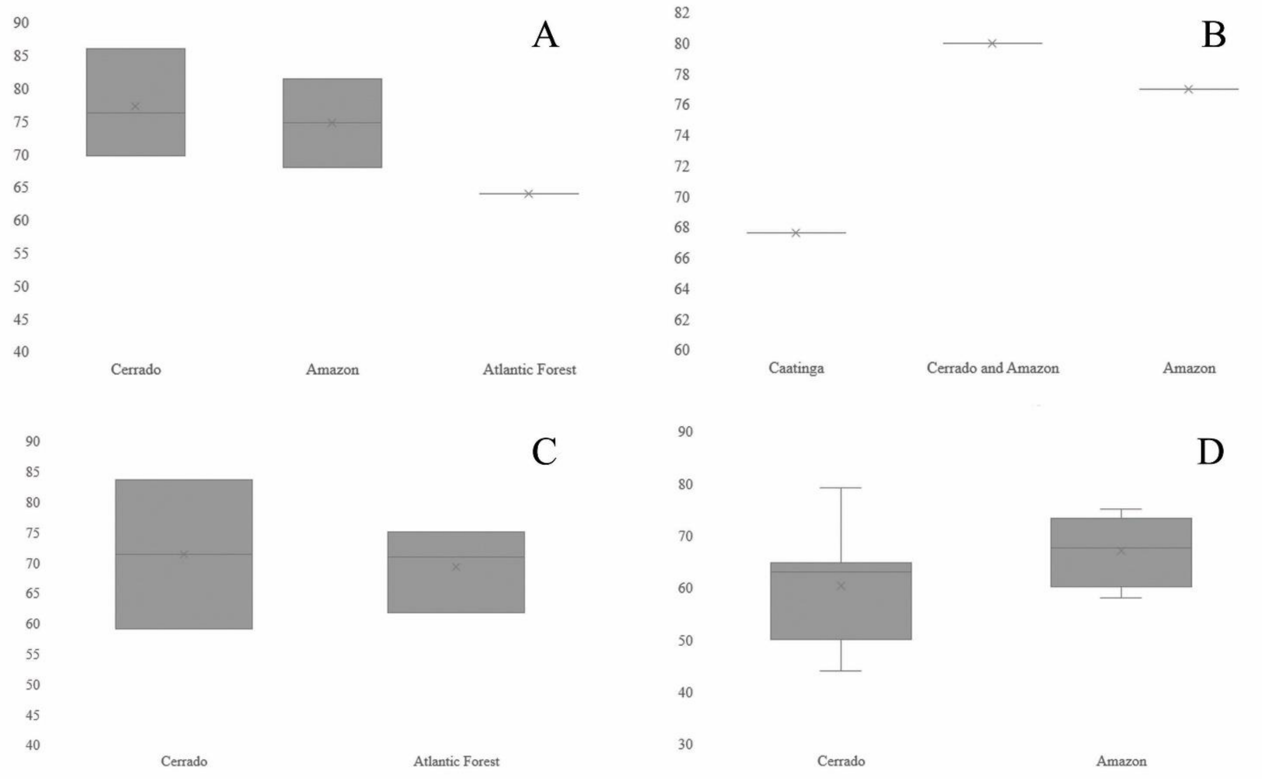
20 µm

FIGURE 6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Cerrado
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Atlantic Forest
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74
72
70
68
66
64
62
60
Caatinga
Cerrado and Amazon
Amazon
C
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85
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75
70
65
60
55
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45
40
Cerrado
Atlantic Forest
D
90
80
70
60
50
40
30
Cerrado
Amazon

Table 1: Female specimens of *Tropidurus* lizards (torquatus group) evaluated in this study. Samples (N) and Voucher numbers. Acronims: **CHUNB** (Coleção Herpetológica da Universidade de Brasília), **INPA-HERPETO** (Coleção de Anfíbios e Répteis do Instituto Nacional de Pesquisas da Amazônia), **MPEG** (Museu Paraense Emílio Goeldi), **UFMT-R** Coleção Zoológica da Universidade Federal de Mato Grosso Setor Reptilia), **ZUEC-REP** (Museu de Zoologia da Universidade Estadual de Campinas "Adão José Cardoso"), **ZUFG** (Coleção Zoológica da Universidade Federal de Goiás).

Species	**Biome**	**Region**	**Locality**	**N**	**Collection date (month/year)**	**Voucher**
Tropidurus torquatus	Cerrado	Centro-oeste	Brasília - DF	1	12/2001	CHUNB 48038
Tropidurus torquatus	Cerrado	Centro-oeste	Cuiabá - MT	74	11-12/2005, 01,03,06,10-11/2006, 01-07/2007, 06-12/2012, 01-05/2013	UFMT-R 2880, 2881, 3040, 3188, 3189, 3551, 3963, 4185, 4257, 4567, 4881, 4890, 4905, 5334, 5468, 5469, 5723, 5724, 5827, 5830, 5911, 11589, 11592, 11595, 11597-11602, 11604, 11607, 11608, 11612, 11613, 11617, 11619, 11621-11623,11628, 11629, 11633, 11634, 11638, 11639, 11645-11648, 11651, 11652, 11654, 11656, 11658, 11660-11662, 11664, 11665, 11667, 11670-11673, 11682-11684, 11688, 11690-11693
Tropidurus torquatus	Cerrado	Centro-oeste	Sítio D' Abadia - GO	2	01/2008	ZUFG 250, 251
Tropidurus torquatus	Cerrado	Norte	Lajeado - TO	1		ZUFG 510
Tropidurus torquatus	Cerrado	Sudeste	Buritizeiro - MG	1	12/2000	CHUNB 44501
Tropidurus torquatus	Cerrado	Sudeste	Unaí - MG	2	09/1997, 04/1999	CHUNB 24706, 32876
Tropidurus torquatus	Amazônia	Norte	Iracema - RR	1	11/2011	INPA-H 31617
Tropidurus torquatus	Amazônia	Norte	Manaus - AM	2	06/2007	INPA-H 19893, 19895
Tropidurus torquatus	Mata Atlântica	Sudeste	Presidente Kennedy - ES	2	09/1997	CHUNB 24985, 24987
Tropidurus hispidus	Caatinga	Nordeste	São José do Ribamar - MA	35	04-05/2007	ZUEC-REP 3195, 3199, 3202, 3203, 3205, 3206, 3213, 3215-3217, 3219, 3221, 3225-3228, 3236, 3241, 3252-3255, 3270-3273, 3275, 3279-3283, 3285, 3286, 3288, 3291
Tropidurus hispidus	Caatinga	Nordeste	Exu - PE	1	07/1993	CHUNB 13465
Tropidurus hispidus	Caatinga	Nordeste	Petrolina - PE	1	09/1991	CHUNB 05832
Tropidurus hispidus	Cerrado e Amazônia	Nordeste	Arari - MA	2	02/1978	MPEG 11540, 11558
Tropidurus hispidus	Amazônia	Nordeste	Santa Luzia do Paruá - MA	4	06/1975, 06/1976	MPEG 10148, 11098, 11128, 11130
Tropidurus hispidus	Amazônia	Norte	Caracaraí - RR	5	07/1993	MPEG 17180, 17206, 17207, 17240, 17251
Tropidurus hispidus	Amazônia	Norte	Manaus - AM	1	07/2008	INPA-H 21223
Tropidurus hispidus	Amazônia	Norte	Marabá - PA	3	05/1969	MPEG 2883, 2885, 2886
Tropidurus itambere	Cerrado	Centro-oeste	Aparecida do Rio Doce - GO	1	12/2006	ZUFG 69

Tropidurus itambere	Cerrado	Centro-oeste	Minaçu - GO	1	05/2008	CHUNB 53175
Tropidurus itambere	Cerrado	Centro-oeste	Pirenópolis - GO	5	07/2000	CHUNB 31676, 31677, 31696, 31731, 31744
Tropidurus itambere	Cerrado	Sudeste	Paracatu - MG	2	10/2001	CHUNB 26898, 26899
Tropidurus itambere	Mata Atlântica	Sudeste	Campinas - SP	5	05/1988, 09/1994, 05,07,10/1995	ZUEC-REP 633, 1795, 1848, 2735, 2736
Tropidurus itambere	Mata Atlântica	Sudeste	Itatiba - SP	2	04/1988	ZUEC-REP 615, 616
Tropidurus itambere	Mata Atlântica	Sudeste	Ourinhos - SP	1	03/2002	ZUEC-REP 2660
Tropidurus itambere	Mata Atlântica	Sudeste	Poços de Caldas - MG	1	08/1998	ZUEC-REP 2163
Tropidurus itambere	Mata Atlântica	Sudeste	Pinheiros - ES	1	07/1974	ZUEC-REP 327
Tropidurus oreadicus	Cerrado	Centro-oeste	Alvorada do Norte - GO	1	03/2004	CHUNB 37605
Tropidurus oreadicus	Cerrado	Centro-oeste	Minaçu - GO	3	06/2001, 12/2002	CHUNB 29640, 48407, 48408
Tropidurus oreadicus	Cerrado	Centro-oeste	Niquelândia - GO	2	01,05/2006	ZUFG 11, 51
Tropidurus oreadicus	Cerrado	Centro-oeste	Pirenópolis - GO	1	07/2000	CHUNB 31702
Tropidurus oreadicus	Cerrado	Centro-oeste	Sítio D' Abadia - GO	1	01/2007	ZUFG 213
Tropidurus oreadicus	Cerrado	Nordeste	Balsas - MA	1	12/2002	CHUNB 43775
Tropidurus oreadicus	Cerrado	Nordeste	Carolina - MA	2	11/2007	CHUNB 52033, 52035
Tropidurus oreadicus	Cerrado	Nordeste	Cocos - BA	5	12/2000, 10/2003, 11/2006, 11/2007	CHUNB 49160, 49163, 50201, 51240, 51489
Tropidurus oreadicus	Cerrado	Norte	Dianópolis - TO	1	09/2003	CHUNB 33306
Tropidurus oreadicus	Cerrado	Norte	Lajeado - TO	6		ZUFG 270, 405, 407, 409, 410, 412
Tropidurus oreadicus	Cerrado	Norte	Mateiros - TO	2	12/1999	CHUNB 24299, 24301
Tropidurus oreadicus	Cerrado	Norte	Palmas - TO	4	12/1999, 07/2001	CHUNB 11302, 24626-24628
Tropidurus oreadicus	Cerrado	Norte	Porto Nacional - TO	1	07/2005	CHUNB 47768
Tropidurus oreadicus	Amazônia	Norte	Macapá - AP	1	09/2001	CHUNB 06467
Tropidurus oreadicus	Amazônia	Norte	Marabá - PA	1	07/1984	MPEG 13690
Tropidurus oreadicus	Amazônia	Norte	Parauapebas - PA	3	07/1986, 07/1992	CHUNB 05706, 06356, 06366
Tropidurus oreadicus	Amazônia	Norte	Porto Velho - RO	9	05,11/2011, 03/2013	INPA-H 32943, 32945, 32947, 32948, 32951, 33015, 33018, 33019, 33023

Table 2: Body length and morphometry of the ovarian follicles of four *Tropidurus* species of *torquatus* group. Average data (± standard deviations) of the snout-vent length (SVL) in millimiters, follicular diameter (FD) in µm, thickness of the granulosa cell layer (TG) in µm, thickness of the zona pellucida (TZ) in µm are presented by species according to the stages of follicular development.

Species	**SVL**	**Structure**	**Oogonia**	**Previtellogenic stages**					**Vitellogenic stages**			
				I	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**
Tropidurus hispidus (*n* = 27)	70.38 (± 9.82)	FD	17.66 (± 3.49)	22.16 (± 5.82)	76.42 (± 38.57)	197.67 (± 16.45)	436.61 (± 155.62)	851.91 (± 252.81)	1078.52 (± 236.30)	1418.99 (± 428.08)	1963.03 (± 788.80)	2799.94 (± 157.98)
		TG	-	-	8.00 (± 6.51)	20.81 (± 5.77)	43.29 (± 18.06)	56.69 (± 22.63)	57.38 (± 16.56)	48.60 (± 9.34)	27.53 (± 2.86)	2.55 (± 0.44)
		TZ	-	-	-	-	0.60 (± 0.37)	1.32 (± 0.94)	2.32 (± 0.78)	3.53 (± 1.57)	6.98 (± 0.85)	4.02 (± 1.30)
		TT	-	-	6.74 (± 3.65)	24.13 (± 11.07)	30.70 (± 12.66)	47.54 (± 26.98)	48.12 (± 19.17)	47.71 (± 9.92)	50.05 (± 4.85)	33.67 (± 3.31)
Tropidurus oreadicus (*n* = 35)	59.90 (± 11.02)	FD	17.34 (± 4.51)	19.06 (± 4.33)	81.06 (± 15.91)	163.08 (± 32.20)	367.73 (± 108.58)	680.83 (± 170.71)	1020.04 (± 313.38)	1521.56 (± 363.34)	2137.89 (± 699.39)	2848.58 (± 431.09)
		TG	-	-	9.98 (± 7.26)	38.83 (± 5.37)	40.65 (± 13.93)	59.88 (± 32.66)	56.49 (± 10.16)	56.58 (± 15.77)	41.40 (± 23.98)	7.60 (± 7.59)
		TZ	-	-	-	-	1.03 (± 0.37)	1.74 (± 0.62)	2.24 (± 1.27)	4.52 (± 1.44)	4.69 (± 1.45)	3.60 (± 2.02)
		TT	-	-	6.06 (± 5.55)	21.64 (± 1.66)	29.91 (± 12.20)	37.40 (± 15.73)	41.46 (± 13.38)	52.71 (± 22.15)	60.45 (± 34.03)	45.80 (± 23.31)
Tropidurus itambere (*n* = 10)	66.20 (± 13.28)	FD	13.40 (± 2.29)	19.06 (± 3.56)	-	-	323.19 (± 116.90)	941.79 (± 397.99)	1041.81 (± 170.90)	1731.16 (± 317.04)	2362.10 (± 319.79)	2831.74 (± 230.46)
		TG	-	-	-	-	40.38 (± 10.48)	83.95 (± 33.96)	64.42 (± 15.04)	74.75 (± 16.04)	32.77 (± 5.07)	6.98 (± 3.21)

		TZ	-	-	-	-	0.90 (± 0.47)	2.41 (± 1.13)	4.27 (± 1.63)	5.10 (± 1.85)	6.58 (± 1.63)	7.63 (± 3.16)
		TT	-	-	-	-	38.04 (± 9.51)	59.46 (± 19.65)	46.64 (± 16.09)	45.63 (± 14.13)	82.72 (± 34.90)	51.19 (± 0.08)
Tropidurus torquatus (*n* = 46)	88.87 (± 15.65)	FD	18.36 (± 7.10)	19.66 (± 14.36)	102.12 (± 32.46)	205.49 (± 65.80)	426.38 (± 253.31)	727.79 (± 385.05)	916.77 (± 473.04)	1211.77 (± 679.12)	1921.71 (± 860.00)	3443.88 (± 1245.23)
		TG	-	-	14.15 (± 7.56)	32.47 (± 14.58)	40.90 (± 16.59)	55.33 (± 26.22)	57.76 (± 25.49)	45.16 (± 27.06)	19.58 (± 14.32)	5.45 (± 3.63)
		TZ	-	-	-	-	1.04 (± 0.73)	1.81 (± 1.00)	2.70 (± 1.70)	2.83 (± 2.03)	3.50 (± 2.24)	4.22 (± 1.59)
		TT	-	-	7.39 (± 4.06)	28.23 (± 12.10)	41.47 (± 16.00)	51.81 (± 34.93)	52.89 (± 31.82)	50.51 (± 35.39)	53.33 (± 39.59)	49.46 (± 28.58)

Table 3: Mean snout-vent lenght and range of (SVL), minimum size at sexual maturity and reproductive aptitude of females *Tropidurus* species (*torquatus* group) evaluated in this study. Samples (N), reproductive individuals (*N) and non-reproductive (nr) individuals

Species	**Locality**	**N(N*)**	**SVL**		**Sexual Maturity**	**Clutch Size**		**Structure Observed**	**Reproductive Aptitude**
			Mean (SD)	**Range**		**Mean (SD)**	**Range**		
Tropidurus torquatus	Brasília - DF	1 (0)	81 (0.0)	81	nr	0	0	no data	no data
Tropidurus torquatus	Cuiabá - MT	74 (44)	90.38 (12.9)	53.07 - 154.57	69,68	5.06 (2.25)	Eggs (4-6)/Fol (2-11)	Eggs and Folicle	August – March
Tropidurus torquatus	Sítio D' Abadia - GO	2 (1)	79.95 (5.33)	76.18 - 83.72	76,18	6 (0.0)	Eggs(0-6)/Fol(0)	Eggs and Folicle	January
Tropidurus torquatus	Lajeado - TO	1 (0)	82.79 (0.0)	82,79	nr	0	0	no data	no data
Tropidurus torquatus	Buritizeiro - MG	1 (1)	86 (0.0)	86	86	0	0	Mature Folicle	December
Tropidurus torquatus	Unaí - MG	2 (1)	70 (0.0)	70	nr	0	0	Previtellogenic Folicle	September
Tropidurus torquatus	Iracema - RR	1 (1)	81.34 (0.0)	81,34	81,34	6 (0.0)	Eggs(0)/Fol(0-6)	Eggs and Folicle	November
Tropidurus torquatus	Manaus - AM	2 (2)	69.41 (1.98)	68.01 -70.02	68,01	4 (0.0)	Eggs(0)/Fol (0-4)	Eggs and Folicle	June
Tropidurus torquatus	Presidente Kennedy - ES	2 (1)	64	64	64	no data	no data	Mature Folicle and Corpus Luteum	September
Tropidurus hispidus	São José do Ribamar - MA	35 (4)	66.85 (6.58)	44.75 - 78.02	67,59	3.5 (1.73)	Eggs (0)/Fol (1-5)	Eggs and Folicle	April – May
Tropidurus hispidus	Exu - PE	1 (0)	77 (0.0)	77	nr	0	0	Previtellogenic Folicle	no data
Tropidurus hispidus	Petrolina - PE	1 (0)	86 (0.0)	86	nr	0	0	Previtellogenic Folicle	no data
Tropidurus hispidus	Arari - MA	2 (1)	82 (2.83)	80 - 84	80	no data	no data	Oviductal eggs	February
Tropidurus hispidus	Santa Luzia do Paruá - MA	4 (0)	74.75 (4.72)	68 - 78	nr	0	0	Oogonia	no data
Tropidurus hispidus	Caracaraí - RR	5 (1)	72.8 (14.06)	56 - 94	77	no data	no data	Oviductal eggs	July
Tropidurus hispidus	Manaus - AM	1 (0)	79.35 (0.0)	79,35	nr	0	0	no data	no data
Tropidurus hispidus	Marabá - PA	3 (0)	61.33 (10.01)	50 - 69	nr	0	0	no data	no data
Tropidurus itambere	Aparecida do Rio Doce - GO	1 (1)	83.59 (0.0)	83,59	83,59	8 (0.0)	Eggs (0)/Fol(0-8)	Mature Folicle	December
Tropidurus itambere	Minaçu - GO	1 (0)	45 (0.0)	45	nr	0	0	Previtellogenic Folicle	no data
Tropidurus itambere	Pirenópolis - GO	5 (0)	52.8 (7.39)	41 - 61	nr	0	0	no data	no data
Tropidurus itambere	Paracatu - MG	2 (1)	63 (5.66)	59 - 67	59	no data	no data	Mature Folicle and Corpus Luteum	October
Tropidurus itambere	Campinas - SP	5 (3)	69.07 (12.41)	54.96 - 81.43	75,11	5.66 (0.57)	Eggs (0-5)/ Fol(0-6)	Eggs and Folicle	September – October
Tropidurus itambere	Itatiba - SP	2 (0)	74.98 (5.77)	70.9 -79.07	nr	0	0	no data	no data

Tropidurus itambere	Ourinhos - SP	1 (0)	73.79 (0.0)	73,79	nr	0	0	no data	no data
Tropidurus itambere	Poços de Caldas - MG	1 (0)	61.7 (0.0)	61,7	61,7	no data	no data	Vitellogenic and Atresic Folicle	August
Tropidurus itambere	Pinheiros - ES	1 (1)	70.96 (0.0)	70,96	70,96	4 (0.0)	Eggs(0)/Fol(0-4)	Mature Folicle	July
Tropidurus oreadicus	Alvorada do Norte - GO	1 (0)	47 (0.0)	47	nr	0	0	Previtellogenic and Atresic Folicle	no data
Tropidurus oreadicus	Minaçu - GO	3 (1)	61 (16.09)	48 - 79	56	no data	no data	Vitellogenic and Atresic Folicle	June
Tropidurus oreadicus	Niquelândia - GO	2 (1)	72.91 (3.21)	70.64-75.19	75,19	no data	no data	Atresic and Previtellogenic Folicle, Corpus Luteum, and Oogonia	May
Tropidurus oreadicus	Pirenópolis - GO	1 (1)	64 (0.0)	64	64	no data	no data	Mature Folicle	July
Tropidurus oreadicus	Sítio D' Abadia - GO	1 (1)	58.84 (0.0)	58,84	58,84	no data	no data	Vitellogenic and Atresic Folicle	January
Tropidurus oreadicus	Balsas - MA	1 (1)	63 (0.0)	63	63	no data	no data	Mature Folicle	December
Tropidurus oreadicus	Carolina - MA	2 (2)	65.5 (0.71)	65 - 66	65	no data	no data	Eggs	November
Tropidurus oreadicus	Cocos - BA	5 (5)	66.6 (4.98)	63 - 75	63	no data	no data	Eggs	October– December
Tropidurus oreadicus	Dianópolis - TO	1 (1)	63 (0.0)	63	63	no data	no data	Eggs	September
Tropidurus oreadicus	Lajeado - TO	6 (2)	77.26 (2.67)	72.33 - 79.82	79,15	4.0 (0.0)	Eggs(0-4)/Fol(0-1)	Eggs and Folicle	no data
Tropidurus oreadicus	Mateiros - TO	2 (1)	42 (4.24)	39 - 45	45	no data	no data	Previtellogenic and Vitellogenic Folicle	December
Tropidurus oreadicus	Palmas - TO	4 (2)	50.25 (6.07)	45 - 59	48	no data	no data	Previtellogenic and Vitellogenic Folicle	July and December
Tropidurus oreadicus	Porto Nacional - TO	1 (1)	44 (0.0)	44	44	no data	no data	Vitellogenic and Atresic Folicle	July
Tropidurus oreadicus	Macapá - AP	1 (1)	75 (0.0)	75	75	no data	no data	Vitellogenic Folicle	September
Tropidurus oreadicus	Marabá - PA	1 (1)	67 (0.0)	67	67	no data	no data	Vitellogenic Folicle	July
Tropidurus oreadicus	Parauapebas - PA	3 (1)	58.33 (2.52)	58 - 61	58	no data	no data	Previtellogenic, Vitellogenic, and Atresic Folicle	July
Tropidurus oreadicus	Porto Velho - RO	9 (7)	71.93 (6.84)	56.62 - 80.39	68,28	4.14 (1.34)	Eggs (0)/Fol (2-6)	Vitellogenic, Mature and Atresic Folicle	March, May and November

6. CONCLUSÕES

As espécies de *Tropidurus* do grupo *torquatus* (*Tropidurus toquatus*, *T. hispidus*, *T. itambere* and *T. oreadicus*) apresentaram células germinativas bem conservadas no seu aspecto morfológico. Espermatogênese apresentou quatro fases (Quiescência-Proliferação-Espermiação-Regressão), porém com padrão de espermiação extendido para as espécies *T. torquatus* e *T. hispidus* e sazonal para as espécies *T. itambere* e *T. oreadicus*. O epitélio e atividade secretora do ducto epididmal variou durante as diferentes fases da espermatogênese, sendo associado a maturação e retenção do espermatozoide durante a regressão testicular.

O ovário foi constituído por uma cama germiantiva e o estroma sujacente, onde folículos em vários estágios de desenvolvimento com um arranjo hierárquico. O desenvolvimento folicular apresentou duas fases, iniciando com a pre-vitelogênese, onde os folículos adquiriram características de epitélio folicular estratificado e multicelular. A vitelogênese foi o estágio subsequente caracterizado pela crescente incorporação de vitelo ao folículo, regressão do epitélio folicular para a forma pavimentosa e unicelular. Apenas a espessura da camada granulosa no final da vitelogênese apresentou diferenças significativas entra as espécies. Ovulação foi caracterizada pela presença de folículo pós-ovulatório com a teca associada ao restante do epitélio folicular. Três fases foram identificadas de acordo com o aspecto morfológico do corpo lúteo correspondendo a: luteogênese, maturidade luteal e regressão. A atresia foi detalhada em suas modificações estruturais durante a regressão folicular, sendo importante componente de regulação da quantidade de folículos disponíveis para maturação durante o período reprodutivo, com ocorrencia frequente nas quatro espécies do grupo *torquatus*.

Maturidade sexual foi um importante aspecto avaliado na aptidão reprodutiva, sendo evidenciado baixa variação nessa medida entre as espécies de *Tropidurus* nesse estudo. Porém, quando avaliado entre populações de diferentes biomas apresentou diferenças sutis, sendo importante aspecto relacionado a variação do tamanho de ninhada e a estratégia reprodutiva e comportamental desse grupo de espécies.

A aptidão reprodutiva nos machos das quatro espécies do grupo *torquatus* foi relacionada a retenção do espermatozoide no ducto epididmal, evidenciando seu importante papel na maturação e viabilidade para a reprodução por maior tempo. A espermiação foi a fase mais extensa da espermatogênese, ocorrendo na maioria dos meses para as espécies *T. torquatus* e *T. hispidus*. Porém, para *T. itambere* e *T. oreadicus* a espermiação foi concentrada em um período específico, sendo provavelmente relacionado com as condições ideais para a reprodução das fêmeas, as quais apresentaram reprodução sazonal. Para as fêmeas a aptidão apresentou variação na produção de folículos e ovos ao longo dos meses, sendo a produção prolongada para *T. torquatus* e sazonal para as espécies *T. hispidus*, *T. itambere* e *T. oreadicus*. Conclui-se que nos aspectos da gametogênese, maturidade sexual e tamanho de ninhada, as quatro espécies do grupo *torquatus* apresentaram características altamente conservada, porém com variação da aptidão reprodutiva em nível intra e interespecíficos, o que se traduz em diferentes estratégias reprodutivas utilizada pelo gênero *Tropidurus* no Brasil.

7. ANEXOS

DECLARAÇÃO

As cópias de artigos de minha autoria ou de minha coautoria, já publicados ou submetidos para a publicação em revistas científicas ou anais de congressos sujeitos a arbitragem, que constam da minha Tese de Doutorado, intitulada **“APTIDÃO REPRODUTIVA DE QUATRO ESPÉCIES DE LAGARTOS DO GÊNERO TROPIDURUS DO GRUPO TORQUATUS (TROPIDURIDAE) NO BRASIL”**, não infringem os dispostos da Lei nº 9.610/98, nem o direito autoral de qualquer editora.

Campinas, 11 de Setembro de 2017.

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Em observância ao **§5º do Artigo 1º da Informação CCPG-UNICAMP/001/15**, referente a Bioética e Biossegurança, declaro que o conteúdo de minha Tese de Doutorado, intitulada "***APITIDÃO REPRODUTIVA DE QUATRO ESPÉCIES DE LAGARTOS DO GÊNERO TROPIDURUS DO GRUPO TORQUATUS (TROPIDURIDAE) NO BRASIL***", desenvolvida no Programa de Pós-Graduação em Biologia Celular e Estrutural do Instituto de Biologia da Unicamp, não versa sobre pesquisa envolvendo seres humanos, animais ou temas afetos a Biossegurança.

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