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Núcleo de Ciências Agrárias e Desenvolvimento Rural
Empresa Brasileira de Pesquisa Agropecuária - Embrapa Amazônia Oriental
Universidade Federal Rural da Amazônia
Programa de Pós-Graduação em Ciência Animal

Gerson Paulino Lopes

Sistema reprodutor feminino de três espécies do gênero *Saimiri* Voigt, 1831 (Primates: Cebidae): Observações macroscópicas e histológicas

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Área de concentração: Produção Animal

Orientador: Dra. Sheyla Farhayldes Souza Domingues
Coorientador: Dr. Helder Lima de Queiroz

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Banca Examinadora

Profa. Dra. Sheyla Farhayldes Souza Domingues (Orientadora)
Universidade Federal do Pará

Profa. Dra. Simone do Socorro Damasceno Santos (Membro Titular)
Universidade Federal do Pará

Profa. Dra. Rossineide Martins da Rocha - (Membro Titular)
Universidade Federal do Pará

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A origem do processo sexual continua a ser um dos problemas mais difíceis em biologia. Não posso tentar encontrar uma resposta para ele nesse contexto, mas posso explicar em que reside a dificuldade […] A recombinação genética expande enormemente as possibilidades de modificação evolutiva. Mas essa é uma vantagem prospectiva em longo prazo e não imediata. A seleção natural não possui capacidade de antevisão. Uma característica não é selecionada apenas por vir a ter efeitos benéficos em determinado momento futuro. São apenas as vantagens atuais que contam.

John Maynard Smith

RESUMO

Os primatas platirrinos apresentam variação em seus aspectos reprodutivos, suas estratégias, comportamentos, fisiologia e morfoanatomia. Algumas dessas variações podem ser consequências da coevolução dos elementos constituintes da genitália de ambos os sexos, por meio da seleção sexual. Diferenças morfológicas podem representar alto grau de especialização dos órgãos genitais intraespecíficos, o que acarreta em um mecanismo do tipo “chave-fechadura”, que pode constituir um dos mecanismos de isolamento copulatório e reprodutivo, com implicações nos processos de especiação. Nas fêmeas, barreiras anatômicas e fisiológicas dentro da vagina, cérvice, útero, junção útero-tubárica e tubas uterinas podem ser obstáculos aos gametas masculinos em direção à fecundação e, podem influenciar o resultado de uma potencial competição espermática. Em primatas neotropicais, algumas descrições morfológicas apontaram diversas semelhanças e diferenças entre os componentes do sistema reprodutor feminino. Em primatas do gênero *Saimiri* foram realizadas descrições anatômicas para *Saimiri sciureus*, *Saimiri oerstedii* e *Saimiri collinsi*. Recentemente, formas anteriormente identificadas como *S. sciureus* e consideradas como subespécies desta, foram elevados ao nível de espécie. Entre eles estão *Saimiri macrodon* e *Saimiri cassiquiarensis.* Estas espécies apresentam ampla distribuição na Amazônia e ocorrem em peripatria com *Saimiri vanzolinii* na Reserva Mamirauá, Amazônia Central. Os limites da distribuição geográfica das três espécies são bem delineados, mas os mecanismos que promovem o isolamento reprodutivo ainda não estão esclarecidos. A possibilidade de existência de híbridos e a invasão progressiva de *S. cassiquiarensis* na área de ocorrência de *S. vanzolinii* são motivos de preocupação. Alguns grupos mistos foram observados na zona de contato entre *S. vanzolinii* e *S. cassiquiarensis*. A ausência de barreiras geográficas demanda que segregação entre estas espécies seja elucidada por outros parâmetros, como o isolamento reprodutivo pela incompatibilidade morfológica. Nesse contexto, descrevemos o sistema reprodutor feminino das referidas espécies a fim de avaliar a possibilidade de que a morfologia genital constitua um mecanismo de isolamento reprodutivo entre elas, através da descrição anatômica, topográfica e histológica da genitália externa e dos órgãos internos do sistema reprodutor feminino. Verificamos que essas espécies compartilham muitas semelhanças na maioria dos órgãos analisados. Embora tenham sido identificadas algumas diferenças importantes que podem desempenhar um papel relevante na evolução dos componentes do sistema reprodutivo dessas espécies, essas diferenças não são suficientes para compor um mecanismo de isolamento reprodutivo para estas três espécies de *Saimiri*. Nossas descrições fornecem informações importantes que podem auxiliar na construção de estratégias de conservação para estas e outras espécies do gênero *Saimiri*. Bem como subsidiar o desenvolvimento de biotecnologias da reprodução, especialmente espécies ameaçadas, como é o caso de *S. vanzolinii* e, elucidar questões sobre aspectos evolutivos dos componentes do sistema reprodutor destas espécies e de outros primatas.

Palavras-chave: Primatas. biologia reprodutiva. morfologia.

ABSTRACT

The platyrrhine primates show variation in their reproductive aspects, their strategies, behaviors, physiology and morphoanatomy. Some of these variations may be consequences of the coevolution of the constituent elements of the genitalia of both sexes through sexual selection. Morphological differences may represent a high degree of specialization of the intraspecific genital organs, which entails a "lock-key" mechanism, which may constitute one of the mechanisms of copulatory and reproductive isolation, with implications in the speciation processes. In females, anatomical and physiological barriers within the vagina, cervix, uterus, uterus-tubal junction, and uterine tubes may be obstacles to male gametes towards fertilization and may influence the outcome of potential sperm competition. In neotropical primates, some morphological descriptions pointed out several similarities and differences between the components of the female reproductive system. *Saimiri sciureus*, *Saimiri oerstedii* and *Saimiri collinsi* were described in primates of the *Saimiri* genus. Recently, previously identified forms as *S. sciureus* and considered as subspecies of this, were elevated to the level of species. Among them are *Saimiri macrodon* and *Saimiri cassiquiarensis*. These species are widely distributed in the Amazon and occur in peripatria with *Saimiri vanzolinii* in the Mamirauá Reserve, Central Amazonia. The limits of the geographical distribution of the three species are well delineated, but the mechanisms that promote the reproductive isolation are still not clear. The possibility of hybrids and the progressive invasion of *S. cassiquiarensis* in the area of *S. vanzolinii* occurrence are of concern. Some mixed groups were observed in the contact zone between *S. vanzolinii* and *S. cassiquiarensis*. The absence of geographical barriers requires that segregation between these species be elucidated by other parameters, such as reproductive isolation due to morphological incompatibility. In this context, we describe the female reproductive system of these species in order to evaluate the possibility that genital morphology constitutes a mechanism of reproductive isolation between them, through the anatomical, topographic and histological description of the external genitalia and internal organs of the female reproductive system. We found that these species share many similarities in most of the organs analyzed. Although some important differences have been identified that may play a relevant role in the evolution of the components of the reproductive system of these species, these differences are not enough to compose a mechanism of reproductive isolation for these three species of *Saimiri*. Our descriptions provide important information that can assist in the construction of conservation strategies for these and other species of the *Saimiri* genus. As well as subsidizing the development of reproductive biotechnologies, especially endangered species such as *S. vanzolinii*, and elucidating questions about evolutionary aspects of the components of the reproductive system of these species and other primates.

Key-words: Primates. reproductive biology. morphology.

SUMÁRIO

1 INTRODUÇÃO GERAL

Os primatas platirrinos apresentam variação em seus aspectos reprodutivos, suas estratégias, comportamentos, fisiologia e morfoanatomia (CAMPBELL, 1972; EMLEN & ORING, 1977; BRADBURY & ANDERSSON, 1987; ANDERSSON, 1994). Algumas dessas variações podem ser consequências da coevolução dos elementos constituintes da genitália de ambos os sexos, por meio da seleção sexual (DARWIN, 1871; 1876; EBERHARD, 1985; DIXSON & MUNDY, 1994; DIXSON, 1998; DIXSON & ANDERSON, 2001; DIXSON, 2012).

Diferenças morfológicas podem representar alto grau de especialização dos órgãos genitais intraespecíficos, o que acarreta em um mecanismo do tipo "chave-fechadura", que pode constituir um dos mecanismos de isolamento copulatório e reprodutivo, com implicações nos processos de especiação (FOODEN, 1967; HERSHKOVITZ, 1977; HOSKEN & STOCKLEY, 2004). Nas fêmeas, barreiras anatômicas e fisiológicas dentro da vagina, cérvice, útero, junção útero-tubárica e tubas uterinas podem ser obstáculos aos gametas masculinos em direção à fecundação e, podem influenciar o resultado de uma potencial competição espermática (EBERHARD, 1985; DIXSON & ANDERSON, 2001).

Em primatas neotropicais, algumas descrições morfológicas apontaram diversas semelhanças e diferenças entre os componentes do sistema reprodutor feminino. Na família Cebidae Bonaparte, 1831, descrições sobre o gênero *Saimiri* Voigt, 1831 foram realizadas para *Saimiri sciureus* (Linnaeus, 1758) (MARTIN, 1833; VALLOIS, 1912; POCOCK 1920; WISLOCKI, 1932; SANDERSON, 1949; HILL, 1960; COLBORN et al., 1967; HERTIG, et al., 1971; HAFEZ & JASZCZAK, 1972), *Saimiri oerstedii* (Reinhardt, 1872) (WISLOCKI, 1936) e *Saimiri collinsi* Osgood, 1916 (BRANCO et al., 2010).

Embora a maioria dos estudos publicados refira-se à espécie *S. sciureus*, estes podem não ter sidos realizados com a referida espécie, pois os autores não fazem referência à procedência dos animais. Dessa forma, os espécimes foram inconsistentemente identificados como *S. sciureus*, sem qualquer designação subespecífica (BOINSKI & CROPP, 1999), ou não puderam ser identificados posteriormente através de dados genéticos (LAVERGNE et al., 2003).

Diversos grupos de animais alocados em zoológicos e instituições de pesquisas são de origem híbrida comprovada (ARIGA et al., 1978; FOGLE, 1990; SCHREIBER et al., 1998; BOINSKI & CROPP, 1999; LAVERGNE et al., 2003). Embora alguns estudos que utilizaram

PCR tenham sidos desenvolvidos para distinguir as espécies de *Saimiri* em cativeiro (VANDEBERG, et al., 1990; SCHREIBER et al., 1998; LAVERGNE et al., 2003; OSTERHOLZ, et al., 2008), eles só foram implementados por pesquisadores que já estavam focados em investigações em sistemática molecular e filogenia, e não para solucionar as dúvidas sobre a correta identificação dos animais usados como modelos de pesquisas em cativeiro (WARD & VALLENDER, 2012). A correta identificação da origem e do patrimônio genético dos espécimes, antes de serem utilizados em estudos no cativeiro ou em laboratório, é de grande importância para interpretação e divulgação dos resultados (WARD & VALLENDER, 2012).

Recentemente, formas anteriormente identificadas como *S. sciureus* e consideradas como subespécies desta, foram elevados ao nível de espécie. Entre eles estão *Saimiri macrodon* (Elliot, 1907) e *Saimiri cassiquiarensis* (Lesson, 1840) (CARRETERO-PINZÓN et al., 2009; LAVERGNE et al., 2010; CHIOU et al., 2011). O arranjo sistemático e taxonômico atual abre a necessidade de novos estudos com a identidade confirmada das espécies analisadas.

S. macrodon e *S. cassiquiarensis* apresentam ampla distribuição na Amazônia. Estas duas espécies ocorrem em peripatria com *Saimiri vanzolinii* Ayres, 1985 na Reserva de Desenvolvimento Sustentável Mamirauá (RDSM) na Amazônia Central (PAIM et al., 2013). Os limites da distribuição geográfica das três espécies são bem delineados, mas os mecanismos que promovem o isolamento reprodutivo ainda não estão esclarecidos (PAIM et al., 2013). *S. vanzolinii* é endêmico da RDSM e provavelmente apresenta a menor distribuição dentre todos os primatas neotropicais (AYRES, 1985; PAIM et al., 2013).

O status de conservação de *S. macrodon* e *S. cassiquiarensis* é considerado pouco preocupante pela Lista vermelha de espécies ameaçadas da International Union for Conservation of Nature (BOUBLI et al., 2008ab). Entretanto, *S. vanzolinii* é considerado como vulnerável à extinção (BOUBLI & RYLANDS, 2008). A possibilidade de existência de híbridos e a invasão progressiva de *S. cassiquiarensis* na área de ocorrência de *S. vanzolinii* são motivos de preocupação (RYLANDS et al., 2013).

Alguns grupos mistos foram observados na zona de contato entre *S. vanzolinii* e *S. cassiquiarensis* (PAIM et al., 2013). A ausência de barreiras geográficas demanda que segregação entre estas espécies seja elucidada por outros parâmetros, como o isolamento reprodutivo pela incompatibilidade morfológica, por exemplo. Nesse contexto, o presente estudo almejou oferecer subsídios para a melhor compreensão sobre a diferenciação morfológica genital feminina destas três espécies peripátricas. Avaliar a possibilidade de que a morfologia genital constitua um mecanismo de isolamento reprodutivo entre elas, através da descrição anatômica, topográfica e histológica da genitália externa e dos órgãos internos do sistema reprodutor feminino.

1.1. TAXONOMIA DOS PLATYRRHINI

Os primatas neotropicais pertencem a Pavordem Platyrrhini e neste táxon está incluído o maior número de espécies entre todos os primatas (GROVES, 2005; RYLANDS, MITTERMEIER & SILVA-JÚNIOR, 2012). Revisões recentes propõem a existência de 20 gêneros, 204 espécies e subespécies (RYLANDS & MITTERMEIER, 2009; RYLANDS, MITTERMEIER & SILVA-JÚNIOR, 2012). Schneider (2000) classificou os Platyrrhini em três famílias: Atelidae Gray, 1825, Pitheciidae Mivart, 1865 e Cebidae Gray, 1831 e, este é o arranjo mais parcimonioso para este táxon. Dentro da família Cebidae há três subfamílias que são Callitrichinae Gray, 1821, Aotinae Elliot, 1913 e Cebinae Bonaparte, 1831. Na subfamília Cebinae há os gêneros *Cebus* Erxleben, 1777, *Sapajus* Kerr, 1792 e, *Saimiri* (SCHNEIDER, 2000; ALFARO, SILVA-JÚNIOR & RYLANDS, 2012).

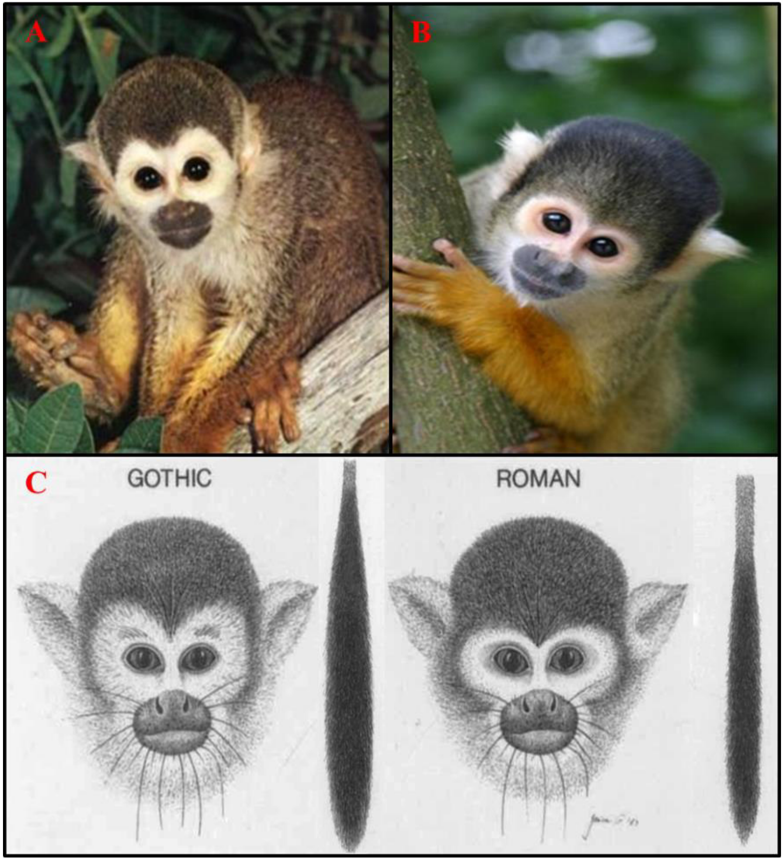
1.2 O GÊNERO *SAIMIRI*

Os “macacos de cheiro” ou “squirrel monkeys” como são conhecidos popularmente os primatas do gênero *Saimiri* estão distribuídos na Amazônia brasileira e, em outros países da América do Sul como Bolívia, Colômbia, Equador, Guianas, Peru, Suriname e Venezuela. Há ocorrência também na América Central, na Costa Rica e no Panamá (BALDWIN & BALDWIN, 1981; HERSHKOVITZ 1984; JACK, 2007).

Saimiri spp. ocorrem em diferentes habitats, como florestas tropicais sazonalmente alagadas (PERES, 1993; 1997; HAUGAASEN & PERES, 2005; BICCA-MARQUES, SILVA & GOMES, 2011), florestas de galerias, florestas primárias e secundárias, fragmentos florestais isolados e degradados (BALDWIN & BALDWIN, 1981; SUSSMAN & PHILIPS-CONROY, 1995; DEFLER, 2004; BICCA-MARQUES, SILVA & GOMES, 2011).

Os macacos-de-cheiro apresentam pelagem cuja coloração é caracterizada como amarelo-acinzentado na coroa e dorso, os pés, pernas, braços e mãos variam de amarelos a alaranjados; a porção distal da cauda se caracteriza como pincel (Figura 1) (HERSHKOVITZ, 1984). De acordo com Maclean (1964) e Hershkovitz (1984) o gênero *Saimiri* é constituído por dois grupos de espécies, que podem ser distinguidos por atributos externos como o formato dos arcos supraciliares, espessura do pincel caudal, comportamento e distribuição geográfica. O grupo "gótico" apresenta pelos brancos ao redor dos olhos e pelos escuros na cabeça que formam um profundo "V" entre os olhos (MAcLEAN, 1964; HERSHKOVITZ, 1984). O grupo "romano" é caracterizado por pelos brancos ao redor dos olhos e pelos escuros na cabeça que formam um padrão arredondado entre os olhos (Figura 1) (MAcLEAN, 1964; HERSHKOVITZ, 1984).

Figura 1 – Exemplares do gênero *Saimiri* com diferenças entre os arcos superciliares e o pincel caudal entre os grupos gótico e romano.



Fonte: Figura A: http://educ.csmv.qc.ca/mgrparent/vieanimale/mam/saimiri/saimiri.htm. Figura B: http://www.ot-lalondelesmaures.fr/index.html?pID=558&bID=252. Figura C: Adaptado de Hershkovitz (1984).

A taxonomia de *Saimiri* é controversa e tem sido objeto de investigação de vários autores (ELLIOT, 1913; LÖNNBERG, 1940; HILL, 1960; HERSHKOVITZ, 1984; THORINGTON, 1985; VANDEBERG et al., 1990; SILVA-JÚNIOR, 1992; COSTELLO et al., 1993; SILVA et al., 1993; BOINSKI & CROOP, 1999; RYLANDS et al., 2000; GROVES, 2001; 2005; LAVERGNE et al., 2010; CHIOU et al., 2011). Dentre esses autores, a classificação de Hershkovitz (1984) tem sido sustentada por evidências moleculares e genéticas (VANDEBERG et al., 1990; BOINSKI & CROPP, 1999; LAVERGNE et al., 2010; CHIOU et al., 2011).

De acordo com Hershkovitz (1984) as espécies e subespécies do gênero *Saimiri* são:

1) *Saimiri boliviensis* (I. Geoffroy & de Blainville, 1834) – com duas subespécies – *S. boliviensis boliviensis* (I. Geoffroy & de Blainville, 1834) e *S. boliviensis peruviensis* Hershkovitz, 1984;

2) *Saimiri sciureus* – com quatro subespécies – *S. s. sciureus* (Linnaeus, 1758), *S. s. macrodon* (Elliot, 1907), *S. s. albigena* (von Pusch, 1941), *S. s. cassiquiarensis* (Lesson, 1840);

3) *Saimiri oerstedii* (Reinhardt, 1872) – com duas subespécies – *S. o. oerstedii* (Reinhardt, 1872) e *S. o. citrinellus* Thomas, 1904;

4) *Saimiri ustus* I. Geoffroy, 1843.

No arranjo taxonômico proposto por Groves (2005) foi incluído o táxon *S. vanzolinii* descrito por Ayres (1985).

Chiou et al. (2011) concluíram que *S. s. macrodon* é uma espécie distinta de *S. sciureus*. Em 2009 Carretero-Pinzón, Ruiz-García & Defler (2009) sugeriram que *S. s. cassiquiarensis* deve ser considerada como espécie distinta de *S. sciureus*. Neste estudo será utilizada a nomenclatura proposta por Groves (2005), Carretero-Pinzón, Ruiz-García & Defler (2009) e Chiou et al. (2011).

1.2.1 Biologia reprodutiva do gênero *Saimiri*

Saimiri spp. são primatas de pequeno porte que apresentam dimorfismo sexual no tamanho corporal, com os machos 30-35% maiores que as fêmeas (BALDWIN & BALDWIN, 1981; BOINSKI, 1999; INGBERMAN, STONE & CHEIDA, 2008). A maturidade sexual é atingida entre dois anos e meio a quatro anos nas fêmeas e, entre dois anos e meio a seis anos nos machos (BALDWIN, 1969; SCOLLAY, 1980; TAUBE, 1980; ROBINSON & JANSON, 1987; BOINSKI, 1992).

O ciclo reprodutivo é considerado curto se comparado a outras espécies de primatas, com duração entre sete e 12 dias (DUKELOW, 1983; 1985). Não há ocorrência de menstruação e, apesar de a citologia vaginal ser utilizada como indicador da fase do ciclo, não é um método considerado seguro para o gênero *Saimiri* (DUKELOW, 1983; 1985).

Saimiri spp. apresentam sazonalidade marcante em relação ao ciclo reprodutivo (DUKELOW 1978; COE & ROSENBLUN, 1978; BALDWIN, 1985; MENDOZA, 1999; SCHIML et al., 1999). Os fatores que determinam essa sazonalidade ainda não são totalmente conhecidos, mas parece haver uma complexa relação entre fatores socioambientais e fisiológicos (COE & ROSENBLUM, 1978; DUKELOW, 1983; TREVINO, 2007). Os nascimentos ocorrem geralmente no período chuvoso, que é o período em que há maior abundância de alimentos (DU MOND & HUTCHINSON, 1967; BALDWIN, 1970; ROBINSON & JANSON, 1987; DEFLER, 2004; STONE, 2004; IZAR et al., 2009). Porém há registros de nascimentos na estação seca (HEARN, 1983; BOINSKI, 1987; PINHEIRO, 2010).

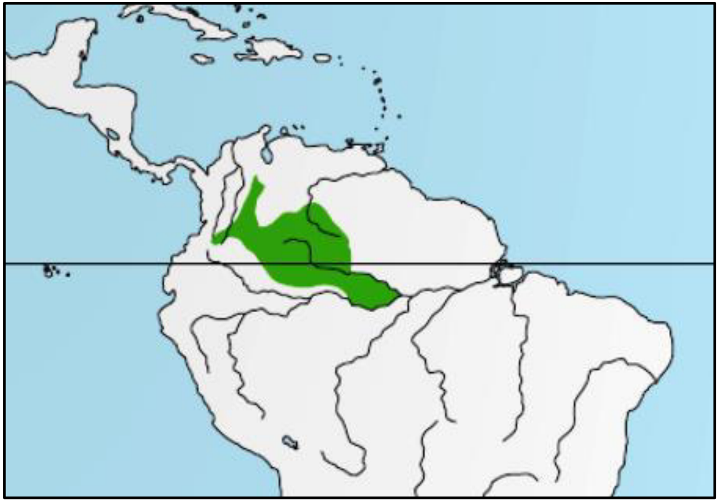
Um único filhote nasce após um período de gestação de 145 a 170 dias (ROBINSON & JANSON, 1987; DEFLER, 2004; STONE, 2004). A massa corporal ao nascimento é de 16-20% do peso da mãe e, é considerada a maior proporção entre os primatas antropoides (DUKELOW, 1983; BALDWIN, 1985). Essa proporção representa custo energético alto para a fêmea, o que pode justificar a concepção de um único filhote por ano (BALDWIN, 1985).

A organização social possivelmente sofre influência da competição por recursos alimentares (BOINSKI et al., 2002; INGBERMAN, STONE & CHEIDA, 2008). O tamanho de grupo, composto por machos adultos, fêmeas adultas, juvenis e infantes pode ter entre 10 e 300 indivíduos (BALDWIN & BALDWIN, 1981; BALDWIN, 1985; BOINSKI et al., 2002; STONE, 2007).

1.2.2 Aspectos gerais de *Saimiri cassiquiarensis*

Espécie do grupo gótico com distribuição na margem norte do Rio Solimões/Amazonas. Abrange parte da Amazônia colombiana (entre os Rios Vichada e Apoporis) e brasileira (oeste do Rio Negro e Demini, norte do Rio Japurá) e, sul da Venezuela (Figura 2) (RYLANDS et al., 2013). Apresentam comprimento corporal e peso respectivamente entre 250-370 mm e 650-1125 g nos machos e, 280-340 mm e 550-1200 g nas fêmeas (RYLANDS et al., 2013). O “status” de conservação da espécie é considerado como pouco preocupante pela Lista vermelha de espécies ameaçadas da IUCN (BOUBLI et al., 2008a).

Figura 2 – Distribuição geográfica de *S. cassiquiarensis.*



Fonte: Rylands et al. (2013).

1.2.3 Aspectos gerais de *Saimiri macrodon*

Esta espécie pertence ao grupo gótico e tem distribuição no oeste da Amazônia brasileira (oeste dos Rios Japurá e Juruá). Sua distribuição se estende ao oeste e sul da Colômbia, leste do Equador e, ao norte e leste do Peru (Figura 3) (RYLANDS et al., 2013). O comprimento corporal é entre 250-320 mm, os machos pesam entre 835 g e 1380 g e, as fêmeas entre 590 g e 1150 g (RYLANDS et al., 2013). *S. macrodon* tem “status” de conservação considerado como pouco preocupante pela Lista vermelha de espécies ameaçadas da IUCN (BOUBLI et al., 2008b).

Figura 3 – Distribuição geográfica de *S. macrodon.*

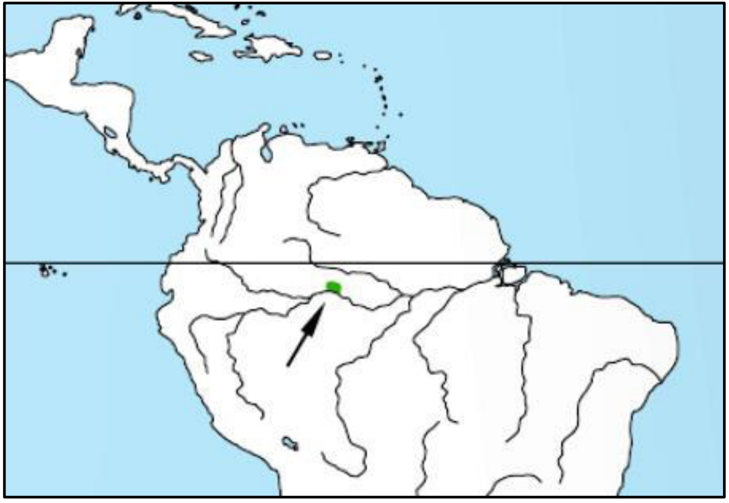
Fonte: Rylands et al. (2013).

1.2.4 Aspectos gerais de *Saimiri vanzolinii*

S. vanzolinii pertence ao grupo romano e apresenta uma das menores distribuições geográficas entre os primatas neotropicais (PAIM et al., 2013). Sua distribuição restringe-se ao norte do Rio Solimões, sudeste da RDSM na Amazônia brasileira (Figura 4) (RYLANDS et al., 2013). Esta distribuição constitui-se em extremo endemismo da espécie (PAIM et al., 2013). O comprimento corporal é entre 260-300 mm nos machos e, 220-260 mm nas fêmeas; os machos pesam em média 950 g e as fêmeas 650 g (RYLANDS et al., 2013). A espécie é considerada como vulnerável à extinção pela IUCN (BOUBLI & RYLANDS, 2008).

A população de *S. vanzolinii* é estimada entre 5.500-10.900 indivíduos (RYLANDS et al., 2013). A possibilidade de híbridos e invasão progressiva de sua distribuição por *S. cassiquiarensis* são motivos de preocupação (RYLANDS et al., 2013).

Figura 4 – Distribuição geográfica de *S. vanzolinii*



Fonte: Rylands et al. (2013).

1.3 O SISTEMA REPRODUTOR DAS FÊMEAS DE PRIMATAS NEOTROPICAIS

O sistema reprodutor das fêmeas compreende a genitália externa ou vulva, gônadas e órgãos acessórios (HILL, 1957). De acordo com Hill (1957) há grande diversidade na anatomia do sistema reprodutor dos Platyrrhini, com semelhanças paradoxais na genitália externa entre os sexos. A genitália externa é utilizada para cópula, excreção, reconhecimento, estímulo sexual, ameaça, exibição e marcação territorial (SANDERSON, 1957).

A genitália externa é composta pelo clitóris, abertura vulvar (rima do pudendo) e lábios grandes e pequenos (HILL, 1960). A rima do pudendo está localizada entre os lábios (POCOCK, 1920; HILL, 1960). Os lábios adjacentes à rima do pudendo são os pequenos lábios (HILL, 1960). Grandes lábios foram descritos na maioria dos primatas neotropicais (KLAATSCH, 1892; BOLK, 1907; SANDERSON, 1949; WISLOCKI, 1936; HILL, 1952; 1957; 1958; 1960; HERSHKOVITZ, 1977; VERAS, 2004; ANKEL-SIMONS, 2007). Porém nem todos fazem distinção entre pequenos e grandes lábios (POCOCK, 1920; HILL, 1960; HERSHKOVITZ, 1977).

Em todos os primatas os grandes lábios são homólogos ao escroto masculino (KLAATSCH, 1892; WISLOCKI, 1936). Hill (1960) relata que nos membros da família Atelidae e subfamília Cebinae o sexo é indistinguível externamente, principalmente nas formas juvenis. Isso é causado pelas glândulas presentes nos lábios que conferem a estes à aparência superficial de um escroto (HILL, 1960).

A hipertrofia do clitóris dificulta a sexagem de certos táxons (POCOCK, 1920; WISLOCK, 1936; HILL, 1957; DIXSON, 2012). O clitóris é descrito como grande, caloso, pendular e semelhante a um pênis em Cebinae e Atelidae (POCOCK, 1920; WISLOCKI, 1936; SANDERSON, 1949; HILL, 1960). Nas outras famílias e subfamílias o clitóris é descrito como pequeno e, geralmente é encoberto pelos lábios (WISLOCKI, 1936; HILL, 1960).

A vagina nos Platyrrhini é um canal comprimido dorsoventralmente, com variações ao longo da mucosa e no comprimento total (ECKSTEIN, 1958; HILL, 1952; 1960). Em algumas espécies o colo do útero (cérvice) é desenvolvido e se projeta para o interior da vagina (HILL, 1960; HAFEZ & JASZCZAK, 1972). Os Platyrrhini possuem útero simples (ANKEL-SIMONS, 2007) e o tamanho deste apresenta ampla variabilidade nas diferentes espécies (ECKSTEIN, 1958; HILL, 1960).

A tuba uterina é dividida em quatro segmentos: intramural, istmo, ampola e infundíbulo (WISLOCKI, 1932). O grau de convolução das tubas varia entre as espécies (TURNQUIST & HONG, 1995). As tubas estão localizadas na margem superior do ligamento largo entre as pregas do ligamento que é o mesossalpíngeo (HILL, 1960). O infundíbulo apresenta prolongamentos em forma de franja que são as fímbrias da tuba (WISLOCKI, 1932).

O formato dos ovários é variável nas diferentes espécies (DEMPSEY, 1939; ECKSTEIN, 1958; HILL, 1960) e, foram descritos como compactos e relativamente grandes em relação ao tamanho do corpo (ECKSTEIN, 1958; HILL, 1960).

1.3.1 Estudos anatômicos e histológicos do sistema reprodutor feminino com primatas do gênero *Saimiri*

No gênero *Saimiri* já foram realizados estudos envolvendo os órgãos do sistema genital nas espécies *S. sciureus*, *S. collinsi* e *S. oerstedii* (MARTIN, 1833; VALLOIS, 1912; POCOCK 1920; WISLOCKI, 1932; 1936; SANDERSON, 1949; HILL, 1960; COLBORN, BO & LANG, 1967; HERTIG, et al., 1971; HAFEZ & JASZCZAK, 1972; WALKER et al., 2009; BRANCO et al., 2010; ALMEIDA et al., 2012). A seguir estão as descrições realizadas com as espécies supracitadas.

1.3.2 *Saimiri sciureus*

Em *S. sciureus* estudos anatômicos foram realizados por Martin (1833), Bolk (1907), Vallois (1912), Pocock (1920), Wislocki (1932), Hill (1960), Colborn, Bo & Lang (1967) e Hafez & Jaszczak (1972). As fêmeas adultas apresentam lábios definidos (HILL, 1960). Os lábios pequenos estão localizados imediatamente adjacentes à rima do pudendo, eles são opacos, rugosos e com muitas glândulas (HILL, 1960). Os pequenos lábios não são cercados por grandes lábios muito evidentes (HILL, 1960). O clitóris é hipertrofiado e protegido acima por um prepúcio em forma de capuz, a glande do clitóris é dura e calosa (HILL, 1960).

A vagina é uma câmara com comprimento médio de 12 mm, com grossas paredes rugosas (HILL, 1960). Fórnices dorsal e ventral estão presentes, o dorsal é ligeiramente mais profundo, e está em contato com o peritônio no fundo da fossa peritoneal (HILL, 1960). A passagem se abre para o exterior, na extremidade dorsal da rima do pudendo (HILL, 1960).

O canal cervical é irregular por conta de projeções musculares espessas (colículos) projetados em seu lúmen das paredes dorsal e ventral (HILL, 1960). A extremidade distal da porção cervical é dilatada e forma um tipo de vestíbulo (COLBORN, BO & LANG, 1967). O diâmetro anteroposterior da cérvice na sua junção ao fundo de saco vaginal mede 9 mm e a distância do óstio interno ao óstio externo mede 10 mm (COLBORN, BO & LANG, 1967).

O epitélio pavimentoso estratificado não queratinizado da vagina se estende ao vestíbulo cervical (COLBORN, BO & LANG, 1967; HAFEZ & JASZCZAK, 1972). A localização da junção escamo-colunar não era homogênea entre os animais (COLBORN, BO & LANG, 1967; HAFEZ & JASZCZAK, 1972). A morfologia do epitélio cervical e a quantidade de material PAS positivo variaram com o estado hormonal das fêmeas (COLBORN, BO & LANG, 1967). Em fêmeas ovariectomizadas o epitélio pavimentoso estratificado não queratinizado não apresentou grânulos mucosos, porém nos animais tratados com estrogênio e progesterona a quantidade de grânulos foi marcante (COLBORN, BO & LANG, 1967).

O útero é proporcionalmente pequeno (MARTIN, 1833) e apresenta a forma piriforme comprimida dorsoventralmente habitual, com uma ligeira constrição entre o corpo e a cérvice (HILL, 1960). O órgão é ligeiramente retroflexo e sua cavidade é semelhante a uma fenda e apresenta paredes dorsal e ventral, um orifício interno e um externo e o canal cervical (HILL, 1960). A distância do fundo até o orifício externo varia de 11,5 a 28 mm (HILL, 1960). A maior espessura, dorsoventralmente, é de 5 mm no fundo; o diâmetro transversal entre a terminação das tubas uterinas é de 7 mm (HILL, 1960).

Hill (1960) descreveu a tuba uterina como tendo formato espiralado, porém Wislocki (1932) descreveu-a como retilínea. O infundíbulo é muito comprimido médio-lateralmente e oculta parcialmente o ovário correspondente (HILL, 1960). As fímbrias observadas foram poucas e curtas (HILL, 1960).

Os ovários foram descritos como grandes (MARTIN, 1833) e apresentam polos cranial e caudal, superfícies medial e lateral e margens dorsal e ventral (MARTIN, 1833; HILL, 1960). De polo a polo, o comprimento é de 8,8 mm, médio-lateralmente o diâmetro é de 2,8 mm e, dorsoventralmente é de 4,1 mm (MARTIN, 1833, HILL, 1960).

Os ligamentos ovarianos fortes são conectados com o polo caudal do ovário (MARTIN, 1833; HILL, 1960). O ligamento próprio é longo e, mantém os ovários longe do útero em uma posição mais longitudinal (WISLOCKI, 1932). As posições relativas de ovário e tuba uterina são primitivas e, correspondem exatamente ao descrito para o típico mamífero quadrúpede (HILL, 1960).

Hertig et al. (1971) realizaram estudo histológico do ovário de *S. sciureus* e descreveram que a região medular em fêmeas adultas tem uma aparência parenquimatosa devido à presença de dois tipos de tecido: tecido intersticial glandular e um tecido de aparência luteinizada. O tecido de aparência luteinizada ocorre em massas irregulares, suas células apresentam núcleo excêntrico, cromatina frouxa e citoplasma acidófilo abundante (HERTIG, et al., 1971). O tecido intersticial glandular é formado por faixas que circundam o tecido de aparência luteinizada, suas células têm núcleo central e citoplasma com grânulos sudanofílicos (HERTIG, et al., 1971). Hertig et al. (1971) denominaram de "massas discretas luteinizadas" o tecido de aparência luteinizada e de "tecido intersticial difuso" o tecido intersticial glandular.

Walker et al. (2009) investigaram as mudanças citológicas nos ovários associadas à idade em *S. sciureus*. Os ovários sofrem alterações microestruturais evidentes com a idade. Ao nascimento, o córtex ovariano é relativamente abundante em folículos primordiais. Com o avanço da idade os folículos primordiais são cada vez mais confinados à região cortical exterior, enquanto na medula há aumento do volume relativo. Em fêmeas maiores que dois anos e meio de idade os ovários mostram sinais de crescimento ativo e maturação folicular, atresia e luteinização. Ao mesmo tempo, há esgotamento inexorável de folículos primordiais em animais com idade superior a oito anos (WALKER et al., 2009).

1.3.3 *Saimiri collinsi*

Apesar de os estudos de Branco et al., (2010) e Almeida et al., (2012) se referirem em seus estudos à espécie *S. sciureus*, os espécimes utilizados nestes estudos eram do Centro Nacional de Primatas (CENP). Entretanto, os espécimes do CENP são provenientes da Ilha do Marajó no Estado do Pará, neste caso, o táxon que ocorre na localidade é *S. collinsi* (CARRETERO-PINZÓN, RUIZ-GARCÍA & DEFLER, 2009; LAVERGNE et al., 2010). Desta forma, será utilizado aqui o nome *S. collinsi* para os referidos estudos.

Em *S. collinsi* a vulva é delimitada pelos lábios vulvares, o clitóris é bem desenvolvido com aspecto fálico (BRANCO et al., 2010). O vestíbulo vaginal é bastante curto, com espessa parede muscular e, estende-se da rima da vulva até o óstio da vagina (BRANCO et al., 2010). A vagina é um tubo muscular com aspecto alongado e achatado dorsoventralmente com comprimento médio de 10,3 mm, com paredes finas, estando disposta entre o vestíbulo vaginal e a cérvice; a mucosa vaginal apresenta um pregueamento longitudinal mediano (BRANCO et al., 2010).

A cérvice é relativamente curta, com uma espessa parede muscular e comprimento médio de 5 mm (BRANCO et al., 2010). O útero é simples, com fundo globoso, e comprimento médio de 63 mm (BRANCO et al., 2010). Tanto a cérvice quanto o corpo do útero estão localizados na cavidade abdominal (BRANCO et al., 2010).

A tuba uterina é um órgão tubular muito fino e retilíneo, sem presença das fímbrias ovarianas (BRANCO et al., 2010). O comprimento médio foi de 15,3 mm para a tuba uterina direita e 15,7 mm para a esquerda (BRANCO et al., 2010).

Os ovários estão dispostos bilateralmente ao útero, com formato elipsoide, superfície lisa. Cada ovário apresenta uma face medial e outra lateral, uma extremidade tubária e outra uterina, uma margem livre e outra mesovárica (BRANCO et al., 2010). As mensurações dos ovários revelaram que, quando comparados entre si, há uma discreta variação na média das dimensões entre eles. O ovário direito apresentou 37 mm de largura, 60 mm de comprimento e 33 mm de altura. O esquerdo apresentou 40 mm de largura, 63 mm de comprimento e 30 mm de altura (BRANCO et al., 2010).

Almeida et al. (2009) descreveram as características morfológicas, realizaram a morfometria e, estimaram a população de folículos ovarianos primordiais, primários e secundários de *S. collinsi* senis, com mais de oito anos de idade. Folículos primordiais foram a maioria dos folículos presentes no ovário, bem como os folículos caracterizados como normais compuseram a maioria da população folicular. A degeneração de folículos primordiais e primários resultou na redução significativa do diâmetro do oócito, mas não do folículo. Entretanto a degeneração de folículos secundários foi caracterizada pela redução do diâmetro do folículo. Foi observada a presença de aglomerados encapsulados de células da granulosa, que pode ser uma característica do envelhecimento em ovários de macacos-de-cheiro (ALMEIDA et al., 2009).

1.3.4 *Saimiri oerstedii*

Em *S. oerstedii*, Wislocki (1936) realizou estudos anatômicos dos lábios vulvares. De acordo com o referido autor o único espécime era uma fêmea adulta. Esta apresentou a vulva delimitada por pequenos lábios bem definidos e, um curto clitóris, incluído em um prepúcio. Os grandes lábios eram bem desenvolvidos, coberto por pelos esparsos e, análogo ao escroto humano, são compostos de pele enrugada (WISLOCKI, 1936). O períneo era relativamente menor que nos machos e, eram cobertos por pelos esparsos de coloração amarela (WISLOCKI, 1936).

Os pequenos lábios não apresentaram pelos e, foram descritos como duas dobras de tecido medial aos grandes lábios. Apresentaram epitélio pavimentoso estratificado, com numerosas glândulas sebáceas e, algumas glândulas sudoríparas (WISLOCKI, 1936). Eles apresentaram uma aparência úmida, avermelhada, semelhante a uma membrana mucosa (WISLOCKI, 1936). Cada lábio se divide anteriormente em duas lamelas que se fundem como pregas, para formar o frênulo do clitóris e o prepúcio (WISLOCKI, 1936). Estudos histológicos dos outros componentes do sistema genital das fêmeas são inexistentes até mesmo para *S. sciureus* a mais conhecida das espécies de *Saimiri*.

Esta dissertação está dividida em três capítulos em formato de artigos. No primeiro capítulo foram feitas as descrições anatômicas e histológicas dos órgãos tubulares do sistema reprodutor feminino das três espécies. O segundo capítulo trata-se de um relato de caso sobre a agenesia de um dos ovários na espécie ameaçada de extinção *Saimiri vanzolinii*. O terceiro capítulo aborda a estimativa da população folicular nas três espécies.

1.4 OBJETIVO GERAL

Descrever o sistema reprodutor feminino de *S. macrodon*, *S. cassiquiarensis* e *S. vanzolinii*.

1.4.1 Objetivos específicos

Descrever aspectos topográficos, anatômicos e histológicos do sistema reprodutor feminino das três espécies;

Buscar diferenças entre topografia, anatomia e histologia do sistema reprodutor feminino destas espécies e o anteriormente descrito para outras espécies de *Saimiri*;

Estimar a população e realizar a morfometria de folículos pré-antrais nas três espécies.

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CAPÍTULO 1

Comparative Characterization of the External Genitalia and Reproductive Tubular Organs of Three Species of the Genus *Saimiri* Voigt, 1831 (Primates: Cebidae)

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ORIGINAL ARTICLE

Comparative Characterization of the External Genitalia and Reproductive Tubular Organs of Three Species of the Genus *Saimiri* Voigt, 1831 (Primates: Cebidae)

G. P. Lopes[1]*, A. B. Brito[1], F. P. Paim[2], R. R. Santos[1], H. L. Queiroz[2] and S. F. S. Domingues[1]

Addresses of authors: [1] Postgraduate Program in Animal Science, Federal University of Pará, Belém, Pará, Brazil;
[2] Research Group on Ecology of Terrestrial Vertebrates, Institute for Sustainable Development Mamirauá, Tefé, Amazonas, Brazil

***Correspondence:**
Tel.: +55 97 98111 5960;
fax: +55 (097) 33439700;
e-mail: gersonlps@hotmail.com

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Summary

Morphological information on the reproductive system allows the understanding of ecological and behavioural aspects of different species as well as supports the development of conservational strategies. Unfortunately, for many species, not enough relevant and precise information is available. In the present study, we describe for the first time the macroscopic and histological aspects of female genital organs and external female genitalia of *Saimiri macrodon*, *Saimiri cassiquiarensis* and *Saimiri vanzolinii*. We perform a comparison between these three peripatric species and investigate the possibility of their reproductive morphology to act as a factor of reproductive isolation. We have found that these species share many similarities in most of the analysed organs. Although some important differences were identified that may play an important role in the evolution of the components of the reproductive system of these species, those differences are not enough to compose a mechanism of reproductive isolation for these three species of *Saimiri*. The results of this study may be used to support the development of biotechnological approaches of reproduction and strategies for conservation programmes and management of threatened species of this genus, particularly *S. vanzolinii*, considered to be a vulnerable species to extinction.

Introduction

Platyrrhini primates present many species-specific variations in their reproductive features including behaviour, physiology, morphology and anatomy (Campbell, 1972; Emlen and Oring, 1977; Bradbury and Andersson, 1987; Andersson, 1994). Some of these variations may be consequences of the coevolution of constituents of the genitalia in both genders, by means of sexual selection (Darwin, 1876; Eberhard, 1985; Dixson, 2012). For instance, morphological differences may represent a high degree of specialization of the intraspecific genitalia, resulting in a ‘lock and key’ system, which can comprise one of the mechanisms implicated in the speciation processes (Fooden, 1967; Hershkovitz, 1977; Hosken and Stockley, 2004).

Regarding females, anatomical and physiological barriers inside the vagina, cervix, uterus, utero–tubal junction and uterine tubes may constitute obstacles to male gametes towards fertilization and can influence the outcome of a potential sperm competition (Eberhard, 1985; Dixson and Anderson, 2001). Morphological description on Neotropical primates points several species differences when comparing the components of the female reproductive system. In the Cebidae family, descriptions of the genus *Saimiri* (squirrel monkeys) were performed for *Saimiri sciureus* (Martin, 1833; Pocock, 1920; Wislocki, 1932; Colborn et al., 1967; Hertig et al., 1971; Hafez and Jaszczak, 1972), *Saimiri oerstedii* (Wislocki, 1936) and *Saimiri collinsi* (Branco et al., 2010).

As observed, most published studies refer to the species *S. sciureus*, but without make reference of the origin of these animals. Hence, one must bear in mind that due to a paucity of proper analyses, there is the risk that those specimens were inconsistently identified as *S. sciureus*, without designation of subspecies (Boinski and Cropp, 1999), or their subsequent identification through genetic was simply not possible (Lavergne et al., 2003). Several groups of squirrel monkeys allocated in zoos or research institutions were proven to have hybrid origins (Ariga et al., 1978; Fogle, 1990; Schreiber et al., 1998; Boinski and Cropp, 1999; Lavergne et al., 2003). With the aim to study phylogeny and molecular biology in captive squirrel monkeys, PCR analysis has been conducted and helped to distinguish *Saimiri* species in captivity (Vandeberg et al., 1990; Schreiber et al., 1998; Lavergne et al., 2003; Osterholz et al., 2008), but without full characterization. Therefore, a detailed identification of the origin and genetic profile of specimens before being used in studies in either captivity or in the laboratory is of great importance for the interpretation and dissemination of results (Ward and Vallender, 2012).

Recently, groups previously identified as a subspecies of *S. sciureus* were relocated to species level. Among them are *Saimiri macrodon* and *Saimiri cassiquiarensis* (Carretero-Pinzón et al., 2009; Lavergne et al., 2010; Chiou et al., 2011; Ruiz-García et al., 2014; Alfaro et al., 2015; Mercês et al., 2015). *S. macrodon* and *S. cassiquiarensis* show a wide distribution in the Amazon, being peripatric with *Saimiri vanzolinii* in the Reserve of Sustainable Development Mamirauá (RDSM) in the central Amazon. The range of the geographical distribution for the three species is well defined, but the mechanisms that promote reproductive isolation remain unclear (Paim et al., 2013).

Saimiri vanzolinii is endemic to the RDSM and probably has the lowest distribution among all Neotropical primates (Paim et al., 2013). The conservation status of *S. macrodon* and *S. cassiquiarensis* is listed as of least concern by the Red List of Threatened Species of the International Union for Conservation of Nature (Boubli et al., 2008a,b). However, *S. vanzolinii* is considered to be a vulnerable species (Boubli and Rylands, 2008), because of the possibility of hybrids and the progressive invasion of their area of occurrence by *S. cassiquiarensis* (Rylands and Mittermeier, 2013). Some mixed groups were observed in the contact zone between *S. vanzolinii* and *S. cassiquiarensis* (Paim et al., 2013). The absence of geographical barriers demands the segregation of these species to be carried out through other parameters such as reproductive isolation by morphological incompatibility, for example.

Based on the hypothesis that female genital morphology constitutes a mechanism of reproductive isolation between those three peripatric *Saimiri* species, our aim in the present study was to provide comparative characterization of the female external genitalia and reproductive tubular organs from *S. cassiquiarensis*, *S. macrodon* and *S. vanzolinii*. This study will provide support for conservational interventions and will contribute to the proper description of the anatomy and histology of female genital organs and external female genitalia from these three species.

Materials and Methods

Study area

The specimens used in this study were collected from RDSM (Fig. 1). The samples used belong to the Mastozoological Section of the Collection of Biological Material at the Institute for Sustainable Development Mamirauá (IDSM). All experimental procedures of this study were approved by the Research Ethics Committee and the IDSM Ethics Committee, under protocol number 002/2012. The licence for collection was granted by the Brazilian Institute of Environment and Renewable Natural Resources, through the System of Authorization and Information on Biodiversity (SISBIO 29906-1).

Animals

Females were captured by shooting them to avoid handling stress. All specimens were deposited in the Mammal Collection of the IDSM for taxonomical studies. The entire carcasses were available for age estimation. The approximate age of the females was estimated based on the phenotype of dental chronology (Long and Cooper, 1968; Smith, 1989), and they were then classified as juveniles (age ranging from 0 to 1.5 year old), adults (age ranging from 1.5 to 5 years old) or senile (older than 5 years) (Table 1). No pathologies or abnormal findings were reported. Subsequently, different research groups from the institute were responsible for further analysis according to their expertise. Our group was responsible for the morphological studies of the female genital organs and external genitalia.

Female genital organs and external genitalia of five female specimens were used: two *S. macrodon* females, two *S. cassiquiarensis* females and one *S. vanzolinii* female. Briefly, among the studied females, one specimen of *S. macrodon* was juvenile and the other was a pregnant adult, in the middle third of pregnancy. The two *S. cassiquiarensis* females were adults; one of them was pregnant, in the first third of pregnancy. The *S. vanzolinii* female was senile.

Macroscopic analysis

We performed the anatomical and topographical description and the photographic record of the organs. The opening of the abdominal cavity was performed through

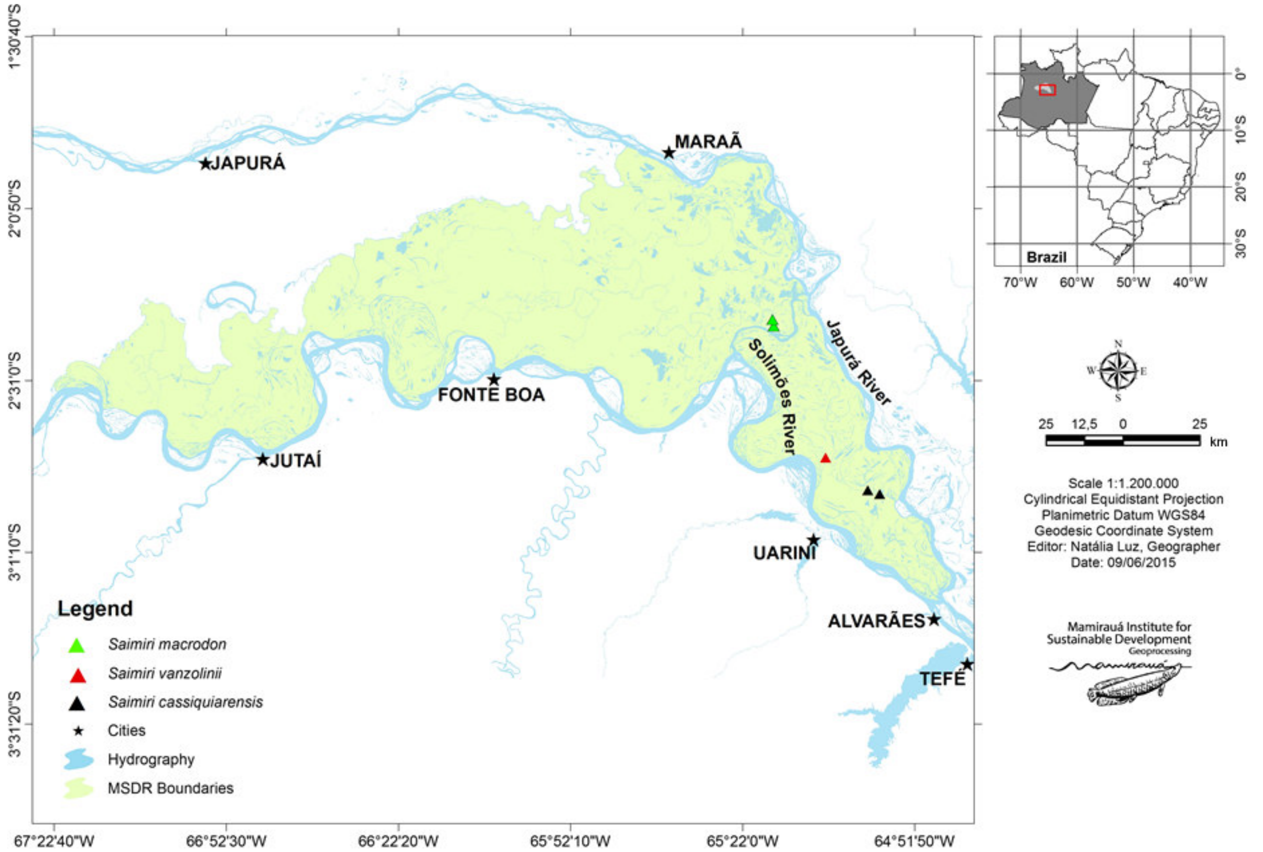

Fig. 1. Distribution of capture areas for *Saimiri vanzolinii, Saimiri cassiquiarensis* and *Saimiri macrodon*. Source: Mamirauá Institute for Sustainable Development Geoprocessing, 2013.

Table 1. Measurement (mm) of body length, vagina, uterus, cervix and fallopian tubes (right and left) in *Saimiri cassiquiarensis*, *Saimiri macrodon* and *Saimiri vanzolinii*. Ratios of organ/body length are presented as (%)

	S. cassiquierensis		*S. macrodon*		*S. vanzolinii*
	Animal 1	Animal 2	Animal 1	Animal 2	Animal 1
Reproductive age	Adult (pregnant)	Adult	Adult (pregnant)	Juvenile	Senile
Body length (mm)	265	218	294	232	240
Vagina, mm (%)					
Fresh tissue	24.07 (9.88)	17.02 (7.81)	16.96 (5.77)	16.03 (6.91)	16.16 (6.73)
Sagittal section	28.17 (10.66)	21.12 (12.96)	28.26 (9.61)	17.03 (12.18)	20.96 (8.73)
Uterus, mm (%)					
Fresh tissue	14.22 (5.93)	10.16 (3.83)	26.97 (9.17)	03.55 (1.53)	07.50 (3.44)
Sagittal section	13.15 (5.48)	10.35 (3.91)	28.50 (9.69)	04.67 (2.01)	05.86 (2.69)
Cervix, mm (%)					
Fresh tissue	09.24 (3.49)	09.56 (4.39)	12.54 (4.27)	04.97 (2.14)	10.47 (4.36)
Sagittal section	09.78 (3.69)	09.98 (4.58)	12.90 (4.39)	04.34 (1.87)	08.50 (3.54)
Left tube, mm (%)					
Sagittal section	26.59 (10.03)	25.78 (11.83)	26.94 (9.16)	28.42 (12.25)	16.35 (6.81)
Right tube, mm (%)					
Sagittal section	27.03 (10.2)	26.83 (12.31)	27.33 (9.30)	28.89 (12.45)	26.30 (10.96)

a ventral midline incision. Biometrics of the internal organs (vagina, cervix, uterus and uterine tubes) was performed using a digital calliper with 0.01 mm resolution. In order to obtain the length of the tubes, we used a nylon fibre following the natural morphology of the studied organs. The measurement of the vagina was made from the vestibule to the junction with the uterus, and the uterus was measured from the bottom to the internal ostium. The measurements of the cervix were made from the internal ostium, until the end of the infravaginal portion of the cervix. For a better delimitation, measurements were taken when the organ was still intact and after sagittal section.

Histological analysis

Tissue samples of the vulva, clitoris, vagina, uterus, cervix and uterine tubes were collected and fixed in 10% formaldehyde for 24 h. The ratio of fixative to tissue volume was 20:1. After fixation, the material was dehydrated in ethanol in a series of increasing concentrations (70, 80, 90 and 100%), with soaking for about 60 min at each concentration. The diaphonization was carried out in two xylene baths, 60 min each. The samples were immersed in two baths of histological paraffin at 60°C for 45 min each. Subsequently, samples were set in standard blocks following pre-established guidelines for each segment: cross-section of clitoris, cross-section of vulva, cross-section of vagina, longitudinal section of uterus, longitudinal section of cervix, cross-sections of portions of the uterine tubes. Serial sections of 5 μm thickness were mounted on glass slides and stained with haematoxylin and eosin for routine histological analysis, periodic acid–Schiff to visualize cervical glycoproteins or Masson's trichrome staining to visualize collagen and muscle fibres throughout the reproductive tract. All sections were analysed by using a light microscope (Olympus, Tokyo, Japan) at magnifications of ×40, ×100, ×400 or ×1000.

Results

External genitalia

In all studied females, the labia majora were well delimited macroscopically, showing a rough cutaneous aspect and sparsely covered with hair. In the specimens of *S. macrodon*, the pregnant female had labia majora with a swollen aspect (Fig. 2a), while in the young female, the labia were barely evident (Fig. 2b). In *S. cassiquiarensis*, the labia were protruding, and their shape and arrangement corresponded to the position, shape and size of a scrotum (Fig. 2c,d). In all three species, the labia majora had a yellowish colour, with the presence of black dotted pigmentation in varying amounts between animals. The pregnant female *S. macrodon* had higher amounts of pigmentation, followed by the juvenile female of the same species, which presented an intermediate pigmentation (Fig. 2a,b). Females *S. cassiquiarensis* had lower amount

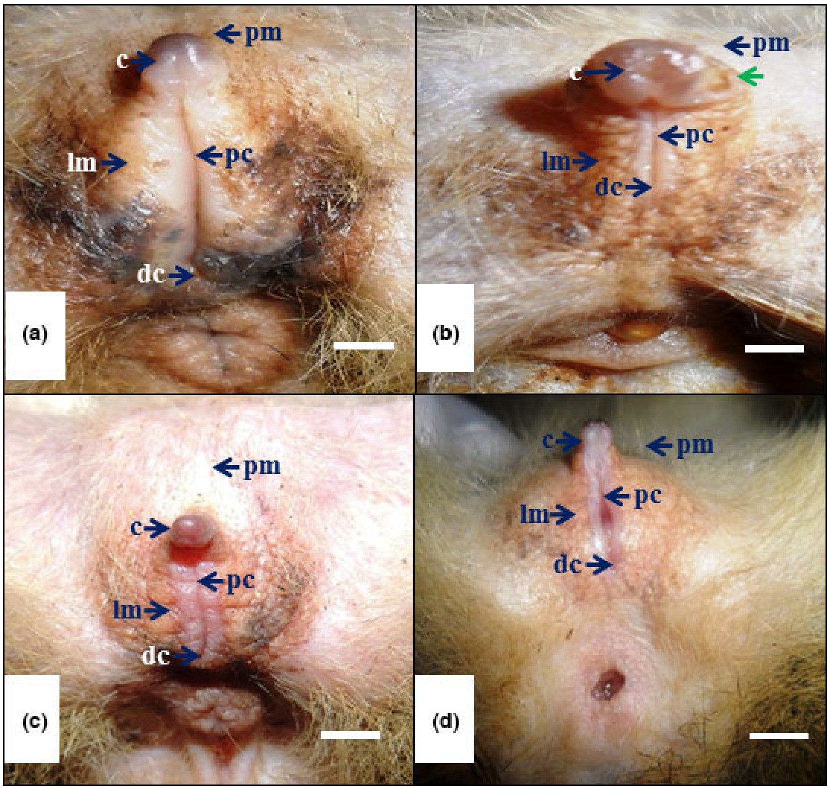

Fig. 2. External genitalia of the females of *Saimiri*. (a) Pregnant adult *S. macrodon* female. (b) Juvenile *S. macrodon* female. (c) Pregnant adult *S. cassiquiarensis* female. (d) Adult *S. cassiquiarensis* female. Caption: pm, pubic mound; c, clitoris; pc, pudendal cleft; lm, labia majora; dc, dorsal commissure. Scale bar: 1 cm.

of pigmentation in the labia majora (Fig. 2c,d). Between the labia majora, it was located the pudendal cleft, which showed a pale pink colour (Fig. 2a–d). Adjacent to the prudential cleft, the skin of the labia showed a pale pink colour, similar to a mucosa. However, this structure showed no folds and was not concealed by the labia majora, but continuous to them, and was not characterized as labia minora. A longitudinal hiatus with smooth edges continuous with the cleft was observed; no lips were formed. A hood-shaped prepuce was also identified. The clitoris had a conical shape and was enlarged in every specimen analysed. The clitoral glands had a smooth surface, callous aspect and dark colour (Fig. 2a–d).

Histological analysis revealed that the outer surface of the labia majora was lined by keratinized stratified squamous epithelium (Fig. 3a–i). There were blood vessels, hair follicles, sebaceous and sweat glands and adipose tissue on the lamina propria of all samples. In *S. macrodon*

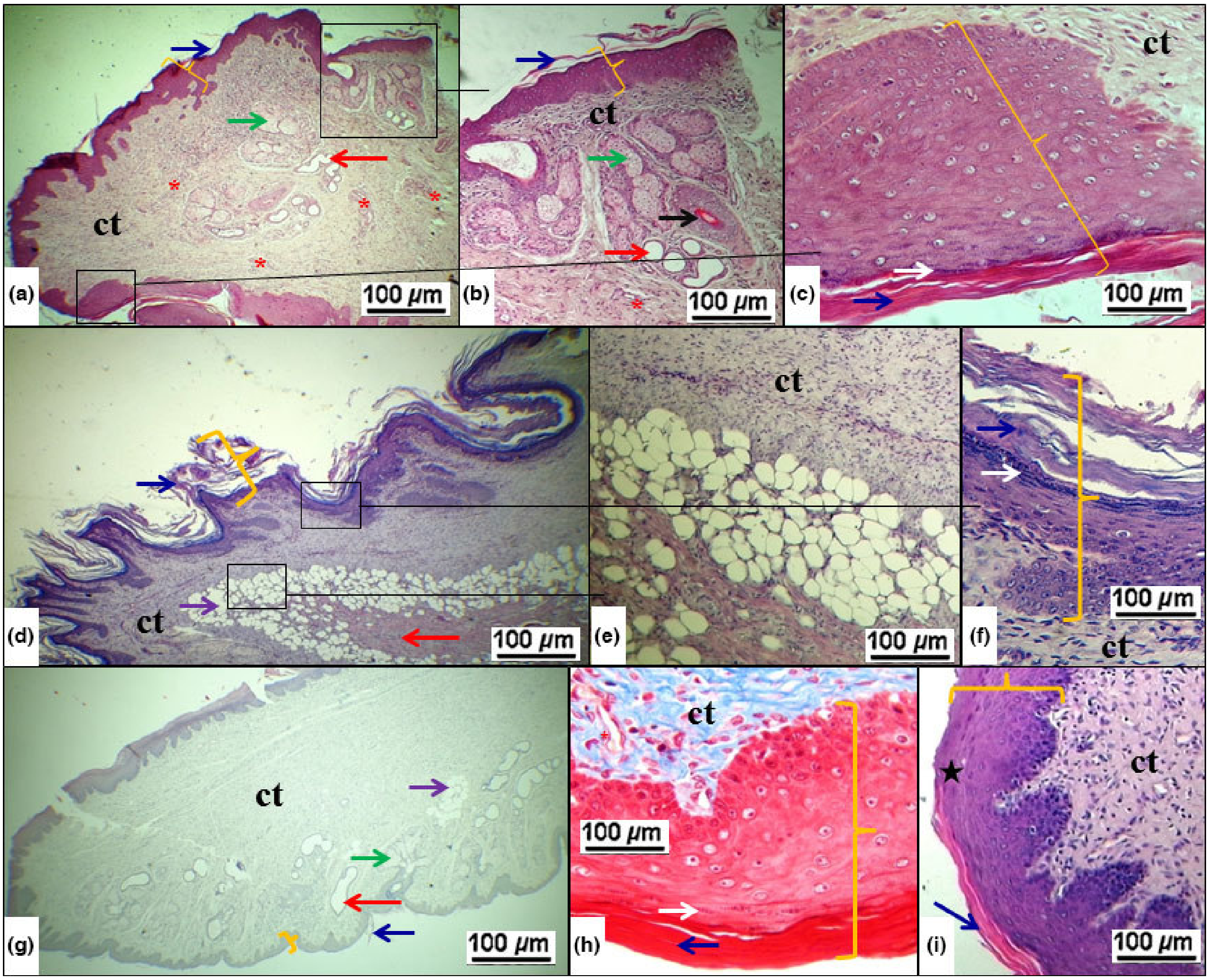

Fig. 3. Photomicrograph of the labia majora in *Saimiri*. (a–c) Adult *S. macrodon*. (d–f) Juvenile *S. macrodon*. (g–i) Adult *S. cassiquiarensis*. Yellow brackets delimit the keratinized stratified squamous epithelium. Blue arrows: stratum corneum; green arrows: sebaceous glands; red arrows: sudoriparous glands; white arrows: stratum granulosum; lilac arrows: adipose tissue; black arrows: hair follicles. Caption: ct: connective tissue. Panel a (×40). Panel b shows a higher magnification (×100) of the indicated top right portion of (a) (×40) showing sudoriparous glands, sebaceous glands and hair follicles. Panel c is a higher magnification (×100) of the bottom left area in (a), with special attention to the granular layer (white arrow). Panel d (×40). Panel e is a higher magnification (×100) of the indicated area at the bottom of Panel d which shows a greater increase in adipose tissue. Panel f is a higher magnification (×100) of the indicated area on the top of Panel d highlighting the granular layer (white arrow). Panel g (×40). Panel h is a magnification (×100) showing the epithelium of the labia in *Saimiri cassiquiarensis* showing a least developed stratum corneum that in *Saimiri macrodon*. In Panel i, there is a magnification (×100) of the transition from keratinized stratified squamous epithelium of the outer surface to the inner surface of the labia majora to the non-keratinized stratified squamous epithelium of the vagina (star). Staining: a–g, i – haematoxylin & eosin; h – Masson's trichrome.

females, the granular layer appeared to be more developed (Fig. 3c,f) than in the females *S. cassiquiarensis* (Fig. 3h). The lamina propria of the mucosa was formed by irregular dense connective tissue, rich in collagen fibres. The connective tissue invaded the epithelium and formed buds. The juvenile female *S. macrodon* showed a greater amount of adipose tissue in the lamina propria (Fig. 3d,e) when compared to the other specimens. The internal surface was covered by non-keratinized stratified squamous epithelium, which was continuous with the vaginal epithelium (Fig. 3i).

The clitoris was lined by keratinized stratified squamous epithelium (Fig. 4a), and the glands had an epithelium with a thick stratum corneum (Fig. 4b,c), which provided the callous aspect macroscopically identified (Fig. 2). The lamina propria was composed of irregular dense connective tissue, with rich vascularity. The connective tissue overran the epithelium and formed buds in which Meissner corpuscles were observed (Fig. 4c).

Vagina

In all three species, the vagina consisted of a long channel, with a dorsoventrally compressed lumen. One-third of its length was found in the pelvic cavity, while the other two-thirds were at the final portion of the abdominal cavity. The disposition was ventral to the rectum, with its cranial portion being covered by the urinary bladder (Fig. 5).

In the caudal portion of the vagina was located the vaginal vestibule, while the cranial region was connected with the uterine cervix (Fig. 6). Unfortunately, in such a closed organ, it was possible to precisely identify the junction between the vagina and the cervix only with a sagittal section, since the cervix penetrated the cranial region of the vagina, and also a circular slit formed around the cervix, the vaginal fornix (Fig. 6). The ventral portion of the fornix was slightly deeper than the dorsal portion. There was an observable difference between the proportions in the length of the vagina only when comparing the juvenile female *S. macrodon* and the adult female *S. cassiquiarensis* (Table 1). The entire surface of the vaginal mucosa showed folds in all studied species. Such folds were arranged transversely in the cranial region and longitudinally in the rest of the mucosa (Fig. 6).

Histologically, the vagina consisted of a fibromuscular tube, with three layers: the tunica mucosa, the tunica muscularis and the tunica adventitia. The mucosal layer was comprised of non-keratinized stratified squamous

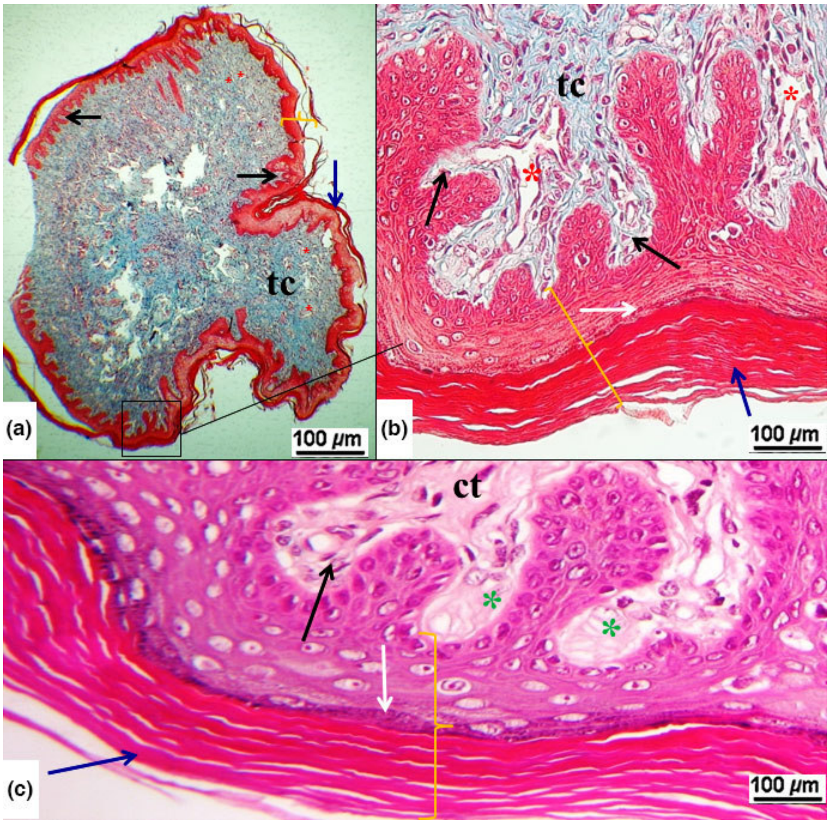

Fig. 4. Photomicrograph of the glands of the clitoris from *Saimiri cassiquiarensis*. Panel a: histological section of a clitoris (×40); Panel b: higher magnification of the indicated area in Panel a (×100); Panel c: higher magnification showing the presence of the Meissner's corpuscles (×100). Brackets delimit the keratinized stratified squamous epithelium. Blue arrows: stratum corneum; black arrows: papillae; white arrows: granular layer; asterisks: Meissner's corpuscles. Legend: ct – connective tissue. Staining: a, b – Masson's trichrome, c – haematoxylin & eosin.

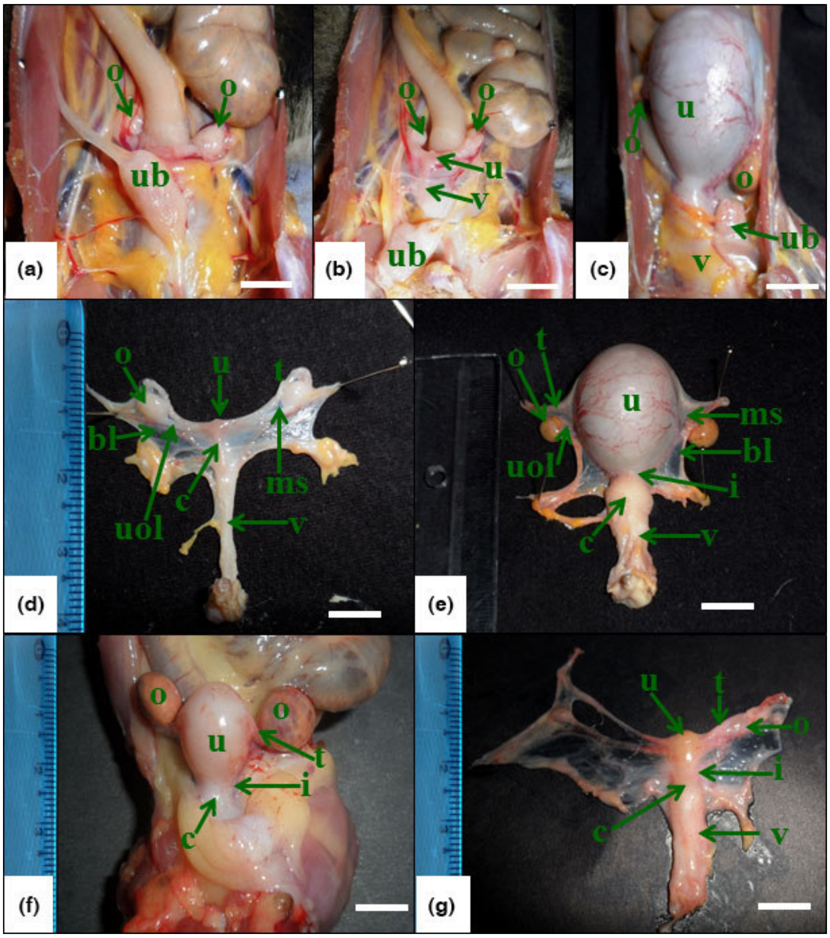

Fig. 5. Frontal view of the reproductive tract of the three *Saimiri* species. Panels a–e: *S. macrodon*. Panel f: *S. cassiquiarensis*. Panel g: *S. vanzolinii*. Panels a–c: organs '*in situ*'. Panel a: non-pregnant female – urinary bladder covered the organ. Panel b: view of the uterus after the removal of the urinary bladder. Panel c: pregnant female, where the urinary bladder did not cover the uterus. Panels d–g: reproductive tract '*ex situ*'. Caption: ub, urinary bladder; c, cervix; i, isthmus; bl, broad ligament; uol, utero-ovarian ligament; ms, mesosalpinx; o, ovaries; t, uterine tube; u, uterus; v, vagina. Scale bar: 1 cm.

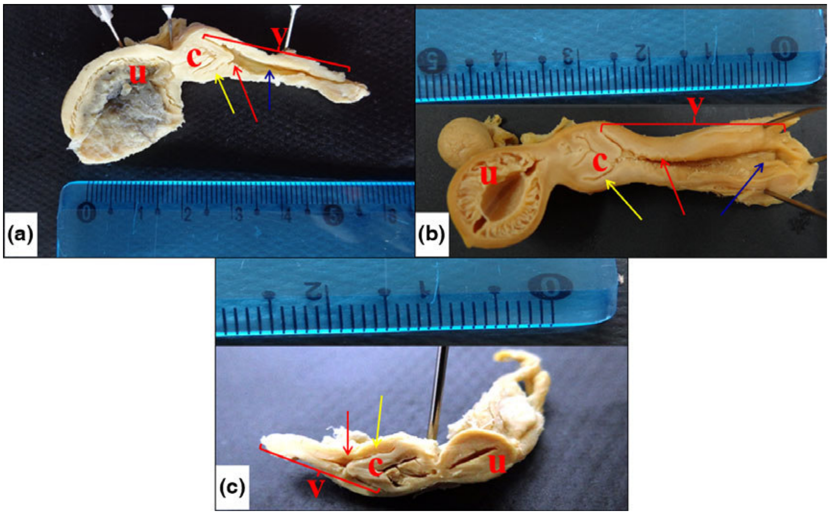

Fig. 6. Photograph of the reproductive tract '*ex situ*' of *Saimiri*, highlighting the vagina. Panel a: *S. macrodon*; Panel b: *S. cassiquiarensis*; Panel c: *S.* vanzolinii. Caption: u, uterus, v, vagina, c, cervix. Blue arrows: longitudinal folds; red arrows: cross-folds; yellow arrows: vaginal fornix.

epithelium and the underlying lamina propria, and desquamation of the vaginal lumen cells was observed in all three species (Fig. 7a,b). The thickness of the layers of the epithelium was variable along the vaginal canal. Similar to the labia, the connective tissue of the lamina propria invaded the epithelium and formed buds (Fig. 7c). The buds give an irregular appearance to the junction between the epithelium and the connective tissue. When the buds were

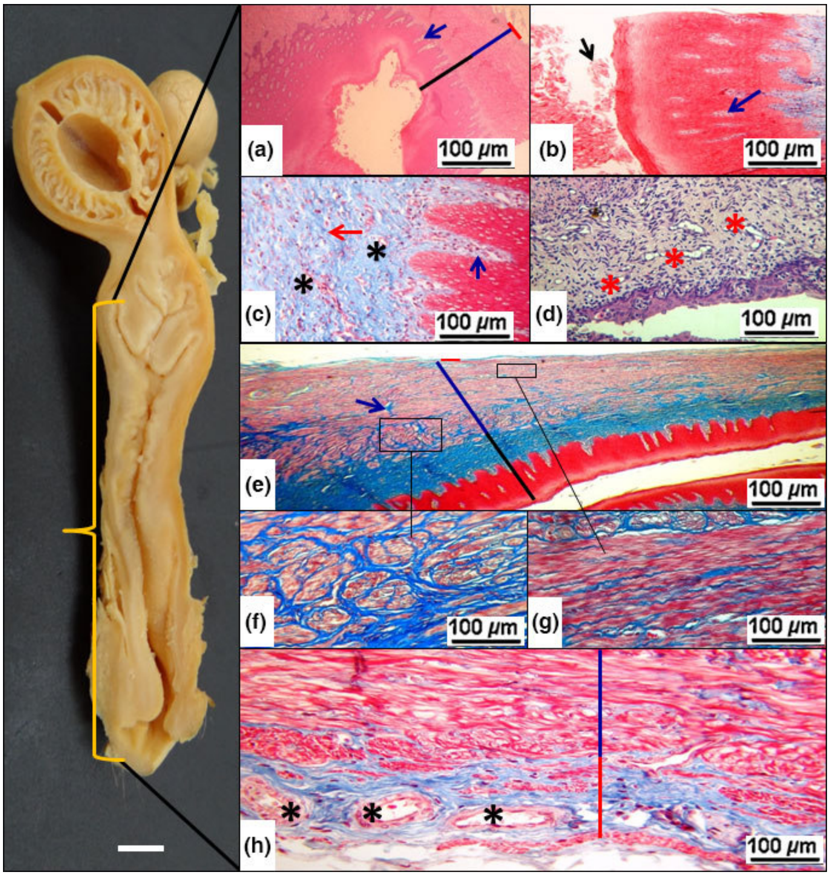

Fig. 7. Photograph and photomicrographs of the vagina in *Saimiri*. Panel a (×40): Bars indicate the three vaginal layers (Black bar: mucosal layer; blue bar: muscle layer; red bar: tunica adventitia); blue arrow: papilla. Panel b (×100): cells in desquamation from vaginal epithelium (black arrow) and indication of the connective tissue of the lamina propria in the epithelium (blue arrow). Panel c (×100): lamina propria indicating the connective tissue; blue arrow: loose connective tissue on the apex of the papilla; red arrow: dense irregular connective tissue; asterisks: numerous blood vessels present in this layer. Panel d (×40): asterisks indicate vascularization of the lamina propria. Panel e (×100): Black bar: mucosa; blue bar: muscle layer; yellow bar: tunica adventitia; blue arrow: dense irregular connective tissue within the muscle layer. Panel f (×400): selected area from panel e with muscle layer showing the circular inner layer which is observed in cross-section. Panel g (×400): selected area from panel e showing the arrangement of the longitudinal smooth muscle fibres. Panel h (×400): Black bar: tunica adventitia; asterisks: blood vessels. Staining: a, d, haematoxylin & eosin; b, c, e–h, Masson's trichrome. Scale bar: 1 cm.

sectioned transversely or obliquely, they appeared as islands within the epithelium (Fig. 7b). The apex of the papillae was composed of loose connective tissue, while the rest of the lamina propria consisted of irregular dense connective tissue, supported by collagen fibres (Fig. 7c). Some of the collagen fibres extended into the muscle layer (Fig. 7e). The lamina propria was well vascularized, where venules and larger vessels were observed (Fig. 7c,d). The muscular layer was composed of smooth muscle fibres (Fig. 7d,e), consisting of two different layers: the inner layer, with a circular arrangement, and the outer layer, with a longitudinal arrangement (Fig. 7f,g). However, these arrangements were not clearly defined, since there were fibres spreading in all directions. Muscle fibres were interlaced with collagen fibres (Fig. 7e). The tunica adventitia showed irregular dense connective tissue, whose fibres entered the muscle layer in a similar way as observed in the lamina propria (Fig. 7h). Blood vessels were also apparent. There were no signs of glands in the vagina of the studied specimens.

Cervix and uterus

The cervix was located in the abdominal cavity, arranged ventrally to the rectum and dorsally to the urinary bladder. Its caudal portion was delimited by the vagina and the cranial portion by the uterus. Uterine body and cervix could be distinguished by sagittal section (Fig. 8). There was a vestibule-like chamber in the caudal portion of the endocervical canal of all females, in the space between the first colliculus and the infravaginal portion. Colliculi are fibromuscular projections which formed crypts throughout the endocervical canal. They were blind-ended and showed variable lengths (Fig. 8). The colliculi were joined at the base and formed a continuous circumferential projection towards the vestibule. *S. vanzolinii* female had a single pair of large or primary colliculi, whereas in the cervix of the other species, we distinguished two sets of colliculi, a pair of primers and a pair of side colliculi (Fig. 8).

The non-keratinized stratified squamous epithelium of the vagina was continuous with the epithelium of the cervix. The transition from non-keratinized stratified squamous epithelium to simple columnar epithelium (squamous–columnar junction) occurred in the cranial face of the first colliculus (Figs 9h,i and 10d). As observed in the vaginal epithelium, papillae were also detected in the epithelium of the infravaginal portion of the cervix (Fig. 10b).

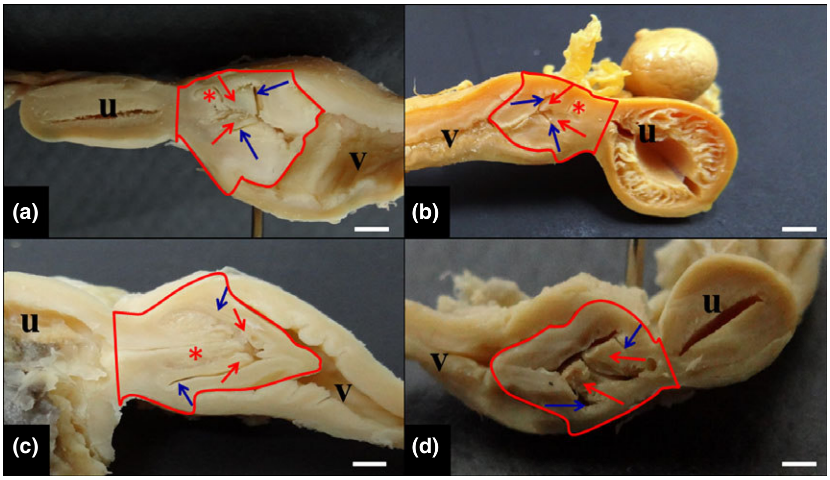

Fig. 8. '*Ex situ*' reproductive tract of the three *Saimiri* species evidencing the complexity of the cervix. Panels a, b: *S. cassiquiarensis*. Panel c: *S. macrodon*. Panel d: *S. vanzolinii*. Caption: u, uterus; v, vagina. Delimited area in red corresponds to the cervix. Red arrows: colliculus; blue arrows: crypts formed by colliculus; asterisks: secondary colliculi.

Several cervical glands, simple and branched, were distributed from the inner ostium of the uterus to the inner face of the first colliculus at the squamous–columnar junction (Figs 9 and 10). The lining of the glands consisted of simple columnar epithelium. There were no glands in the infravaginal portion of the cervix.

Saimiri macrodon at the middle third of pregnancy presented glands and crypts formed by columnar cells with small nuclei, connected to the primary colliculi and displaced towards the basal lamina (Fig. 10i). A large amount of mucus was observed in the lumen of these glands. However, in the glands of the secondary colliculi, close to the uterus and in the final portion of the uterus, the observed cells had round nuclei and no clear shift towards the basal lamina (Fig. 10g,h). In the crypts and lumen of the glands, only a small amount of mucus was observed (Fig. 10g). In the other females, the simple columnar cells of the cervical glands and mucosal folds were located deeply in the pregnant female (Fig. 9f). The cells in the mucosal folds had larger nuclei. In addition, a small amount of mucus was observed in the lumen and crypts of the glands. The endocervical canal in the pregnant female was much tighter than in other females and showed a large amount of mucus in its lumen (Fig. 10a).

The lamina propria of the cervix was composed of irregular dense connective tissue (Fig. 9b). The lamina propria on the infravaginal portion had a thickness similar to that of the end of the vagina (Fig. 9a). However, the remainder appeared as a thin layer (Fig. 9b). The cervical muscle layer was thick, and the arrangement of the muscle layers on the walls that cover the colliculi was well defined, with an inner circular layer and a longitudinal outer layer, both with similar thickness (Fig. 9d). In the region of the uterine isthmus (the boundary between uterus and cervix), the circular muscle layer was predominant, and only a thin longitudinal outer layer was present (Fig. 9e).

In all three species, the uterus was located in the abdominal cavity (Fig. 5a–c). In the two pregnant females, the urinary bladder did not cover the uterus (Fig. 5c), while in the other females, the uterus was fully covered by the urinary bladder (Fig. 5a). The uterus was simple, with an inverted pear shape (Fig. 5). In the cranial region, above the insertion of the tubes, stood the globe-shaped fundus of the uterus. At the end of the uterine body was a slight constriction of the boundaries between the body and cervix (uterine isthmus) (Fig. 5e–g).

In the uterus, it was possible to observe the uterosacral, round and broad ligaments, as well as the utero-ovarian ligaments (Fig. 5d,e). The broad uterine ligament bordered the uterine body similarly to a membrane, until it fused with the peritoneum in the cervical region. This ligament was attached to the lateral margin of the uterus, beginning at the junction with the oviduct. The round ligament was curved sideways and entered into the inguinal canal.

The perimetrium consisted of mesothelium and a thin underlying layer of irregular dense connective tissue (Figs 11j, 12c and 13f). The myometrium was highly vascularized, organized in bundles of smooth muscle fibres and randomly oriented. They could show a circular, longitudinal or diagonal direction, throughout the uterus, when viewed in sagittal section (Figs 11c,i,j, 12c,d and 13d–g). Muscle fibres were interspersed with collagen fibres (Figs 11–13).

The endometrium presented an epithelial layer and the lamina propria, where the endometrial glands were found. Both endometrial epithelium and glands were formed by simple columnar-type cells, in most specimens (Figs 11b and 12b). The exception was the non-pregnant female

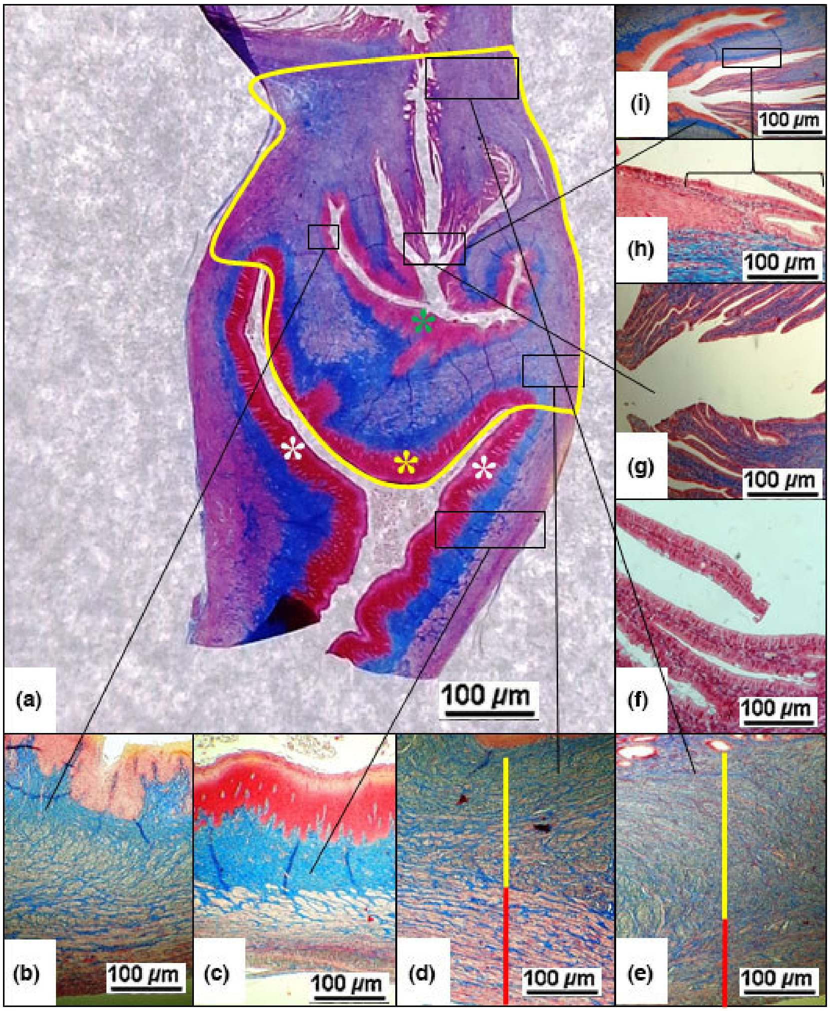

Fig. 9. Photomicrographs (a–i) of the cervix from *Saimiri cassiquiarensis*. Panel a (×6): Delimited area in yellow corresponds to the cervix; white asterisks: vaginal epithelium; yellow and green asterisks: cervical epithelium. Panel b (×100): inset magnification of part of Panel a in which the lamina propria of the cervix is thinner than the lamina propria of the vagina as shown in Panel c (×100). Panel d (×100): inset magnification of the cervical muscle layer; note the two defined muscle layers of similar thickness – the inner layer with a circular arrangement (yellow bar), and the outer layer showing a longitudinal arrangement (red bar). In (e) (×400), observe the thick circular muscle layer (yellow bar) and the thin longitudinal muscle layer (red bar) in the uterine isthmus. Panel f (×100): low columnar cells lining the glands and cervical mucosa, extending from the uterus to the cranial face of the first colliculus. Panels g and h (×100): cervical glands and the crypts formed by the folds of the mucosa. Panel i (×100): inset magnification showing the transition from non-keratinized stratified squamous epithelium to simple columnar epithelium. Staining: a–i, Masson's trichrome.

S. cassiquiarensis, who had a pseudostratified columnar epithelium (Fig. 13c,c1). The lamina propria of the endometrium was constituted by loose connective tissue richly vascularized (Fig. 12b).

The juvenile female *S. macrodon* had less developed endometrium with fewer endometrial glands than the other females (Fig. 11a–c). In the pregnant female of *S. macrodon*, the endometrium was less thick than the myometrium, and the endometrial glands had less columnar cells (Fig. 11i). In the placenta, it was possible to distinguish the stratum basale, the chorionic villi and abundant vasculature (Fig. 11e–g). Endometrium of *S. vanzolinii* presented small glands and was less thick than the myometrium (Fig. 12a).

In the non-pregnant female *S. cassiquiarensis,* the endometrium was well developed (Fig. 13a). In the pregnant female, the endometrium had reached a higher secretory development (Fig. 13h) with tortuous glands (Fig. 13m). It was possible to observe a thin layer of chorion and chorionic villi in the endometrium (Fig. 13l).

Uterine tubes

The uterine tubes consisted of two tubular structures (Fig. 14a) located on the upper margin and in between the folds of the broad ligament, the mesosalpinx. The tubes extended from the side of the uterine fundus, towards the cranial pole of each ovary. Four portions are usually recognized in the uterine tubes: infundibulum, ampulla, isthmus and uterine portion (intramural).

The ostium uterinum tubae was characterized by a small opening at junction of the uterine tubes with the uterus (intramural). The isthmus was the narrowest and straight portion connected to the ampulla (Fig. 14c). The ampulla was the longest and widest part of the tubes (Fig. 14b). The end of the uterine tube opens into the infundibulum, which had the shape of a funnel. In it, there were numerous finger-like processes, the fimbriae (Fig. 14b). The fimbriae were found in both *S. cassiquiarensis* females, but it was not possible to find this structure in the *S. vanzolinii* specimen. The presence of

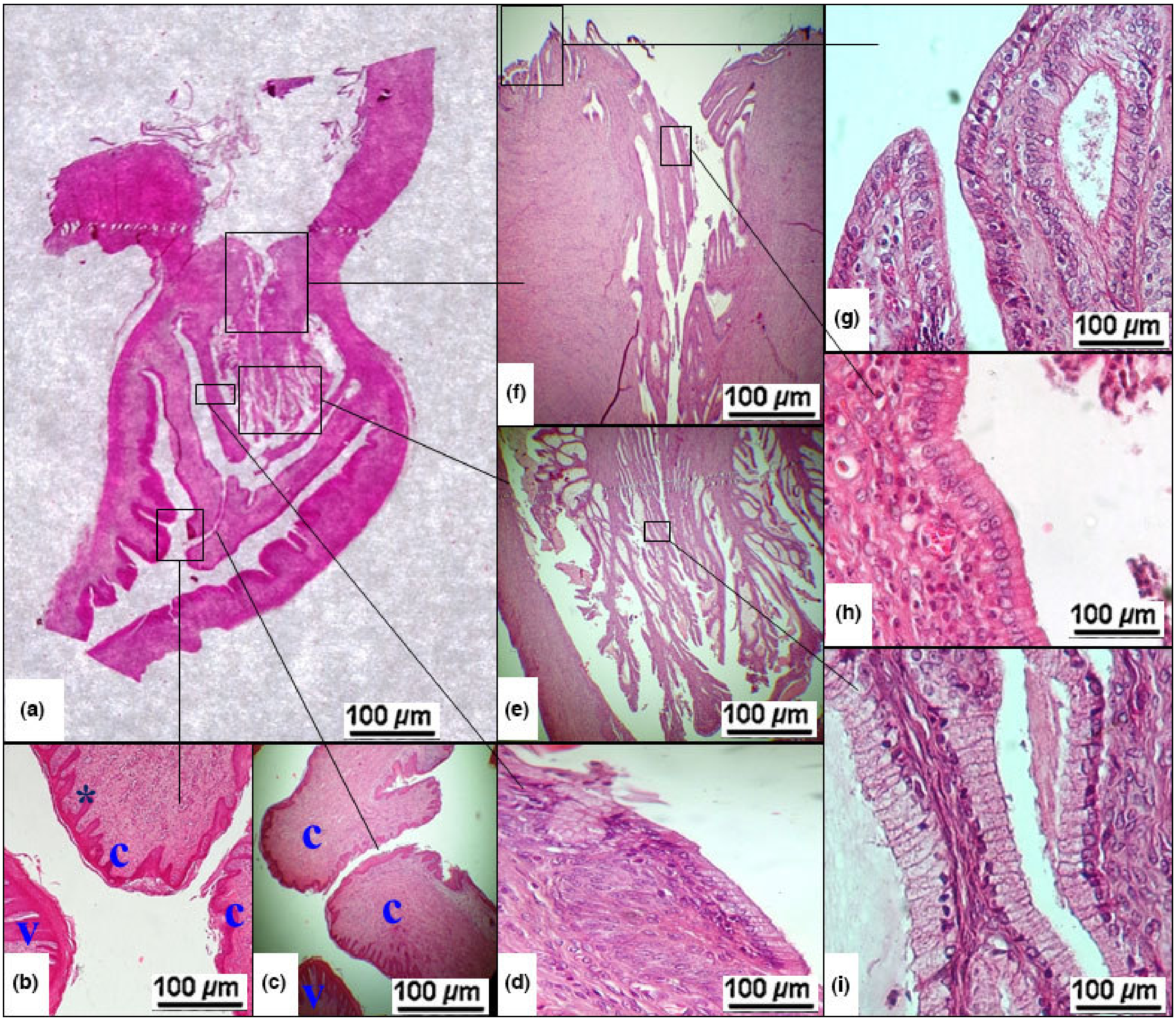

Fig. 10. Photomicrographs of the cervix from pregnant *Saimiri macrodon*. Panel a (×6): Cervix. Indicated areas are addressed at higher magnification in the following panels. Panel b (×100): similarity between the non-keratinized squamous epithelium of the vagina and the similar epithelium of the infravaginal portion of the cervix; and the similarity between the projections (papillae) of the irregular dense connective tissue that overruns the epithelium. (see * representing this similarity) Panel c (×40): the opening of the cervical canal (arrow). Panel d (×400): squamous–columnar junction in the inner face of the first colliculus. Panel e (×100): a detail of the region in which there is a large production of mucus in the endocervical glands. Panel f (×40) shows the cervical canal near the entrance of the uterus; note the continuousness of the uterine glands, which are similar to the cervical glands. Panels g and h (×400): columnar cells present in the glands at the end portion of the uterus and cranial portion of the cervical canal; note the round and displaced nuclei of the basal lamina, and the presence of mucus. Panel i (×400): columnar cells present in the primary colliculus; the cells presented small and displaced nuclei towards the basal lamina. Caption: c, cervix; v, vagina; p, papillae; staining: periodic acid–Schiff.

fimbriae in the young female *S. macrodon* was observed, but this structure occurred to a lesser extent than in the adult specimen. Inside the infundibulum was observed that a bend of the broad ligament (anterior mesosalpinx) held the uterine tubes in place, while the anterior mesosalpinx was extended along the back of the tube until it merges with the uterus.

The tubes followed a straight course in *S. cassiquiarensis* and *S. vanzolinii* (Fig. 5f,g), whereas in *S. macrodon*, they were straight in the isthmus portion and spiral in the mid-caudal region of the ampulla and along the infundibulum (Fig. 5d,e). The left uterine tube in the female *S. vanzolinii* was smaller than the right tube (Table 1).

Histologically, three layers were observed on the walls of the uterine tubes: the inner layer (mucosa), the intermediate layer (muscle) and outer layer (serous) (Fig. 14a–d). The mucosa showed longitudinal folds

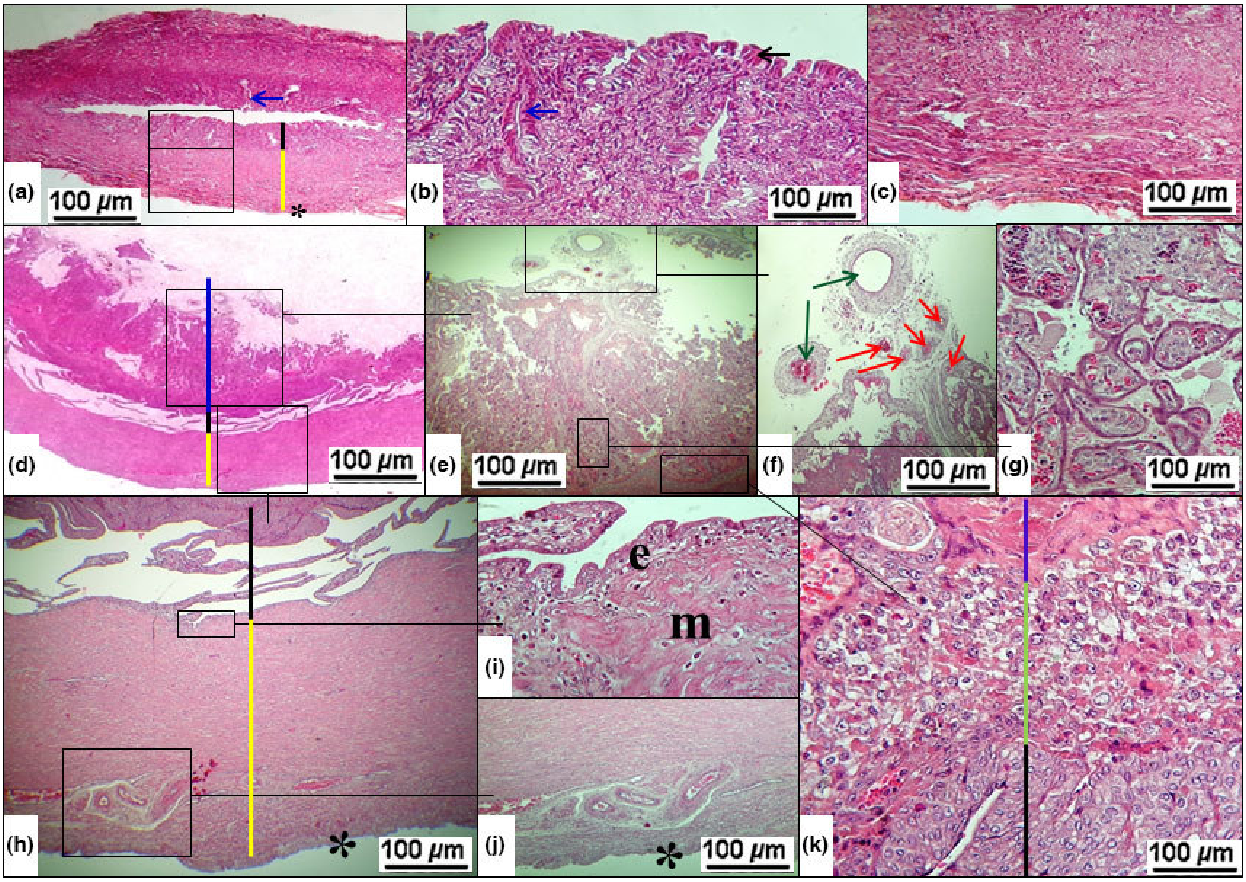

Fig. 11. Photomicrograph of the uterus of *Saimiri macrodon*. Panels a–c: juvenile female. Panel d–k: pregnant female. Observe the structure of the uterine wall in panels a and d: Perimetrium (asterisk), myometrium (yellow bar), endometrium (black bar) and placenta (blue bar). In panels a (×40) and b (×100), blue arrows indicate the endometrial glands. (b) Inset magnification of the endometrium delimited in the upper area of panel a to show the columnar cells of the endometrium (black arrow) and the endometrial glands (green arrow). Panel c (×100): inset magnification of the myometrium delimited at the bottom of panel a to show the myometrium and arrangement of muscle fibres. Panel d (×100): placenta. Panel e (×100): magnification of the upper delimited area in panel d. Panel f (×100): magnification of a portion of the placenta showing arteries (green arrows) and veins (red arrows). Panel g (×400): magnification indicating the chorionic villi. Panel h (×400): wall of the pregnant uterus. The black bar indicates the endometrium, the yellow bar indicates the myometrium, and the asterisk indicates the perimetrium. Panel i (×400): magnification of the delimited area on top of panel h showing the myometrium (m) underlying the endometrium (e); note the random arrangement of smooth muscle fibres and low columnar cells of the endometrium. Panel j (×100): magnification of the delimited area on top of Panel h showing the arrangement of the muscle fibres and irrigation of the myometrium, and the perimetrium (asterisk). Panel k (×400): magnification of the delimited area in the lower portion of the panel e showing the boundaries between the chorionic villi (blue bar), the stratum basale (green bar) and the endometrium (black bar). Staining: haematoxylin & eosin.

protruding into the lumen. It was highly vascularized and sustained by a thin layer of loose connective tissue, rich in collagen fibres (Fig. 14b). Along the uterine tube, the folding of the mucosa was variable. The folds were more evident in the ampulla and in the infundibulum (Fig. 14a,b). In the distal portion of the infundibulum, the folding occurred in the shape of fringes, which are the tube's fimbriae (Fig. 14e). Collagen fibres were observed below the epithelium of the folds (Fig. 14c). In the isthmus, the folding was simple, with few folds, and close to the uterus it had become small. The fimbriae were highly vascularized, and ciliated cells were more numerous in this region (Fig. 14f). It was possible to observe the presence of collagen fibres in all folds. In all specimens, this layer was covered by a simple columnar epithelium; ciliated cells were found in all regions of the tube. The ciliated cells found in the isthmus were fewer than in the other portions. The muscle layer consisted of smooth muscle fibres arranged in an outer layer, with longitudinally oriented fibres, and an inner layer of muscle fibres, oriented in a circular manner (Fig. 14b). Numerous blood vessels were visible in this layer. The

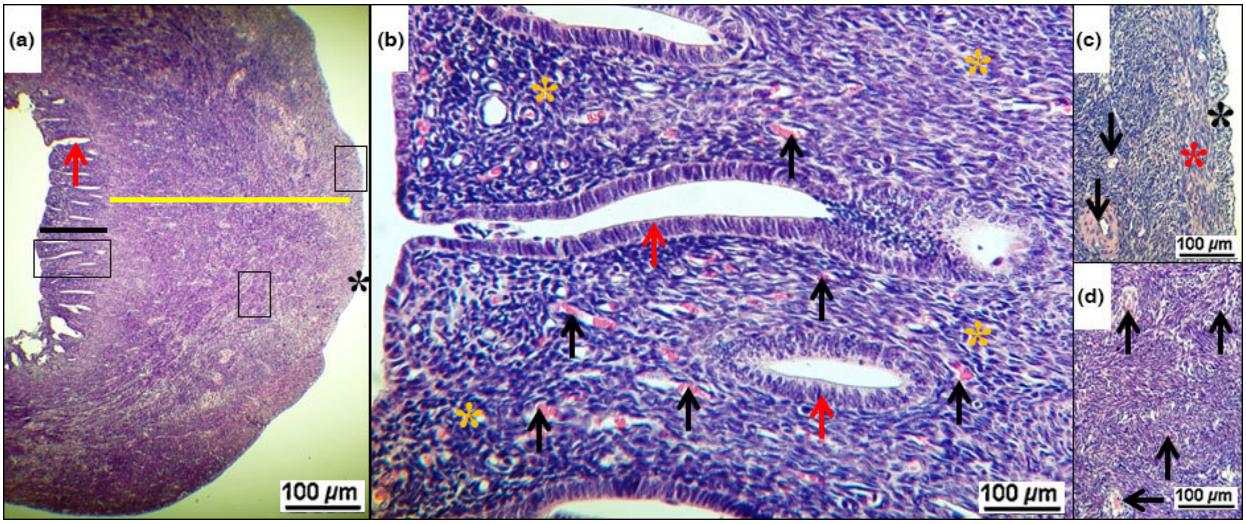

Fig. 12. Photomicrograph of the uterus of *Saimiri vanzolinii*. Note the structure of the uterine wall in Panel a (×40): Perimetrium (asterisk), myometrium (yellow bar) and endometrium (black bar). Red arrows: endometrial glands. Panel b (×400): magnification of the indicated area in the endometrium; note the rich vascularization (black arrows), the endometrial glands (red arrows) and the loose connective tissue (asterisk). Panel c (×100): inset of the indicated top right area in panel a showing irrigation of the myometrium (black arrows) and the longitudinal arrangement of muscle fibres (red asterisk) adjacent to the perimetrium (black asterisk). Panel d (×100): inset of the centre area of panel a showing the random arrangement of the myometrium fibres, as well as its irrigation (black arrows). Staining: haematoxylin & eosin.

serosal layer was composed of flattened mesothelial cells and an underlying narrow layer of irregular dense connective tissue.

Discussion

This study is the first to describe anatomical and histological aspects of the female genital organs of the rare and threatened *S. vanzolinii* as well as of *S. macrodon* and *S. cassiquiarensis*. In this study, there is the certainty of unambiguous identification of two other species analysed, since the origin of the animals is known. Therefore, it can be stated that also the same aspects of *S. macrodon* and *S. cassiquiarensis* are herein described for the first time.

The pseudoscrotal formation observed in some primate species can be explained by the fact that the labia majora are homologous to the male scrotum (Wislocki, 1936), but it must be considered that, despite this homology, not all species share this pseudoscrotal formation.

The hypertrophy of the clitoris of the three analysed species is a feature already known in members of Cebinae subfamily, as well as in some Atelidae (e.g. *Ateles*) (Hill, 1952, 1960; Napier and Napier, 1967). This characteristic, along with a pseudoscrotal formation of the lips, in many cases may hinder the rapid visual determination of sex in these taxa (Pocock, 1920; Wislocki, 1936; Hill, 1952, 1960; Dixson, 2012). One possible explanation for the development and maintenance of the hypertrophy of the clitoris in females of the species of *Saimiri* is that this would be a structure used in the expression of the behaviour of dominance hierarchy and connection between females. Dominant females exhibit the clitoris, similar to males that expose the penis, as a demonstration of dominance over other group members (Winter and Ploog, 1967; Alvarez, 1975). These two behaviours are probably similar, since the area of the female brain that is activated during the exhibition of the clitoris is the same area that is activated in males during erection (Maurus et al., 1965).

The differences in the length of the vagina are due to the fact that, macroscopically, with a closed organ, it is not possible to fully measure the vagina, since the distal portion of the cervix protruded into the vaginal canal. The description of primates' vaginas as 'long' refers to the fact that some of the uteri of some Platyrrhini are located in the abdominal cavity (Hill, 1960; Cui and Matthews, 1994). This location of the uterus seems to be an apomorphy of Neotropical primates. The transverse folds may play an important role in sexual selection for *Saimiri*, since the gametes can enter and be retained in these folds, acting as a barrier in sperm competition and also as a place of gamete reserve. However, this hypothesis remains to be further investigated in order to allow corroboration.

There are differences in the anatomy of the cervix in several species of primates (Colborn et al., 1967; Hafez and Jaszczak, 1972). In studies with animals identified as

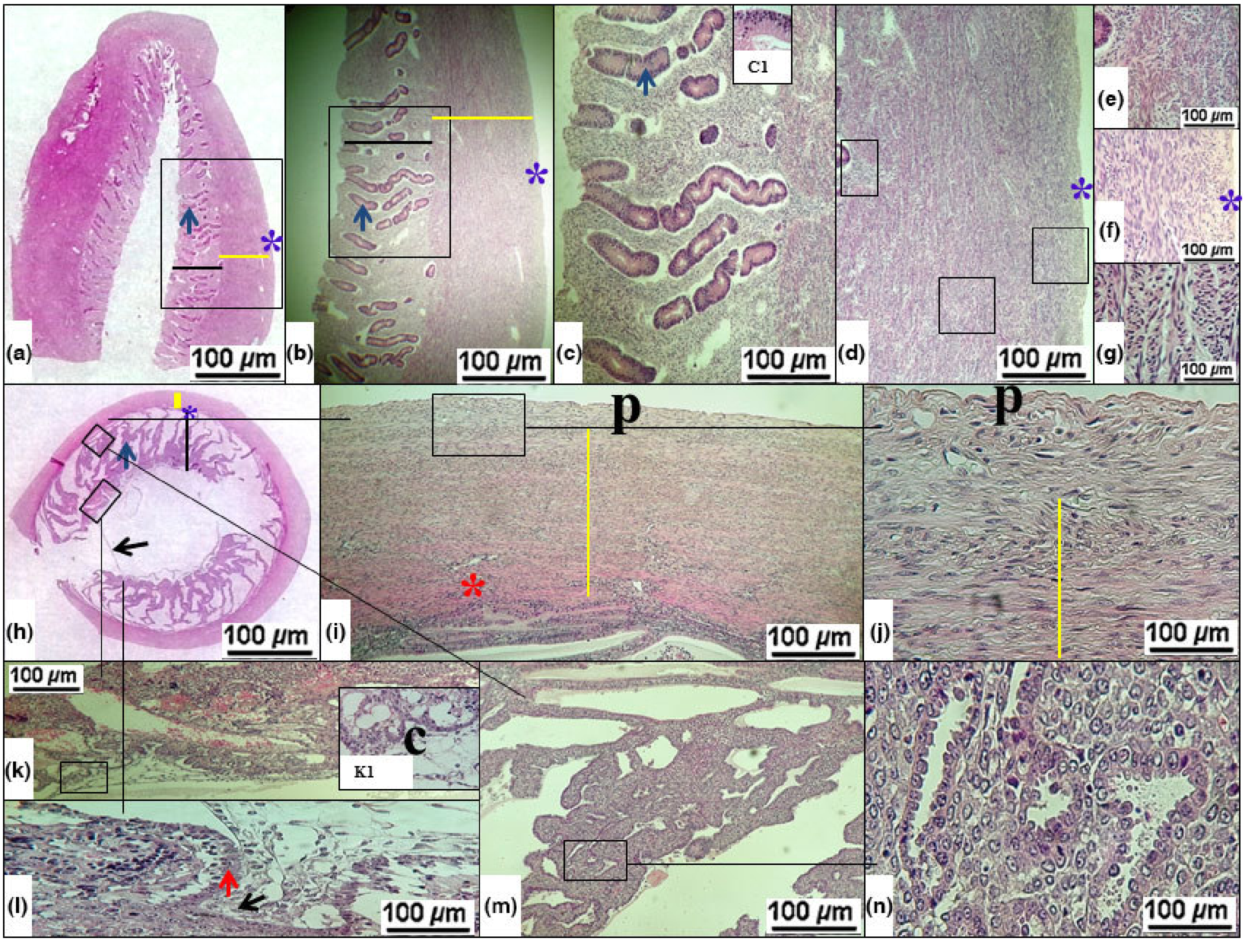

Fig. 13. Photomicrograph of the uterus of *Saimiri cassiquiarensis.* Panel a–g: non-pregnant uterus. Panel h–n: pregnant uterus. Note the structure of the uterine wall in panels a, b and h. Perimetrium (blue star), myometrium (yellow bar) and endometrium (black bar). Blue arrows: endometrial glands. Panel a (×6): endometrium. Panel b (×100): magnification panel a; note the columnar cells of the endometrial glands, also presented at a higher magnification in Panel c (×400). Panel d (×40): note the arrangement of muscle fibres of the myometrium and perimetrium (asterisk). Panel e: (×100): magnification of the arrangement of muscle fibres near the endometrium in the indicated left area of panel d. Panel f (×100): magnification of the indicated left area in panel d showing the arrangement of the muscle fibres adjacent to myometrium (asterisk). Panel g (×400): magnification of the centre area of panel d showing the arrangement of muscle fibres; note their irregular pattern. Panel h (×6): developed endometrium with the corium allantois (black arrow) and the endometrial glands (blue arrow). Panel i (×40): magnification showing the myometrium (yellow bar) and perimetrium (p). Panel j (×100): magnification of the indicated area of Panel i showing the perimetrium (p) and the arrangement of the muscle fibres in the myometrium (yellow bar). Panel k (×40): magnification of the delimited area in panel h, showing endometrium vascularization. Panel k1 (×100): expansion of the enclosed area in panel k pointing the corium. Panel l (×100): inset of the indicated area of panel h showing the attachment of the corium (black arrow) to the endometrium (red arrow). Panel m (×100): magnification of the indicated area in panel h showing the convoluted aspect of the endometrial glands. Panel n (×400): magnification of the endometrial glands. Staining: haematoxylin & eosin.

S. sciureus, this portion of the uterus has been described as long and well developed, with an average length of 10 mm, similar to the lengths herein recorded (Colborn et al., 1967; Hafez and Jaszczak, 1972). However, Branco et al. (2010) described the cervix in captive *S. collinsi* as short, with an average length of 5 mm. It should be noted, however, that these authors measured the closed organ, and possibly the measurement was made only to the supravaginal portion of the organ. It is believed that differences in the cervix between species may be the result of the duration of mating, the frequency of insertion of the penis into the female as well as the ejaculatory push and copulatory posture (Hafez and Kanagawa, 1972; Hafez, 1973). These morphological, anatomical and behavioural differences present the key–lock hypothesis to explain the specialization of intraspecific genitals. It

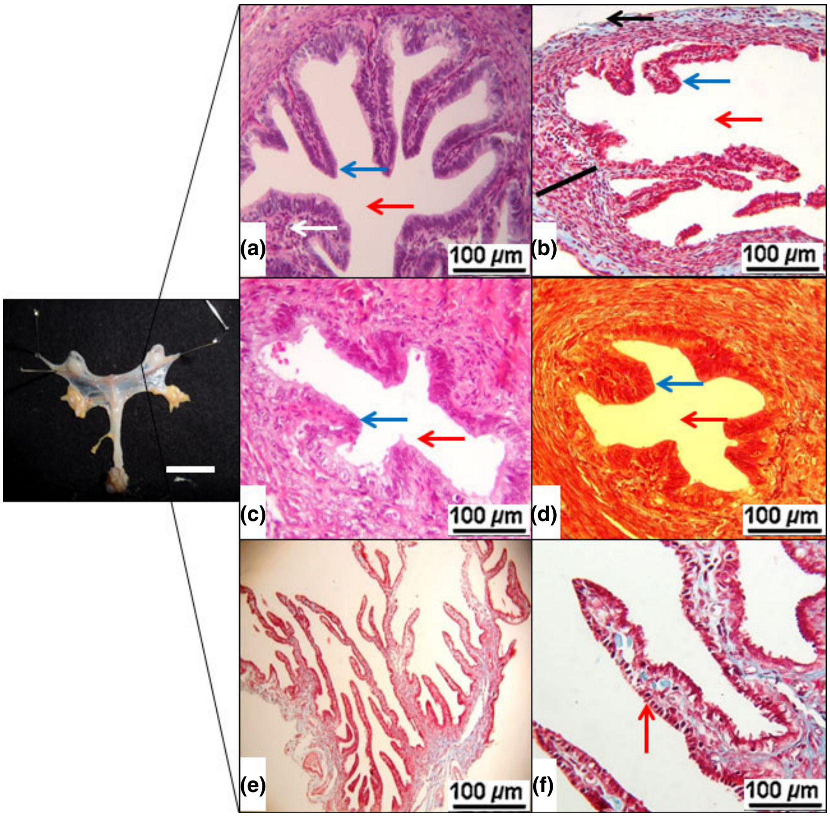

Fig. 14. Photograph and photomicrograph of the uterine tube of *Saimiri*. Panel a (×100): Infundibulum; Panel b (×100): Ampulla; Panel c (×100): Isthmus. Panel d (×400): Intramural. Panel e (×100): Fimbriae; Panel f (×1000): Ciliated cells of the fimbriae (green arrow). Red arrows: lumen; blue arrows: mucosal folds; white arrow: lamina propria of the mucosa; black arrow: serosa. Black diagonal bar: muscle layer. Staining: a and c, haematoxylin & eosin; b, d, e and f, Masson's trichrome. Scale bar: 1 cm.

would act as copulatory isolation mechanism against hybridization in sympatric, peripatric or parapatric species (Fooden, 1967; Hershkovitz, 1977), consolidating the process of speciation (Hosken and Stockley, 2004). The shape and size of the cervix and other structures of the female reproductive tract in the analysed species suggest that there are no incompatibilities that may pose a reproductive isolation barrier between them. The difference in the amount of colliculi between *S. vanzolinii* and other species may be a derived characteristic related to the presence of more than one colliculus. *S. cassiquiarensis* and *S. macrodon*, as well as *S. sciureus* (Colborn et al., 1967; Hafez and Jaszczak, 1972), which are older species than *S. vanzolinii* (Alfaro et al., 2015), had more than one colliculus. This difference may also be associated with the fact that the latter species are more distant phylogenetically related to the other two species described herein (Alfaro et al., 2015).

Importantly, hybridization is common among *Saimiri* species, in captive or natural environments (Ariga et al., 1978; Thorington, 1985). There seems to be hybridization between *S. macrodon* and *S. cassiquiarensis* at the Mamirauá Reserve as well as between *S. cassiquiarensis* and *S. vanzolinii* as identified by genetic analysis (Paim et al., 2013). All of these factors indicate that the genital morphology mechanisms for reproductive isolation in the analysed species of the genus *Saimiri* have not evolved completely. However, similar to other primate species, other mechanisms, such as ecological and behavioural differences, may act as potential barriers to this reproductive isolation (Fleagle and Mittermeier, 1980; Porter, 2001).

The cervix is considered a place of reserve and selection of gametes (Mattner and Braden, 1963; Hafez and Jaszczak, 1972), also acting as a barrier to prevent excessive number of incoming sperm to the site of fertilization (Hafez and Jaszczak, 1972). It should be considered that the crypts formed by colliculi form blind ends, which could be mistaken with the entry of the cervical canal. We suggest that this would result in an error in the insertion of sperm in case of direct artificial insemination in the cervix. Mechanisms of sperm selection are adaptations that play a role in post-copulatory competition among males, especially in primates whose social and reproductive systems are multimale and multifemale (Moller, 1988; Clutton-Brock, 1989), as seems to be the case for the species of *Saimiri* (Izar et al., 2009; Stone, 2014). According to Hafez and Jaszczak (1972), one hour after intercourse, there are different concentrations of gametes throughout the several cervical crypts in some primates. At the same time, the distribution of sperm throughout the cervix is affected by biophysical and biochemical characteristics of the cervical mucus (Hafez and Jaszczak, 1972).

The extension from the vaginal epithelium up to the cervical canal was also recorded by Colborn et al. (1967) and Hafez and Jaszczak (1972) in animals and then identified as *S. sciureus*. However, in those studies, the site of the squamous–columnar junction was variable. In the species described herein, this feature was limited to the cranial face of the first colliculus. Nonetheless, it is emphasized that this characteristic is influenced by age, pregnancy and hormonal state (Sonoda et al., 1998; Wood, 2008; Harbison et al., 2013).

The developed musculature, showing a different layout in the isthmus and the rest of the cervix, aids as contraction strength to close the sphincter at the time of pregnancy, or during the period in the oestrous cycle in which the uterus needs to be closed. In the muscle layers that overlap the colliculi, the well-defined longitudinal and circular musculature presses the infravaginal portion of the cervix and the colliculi, which consequently reduces the intracervical lumen during pregnancy. This can be enhanced by the presence of large amounts of mucus in the cervical glands in pregnant females, which plays a crucial contribution to the sealing of the channel, so that foreign bodies cannot enter the uterus during pregnancy (Iacobelli et al., 1971; Nasir-ud-Din et al., 1979, 2003). The random pattern of smooth muscle cells of the myometrium is associated with the key role of the contractions of these muscles during pregnancy and childbirth, being regulated by hormonal factors, cellular and molecular (Brainard et al., 2007; Spencer et al., 2012; Gao et al., 2014).

The non-pregnant uterus in *Saimiri* is proportionally small in relation to body size (Martin, 1833; Hill, 1960). As in all Neotropical primates, the uterus is a single tube, the *simplex* uterus, with a globoid fundus (Wislocki, 1932; Eckstein, 1958; Hill, 1960; Monteiro et al., 2003; Pissinatti et al., 2008; Veras et al., 2009). The difference in the proportions between the uteri analysed in this study was influenced by the pregnancy which was already in its middle third. The size of the uterus is variable in Platyrrhini monkeys (Wislocki, 1932; Hill, 1952, 1960; Eckstein, 1958). The longest on record for a non-pregnant uterus of New World primates was 50 mm in *Ateles* (Eckstein, 1958). However, Branco et al. (2010) reported that captive specimens of *S. collinsi* had an average length of the uterus of 63 mm (excluding the cervical region). This length is much larger than the specimens of this study, where the greatest uterine length was 10.35 mm. The measurement of Branco et al. (2010) may be misleading, since no Platyrrhini study shows this uterine length, not even the largest Neotropical primates, such as *Alouatta*, *Ateles*, *Brachyteles* and *Lagothrix*.

This study revealed that the anatomy described for *Saimiri sciureus* presented some differences from the species studied herein, besides other aspects not described for that species. The three species showed more similarities than differences in most structures, but it is noteworthy that such differences may be due to individual variation. The differences are apparently not relevant as mechanisms of reproductive isolation, but some characteristics of the cervix may play a role in post-copulatory competition of males and their sperm, in a context of polygyny and polyandry. Furthermore, the observed differences regarding *S. vanzolinii* senile female are probably ageing changes (Lapin et al., 1979; Verguts et al., 2013).

The development of reproductive biotechnologies is often ineffective for many species, for the little knowledge of the reproductive aspects of the species is one of the main difficulties of *in situ* and *ex situ* conservation programmes based on such biotechnology. Thus, the present description provides important information that can help create conservation strategies for these and other species of the genus *Saimiri,* as well as subsidize the development of reproductive biotechnologies or techniques of assisted reproduction and shed some light on important questions over evolutionary aspects of the components of the reproductive system of these species and other primates.

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Conflicts of Interest

The authors state that there are no conflict of interests that might be prejudicial to the impartiality of this research.

Author Contributions

GPL and ABB took part in the preparation, processing and description of samples. GPL, ABB, FPP, RRS, HLQ and SFSD contributed to the data analysis and writing of the article.

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CAPÍTULO 2

Unilateral ovarian absence in a Black-headed Squirrel Monkey (*Saimiri vanzolinii* Ayres, 1985), a threatened Neotropical primate species

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CASE REPORT

Unilateral ovarian absence in a Black-headed Squirrel Monkey (*Saimiri vanzolinii* Ayres, 1985), a threatened neotropical primate species

Gerson P. Lopes[1,2], Adriel B. Brito[1], Regiane R. Santos[1],
Sheyla F. S. Domingues[1], Fernanda P. Paim[,2,3] Helder L. Queiroz[1,2]

[1] Laboratory of Amazon Animal Biotechnology and Medicine, Federal University of Pará, Castanhal, PA, Brazil.

[2] Mamirauá Institute for Sustainable Development, Tefé, AM, Brazil.

[3] *Federal University of Minas Gerais, Belo Horizonte, MG, Brazil.*

Correspondence

Corresponding author: Rodovia BR-316 km 7 s/n, Levilândia, CEP 67030-000, Ananindeua, Pará, Brasil. Tel.: +55 91 32130441; fax: +55 91 32650304

E-mail address: r.rodriguesdossantos@pq.cnpq.br

ABSTRACT

Ovarian agenesis is an unusual anomaly with traumatic or congenital origin. In the present case report, we describe our findings in a senile *S. vanzolinii* female. Since this neotropical primate species is listed as vulnerable, with limited geographic distribution in the Brazilian Amazonia, ovarian agenesis may be important to its conservation.

Introduction

Ovarian agenesis either or not accompanied by the absence of fallopian tube (oviduct) is a rare phenomenon. It has congenital or traumatic origin [5, 7, 18]. Congenital ovarian agenesis is often associated to alterations on the M*üllerian duct differentiation [7, 14, 18*], while the traumatic origin is linked to torsion followed by ovarian necrosis [6, 14, 16]. In both cases, unilateral ovarian agenesis is commonly asymptomatic. Reports describing ovarian agenesis have been published on primates, especially focused on humans [20], with scarce reports in non-human primates [4]. The importance of documentation from reproductive anomalies such ovarian agenesis encompasses not only the formation of a data basis, but this information can also be used to perform a proper analysis of its occurrence and impacts on the natural habitat of the affected females. Herewith we describe a case of unilateral ovarian absence in *Saimiri vanzolinii* (black-headed squirrel monkey), a free living neotropical non-human primate species recently listed as threatened by the IUCN [9].

Case report

All the procedures herein described were approved by Ministério do Meio Ambiente - Sistema de Autorização e Informação em Biodiversidade - SISBIO/ICMBio/MMA nº 29906-1 and by the Ethical Committee in Animal Research of Mamirauá Institute of Sustainable Development (MISD) - nº 002/2012. In 2012, during a field study at MISD, *Saimiri vanzolinii* specimens were selected for genetic studies. All the procedures were performed under the supervision of a veterinary. For this, the animals were firstly anesthetised with ketamine hydrochloride (Cetamine 15 mg/kg; IM; Köning S.A., Avellaneda, Argentina) and xilazine hydrochloride (Anasedan 2mg/kg; IM; Köning S.A.). During the procedures, however, one senile female did not recover from anaesthesia and died. The approximate age (> 6 years-old) of the female was estimated based on dentition considering tooth eruption, intra-osseous tooth formation and tooth wear [19]. Therefore, the animal was submitted to an exploratory necropsy. The female was presenting head and tail alopecia together with a high parasitic infection. It was observed also the presence of a dense fibrous tissue in the coelomic cavity. We performed macro- and microscopic evaluation of the reproductive system. For this, tissues were fixed in 10% formalin.

During inspection, extern genitalia, vagina, cervix and uterus were normal. However, the left oviduct was almost 10 mm shorter that the right one (16.35 mm left oviduct; 26.30 mm right oviduct), and both oviducts were deprived from fimbriae. The left ovary was morphologically normal (length: 5.41 mm; breadth: 3.30 mm; height: 3.21 mm), but at the place of the right ovary it was found an ovoid and soft structure (Figure 1A). The left broad ligament was shorter than the right one. The uterine ligaments were normal without adherences.

At microscopic analysis, the left ovary presented evidences of a normal functioning history by the presence of more than 28.000 ovarian follicles at different developmental stages, and a corpus luteum was present (Figure 1B, C). On the right side, however, the ovoid soft structure was found to be not an ovary, but a structure similar to the oviducts (Figure 1D), because of its similar folded mucosa with ciliated columnar epithelial cells (Figure 1E).

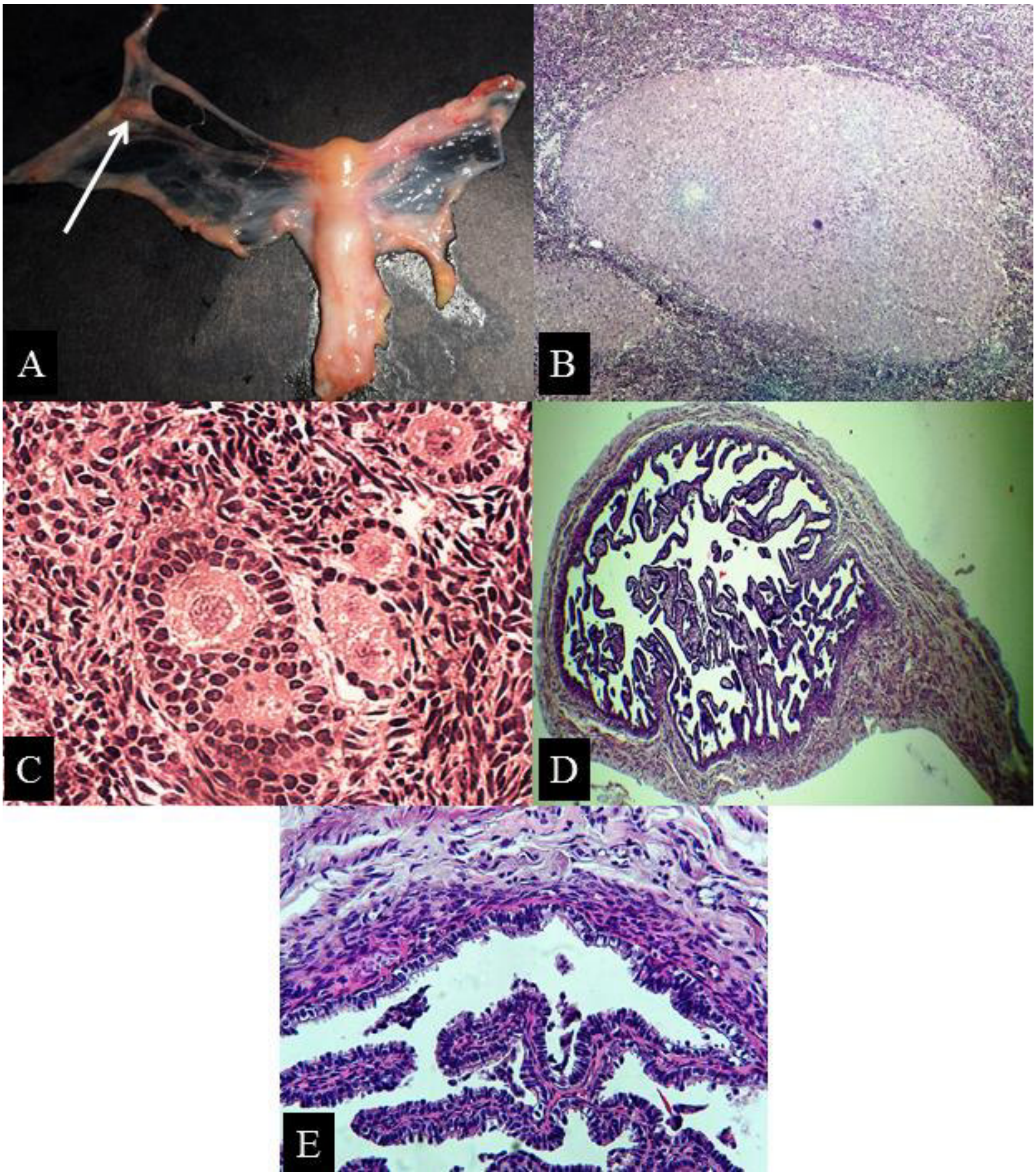

Figure 1. Images depicting the reproductive tract of a senile *S. vanzolinii* female, showing the found ovoid-soft structure occupying the location of the right ovary (A; see white arrow). After routine histology staining (haematoxylin-eosin) the left ovary presented a corpus luteum (B) and ovarian follicles at different developmental stages (C). The ovoid structure, however, showed a folded mucosa with ciliated columnar epithelial cells (D), similar to the microscopic characteristics of the oviduct (E).

Discussion

Herewith we report a case of ovarian agenesis in a *Saimiri* species listed in the IUCN list as vulnerable [9], a threatened category. For our knowledge, this is the second report of ovarian agenesis in a neotropical primate. The first case was observed in a *Sapajus apella* female [4].

Alopecia was observed in the examined female. This can be a physiological or not physiological process. For example, it has been shown in other non-human primate species that alopecia can be influenced by aging, seasonality, pregnancy, hormonal changes, or by stress, endocrine disorders, and nutritional imbalance, bacterial or parasitic infections [2]. In the present case report, the female was probably presenting alopecia due to both processes, i.e. senility and parasitic infection.

The presence of a functioning ovary, i.e. presenting follicles at different developmental stages and corpus luteum, in senile squirrel monkeys has been previously reported [1, 21]. The observed anomaly was restricted to the absent right ovary, which was supplanted by an ovoid structure with histological characteristics of an oviduct. The differences in the length of the oviducts and broad ligaments might be attributed to a developmental malformation [4, 5, 12], probably related to a congenital defect in which the migration of the germ cells did not occur [12], leading to the non-formation of one of the ovaries [15]. Such defect is more unusual than other anomalies attributed to a failure in the Müller duct development, which causes defects in the oviduct, uterus, cervix, vagina, and extern genitalia [3, 17].

In the present studied case, a possible hybridization effect was discarded, once there is no evidence of contact between *S. vanzolinii* and its congener and neighbour species, *S. cassiquiarensis* [14]. Although individuals presenting inefficient reproductive performance may impact their population, absence of one ovarian will not circumvent the generation of descendants [8]. However, it may contribute to a decrease in the size of the population. For instance, women with a single ovary will be not less fertile, but there is no compensatory mechanism for the number of reserve follicles [10]. Thus, similar effects are expected in non-human primates as well. This is an unusual case, and will be mostly accidentally observed in non-human primates, especially those free-living species. Reporting this finding will stimulate other groups to also maintain attention on the reproductive system during unforeseen necropsies.

One could suggest a further ovarian function evaluation through ultrasound using a larger number of females. However, as *Saimiri vanzolinii* is a vulnerable species, we are not allowed to capture many females for such analysis. Also, we do not expect agenesia as a common condition, but as a rare observation as reported for other primates. And, capturing a large number of animals for such a study may lead to undesirable stress and end up with negative effects on their reproductive performance, which is not ethically approved. On the other hand, comparative biology can be used to study more deeply the closest species which are not endangered and share the sabe geographical area, such as *S. cassiquiarensis*. Recently, we have shown similarities among *S. vanzolinii, S. cassiquiarensis*, and *S. macrodon*, when evaluating reproductive female organs [11]. These findings will support the development of reproductive biotechniques for *S. vanzolinii* using, as models, the not threatened species *S. cassiquiarensis* and *S. macrodon*.

The present manuscript is not an attempt to induce a change in the conservation status of *S. vanzolinii*, which is already listed as threatened by IUCN, due to its current restricted geographic distribution and small population size. The present information is relevant because animals with ovarian agenesis can present, in the best of scenarios, a reduced reproductive potential, leading to a decrease in the natural population growth rate of the species. Something that is not desirable in the case of threatened species. Hence, it is an additional cause for caution in relation to the need of protection for *S. vanzolinii*.

Acknowledgments

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CAPÍTULO 3

Population estimate and morphometry of ovarian preantral follicles from three recently recognized squirrel monkey species: a comparative study

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Population estimate and morphometry of ovarian preantral follicles from three recently recognized squirrel monkey species: a comparative study

Running title: Ovarian follicular population in three squirrel monkey species

Gerson P. Lopes[1,2], Regiane R. Santos[1], Débora V. Almeida[1],
Adriel B. Brito[1], Helder L. Queiroz[2], Sheyla F. S. Domingues[1],

[1]Federal University of Pará, Postgraduate Program in Animal Science, Belém, Pará, Brazil
[2]Institute for Sustainable Development Mamirauá, Research Group on Ecology of Terrestrial Vertebrates Tefé, Amazonas, Brazil

*Correspondence to Regiane R Santos
Federal University of Pará, Laboratory of Wild Animal Biology and Medicine
BR 316 Km 61, CEP 687406970, Castanhal, Pará, Brazil.
Email: r.rodriguesdossantos@pq.cnpq.br

ABSTRACT

We describe morphological and morphometrical characteristics of preantral ovarian follicles from three recently recognized *Saimiri* species: *S. macrodon*, *S. cassiquiarensis* and *S. vanzolinii*; the last one a threatened species. Ovaries from four adult monkeys were evaluated: one pair from a pregnant *S.* macrodon, two ovarian pairs from *S. cassiquiarensis* females (one of them pregnant), and one left ovary from a senile *S. vanzolinii*, applying classical histology. Follicular preantral population was quantified and morphology and morphometry of primordial, primary and secondary follicles were evaluated. Follicular preantral population varied among species, being 347,153 in the ovaries of the *S. macrodon*, 270,342 and 278,376 in the ovaries of both adult non–pregnant and pregnant *S. cassiquiarensis* females, and 28,149 in the ovary from a senile *S. vanzolinii*. Most follicles were at primordial and transition stages, except for the senile *S. vanzolinii* female, which presented the lowest percentages of primordial and transition follicles when compared with primary and secondary ones. Most preantral follicles (> 70%) were morphologically normal in the ovaries from all studied S. macrodon and S. cassiquiarensis females, but the ovary of the senile *S. vanzolinii* female presented a significant decrease in the percentage of normal follicles (primordial: 44 61%, transition: 52%, primary: 54%, and secondary: 48%). In general, follicular diameter increased significantly from primordial to transition, and subsequently from primary to secondary follicles. Oocyte diameter started to increase significantly only in primary and secondary follicles depending on the species. For the first time we characterize preantral follicles from three squirrel monkeys species.

Keywords: primate, neotropical, *Saimiri* sp., ovarian follicles, morphology, morphometry.

Introduction

Squirrel monkeys (*Saimiri* spp.) are neotropical primates with a lifespan of approximately 30 years (Helvacioglu *et al*., 1994). Female reproductive life starts at the age 2.5 to 3.5 years (Baldwin, 1969; Taube, 1980; Boinski, 1992), and at approximately 8 years old a massive depletion in the number of reserve gametes is initiated, resulting in the complete follicular loss when the females are circa 20 years old (Walker *et al*., 2009). Monkeys from this genus present a seasonal reproduction and an extremely short ovarian cycle, lasting for 7 to 9 days (Lang 1967, Wolf *et al*., 1977; Schiml *et al*., 1999; Trevino, 2007), differently from other neotropical primates that present longer ovarian cycles, such as 18–23 days, for *Sapajus apella* (Nagle and Denari, 1983), 20–24 for *Ateles geoffroyi* (Campbell *et al*. 2001), 21 days for *Brachyteles hypoxanthus* (Strier and Ziegler 1994, 1997), 24–30 days for *Callithrix jacchus* (Kendrick and Dixson 1983; Dixson, 2001), 15 days for *Aotus trivirgatus* (Dixson, 1983), and 22 days for *Saguinus oedipus* (Brand, 1981). Also, progesterone peak levels are usually higher in *Saimiri* species (399 ng mL^{-1}) (*Saimiri sciureus*; Wolf *et al*., 1977) than in other neotropical primates such as *Sapajus apella* (60–100 ng mL^{-1}) (Nagle and Denari, 1983), suggesting species-specific differences. As observed, information on ovarian cycle and late folliculogenesis is abundant for this genus. However, knowledge related to the early folliculogenesis focusing on preantral follicles of *Saimiri* sp. or other neotropical species is still needed.

Preantral follicles are the reserve of female gametes, and will decrease with age until females become senile and enter menopause. These follicles represent 90% of the total ovarian follicle population (Gougeon and Chain, 1987). Besides nutritional and health status, the total population of preantral follicles in the ovary is species–specific and age-dependent. For instance, the number of follicles per ovary from an adult women ranges of 76,000 (19 years old) to 27,000 (46 years old) (Gougeon and Chainy, 1987), each ovary from an adult female of *Sapajus apella* contains ca. 51,000 preantral follicles (Domingues *et al*., 2004), while in *Macaca nemestrina* preantral follicular population was found in a range of 30,900 (8 months old) to 9,940 (12–13 years old) (Miller *et al*., 1999), and in *Macaca mulatta* preantral follicular population was found to be 910,000 follicles at birth (Baker and Wai, 1976). Almeida *et al*. (2012) estimated the population of primordial, primary, and secondary follicles in a senile *Saimiri sciureus* as 915, 230 and 115 follicles, respectively. Besides the number of follicles, morphometric characteristics of preantral follicles can vary with species (Gougeon, 1996), which can be used to study female reproduction in different animal species. Furthermore, such information can be used to determine characteristics of follicular atresia, infertility, and menopause (Almeida *et al*., 2012). Knowledge on ovarian morphology and follicular population size may support the development of protocols for the conservation *in*– and *ex–situ* of endangered primate species (Mayor *et al*., 2013). Moreover, it contributes to the understanding of the processes related to formation, growth and maturation of oocytes enclosed in preantral follicles (Fortune, 2003; Scarlecio *et al*., 2014).

Information on population estimates, with morphological and morphometric characterization of preantral follicles are available for limited neotropical primate species, such as *Sapajus apella* (Domingues *et al*., 2004), *Saimiri sciureus* (Walker *et al*., 2009; Almeida *et al*., 2012), and *Alouatta caraya* (Lopes *et al*., 2006), and absent for three *Saimiri* species recently recognized: *Saimiri macrodon*, *Saimiri cassiquiarensis* and *Saimiri vanzolinii*. The last one, a threatened species (IUCN, 2016). Therefore, in the present study, we aimed to estimate the population of preantral ovarian follicles, as well as to characterize the morphology and morphometry of these follicles collected from three recently recognized *Saimiri* species: *Saimiri macrodon*, *Saimiri cassiquiarensis* and *Saimiri vanzolinii*.

Materials and methods

Animals

All animals were captured and collected during field expeditions at the Mamirauá Reserve, Amazonas, Brazil (Lopes *et al.*, 2017). Animals were euthanized as part of a large research program to investigate different biological aspects of these primate species. All experimental procedures of this study were approved by the Research Ethics Committee and the Mamiraua Institute for Sustainable Development Ethics Committee, under protocol number 002/2012. The license for collection was granted by the Brazilian Institute of Environment and Renewable Natural Resources, through the System of Authorization and Information on Biodiversity (SISBIO 29906–1). All the biological material used in this investigation are deposited at the Mammals Section of the scientific collection of Mamiraua Institute for Sustainable Development.

The ovarian samples were obtained from the study animals at IDSM and were separately maintained in paraffin sections for histological analysis (Lopes *et al.*, 2017). Ovarian sections from four adult squirrel monkeys were evaluated: one pair from an adult pregnant *S. macrodon* (pregnant), two pairs of adult *S. cassiquiarensis* females (one of them pregnant), and one left ovary from a senile *S. vanzolinii*. The approximate age of the females was estimated based on the phenotype of dental chronology (Smith, 1989), and they were classified as adults (ages ranging from 1.5 to 5 years old) or senile (older than 5 years). No pathologies or abnormal findings regarding the ovarian donors were reported.

Histological analysis

The ovaries were fixed in 10% formalin and subsequently prepared for classical histology. Serial sections (5 µm thick) were cut and every 10th section was mounted and stained with hematoxylin and eosin and examined under a converted light microscope (Leica, Wetzlar, Germany).

Preantral follicles were classified according to Domingues *et al.* (2004) in which the oocyte is surrounded by a layer of flattened granulosa cells (primordial follicle), by one layer of flattened and cuboidal granulosa cells (primordial), by one layer of cuboidal granulosa cells (primary), or by two or more layers of cuboidal granulosa cells (secondary). Preantral follicles were characterized as morphologically normal or atretic as described by Domingues *et al.* (2004) and Brito *et al.* (2013), where the normal follicles were those that presented the complete basal membrane, absence of pycnotic bodies in the oocyte nucleus, without signs of oocyte and/or granulosa cells degeneration, as well as no shrunken oocyte, nor detachment between oocyte and granulosa cells.

The total number of each type of follicle was estimated using a correction factor as described by Gougeon and Chainy (1987):

$$\mathbf{N1 = No * St * Ts/So * dn}$$

Where,

Ni is the total corrected number of follicles of one class;

Noisthe number of follicles observed in the analyzed sections;

Stis the total number of sections in the ovary;

Ts is the thickness of the section (5 µm);

Sois the number of observed sections;

and **dn** is the mean diameter of the nucleus of the oocyte for each follicle class.

Follicular, oocyte and oocyte nucleus diameters were measured using a digital camera (Moticam® 5, 5.0) coupled to a computer and a morphometric analysis program (Motic Images Plus 2.0ML, Australia). For this, the follicular dimensions were measured with a micrometric eye lens in an optical microscope (400x). The largest and the smallest diameters of the oocyte nucleus, oocyte and follicle were measured. The mean diameter of each structure was calculated. The thickness of the of the granulosa layer was estimated in all preantral follicles categories, subtracting the follicular diameter from the oocyte diameter (Domingues *et al.*, 2004). Follicles were only considered when the nucleus of the oocyte was visible. At least 400 follicles per animal species were measured.

Statistical analysis

Statistical analysis was performed applying the programs BioEstat 5.3 (Ayres *et al.*, 2007) and StatView 5.0 (SAS Institute Inc., Cary, NC, EUA). Comparisons between the number of follicles per left and right ovary within each species were performed with the Wilcoson–Mann–Whitney test. Morphometric data were compared among different follicular class within a same species using ANOVA and Tukey as a post–hoc test. Results are expressed as mean ± standard error of the means (SEM) and differences were considered significant when $p < 0.05$.

Results

The ovaries were characterized by two regions: cortex and medulla. Most blood vessels were found in the medulla, but the cortical area also presented vascular irrigation, as expected (Fig. 1A). The ovarian cortex was characterized by the presence of ovarian follicles at different developmental stages (Fig. 1B,D), including those multi–oocyte follicles (MOFs) characterized by a follicle containing two or three oocytes (Fig. 1C). This type of follicle was present in all evaluated ovaries (0,35% in *S. macrodon*, 1,15% in *S. cassiquiarensis* and 0,23% in *S. vanzolinii* ovaries), and in all developmental stages from primordial to secondary. All ovaries presented a *corpus luteum* (Fig. 1E).

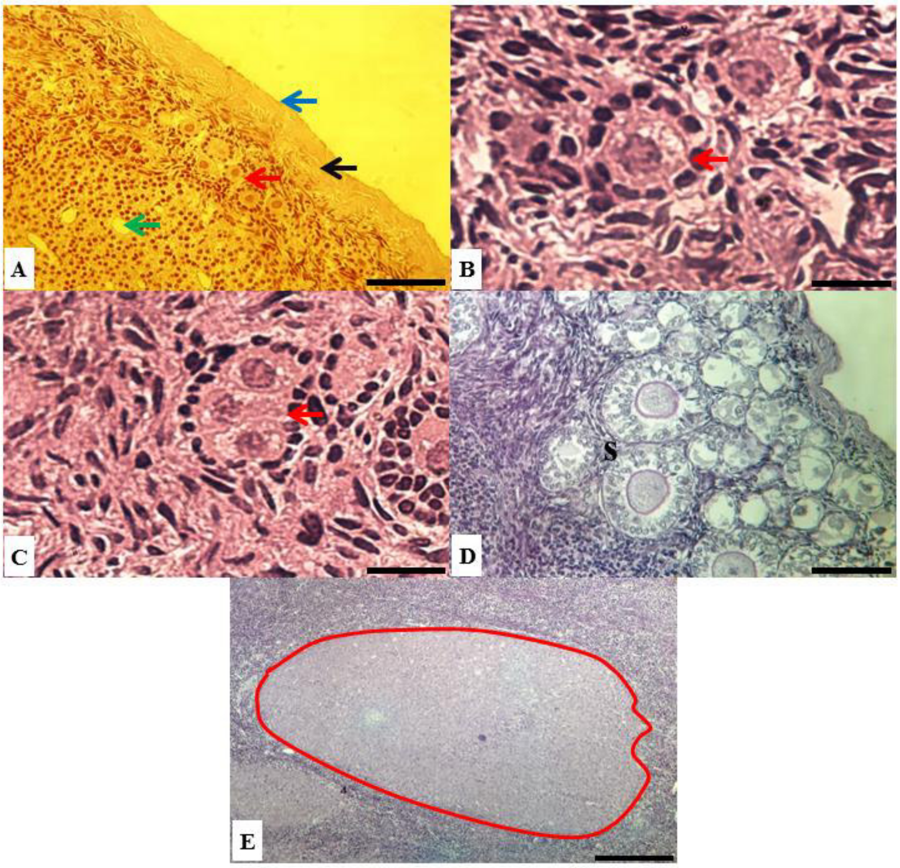

Fig. 1 Photomicrography of *Saimiri* ovaries. Panel A: histological section of ovarian tissue showing epithelium (blue arrow), tunica albuginea (black arrow), cortex (red arrow), blood vesser (green arrow); 40x. Panel B: a transitin follicles is pointed with a red arrow; 100x. Panel C: Multi–Oocyte Follicle (MOF) enclosing three oocytes (red arrow); 40x. Panel D: Preantral follicles at different developmental stages, including secondary follicles (marked with an "s"); 100x. Panel E: A delimited corpus luteum; 40x. Panel F: luteinized tissue as pointed by a red arrow; 40x. Panel G: image zoom of luteinized tissue separated from the ovarian medulla by a blue line; 400x. Hemaetoxylin–eosin staining. Scale bar: 100 µm.

The major population of preantral follicles (mean number of 347,153) was encountered in the ovaries of the *S. macrodon*, which presented a greater number of follicles in the left ovary (426,607) than in the right one (267,699); thus, a difference > 150,000 preantral follicles (Table1). Both adult non–pregnant and pregnant *S. cassiquiarensis* females presented similar preantral follicular population, i.e. a mean number of 240,906 - 299,778 and 237,187 – 319,565 preantral follicles per ovary respectively. From the senile *S. vanzolinii*, only the left ovary was recovered and evaluated, which presented the lowest follicular population (28,149); see Table 1.

Table 1 Mean (±SE) number of ovarian preantral follicles present in the ovaries from *S. macrodon* (pregnant), *S. cassiquiarensis* (pregnant and non–pregnant), and *S. vanzolinii* (senile).

Species	**Ovary**		
	Right (±SE)	**Left (±SE)**	**Mean (±SE)**
S. macrodon (pregnant)	267,699 ± 10,410^{a}	426,607 ±17,789^{b}	347,153 ± 79,454
S. cassiquiarensis (non–pregnant)	299,778 ± 13,172^{a}	240,906 ± 14,854^{a}	270,342 ± 29,436
S. cassiquiarensis (pregnant)	237,187 ± 18,182^{a}	319,565 ± 16,820^{b}	278,376 ± 41,189
S. vanzolinii (senile)	–	28,149 ± 303	–

^{a,b} different letters indicate significant differences between right and left ovaries within the same *Saimiri* species and category; $p < 0.05$

Table 2 depicts the total number of evaluated follicles and the proportions of follicles per class, as well as the number of morphologically normal preantral follicles according to each follicular class, with their respective percentages. The ovaries of *S. macrodon* presented similar percentages of total primordial (32%) and secondary (30%) follicles, being higher than the percentages of transition (20%) and primary (18%) follicles. *S. cassiquiarensis* females presented ovaries containing mostly primordial (38 - 39%) and transition (25 - 27%) follicles, when compared with the percentages of primary (18 - 19%) and (16 - 18%) secondary follicles. The ovary from the senile *S. vanzolinii* female presented the lowest percentages of primordial (15%) and transition (24%) follicles when compared with primary (31%) and secondary (30%) ones. Most preantral follicles (> 70%) were morphologically normal in the ovaries from all studied *S. macrodon* and *S. cassiquiarensis* females. As expected, the ovary of the senile *S. vanzolinii* female presented a significant decrease in the percentage of morphologically normal preantral follicles, especially those classified as transition (52%), primary (54%), and secondary (48%) follicles, when compared with the primordial (61%) ones.

Table 2 Number and mean (±SE) percentage of total and morphologically normal ovarian preantral follicles per class and animal species/category, i.e. in the ovaries from *S. macrodon* (pregnant), *S. cassiquiarensis* (pregnant and non–pregnant), and *S. vanzolinii* (senile).

Species	**Preantral follicular classes**							
	Primordial		**Transition**		**Primary**		**Secondary**	
	Total n (% ± SE)	Normal (% ± SE)	Total n (% ±SE)	Normal (% ±SE)	Total n (% ±SE)	Normal (% ±SE)	Total n (% ±SE)	Normal (% ±SE)
S. macrodon	111,471	81,373	69,118	52,530	61,158	49,538	105,406	82,217
(pregnant)	32 ± 4 a	73 ± 13 A	20± 3 b	76 ± 13 A	18 ± 3b	81 ± 13A	30 ± 4 a	78 ± 12A
S. cassiquiarensis	105,239	88,401	71,822	53,867	49,989	35,492	43,292	33,335
(non–pregnant)	39 ± 5a	84 ± 14A	27 ± 4 b	75 ± 12 A	18 ± 3c	71 ± 13 A	16 ± 2c	77 ± 12 A
S. cassiquiarensis	105,782	93,088	69,582	50,099	53,986	39,950	49,027	37,751
(pregnant)	38 ± 5a	88 ± 14A	25 ±3b	72 ± 12A	19 ± 3c	74 ± 13A	18 ± 3c	77 ± 12A
S. vanzolinii	4,255	2,596	6,874	3,574	8,617	4,653	8,403	4,033
(senile)	15 ± 3a	61 ± 11A	24 ± 3b	52 ± 11B	31 ± 4c	54 ± 12B	30 ± 4 c	48 ± 11B

$^{a–d}$ different lower-case letters indicate significant differences among the percentages of total follicles per follicular class within the same *Saimiri* species and category; $p< 0.05$

$^{a–d}$ different upper-case letters indicate significant differences among the percentages of morphologically normal follicles per follicular class within the same *Saimiri* species and category; $p< 0.05$.

Tables 3, 4 and 5 present the diameters of follicles, oocytes, oocyte nucleus, as well as thickness of granulosa cells layer per follicular class when evaluating ovaries from non–pregnant and pregnant *S. cassiquiarensis,* as non-pregnant *S. macrodon* and senile *S. vanzolinii*, respectively. In ovaries from non–pregnant and pregnant *S. cassiquiarensis* the only remarkable difference observed was the larger follicular and oocyte diameter of secondary follicles from pregnant compared with non-pregnant females (Table 3). However, because only one specimen per group was evaluated, no statistical conclusion can be drawn. Morphometric evaluation resulted in similar results for both females, where follicular diameter increased significantly from primordial to transition, and subsequently from primary and to secondary follicles. Oocyte diameter started to increase significantly only in primary and secondary follicles, and oocyte nucleus increased significantly in diameter only when follicles were at the secondary stage. Thickness of the granulosa cells layer increased significantly in each follicular class after activation (Table 3). In ovaries from *S. macrodon* (Table 4) and *S. vanzolinii* (Table 5), follicular and oocyte diameter increased significantly from primordial to transition, and subsequently to primary and to secondary follicles. The same was observed when the thickness of the granulosa cells' layer was compared within *S. vanzolinii* follicles (Table 5). In *S. macrodon* follicles, diameter of oocyte nucleus, as well as the layer formed by granulosa cells increased in thickness from the transition stage to further primary and secondary ones (Table 4). In *S. vanzolinii* follicles, diameter of oocyte nucleus increased significantly only from the primary to the secondary stage (Table 5).

Table 3 Mean (± SEM) follicular, oocyte, oocyte nucleus diameter and granulosa cells layer thickness (µm) in primordial, transition, primary and secondary follicles from *S. cassiquiarensis* (non–pregnant and pregnant).

Follicular class	**Diameter (µm)**			
non–pregnant	**Follicle** Mean (µm) (range)	**Oocyte** Mean (µm) (range)	**Oocyte nucleus** Mean (µm) (range)	**Granulosa cell layer thickness** Mean (µm) (range)
Primordial	19.7 ± 0.1^{a} (12.7 – 25.0)	14.4± 0.1^{a} (9.8 - 18)	7.8 ± 1.1 ^{a} (5.2 – 10.3)	5.1 ± 0.3 ^{a} (1.8 – 7.2)
Transition	23.1 ± 0.2 ^{b} (17.0 – 30.1)	16,07 ± 0.2^{ab} (10.3 – 21.7)	8.1 ± 0.1 ^{a} (2,47 – 10.8)	7.0 ± 0.2 ^{b} (3.3 – 10.6)
Primary	27.5 ± 0.3 ^{b} (20.3 – 37.0)	17.9 ± 0.3 ^{b} (12.1 – 30.9)	8.4 ± 0.1 ^{a} (5.9 – 10.9)	9.3 ± 0.3 ^{c} (2.5 – 17)
Secondary	156.7 ± 11.0 ^{c} (23.7 – 581.6)	59.5 ± 4.6 ^{c} (14.2 – 320.3)	24.2 ± 1.7 ^{b} (6.9 – 87.8)	83.1 ± 11.0 ^{d} (7.1 – 487.8)
pregnant				
Primordial	19.0± 0.1^{a} (12.7 – 22.5)	13.7± 0.1^{a} (9.4 – 16.5)	7.7 ± 0.1^{a} (5.6 – 8.9)	4.3 ± 0.3 ^{a} (1.8 – 7.2)
Transition	23.6± 0.1 ^{b} (19.9 – 28.6)	15.6± 0.1^{ab} (11.9 – 19.0)	7.6 ± 0.1^{a} (6.2 – 10.1)	6.7± 0.4^{b} (1.5 – 8.0)
Primary	25.6± 0.3 ^{b} (19.5 – 36.0)	17.7± 0.3 ^{b} (12.9 – 30.3)	8.0± 0.1 ^{a} (6.2 – 9.1)	8.9± 0.4 ^{c} (2.5 – 15.0)
Secondary	239.8 ± 13.4 ^{c} (26.7 – 583.8)	90.1± 6.0 ^{c} (14.4 – 318.6)	35.0± 2.3 ^{b} (7.8 – 134.1)	96.6± 18.0 ^{d} (8.2 – 462.2)

$^{a–d}$ different letters indicate significant differences among follicular classes within the same evaluated parameter, i.e. follicular, oocyte, oocyte nucleus, and granulosa cell diameter, and specimen, i.e. non-pregnant or pregnant female; $P < 0.05$

Table 4 Mean (± SEM) follicular, oocyte, oocyte nucleus diameter and granulosa cells layer thickness (µm) in primordial, transition, primary and secondary follicles from *S. macrodon* (pregnant).

Follicular class	**Diameter (µm)**			
	Follicle Mean (µm) (range)	**Oocyte** Mean (µm) (range)	**Oocyte nucleus** Mean (µm) (range)	**Granulosa cell layer thickness** Mean (µm) (range)
Primordial	19.8 ± 0.1 ^{a}	14.3 ± 0.1 ^{a}	7.9 ± 0.1 ^{a}	7.4 ± 0.1 ^{a}
	(15.8 – 21.9)	(10.7 – 16.9)	(5.3 – 9.1)	(4.9 – 10.7)
Transition	23.3 ± 0.2 ^{b}	15.9 ± 0.2 ^{a}	7.9 ± 0.1 ^{a}	7.4 ± 0.1 ^{a}
	(19.6 – 27.6)	(12.4 – 19.2)	(5.2 – 10.5)	(5.0 – 11.3)
Primary	26.6 ± 0.3 ^{c}	17.2 ± 0.3 ^{b}	8.2 ± 0.1 ^{b}	9.4 ± 0.2 ^{b}
	(21.1 – 33.2)	(12.9 – 25.4)	(4.9 – 10.2)	(5.7 – 13.1)
Secondary	230.7 ± 9.4 ^{d}	106.8 ± 4.5 ^{c}	34.2 ± 1.6 ^{c}	123.9 ± 5.7 ^{c}
	(76.3 – 525.5)	(46.6 – 249.7)	(11.5 – 87.8)	(28.5 – 275.7)

$^{a-d}$ different letters indicate significant differences among follicular classes within the same evaluated parameter, i.e. follicular, oocyte, oocyte nucleus, and granulosa cell diameter; $p< 0.05$

Table 5 Mean (± SEM) follicular, oocyte, oocyte nucleus diameter and granulosa cells layer thickness (µm) in primordial, transition, primary and secondary follicles from *S. vanzolinii*.

Follicular class	**Diameter (µm)**			
	Follicle Mean (µm) (range)	**Oocyte** Mean (µm) (range)	**Oocyte nucleus** Mean (µm) (range)	**Granulosa cell layer thickness** Mean (µm) (range)
Primordial	19.8 ± 0.3^{a}	14.9 ± 0.2^{a}	8.1 ± 0.1 ^{a}	4.9 ± 0.1 ^{a}
	(12.7– 21.9)	(10.9– 16.9)	(5.3 – 9.1)	(1.8–7.0)
Transition	22.7 ± 0.3^{b}	15.6 ± 0.2 ^{a}	7.9 ± 0.1 ^{a}	7.0 ± 0.2^{a}
	(17.0– 26.8)	(12.4 – 19.5)	(6.2 – 10.1)	(4.0– 10.3)
Primary	27.1 ± 0.4^{c}	17.3 ± 0.4^{b}	8.3 ± 0.1 ^{b}	9.8 ± 0.3^{b}
	(21.8– 35.0)	(12.1– 25.1)	(6.9 – 10.9)	(5.5– 15.1)
Secondary	86.3 ± 2.9^{d}	35.8 ± 1.5^{c}	17.1 ± 0.5^{c}	50.5 ± 3.3^{c}
	(53.6–160.8)	(12.6–53.6)	(11.1–30.4)	(11.6–116.1)

$^{a-d}$ different letters indicate significant differences among follicular classes within the same evaluated parameter, i.e. follicular, oocyte, oocyte nucleus, and granulosa cell diameter; $p< 0.05$

Discussion

Information regarding the reproductive aspects of threatened species is crucial to increase effectiveness of conservation programs (Andrabi and Maxwell, 2007; Mayor *et al.*, 2013). Obviously, the paucity of biologic material for evaluation is more accentuated for endangered species (Pukazhenthi and Wildt, 2004). This is the first study describing morphological and morphometric aspects of initial folliculogenesis in the threatened species *S. vanzolinii,* as well as other two other saimiri species from the same geographical area (*S. macrodon* e *S. cassiquiarensis*). Characterization of initial follicular development will support the application of reproductive technologies to protect these species.

Follicular population varied between left and right ovaries in each species studied here, as well as among species. It must be also considered that the low number of follicles encountered in the ovary from the senile *S. vanzolinii* female was most probably a physiologic response of ovarian aging (Tardif, 1985; Walker *et al.*, 2009; Almeida *et al.*, 2012). Besides aging, reproductive state, nutrition, and genetic factors are also known to affect follicular population (Erickson, 1966; Erickson *et al.*, 1976; Cahill *et al.*, 1979; Scaramuzzi *et al.*, 1993; Forman *et al.*, 2013).

The remarkably different follicular population from left and right ovaries found in the *S. macrodon* female and in the pregnant *S. cassiquiarensis* female was also previously described in ovaries of *Sapajus apella* (Domingues *et al.*, 2004). These later authors studied the ovaries of four adult *Sapajus apella* females, and recorded more follicles present in the right ovary than in the left one (Domingues *et al.*, 2004). They suggested that in *S. apella* the right ovary has more follicles because it is less functional than the left one, since Nagle *et al.* (1994) observed the lowest ovulation rates in the right ovaries *S. apella*. In women, however, no differences in ovulation rates are observed between right and left ovaries (Lass *et al.*, 1997), although some authors describe a more frequent ovulation rate in the right ovary (Potashnik *et al.*, 1987; Fukuda *et al.*, 2000). Although we found the opposite in the present research, our data are restricted to one animal only. However, all together, it can be confirmed that ovarian follicular population is also variable between right and left ovary in different individuals (Miller *et al.*, 1999).

Primordial, transition, primary and secondary follicles presented morphometric differences, an extra parameter to characterize these preantral follicles (Domingues *et al.*, 2004) as well as to determine their morphological quality. Our results confirm that morphometric information supports follicular classification in different developmental stages (Domingues *et al.*, 2004; Lopes *et al.*, 2006). Differences observed among species were previously found when studying other mammals (Koering, 1983; Gougeon, 1996). The changes in the diameters of oocyte nucleus, oocyte, follicle and granulosa cells layer thickness indicate that preantral follicular development follow two distinct phases (Gougeon&Chainy, 1987; Domingues *et al.*, 2004). In the first phase (activation), the pre-granulosa cells surrounding the oocyte are differentiated to granulosa cells, which is characterized by the change in their flattened to cuboidal structure. In this period, the oocyte size increases (Braw-Tal, 2002). In a second phase, increase in both follicular and oocyte diameters take place together with the proliferation of the granulosa cells (Gougeon and Chainy, 1987; Domingues *et al.*, 2004). Correlations are difficult to interpret, once herein we have not the possibility to obtain several samples per species in the present study.

Multi–oocyte follicles were observed and previously described in other primates (Harrison, 1949; Graham and Bradley, 1971; Domingues *et al.*, 2004; Lopes *et al.*, 2006). It was suggested that MOFs are a result of an incomplete ovarian organogenesis, i.e. defected follicular formation (Domingues *et al.*, 2004). It was believed that all those follicles become atretic (Hartman, 1926; Harrison, 1949). However, MOFs can be ovulated (Bysted *et al.*, 2001; Reynaud *et al.*, 2005; Silva–Santos and Seneda, 2011) as an exceptional phenomenon (Gougeon, 1981). These follicles present a high concentration of estradiol and low progesterone concentration, which may influence steroidogenesis (Stankiewicz *et al.*, 2009).

In conclusion, for the first time the morphometry and morphological characteristics of preantral follicles were described for three recently recognized squirrel monkey species (*S.*

macrodon, *S. cassiquiarensis* and *S. vanzolinii*), and revealed that specific follicular development patterns are present, as well as are age dependent. These differences among species in their early folliculogenesis will be important during the development of reproductive technologies applied to ovarian preantral follicles, especially to protect threatened species such as *S. vanzolinii*.

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Conflict of interest

The authors declare that they have no conflict of interest.

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