UNIVERSIDADE FEDERAL DA PARAIBA

CENTRO DE CIÊNCIAS AGRÁRIAS

ESTUDO MORFOLÓGICO DO SISTEMA REPRODUTOR FEMININO DO BICHO-PREGUIÇA-DE-GARGANTA-MARROM (*Bradypus variegatus,* SCHINZ, 1825)

Samantha Mesquita Favoretto

Médica Veterinária

2014

UNIVERSIDADE FEDERAL DA PARAIBA

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Samantha Mesquita Favoretto

Orientadora: Profa. Dra. Danila Barreiro Campos

Dissertação apresentada ao Programa de Pós-Graduação em Ciência Animal do Centro de Ciências Agrárias da Universidade Federal da Paraíba, como parte das exigências para a obtenção do título de Mestre em Ciência Animal

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SAMANTHA MESQUITA FAVORETTO

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Nascida em 01 de março de 1984, no município de São Paulo, Estado de São Paulo. Ingressou no curso de Medicina Veterinária na Faculdade de Ciências Agrárias e Veterinárias da Universidade Estadual Paulista “Júlio de Mesquita Filho” (UNESP), Campus de Jaboticabal, em janeiro de 2004. Desde o início de sua graduação atuou na área de Animais Silvestres, participando através de sua iniciação científica do Núcleo de Pesquisa e Conservação de Cervídeos (NUPECCE). Obteve o Título de Médica Veterinária em dezembro de 2008. Trabalhou como voluntária no Centro de Triagem de Animais Silvestres-Ibama em Goiânia-GO e em agosto de 2009 ingressou no Programa de Aprimoramento Profissional da Fundação Parque Zoológico de São Paulo. Permaneceu nesta instituição por dois anos e meio, quando ingressou no curso de mestrado do Programa de Pós Graduação em Ciência Animal do Centro de Ciências Agrárias da Universidade Federal da Paraíba.

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ESTUDO MORFOLÓGICO DO SISTEMA REPRODUTOR FEMININO DO BICHO-PREGUIÇA-DE-GARGANTA–MARROM (*Bradypus variegatus* Schinz 1825)

RESUMO GERAL

Apesar de sua ampla distribuição geográfica pouco se sabe sobre o bicho-preguiça-de-garganta-marrom. Mesmo a espécie não sendo considerada ameaçada, nos últimos anos ocorreu diminuição de populações no Brasil e Colômbia. Este estudo teve como objetivo descrever a morfologia do sistema reprodutor feminino do *B. variegatus*, podendo futuramente auxiliar em programas de reprodução assistida para a espécie e, assim, no acréscimo do número de indivíduos tanto cativos como em vida livre. Para a execução do estudo foram obtidas carcaças por meio de doação do Cetas/IBAMA de João Pessoa e do Museu Paraense Emilio Goeldi (SISBIO 37715). Foram estudados seis animais: três animais tiveram a artéria carótida comum canulada e foi realizada a injeção de formol a 10% após descongelamento da carcaça; e três animais foram dissecados logo após a descongelação. Um dos indivíduos não formolizados estava prenhe. Os ovários do bicho-preguiça-de-garganta-marrom, com localização sublombar, caudal aos rins, apresentam forma ovóide e se encontram totalmente inseridos na bolsa ovárica, a qual demonstra apenas uma pequena abertura de comunicação com a cavidade abdominal. Histologicamente os ovários demonstram córtex e medula bem definidos. Folículos, com um único oócito e em vários estágios de desenvolvimento, corpo lúteo e corpos albicantes são encontrados no córtex. As tubas uterinas são órgãos pares de aspecto sinuoso, estão inseridos na mesossalpinge e se estendem dos ovários até o útero sendo sua inserção no útero medialmente aos ovários. São formadas por três camadas, tunica mucosa, tunica muscular e tunica serosa, e apresentam epitélio pseudoestratificado ciliado. O útero do bicho-preguiça-de-garganta-marrom é simples, achatado dorso ventralmente, tem formato piriforme em seu fundo e continua por um longo canal. O útero possui internamente dois tecidos diferentes à macroscopia: em sua parte cranial o tecido é liso e firme a palpação, e o restante é composto por um tecido não tão firme e com pregas longitudinais. As

glândulas endometriais são dispostas de forma dispersa estando dilatadas no animal prenhe. O miométrio é formado por três camadas: muscular longitudinal, camada vascular e camada muscular circular. O epitélio uterino é pseudoestratificado. Foram observadas duas cérvices no bicho-preguiça-de-garganta-marrom, estas de comprimento diminuto em comparação com o útero, assim como diâmetro, sendo que as cérvices também apresentam pregas longitudinais e epitélio pseudoestratificado. As cérvices ligam o útero a um órgão de maior diâmetro e com pregas longitudinais, o seio urogenital, este revestido por epitélio transicional. A vulva do bicho-preguiça-comum não é distinguível ao exame externo, nota-se apenas uma abertura onde desembocam o aparelho urogenital e sistema digestório. Observou-se a mudança do epitélio transicional do seio urogenital para epitélio escamoso não queratinizado e, posteriormente, para o epitélio queratinizado da vulva. A caracterização exata dos órgãos do sistema reprodutor feminino e a definição de qual região configurava-se o útero propriamente dito só se tornou possível através da dissecação do animal prenhe e observação da localização do feto. O sistema reprodutor feminino do bicho-preguiça-de-garganta-marrom, assim como outros sistemas da espécie, demonstrou morfologia única, possuindo algumas semelhanças a outros pilosus como o útero simples e a presença do seio urogenital. Nossos resultados, porém, diferem dos descritos para tatus, espécies da mesma superordem, que possuem morfologia ovariana distinta e, em algumas espécies, folículos contendo mais de um oócito. A presença de uma cervix dupla e útero simples é uma característica única do *B. variegatus*, não sendo observado descrição semelhante na literatura para outras espécies.

Palavras-chave: Xenarthra, pilosus, reprodução, ovário, útero.

FEMALE REPRODUCTIVE SYSTEM OF THE BROWN-THROATED-SLOTH (*Bradypus variegatus* Schinz 1825): MORPHOLOGIC STUDY

ABSTRACT

Despite the wide distribution of the brown-throated sloth, there are few studies about the species. According to IUCN, the species is not considered endangered. However, in recent years, there was a decrease of populations of the species in Brazil and Colombia. The aim of the present study was to describe the morphology of the female reproductive system of *B. variegatus*, providing data that might be applied in programs of assisted reproduction for the species, and thus contribute to increase the number of both captive and wild individuals. Carcasses were donated by Triage Center for Wild Animals - IBAMA (Joao Pessoa – PB Brazil) and by Paraense Emilio Goeldi Museum (Belém- PA Brazil) (SISBIO 37715). Three of the six animals were fixed by injection of 10% formaline into the carotid artery and dissected. In addition, three of the females were dissected immediately after thawing (one of them was pregnant). Ovoid ovaries were positioned caudally to the kidneys and surrounded by the ovarian bursa, which was opened to the peritoneal cavity by a narrow slit. ovaries consisted of an inner medulla and an external cortex surrounded by the tunica albuginea, which was covered by cuboidal epithelium. Follicles at different stages of development, corpus luteum and corpus albicans were found in the ovarian cortex. All follicles contained a single oocyte. Uterine tubes were coiled and filiform paired, extending from the ovaries to the uterus. Uterine tubes consisted of three layers: tunica mucosa, tunica muscularis and tunica serosa and were lined by pseudostratified epithelium. A simplex and dorso-ventrally flattened uterus with a long canal was found in the sloths studied. Macroscopically, the cranial portion possessed a hard tissue and continued through a long canal with longitudinal folds. Endometrial glands were scattered disposed, being more dilated in the pregnant animal. Three layers of tissue forms the myometrium: a longitudinal muscular layer, a vascular and a circular muscular layer. The epithelium of uterus was

pseudostratified. Two cervixes, showing a small length and width when compared to the uterus were observed in the sloths. Cervixes possessed longitudinal folds lined by pseudostratified epithelium and connected the uterus to a large distensible cavity, the urogenital sinus. Urogenital sinus showed mucous glands in the submucosa and a transitional epithelium. Vulva of brown-throated sloth was not distinguishable to the external examination, there was only one opening which debouch the gastrointestinal system and urogenital tract. The urogenital sinus epithelium changed gradually from transitional epithelium to non-keratinized squamous epithelium and then to the keratinized squamous epithelium of the vulva. Definition of the limit between cervix and uterus was only possible by analyzing the pregnant female and position of the fetus within the uterine canal. Reproductive system of *B. variegatus* demonstrated a unique morphology, with some similarities to other pilosus as simplex uterus and urogenital sinus and differences from some armadillos, that possesses a distinct ovarian morphology and some follicles with groups of oocytes. The presence of a duplex cervix and a simplex uterus is a feature only described in this specie.

Keywords: Xenarthra, pilosus, anatomy, ovary, uterus.

CONSIDERAÇÕES GERAIS

O Brasil é um dos países mais ricos em biodiversidade do mundo (MITTERMEIER et al., 2005). Segundo levantamento do Instituto Chico Mendes de Conservação de Biodiversidade estima-se que possua aproximadamente 8.200 espécies descritas de vertebrados, sendo 713 mamíferos, 1.826 aves, 721 répteis, 875 anfíbios e aproximadamente 4.100 peixes (PERES et al., 2011).

Os xenarthras são um dos grupos mais antigos dentre toda a diversidade de fauna no Brasil. Representam um dos quatro principais grupos de mamíferos placentários, entre Afrotherios, Laurasiatérios e Euarchontoglires, e sugere-se que sua origem seja anterior a separação de Godwana (REZENDE et al., 2010). Atualmente, o grupo é classificado como superordem, sendo dividida em duas ordens: Cingulata, sendo esta composta pelos tatus; e Pilosa, formada pelas preguiças e pelos tamanduás. Os Xenarthras possuem características anatômicas e fisiológicas peculiares, como o baixo metabolismo, baixa temperatura corpórea e adaptações morfológicas aos mais diversos tipos de nichos, como o focinho dos tamanduás e a postura característica dos bichos preguiça (MIRANDA e COSTA, 2007).

Assim como outras espécies de animais selvagens, as populações de xenarthras tem sofrido com as ameaças antrópicas como a destruição e fragmentação de habitats, ocorrência de isolamento de populações, disseminação de espécies exóticas e suas doenças, maior contato com animais domésticos, poluição do solo, ar e água, mudanças climáticas e maior pressão sobre os habitats (SOULÉ, 1991). Dentre as 38 espécies existentes de xenarthras nove estão classificadas como vulneráveis ou em perigo de extinção, como é o caso de *B. torquatus*, o bicho-preguiça-de-coleira (CHIARELLO et al., 2011) e o tatu bola (*Tolypeutes tricinctus*-SUPERINA et al., 2010) ambos de ocorrência endêmica no Brasil.

Para a conservação de espécies são descritas duas principais táticas, a primeira, a conservação in situ, envolve a preservação de habitat, geralmente em larga escala, e com isto protegendo uma gama de espécies (MARGULES e

PRESSLEY, 2000), e a segunda (*ex situ*) que tem seu foco na reprodução e propagação de espécies individuais através da reprodução de animais em cativeiro (HOLT e PICKARD 1999, PUKAZHENTHI e WILDT, 2004). A reprodução de animais selvagens *ex situ,* no entanto, muitas vezes tem de fazer uso de biotecnologias da reprodução pois pode não haver compatibilidade entre os indivíduos. Para o uso destas biotecnologias é necessário o conhecimento da anatomia da trato reprodutivo feminino, conhecimento sobre o ciclo estral e acerca das características espermáticas da espécies em questão.

Há poucos estudos sobre as características reprodutivas dos xenarthras. Sabe-se que tamanduás bandeira e mirim apresentam, respectivamente, ciclo estral de 51 (PATZI et al.,1998) e 42 dias (MARGERY et al., 1994), que a gestação dos tamanduás bandeira tem duração de aproximadamente 190 dias e que estes apresentam um filhote por gestação (PATZI et al.,1998). O sistema reprodutivo feminino é constituído por um útero simples, ausência de uma cervix verdadeira e a permanência do seio urogenital no trato reprodutivo inferior (ROSSI et al., 2011).

Os tatus são ainda mais peculiares, possuindo útero bicornuado ou simples dependendo da espécie. Os ovários também podem possuir morfologia distinta na espécie (CETICA et al., 2005). No entanto, a particularidade mais significante dos tatus em relação a fisiologia reprodutiva é a existência da poliembrionia em animais do gênero *Dasypus sp.* (PRODOHL ET AL., 1996).

Com relação aos bichos preguiça observa-se que os trabalhos são escassos, baseando-se principalmente em dados comportamentais. Descreve-se que a gestação normalmente é de apenas um filhote e o útero, assim como dos tamanduás, é simples para animais do gênero *Bradypus sp.* (POMPEU E SILVA ET AL., 2010), porém não há estudos morfológicos e histológicos mais detalhados do sistema reprodutor feminino. Estudos para a determinação do ciclo estral pela dosagem de hormônios fecais foram iniciados e, embora ainda não haja confirmação, sugere-se que a duração do ciclo em *Bradypus variegatus* seja superior a 60 dias (MUHLBAUER ET AL., 2006).

A espécie *B. variegatus* é a espécie de preguiça que possui maior área de ocorrência: desde o norte da Argentina até a região Amazônica. A espécie não está

classificada como ameaçada de extinção, porem nos últimos anos tem ocorrido diminuição da populações em países como Brasil e a Colômbia (CHIARELLO et al., 2011). Devido a sua vasta abrangência e maior número de indivíduos, a espécie pode ser utilizada como base para o estudo de características reprodutivas de outras espécies de preguiça do mesmo gênero. Ainda que de difícil manutenção em cativeiro (MIRANDA e COSTA, 2007), a espécie é facilmente encontrada, mesmo em regiões metropolitanas em que há ainda pequenas áreas de preservação, facilitando a observação de dados comportamentais/reprodutivos.

Revisão acerca de dados comportamentais da espécie, sugerem que a gestação de *B. variegatus* é de 6 meses e o intervalo entre partos é de 10 a 12 meses. Segundo os autores os filhotes são amamentados por um mês, iniciam a ingesta de dieta sólida com quatro dias e permanecem com a mãe por cerca de 6 meses. Parece haver sazonalidade reprodutiva, porém esta varia de acordo com as diferenças climáticas de cada região e entre os anos avaliados (TAUBE et al. 2001). Estudos avaliando o comportamento de animais em Pernambuco indicam que a cópula ocorre logo após a época das chuvas na região (BEZERRA et al., 2008) e que não há espermatogênese na seca, período de novembro a fevereiro (GILMORE et al., 1994 apud HAYSSEN, 2010).

Dados acerca da anatomia dos sistema reprodutor feminino datam de 1928. Wislocki (1928) relata que o *B. variegatus* apresenta ovários pequenos, com a ovulação podendo ocorrer em qualquer um dos ovários e útero simples com formato de pera. A placenta é classificada como zonária discoidal endotélio-corial (POMPEU e SILVA et al., 2010). Os machos de *B. variegatus* apresentam testículos esféricos, abdominais, vesícula seminal rudimentar e a próstata (WISLOCKI 1928).

Devido à ausência de dados mais específicos sobre a anatomia do trato reprodutivo de bicho-preguiça e a necessidade de maiores estudos sobre a reprodução de espécies do gênero para possível aplicação de biotecnologias da reprodução, este estudo teve por objetivo descrever a morfologia e a histologia do sistema reprodutor feminino do bicho-preguiça-comum e compara-la com a descrita para outros xenarthras.

CAPITULO I

Sistema Reprodutor Feminino do Bicho-preguiça-de-garganta-marrom (*Bradypus variegatus* Schinz 1825): Anatomia e Histologia

Manuscrito submetido à revista Anatomia, Histologia, Embryologia

Reproductive System of Brown-throated Sloth (*Bradypus variegatus*, Schinz 1825, Pilosa, Xenarthra): Anatomy and histology

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With 4 figures and 2 tables

Summary

Brown-throated Sloth is listed at IUCN Red List as Least Concern, however in recent years, habitat degradation and fragmentation led to the decline of some populations. For conservation of wildlife species, the ideal would be to maintain a viable population *in situ* and a stable population *ex situ*. To achieve this purpose first is necessary to improve the knowledge about reproductive biology and morphology of target species. The aim of the present study was to describe anatomical and histological features of female reproductive system of the brown throated sloth as well as establish similarities and differences between this species and other xenarthras. Ovaries were ovoid and surrounded by the ovarian bursa, presented an external cortex and an inner medulla. Follicles at different stages of development with a single oocyte, corpus luteum and corpus albicans were found in the ovarian cortex. A simplex uterus with a long canal and two cervixes were present in the sloths studied. Uterus and cervixes presented a ciliated pseudostratified epithelium. The cervixes connected the uterus to urogenital sinus, a distensible cavity lined by

transitional epithelium. Female reproductive tract of *B. variegatus* presented the unique characteristic of a double cervix and showed some similarities to species of the same order (Pilosa) as the presence of a simplex uterus with a long canal and a urogenital sinus.

Introduction

The order Pilosa, superorder Xenarthra, is composed of sloths and anteaters. The sloths are divided in two families: family Megalonychidae *(*two-toed sloth) genus *Choloepus* sp., and family Bradypodidae (three-toed sloth) genus *Bradypus* sp. (Medri et al., 2011).

B. variegatus, the brown-throated sloth, belongs to the genus *Bradypus* sp.. The species ranges from south of Central America to South America and occurs in forested areas of Atlantic forest, Savana and Amazon Biomes (Chiarello et al., 2012). Sloths are arboreal folivorous, having a varied diet composed of at least 13 families of plant species (Hayssen, 2010). Animals possess low metabolic rates as well as low and variable body temperature (Goffart, 1971 apud Taube et al., 2001). They are solitary, being active during the day and night (Hayssen, 2010).

According to International Union for Conservation of Nature, the brown-throated-sloth is classified as Least Concern, due to the slow declining of its populations as well as the wide distribution area of the species and its occurrence in a number of protected areas. However, in recent years, habitat degradation and fragmentation led to the decline of some populations, especially in Colombia and Brazil (Chiarello et al., 2012). In fact, brown-throated sloths are more vulnerable than others sloths to habitat changes duo to their reduced mobility, diurnal habits, small home range (Moreno and Plese, 2006), and low population growth rate (Peres et al., 2008). In Colombia, the illegal trade of sloths has risen in recent years, being another threat to the survival of the species (Moreno and Plese, 2006).

For conservation of threatened wildlife species, there are two major approaches: *in situ* and *ex situ*. The first approach implicates in preservation of

habitats (Pukazhenthi et al., 2006; Margules and Pressey, 2000), and the second has its basis

on breeding individual species at captivity (Holt and Pickard 1999) since captive populations may become the last resort if wild populations of some species are extinct (Pukazhenthi et al., 2006).

In order to support the development of reproductive projects for a species in captivity it is essential knowledge about its reproductive biology, behavior, nutrition, and genetics (Holt et al., 2003). In addition, another important aspect to be known is related to anatomomorphological features of the animal. Unfortunately, there are few studies direct towards wild species which differ enormously in physiology, anatomy and behavior (Comizzoli et al., 2000).

As observed for the majority of wild species, information related to a number of aspects of reproductive morphology and biology are also scarce in sloths. There is no confirmation about seasonality and the length of estrous cycle is not determined, although analyzing fecal hormones some authors suggest that is longer than 60 days (Muhlbauer et al., 2006). It is known that sloths generally give birth to a single offspring, gestational period is 6 months, and inter birth interval length varies from 10 to 12 months (Bezerra, 2008; Taube et al., 2001). Furthermore, female of *B. variegatus* nurses their youngs for about a month but remain with them for six to eight months (Gilmore et al., 2001). Moreover, studies regarding the morphology of brown-throated sloth female reproductive system do not fully address the system and no information about histology are available in the literature. It is known that the species has a pear-shaped uterus with a single cavity (Wislocki, 1928) and that its placenta is classified as corioamniotic, zonary discoidal and endotheliochorial (Amorim et al., 2003).

In order to contribute to future studies in the species, the aim of this study was to describe anatomical and histological features of the female reproductive system of the brown-throated sloth as well as compare the results with available data in the literature for Xenarthra superorder.

Materials and Methods

Six frozen female cadavers of brown throated sloths were donated by Triage Center for Wild Animals - IBAMA (Joao Pessoa – PB, Brazil) and by Paraense Emilio Goeldi Museum (Belém- PA, Brazil) under the license number 37715-2 SISBIO. There were no data related to cause of death or age of the animals and one of the females was pregnant. Cadavers were transported to Center of Agricultural Sciences of Paraiba Federal University. Total body length (TBL) was taken in all individuals by measuring the distance between the occipito-atlantical joint to the base of the tail.

In order to study the gross anatomy of reproductive organs, three of the six animals were fixed by injection of 10% formaline into the carotid artery and dissected. In addition, three of the females were dissected immediately after thawing. Female reproductive systems were analyzed *in situ* and *ex situ* and pictures were taken using a Samsung Galaxy Tab GT-P7500 camera. The length of clitoris, urogenital sinus, cervix, uterus and uterine tubes were measured using a caliper rule. Ovaries were measured in three dimensions (length, depth and width) and ovarian volume was calculated using the formula: $V= 4/3\ \pi\ (r1r2r3)$, where r1 length/2; r2= depth/2; and r3= width/2) (Mayor et al., 2012).

In order to perform histological studies, small fragments of each region of the female reproductive system of two animals were fixed in 10% buffered formaline solution and embedded in paraffin. Sections (5 µm) were stained following the routine hematoxylin-eosin (HE) method. Tissues were examined with a light microscope (Zeiss) connected to a camera (Moticam 2500).

Statistical analyses were conducted using Minitab software (Minitab Statistical Software, Minitab Inc, University of Pennsylvania, USA). Mean values of length, width and depth of right and left ovaries were compared with One-way ANOVA and variables were tested for differences using Tukey Comparison Test. Differences were considered statistically significant at $P<0.05$. Data are presented as mean ± S.D.

Results

Total body length

The average total body length of the studied sloths was 44.8 ± 2.9 cm. Individual values are shown in Table 1 and Table 2.

Ovaries

Ovaries of *B. variegatus* were ovoid structures located under the third lumbar vertebra inside an ovarian bursa (Fig. 1A and 2A). Length, depth and width of the ovaries are demonstrated in Table 1. Ovarian volume was 65.3 ± 48.2 and 77.4 ± 61.5 mm^3 for right and left ovaries, respectively. *There* was no significant *difference* between right and left *ovaries in all variables analyzed.* No protruding follicles were visible on ovaries surfaces in all non-pregnant females, while in the pregnant female a prominent corpus luteum was observed above the surface of the ovary.

Brown-throated sloth did not present the suspensory ligament of the ovary, which was sustained only by the ovarian bursa. Ovarian bursa comprised the mesovarium and mesossalpinx. The structure was a thin membrane that involved almost all ovary, remaining only a narrow slit communication to the peritoneal cavity.

Microscopically, the ovaries consisted of an inner medulla and an external cortex surrounded by the tunica albuginea, which was covered with a cuboidal epithelium (Fig. 2B). Follicles at different stages of development (primordial - oocyte surrounded by a layer of squamous (flattened) granulosa cells; primary - oocyte surrounded by a layer of cuboidal granulosa cells; secondary - oocyte surrounded by more than one layer of cuboidal granulosa cells, with no visible antrum (Myers et al., 2004) and antral follicles (Fig. 2C), corpus luteum (Fig. 2D) and corpus albicans (Fig. 2C) were observed in the ovarian cortex. All follicles contained a single oocyte (Fig. 2C).

Uterine tubes

Uterine tubes of the brown-throated sloth were paired tubes extending from the ovary to the uterus. They were positioned within the ovarian bursa, being filiform and coiled with a funnel-shaped opening near the ovaries (Fig. 2A). Mean length of right and left uterine tubes were 26.1 ± 1.1 and 23.0 ± 8.0 mm (Table 2). We observed that uterine tubes were longer (~2 to 4 folds) in the pregnant female compared with all non-pregnant females. Histologically, the uterine tubes showed tree layers: tunica mucosa, tunica muscularis and tunica serosa (Fig 2E). The folded mucosa was characterized by a ciliated pseudostratified epithelium (Fig. 2F).

Uterus

In all females studied uterus was a pear-shaped organ in the cranial part, with a fundus slightly convex and a long canal. The uterus was suspended bilaterally from the body wall by the mesometrium. The organ was dorso-ventrally flattened and measured 63.8 ± 9.33 mm of length, excluding the cervix (Table 2). The uterus was macroscopically divided in two parts: the cranial part, which possessed a hard tissue, and a long canal comprised of soft tissue with longitudinal folds (Fig 1A). Definition of the limit between cervix and uterus was only possible by analyzing the pregnant female and position of the fetus within the uterine canal (Fig. 1B).

Endometrium of the brown-throated sloth showed a pseudostratified epithelium (Fig. 3C) and a lamina propria with tubular glands scattered disposed. Endometrial glands were dilated and demonstrated hypertrophy in the pregnant female (Fig. 3A). Myometrium was organized into three layers: the inner and outer layers mainly composed of smooth muscle fibers, and the vascularized intermediate layer. The inner layer of smooth muscle was composed of circular fibers, whereas muscle fibers were arranged longitudinally in the outer layer. Covering the myometrium there was a layer of connective tissue, the perimetrium.

Brown-throated sloth presented a double cervix. Cervical canals extended from the internal uterine orifices to the external uterine orifices (Fig. 1C). External orifices of the uterus opened into urogenital sinus, dorsally to external urethral orifice

(Fig. 1B), but approximately the same level. Cervix showed a mean length of 14 ± 6.4 mm (Table 2). Microscopically, the cervix possessed longitudinal folds with dense connective tissue lined by pseudostratified epithelium (Fig. 3F).

Lower portion of the female reproductive system

The cervix of brown-throated sloth ends in the urogenital sinus, a flaccid and distensible cavity that presented a hard tissue and longitudinal folds. The urogenital sinus was cranially limited by the external orifices of the uterus and by the external urethral orifice, and caudally limited by the clitoris and vulva (Fig. 1B). The length of urogenital sinus was 19 ± 1.0 mm (Table 2). Microscopically, the urogenital sinus was lined by transitional epithelium (Fig. 4D) and possessed a lamina propria with dense connective tissue.

Urogenital sinus connected directly to the vulva. Close to the external orifice of urogenital sinus the epithelium changes gradually from transitional epithelium to non-keratinized squamous epithelium and then to the keratinized squamous epithelium of the vulva (Fig. 4E). Mucous glands were present in the vulva submucosa (Fig. 4D).

Clitoris of the brown-throated sloth was a “V” shaped organ with a ventral apex, measuring 3.4 ± 1.0 mm of length (Fig. 4A). The organ was located ventrally within the end of urogenital sinus and was partially covered by the prepuce; *B. variegatus* did not possess a true clitoral fossa. The clitoris consisted of two cavernous bodies encircled by dense collagen fibers and mucous glands. Clitoris was lined by transitional epithelium (Fig. 4C).

Discussion

This is the first complete description of the female genital organs in a sloth species. Moreover, this description provides useful information that may be considered for the development of female reproductive biotechnologies. It was demonstrated that female reproductive system of the brown-throated sloth showed important differences with respect to other species of Xenarthra. Animals presented ovaries with similar features of other pilosus as well as a simplex uterus and the unique feature of a double cervix. As other species of xenarthras, *B. variegatus* presented an urogenital sinus instead of a true vagina.

The presence of a double cervix and a simplex uterus is only described in the species *Bradypus variegatus.* Lagomorphs and some rodents have a double cervix but also possess a double uterus (Vaughan, 2010; Breed et al., 1985). In our study we found that the double cervix was lined by a pseudostratified epithelium. Wislocki (1928) describes this double canal as being two vaginal canals, although other authors argue that no eutherian mammal possess a double vagina (Vaughan, 2010). In addition, instead of squamous stratified epithelium found in most mammalian vaginas, *B. variegatus* possessed the same epithelium found in the uterus, confirming that the double organ was still part of the uterus.

Cervix, as uterus, develops from the embryonic paramesonephric ducts. Caudal parts of these ducts fuse to an extent which varies according to the species; in some rodents, fusion of the ducts is limited to the vagina, and the uterus is maintained as a double organ. In primates, fusion is more extensive, remaining only double uterine tubes (Konig et al., 2007). In *B. variegatus* we could suggest that the fusion occurs only in the middle part of paramesonephric ducts, remaining the uterine tube and cervix as double structures and a simplex uterus.

The simplex uterus observed in *B. variegatus* was a pear shaped organ, dorso-ventrally flattened with a long canal as described for *B. torquatus* (Pompeu and Silva et al., 2010), lesser anteater (Rossi et al., 2011, Pompeu and Silva et al., 2010), anteater (Schauerte et al., 2005 apud Rossi et al., 2011), and several species of armadillos, including *Dasypus hybridus* and *Tolypeutes matacus* (Cetica et al., 2005). In our species, a pseudostratified epithelium was observed lining the uterus, instead

of simple columnar epithelium observed in lesser anteater (Rossi et al., 2011) or simple cubic or columnar epithelium found in armadillos (Cetica et al., 2005). Endometrial glands with scattered distribution were observed in the sloths studied. In the pregnant female, endometrial glands were hypertrofiated and dilated, probably as a response to the increase in progesterone (Ovalle and Nahirney, 2008).

Simplex uterus is associated with one or low number of offspring and is present in evolved eutherian mammals, as primates (Ford, 1987), including humans. Less evolved animals usually present a bicornuate uterus and more than one young per gestation (Kluge, 1977 apud Cetica et al., 2005). Even being considered less evolved species, most xenarthras have similar reproductive characteristics of evolved species, such as simplex uterus and few young per gestation. Exceptions to this feature are armadillos from the genus *Dasypus sp.*, species that give birth to two to twelve monozygotic cubs (Medri et al., 2006) and *Euphractus sexcinctus* (Gucwinska, 1971). *B. variegatus* present a single young per gestation, although two youngs have already being described with a single female, suggesting the occurrence of twins (Bezerra et al., 2008). In our study, we observed a single fetus within the uterus, as described in the literature.

The uterus of *Bradypus variegatus* was a pear-shaped organ and continued through a long canal. In lesser anteater, authors consider that this long canal is an uterovaginal canal that function as a cervix, since the organ has a smaller lumen compared to the uterus (Rossi et al., 2011). In armadillos, because of microscopic similarities (type of epithelium, lamina propria and surrounding muscularis externa), this canal is considered to be the cervix (Cetica et al., 2005). In a comparative study of macroscopic features of the uterus from xenarthras, Pompeu and Silva (2010) described the presence of a long cervix in *Dasypus novemcynctus*, lesser anteater and *B. torquatus*. In our study, we observed that caudally to this long canal, there were two small canals, with an even smaller lumen when compared to the uterus, that we considered the cervixes. In addition, in the pregnant female the fetus occupied the entire canal referred as uterovaginal canal in anteater and armadillos (Rossi et al., 2011; Cetica et al., 2005), excluding the possibility that this organ is the cervix in *B. variegatus*.

At the lower portion of brown-throated sloth reproductive system we observed a cavity with a wide diameter, flaccid and distensible, that presented a hard tissue and longitudinal folds. This cavity was limited cranially by the external orifices of the uterus and by the external urethral orifice, one close to the other, and caudally by the clitoris and vulva. As this organ was lined by transitional epithelium it was not classified as the vagina but as urogenital sinus, an embryologic structure that persisted in some mammals, including marsupials and monotrematas (Senger, 2007) and some xenarthras (Cetica et al., 2005; Rossi et al., 2011). In our animals, the urogenital sinus ends with the change of transitional epithelium to stratified keratinized epithelium of the vulva.

Snoeck et al. (2011) described the use of vaginal cytology to evaluate estrous cycle in *B. torquatus*. During estrous cycle, vaginal stratified epithelium undergoes characteristics changes, becoming keratinized in estrus in dogs and cats (Feldman and Nelson, 2004; Toniollo et al., 1995), goats (Jarosz et al., 1971), rodents (Guimarães et al., 1997) and other species, in order to protect the organ during penis intromission (Rossi et al., 2011). Males of *Bradypus sp*. present a short penis, with a mean length of 1.26 cm (Martins, 2003), probably not occurring a true intromission in the female reproductive system. Thus, considering this observation and the fact that *B. variegatus* possess a urogenital sinus instead of a true vaginal canal we believe that this method of estrous cycle evaluation could not be applied to brown-throated sloth.

In our females, follicles were present within the cortex and presented a single oocyte as also observed in some domestic and wild species (Telfer et al., 1987), including the lesser anteater (Rossi et al., 2011). This morphological feature is different from that described for armadillos. Species *Tolypeutes matacus, Chaetophractus villosus, Chaetophractus vellerosus, Zaedyus pichiy* and *Clamyphorus truncatus*, besides having a distinct ovary morphology, also possess some follicles containing groups of oocytes (Cetica et al., 2005). These characteristic is also described for some marsupials, as the opossum (Gonçalves et al., 2009), rabbits and bitches (Telfer et al., 1987), although the reproductive significance is still not explained (Cetica et al., 2005).

Ovaries measured 0.78, 0.47, and 0.33 cm of length, width and depth, respectively. Barreto e al. (2013) working with the same species found distinct values for ovaries morphometry: 0.37 of length, 0.73 of width and 0.62 of depth. Follicles protruding from the ovaries surface were not founded in our study; however, their presence depends upon the phase of estrus cycle at the time of female evaluation. Follicles growth and develop at proestrus, becoming mature at the estrous (Colville et al., 2010), in other phases of the cycle a protruding follicle would not be encountered.

Reproductive knowledge of the sloths is fundamental for developing appropriate captive management practices for the species and for future studies and application of reproductive biotechnologies. Morphology of the reproductive system of brown-throated-sloth is different from the other species of xenarthra group, with some similarities to anteaters and armadillos, as the simplex uterus and the urogenital sinus. The presence of a double cervix is a unique feature only described in this species.

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Table 1. Morphometry (Mean ± SD) of the ovaries of *B. variegatus.* There was no difference between values from left and right ovaries.

Animals	TBL (cm)	Ovaries (mm)							
		Length		Width		Depth		Volume (mm^3)	
		R	L	R	L	R	L	R	L
1	48.0	9.0	8.5	5.5	6.0	3.5	3.5	90.7	93.4
2	41.5	6.0	6.0	3.5	3.5	2.5	2.5	27.4	27.4
3	46.0	6.5	6.0	4.0	4.0	3.0	3.0	40.8	37.7
4	42.0	7.0	6.0	4.0	3.5	2.0	2.5	29.3	27.4
5	46.0	11.0	12	6.0	7.0	4.0	4.0	138.2	175.8
Mean*	44.7	7.9	7.7	4.6	4.8	3.0	3.5	65.3	77.4
SD	2.52	2.1	2.6	1.1	1.6	0.8	1.1	48.2	61.5
6**	46.5	9.0	9.0	8.0	6.0	3.0	6.0	113	169.6

TBL = total body length. SD= standard deviation, R = right, L = left.

*Mean values of non-pregnant females, ** = pregnant female.

Table 2. Morphometry (Mean ± SD) of the tubular organs of *B. variegatus* reproductive system. There was no difference between values from left and right uterine tubes.

Animals	TBL (cm)	Length (mm)				
		Uterine tubes				
		R	L	Uterus	Cervix	Urogenital sinus
1	48.0	19	17	57.5	17	17
2	41.5	19	17	60	4	11
3	46.0	17.5	17.5	57	16	12
4	42.0	33	29	79.5	12	20
5	46.0	42	34	65	21	35
Mean*	44.7	26.1	23	63.8	14	19
SD	2.52	1.1	8.0	9.33	6.4	1.0
6**	46.5	75	75	150	19	19

TBL = total body length. SD= standard deviation, R = right, L = left.

*Mean values of non-pregnant females, ** = pregnant female.

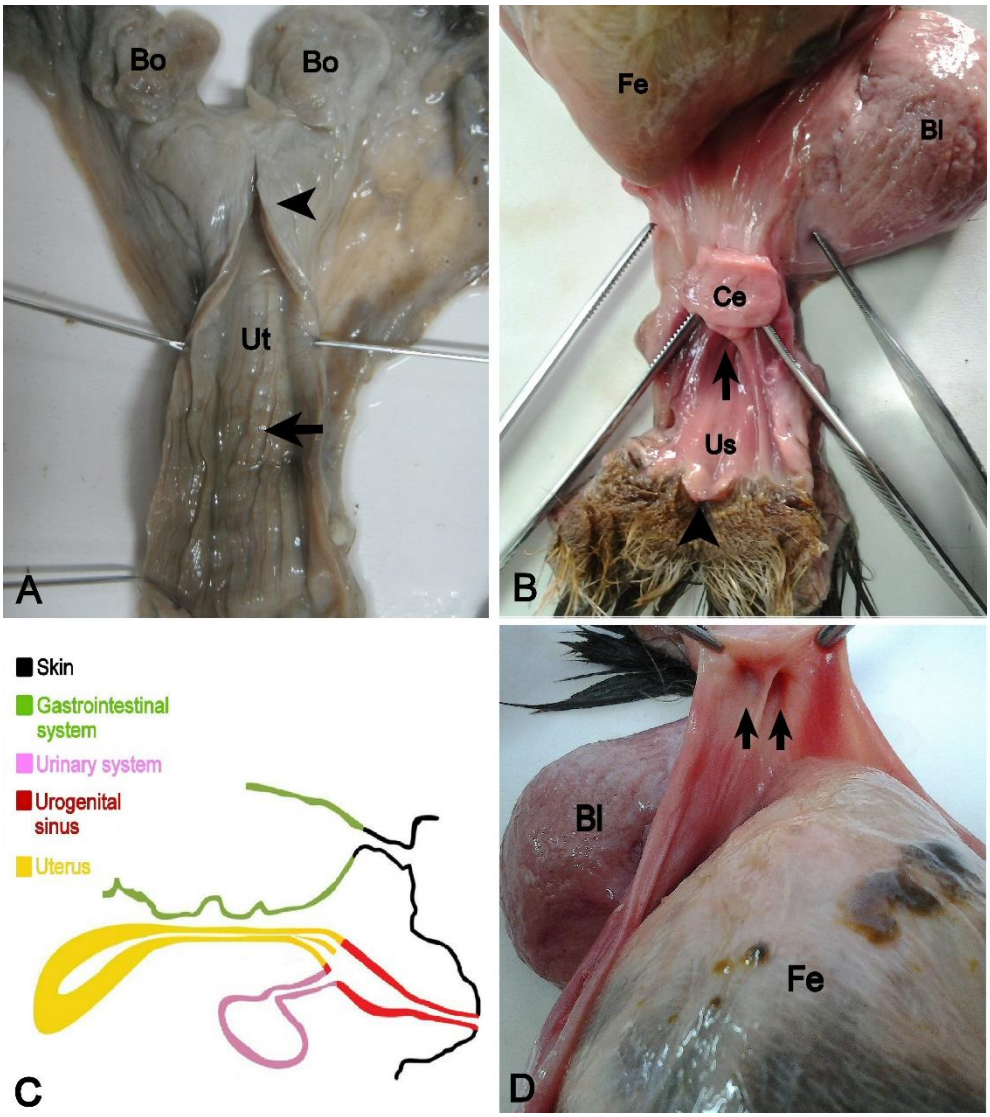

Fig. 1. Gross anatomical features of the female reproductive system of *B. variegatus*. A) Ovaries were surrounded by the ovarian bursa (Bo). The fundus of the uterus (Ut) was comprised of a hard tissue (arrowhead) and presented a long canal with longitudinal folds (arrow). B) Urogenital sinus (Us) of the brown-throated-sloth was delimited cranially by the cervixes (Ce) and the external urethral orifice (arrow), and caudally by the clitoris (arrowhead) and vulva. Fetus (Fe), bladder (Bl), uterus (Ut), instruments point to external orifices of the uterus. C) Schematic drawing of the female genital system of *B. variegatus* adapted from Rossi et al. (2011). D) Internal

view of the uterus of *B. variegatus* showing a pair of internal orifices of the uterus (arrows), which represents the entrance of the double cervix. Fe: fetus; Bl: bladder.

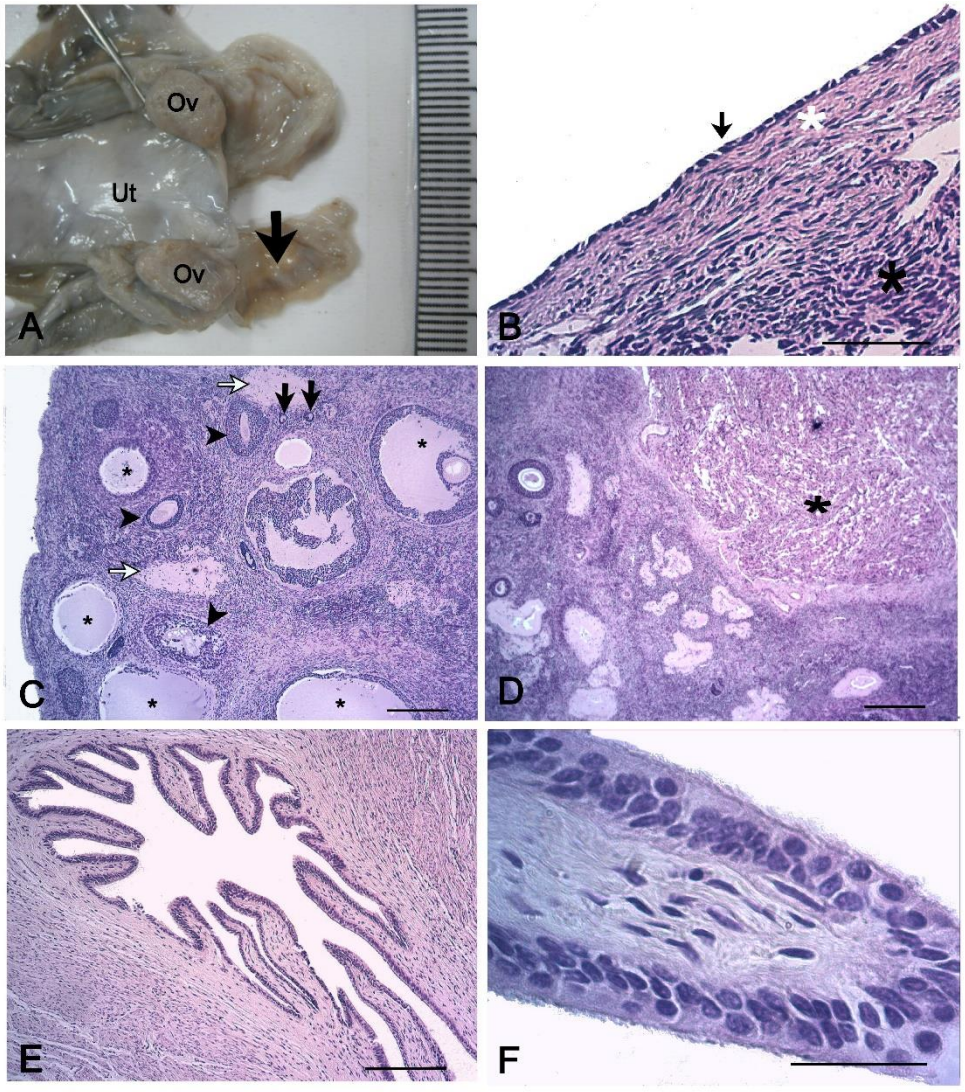

Fig. 2. Gross anatomy and photomicrographies of the ovaries and uterine tube of *B. variegatus*. A). Ovaries (Ov) and uterine tube (arrow) are visible after section of the ovarian bursa. Ut: uterus. B). Tunica albuginea (white asterisk) involved the ovarian cortex (black asterisk) and was covered by cuboidal epithelium (arrow). C) In non-pregnant animals, longitudinal section of the ovary showed antral follicles (asterisks), secondary follicles (arrowheads), primary follicles (black arrow) and corpus albicans (withe arrow). D) A corpus luteum (asterisk) was observed in the ovary of the pregnant female. E) Uterine tubes presented a folded mucosa. F) Mucosa of the uterine tubes possessed a pseudostratified epithelium with ciliate cells. Hematoxilin-eosin Staining. Bars: 200 µm (B), 300 µm (C, D and E), 50 µm (F).

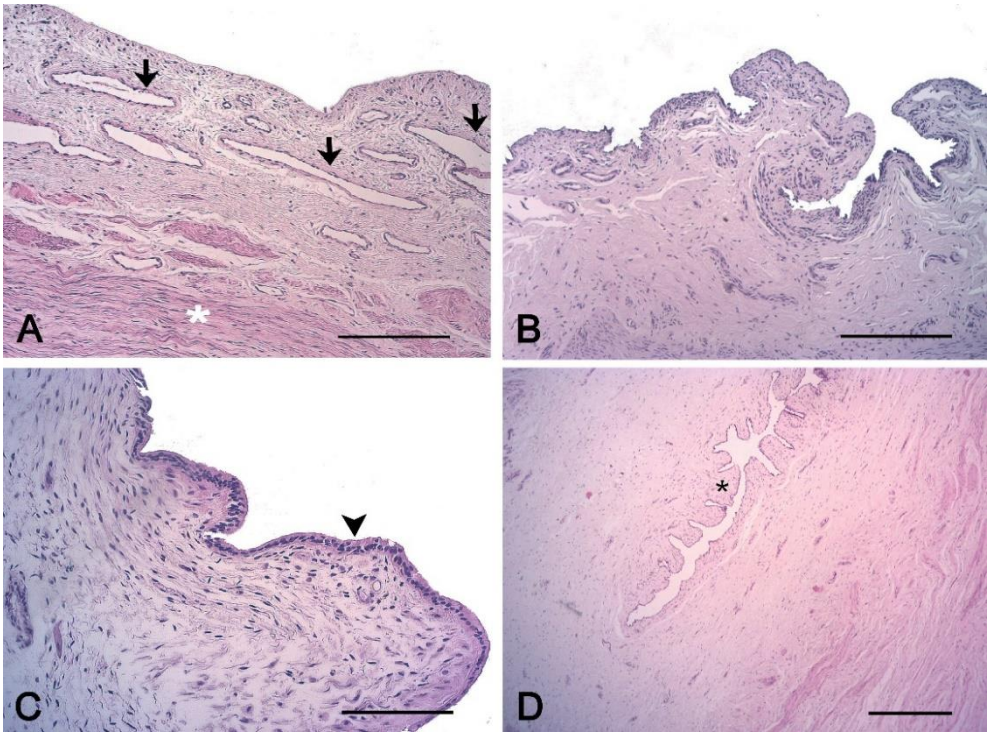

Fig. 3. Photomicrographies of the uterus of *B. variegatus*. A) In the pregnant female, uterus presented dilated endometrial glands (arrows). White asterisk: myometrium layer. B) Dilated glands were not present in non-pregnant female uterus. C) Endometrium was lined by pseudostratified epithelium (arrowhead). D) Cervix consisted of longitudinal folds with dense connective tissue (asterisk) lined by pseudostratified epithelium. Hematoxilin-eosin Staining. Bars: 200 µm (A, B and C) and 400 µm (D).

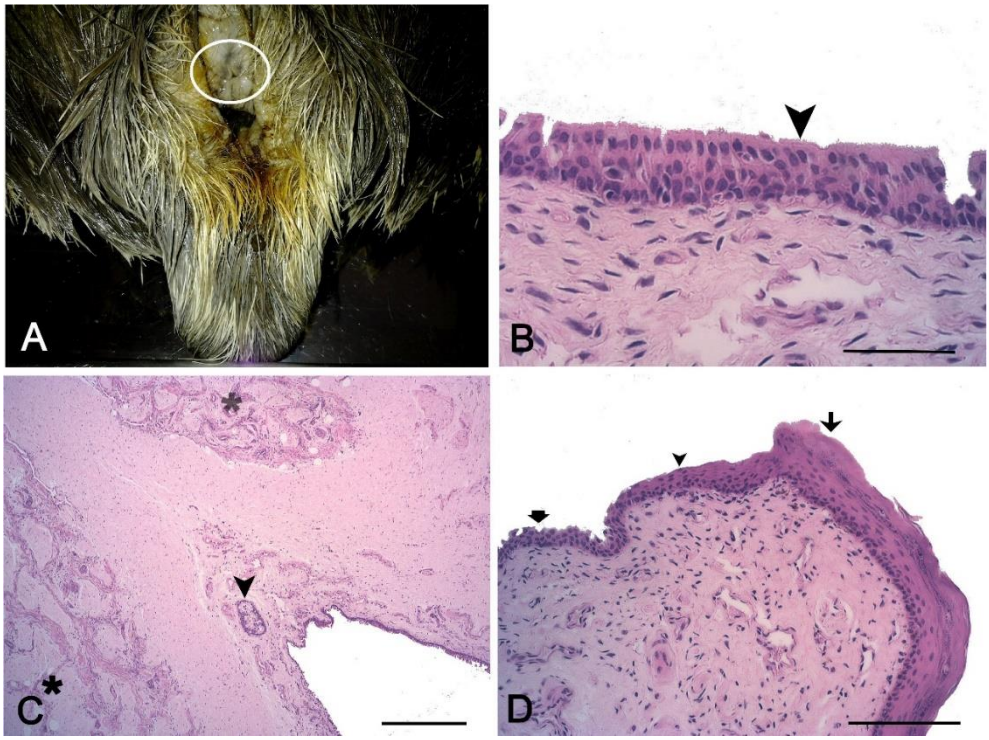

Fig. 4. Gross anatomy and photomicrographies of the lower portion of the reproductive system of *B. variegatus* A) Caudal view of the female perineum area showing the clitoris (white circle). B) Urogenital sinus was characterized by a transitional epithelium (arrowhead). C) Clitoris possessed cavernous tissue (asterisk) and mucous gland (arrowhead). D) Epithelium of urogenital sinus changed gradually from transitional epithelium (short arrow) to non-keratinized squamous epithelium (arrowhead) and then to the keratinized squamous epithelium of the vulva (arrow). Hematoxilin-eosin Staining. Bars: 100 µm (B), 400 µm (C), 200 µm (E).

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