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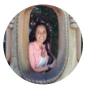

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ORIGINAL ARTICLE

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

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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

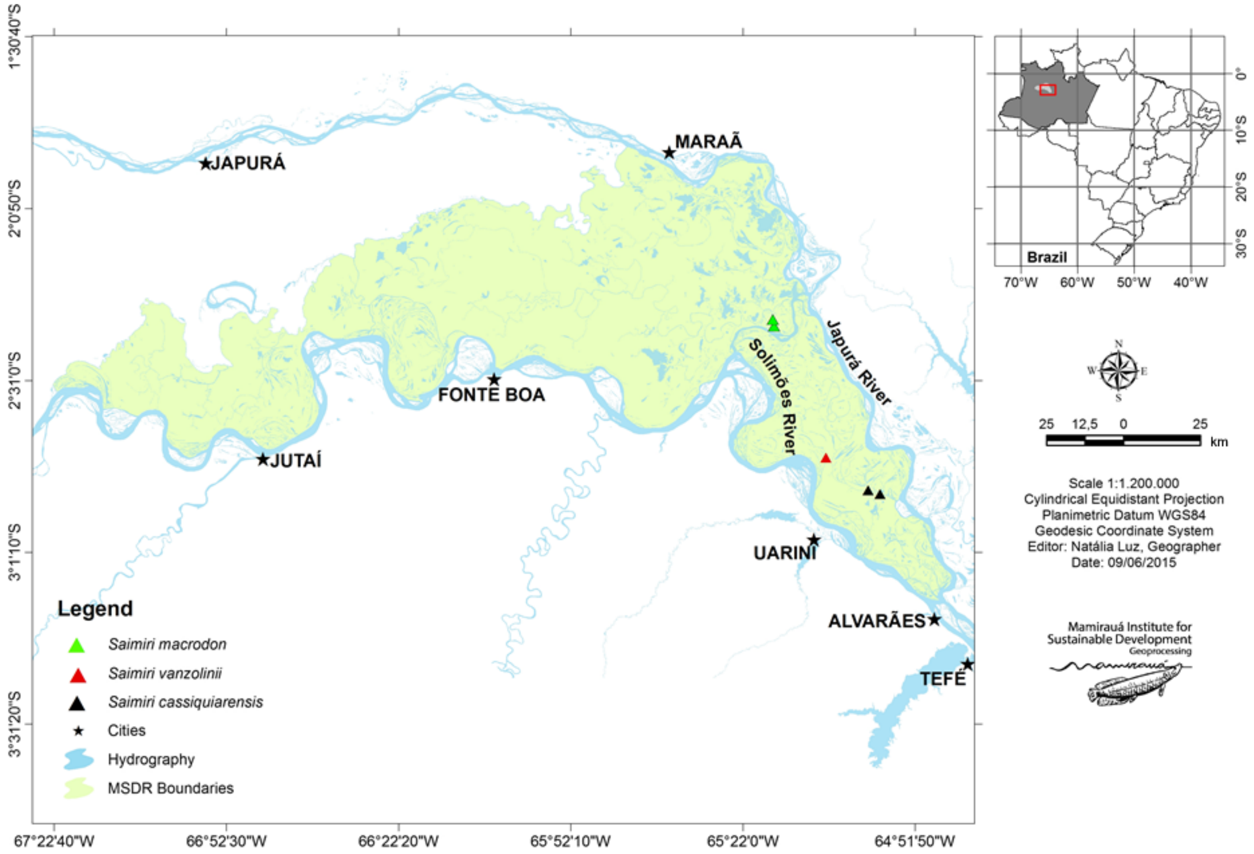

Fig. 1. Distribution of capture areas for *Saimiri vanzolinii, Saimiri cassiquiarensis* and *Saimiri macrodon*. Source: Mamirauá Institute for Sustainable Development Geoprocessing, 2013.

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

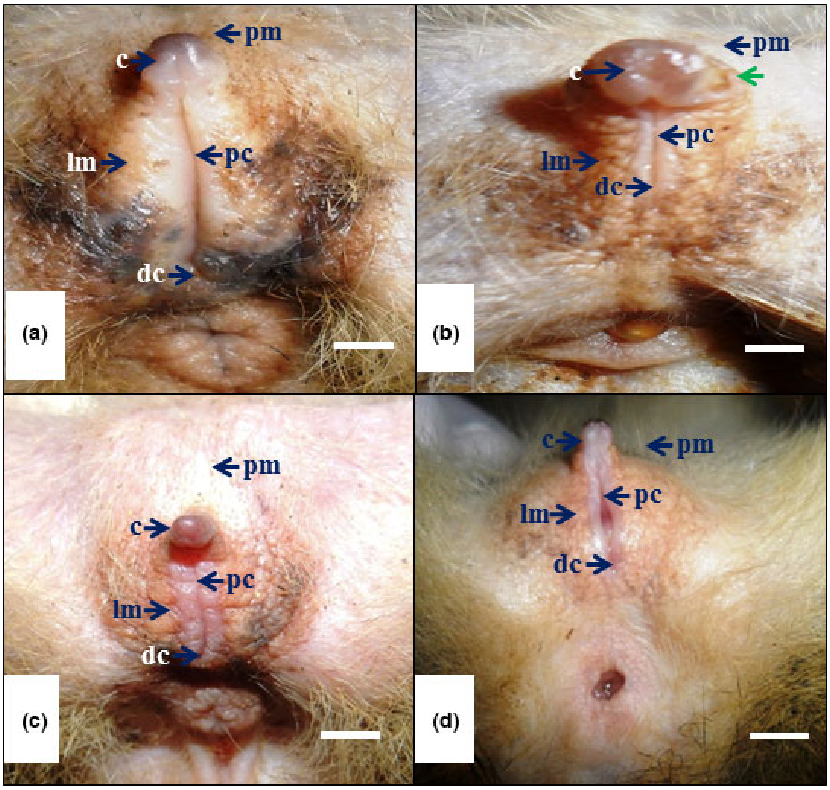

Fig. 2. External genitalia of the females of *Saimiri*. (a) Pregnant adult *S. macrodon* female. (b) Juvenile *S. macrodon* female. (c) Pregnant adult *S. cassiquiarensis* female. (d) Adult *S. cassiquiarensis* female. Caption: pm, pubic mound; c, clitoris; pc, pudendal cleft; lm, labia majora; dc, dorsal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

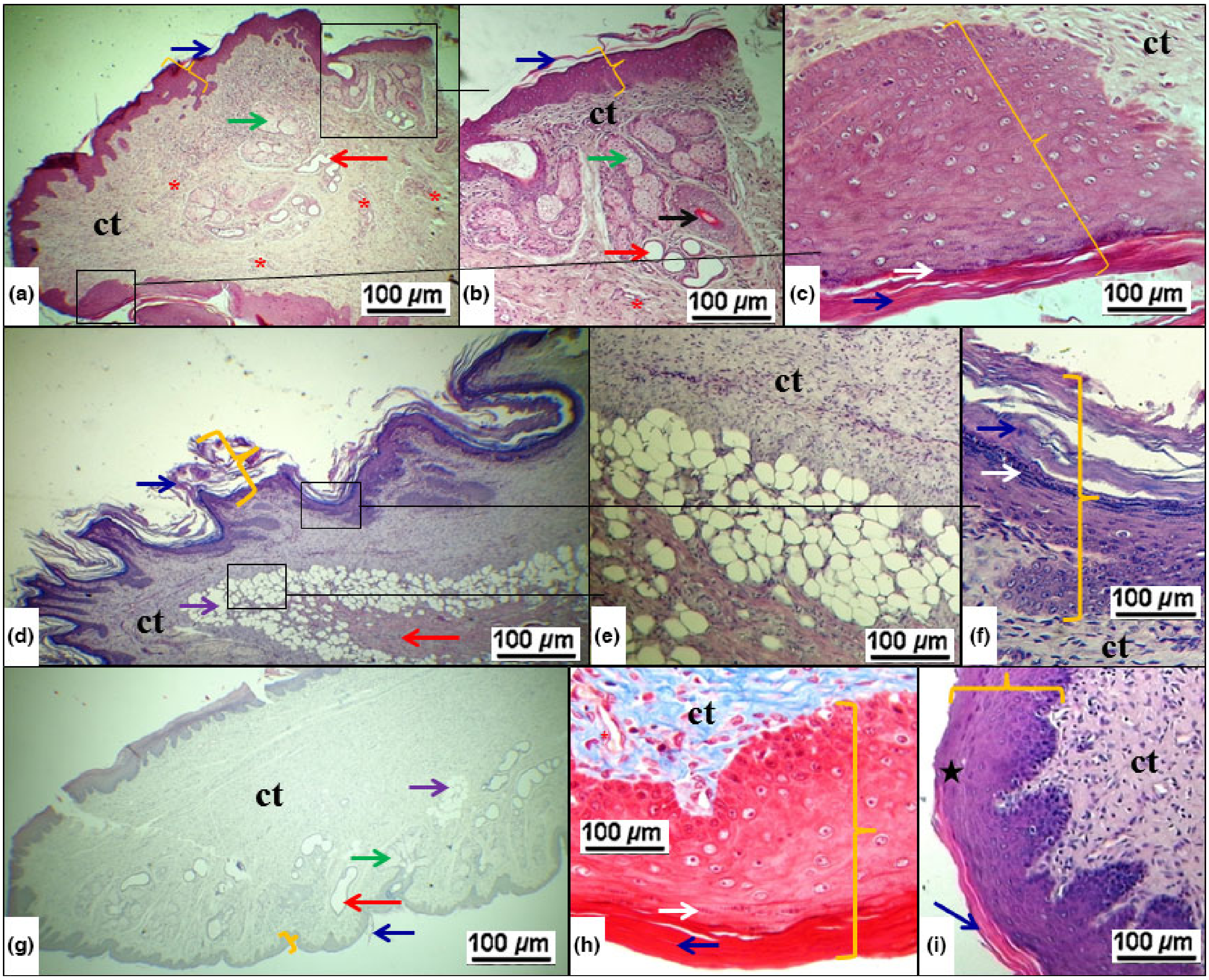

Fig. 3. Photomicrograph of the labia majora in *Saimiri*. (a–c) Adult *S. macrodon*. (d–f) Juvenile *S. macrodon*. (g–i) Adult *S. cassiquiarensis*. Yellow brackets delimit the keratinized stratified squamous epithelium. Blue arrows: stratum corneum; green arrows: sebaceous glands; red arrows: sudoriparous 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

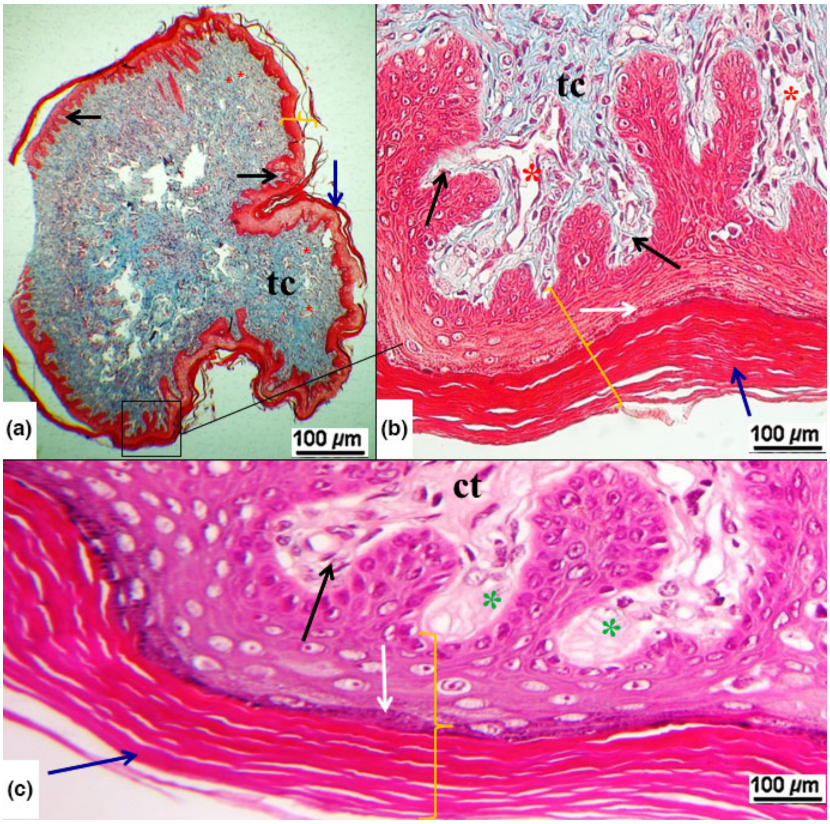

Fig. 4. Photomicrograph of the glands of the clitoris from *Saimiri cassiquiarensis*. Panel a: histological section of a clitoris (×40); Panel b: higher magnification of the indicated area in Panel a (×100); Panel c: higher magnification showing the presence of the Meissner's corpuscles (×100). Brackets 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.

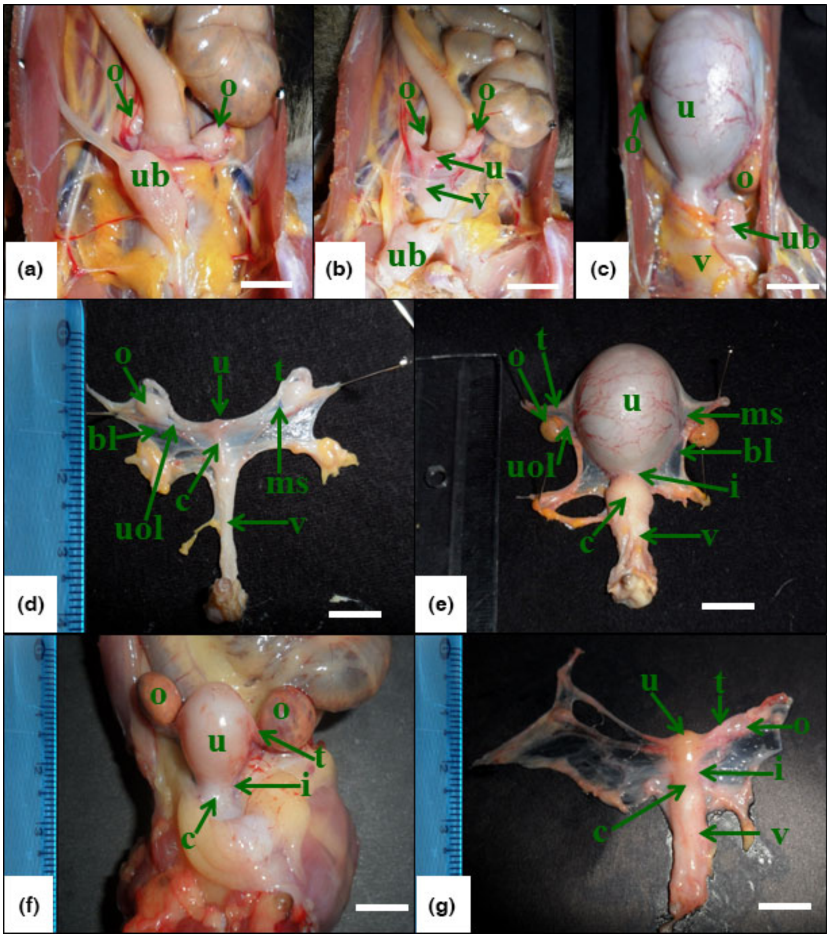

Fig. 5. Frontal view of the reproductive tract of the three *Saimiri* species. Panels a–e: *S. macrodon*. Panel f: *S. cassiquiarensis*. Panel g: *S. vanzolinii*. Panels a–c: organs '*in situ*'. Panel a: non-pregnant female – urinary bladder covered the organ. Panel b: view of the uterus after the removal 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.

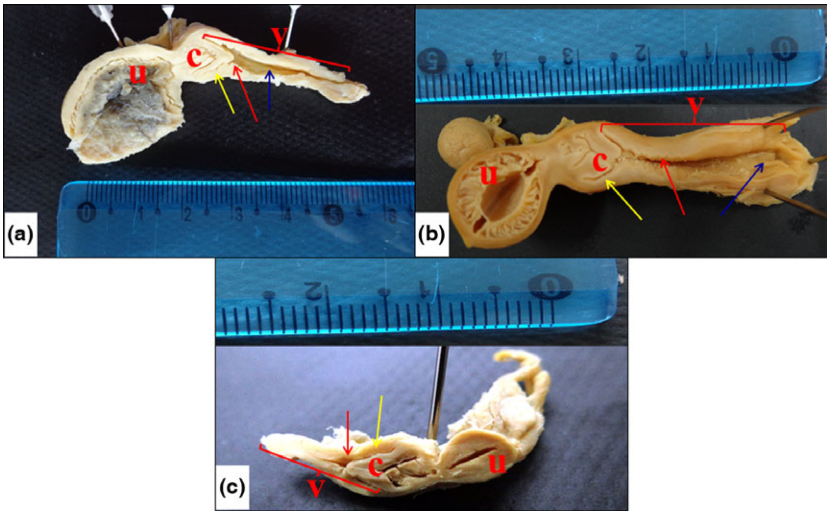

Fig. 6. Photograph of the reproductive tract '*ex situ*' of *Saimiri*, highlighting the vagina. Panel a: *S. macrodon*; Panel b: *S. cassiquiarensis*; Panel c: *S.* vanzolinii. Caption: u, uterus, v, vagina, c, cervix. Blue arrows: longitudinal folds; red arrows: cross-folds; yellow arrows: vaginal fornix.

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

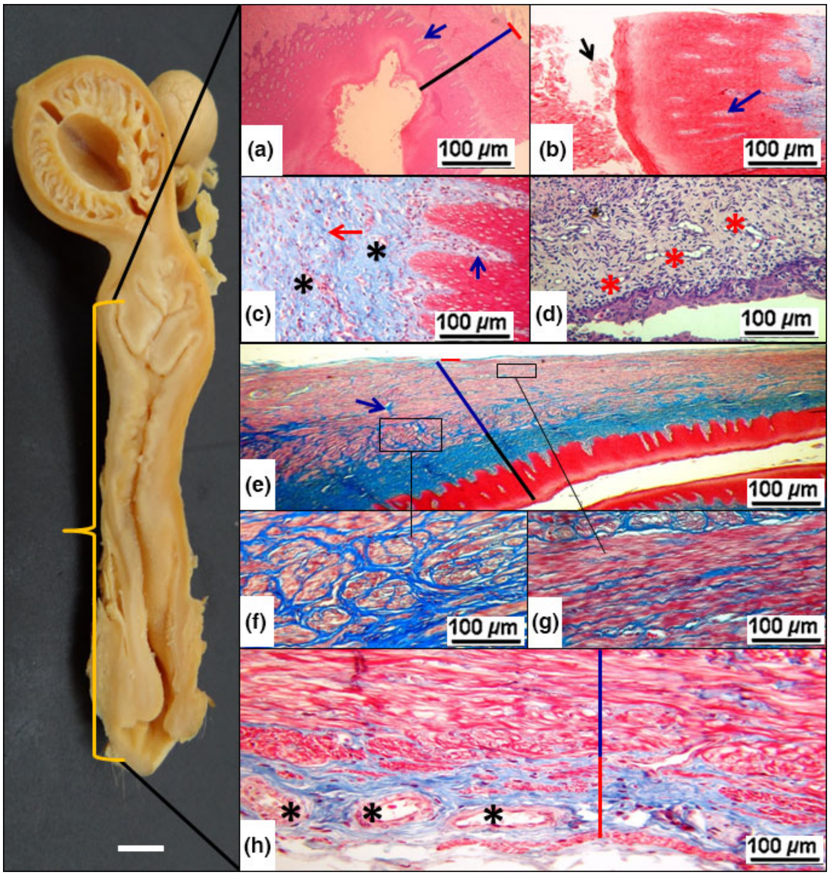

Fig. 7. Photograph and photomicrographs of the vagina in *Saimiri*. Panel a (×40): Bars indicate the three vaginal layers (Black bar: mucosal layer; blue bar: muscle layer; red bar: tunica adventitia); blue arrow: papilla. Panel b (×100): cells in desquamation from vaginal epithelium (black arrow) and 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).

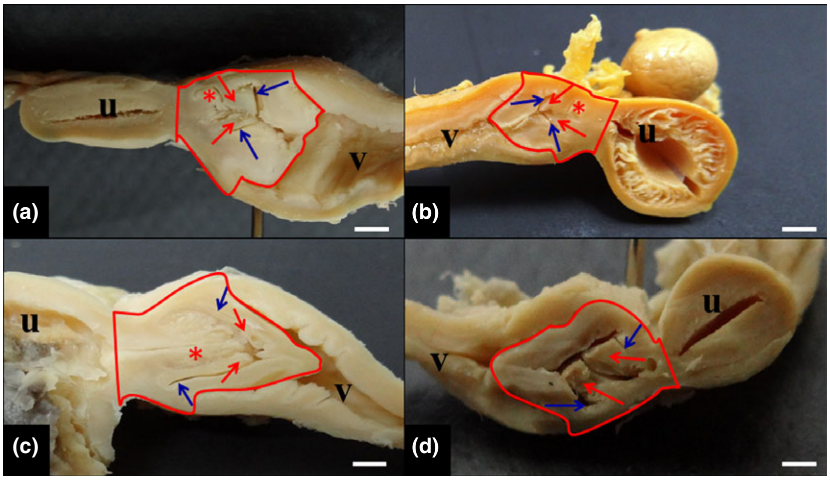

Fig. 8. '*Ex situ*' reproductive tract of the three *Saimiri* species evidencing the complexity of the cervix. Panels a, b: *S. cassiquiarensis*. Panel c: *S. macrodon*. Panel d: *S. vanzolinii*. Caption: u, uterus; v, vagina. Delimited area in red corresponds to the cervix. Red arrows: colliculus; blue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

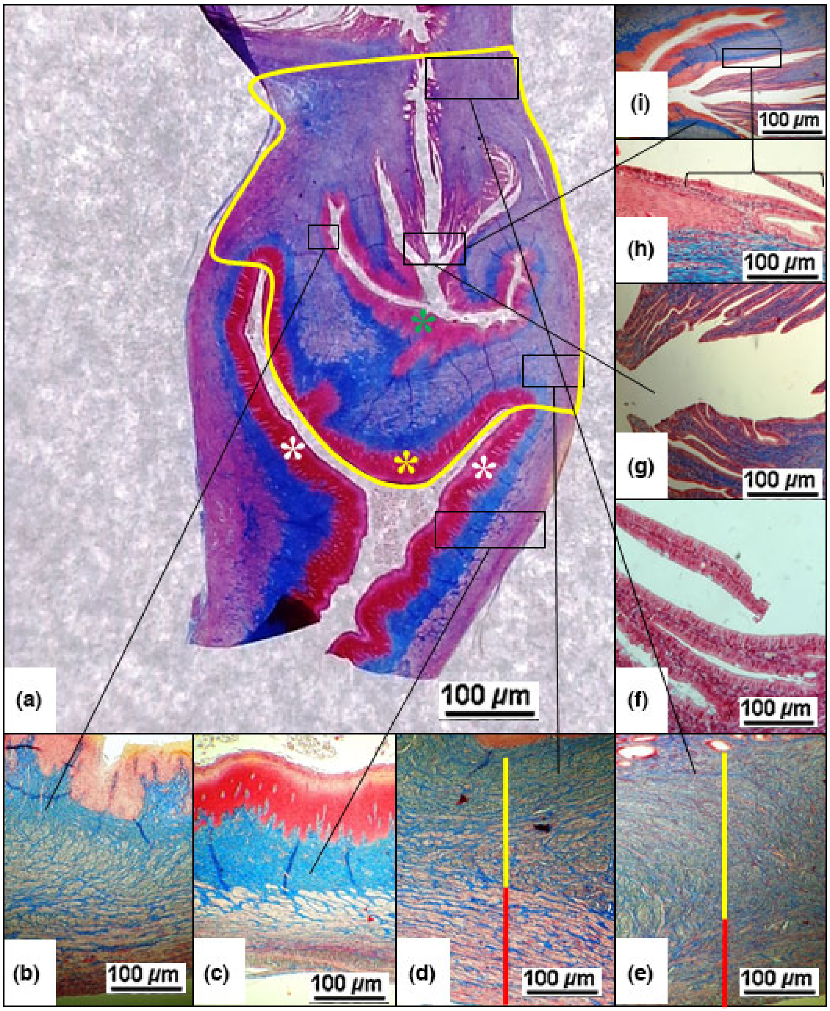

Fig. 9. Photomicrographs (a–i) of the cervix from *Saimiri cassiquiarensis*. Panel a (×6): Delimited area in yellow corresponds to the cervix; white asterisks: vaginal epithelium; yellow and green asterisks: cervical epithelium. Panel b (×100): inset magnification of part of Panel a in which the lamina 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

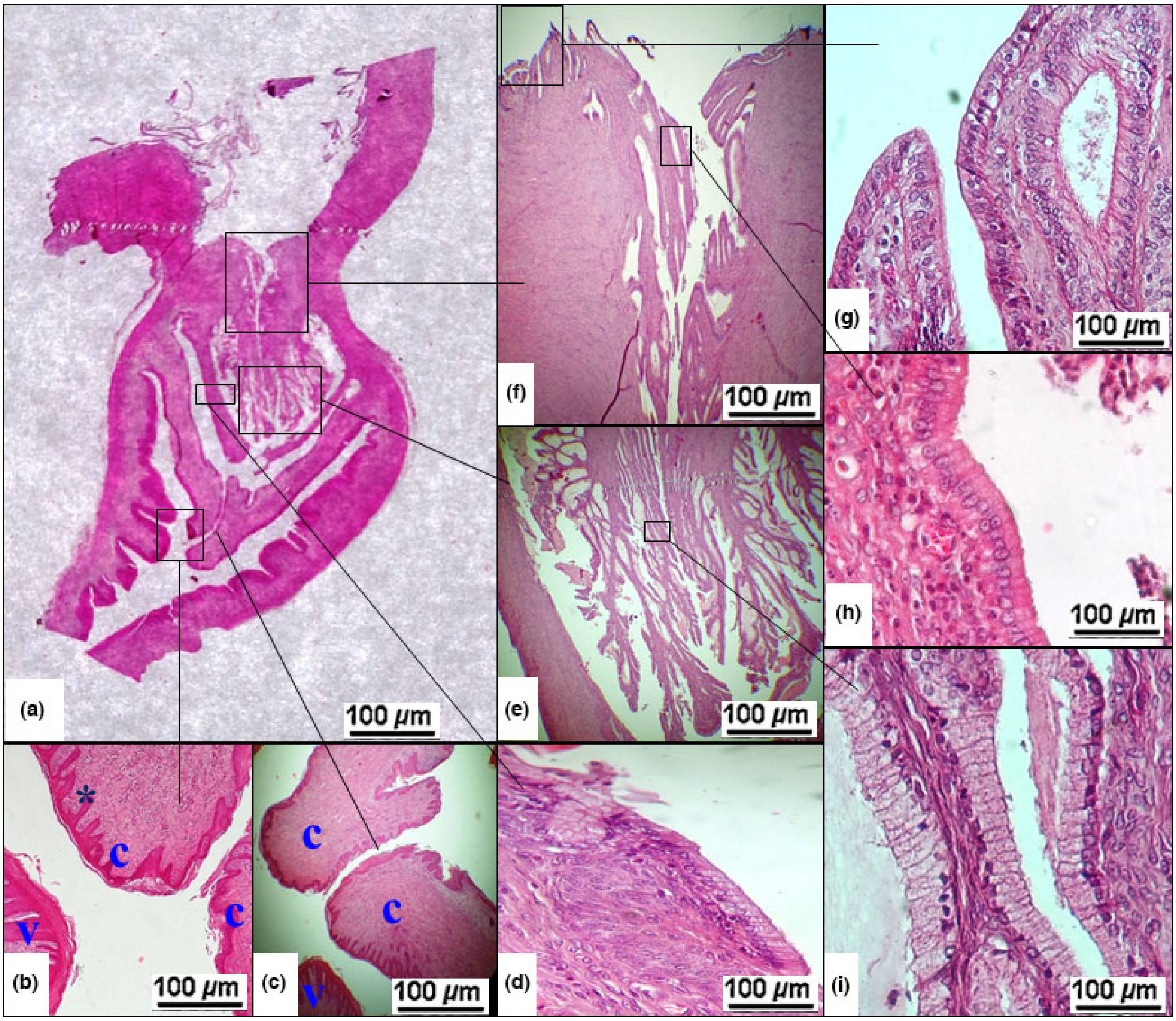

Fig. 10. Photomicrographs of the cervix from pregnant *Saimiri macrodon*. Panel a (×6): Cervix. Indicated areas are addressed at higher magnification in the following panels. Panel b (×100): similarity between the non-keratinized squamous epithelium of the vagina and the similar epithelium of the infravaginal 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

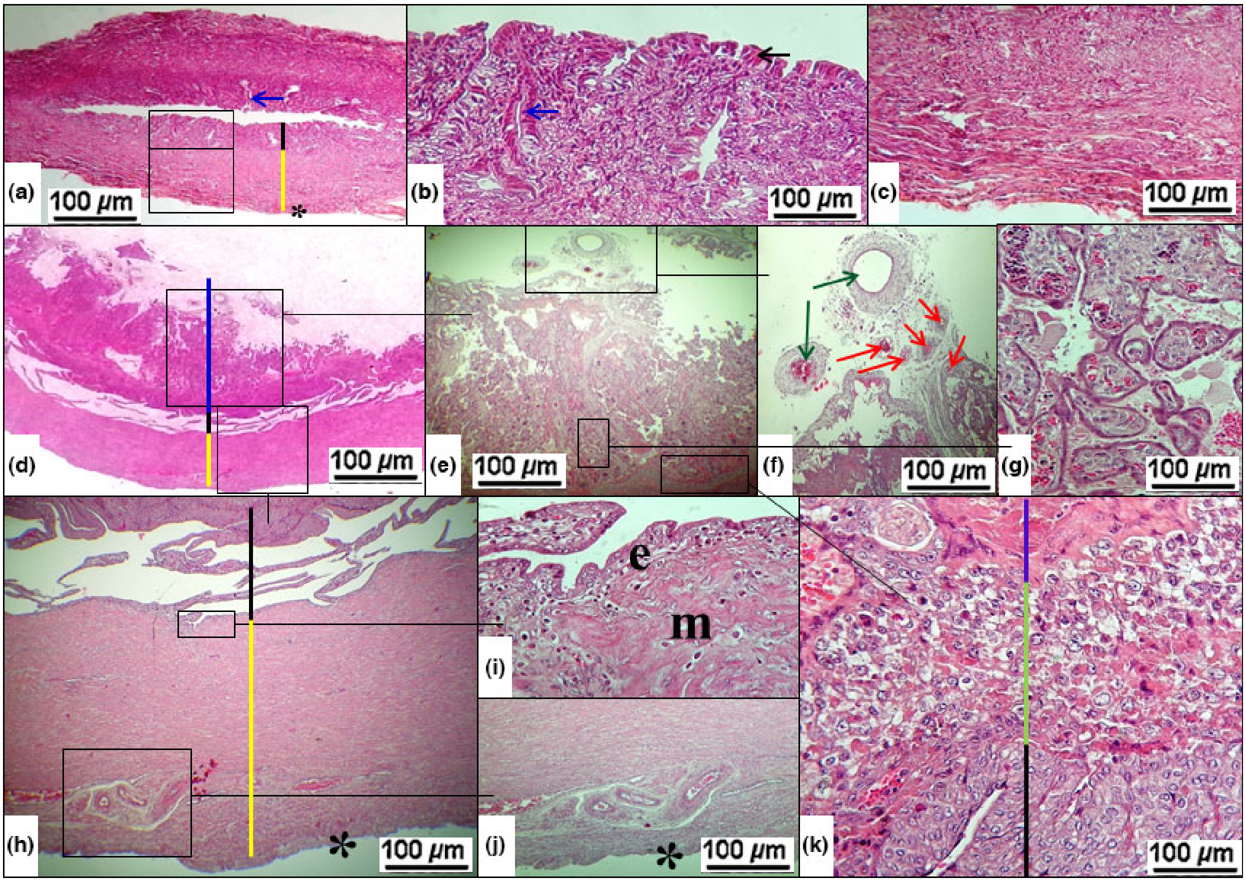

Fig. 11. Photomicrograph of the uterus of *Saimiri macrodon*. Panels a–c: juvenile female. Panel d–k: pregnant female. Observe the structure of the uterine wall in panels a and d: Perimetrium (asterisk), myometrium (yellow bar), endometrium (black bar) and placenta (blue bar). In panels a (×40) and b (×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

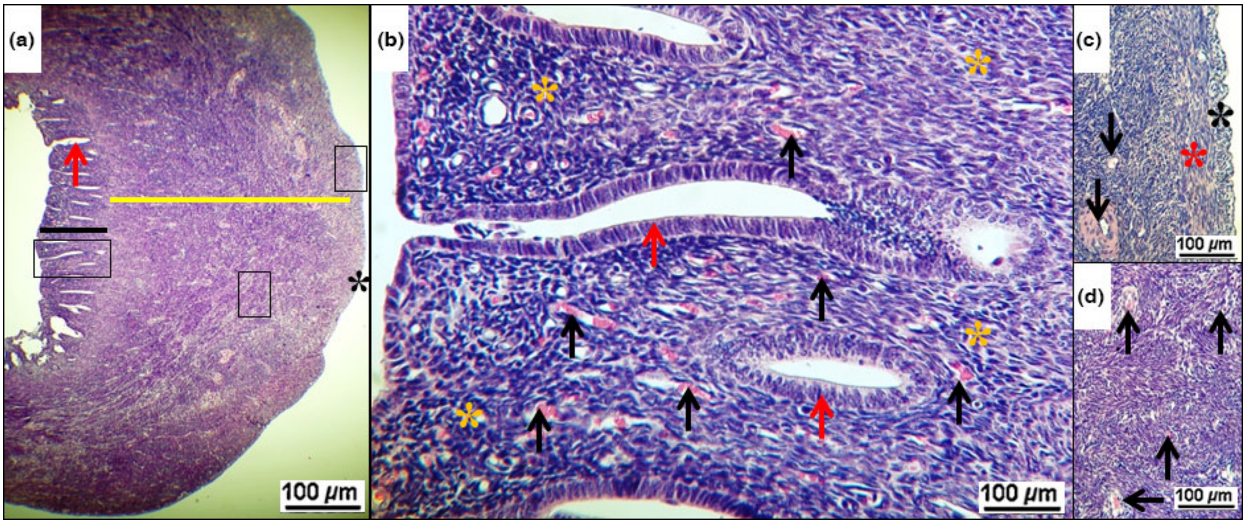

Fig. 12. Photomicrograph of the uterus of *Saimiri vanzolinii*. Note the structure of the uterine wall in Panel a (×40): Perimetrium (asterisk), myometrium (yellow bar) and endometrium (black bar). Red arrows: endometrial glands. Panel b (×400): magnification of the indicated area in the endometrium; 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

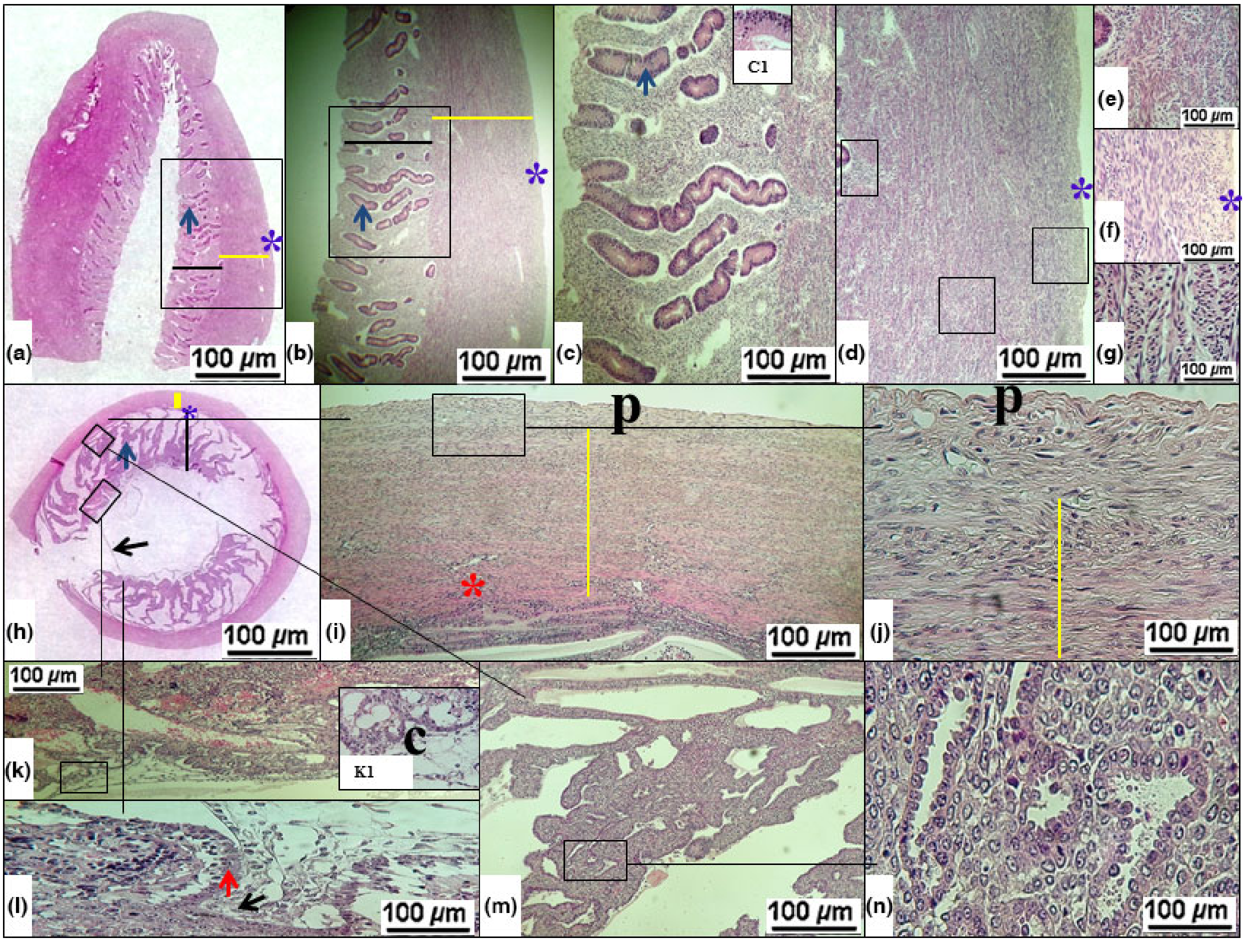

Fig. 13. Photomicrograph of the uterus of *Saimiri cassiquiarensis.* Panel a–g: non-pregnant uterus. Panel h–n: pregnant uterus. Note the structure of the uterine wall in panels a, b and h. Perimetrium (blue star), myometrium (yellow bar) and endometrium (black bar). Blue arrows: endometrial glands. 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

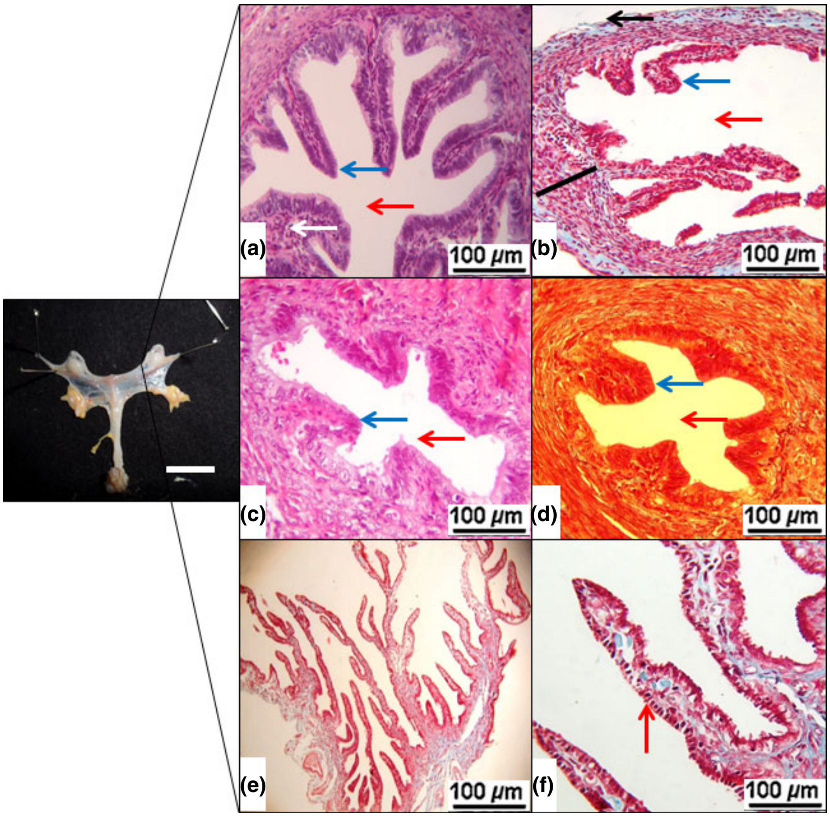

Fig. 14. Photograph and photomicrograph of the uterine tube of *Saimiri*. Panel a (×100): Infundibulum; Panel b (×100): Ampulla; Panel c (×100): Isthmus. Panel d (×400): Intramural. Panel e (×100): Fimbriae; Panel f (×1000): Ciliated cells of the fimbriae (green arrow). Red arrows: lumen; blue arrows: 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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