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Testicular biometry and semen characteristics in captive and wild squirrel monkey species (*Saimiri* sp.)

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ABSTRACT

Differential phenotypic characteristics for taxonomic diagnosis purposes are well determined in the genus *Saimiri* (squirrel monkey). However, data on its reproductive characteristics are lacking. Our aim was to determine testicular biometry and correlate with seminal analysis in captive (*Saimiri collinsi*) and free living (*Saimiri vanzolinii, Saimiri cassiquiarensis*, and *Saimiri macrodon*) squirrel monkeys. Testicular length, width, height, circumference, and volume were measured. Testicular biometry showed no differences between right and left testicles within the same species, as well as among species. Semen collected by electroejaculation was constituted of a liquid and coagulated fraction, or only one of them. No significant difference was observed between mean volumes of liquid (49.2 ± 68.9 μL: *S. collinsi*; 28.3 ± 59.8 μL: *S. vanzolinii*; 5 ± 7.1 μL: *S. cassiquiarensis*; and 0 μL: *S. macrodon*) and coagulated (65.4 ± 142.1 μL: *S. collinsi*; 125.8 ± 142.5 μL: *S. vanzolinii*; 175 ± 176.8 μL: *S. cassiquiarensis*; and 500 μL: *S. macrodon*) fractions within species or when each fraction was compared among the studied species. No correlation between testicular volume and seminal volume was observed when liquid (R = 0.31, *S. collinsi*; R = −0.69, *S. vanzolinii*) and coagulated (R = 0.32, *S. collinsi*; R = −0.37, *S. vanzolinii*) fractions were evaluated. No sufficient data were obtained for the other two species. Seminal quality was similar among species, and the most common defect was coiled tail. The method of electroejaculation yielded satisfactory results on these species, under field conditions.

1. Introduction

Squirrel monkeys (*Saimiri* sp.) are Neotropical primates whose taxonomy was recently revised. Although molecular and morphologic studies have presented conflicting results regarding the taxonomic organization and geographical distribution [1,2], the most updated classification proposed by Mittermeier et al. considers the following species of *Saimiri: Saimiri cassiquiarensis, Saimiri vanzolinii, Saimiri macrodon, Saimiri boliviensis, Saimiri sciureus, Saimiri ustus*, and *Saimiri oerstedii* [3]. In addition, the taxon *Saimiri collinsi* has been recently recognized through molecular studies [1,4].

We are performing the first study on testicular biometry and seminal characterization based on this new taxonomic organization with the following species: *S. collinsi, S. vanzolinii*, *S. cassiquiarensis,* and *S. macrodon*. Phenotypic differences among these species are more related to color and pelage patterns as depicted in Supplementary Figure 1. The conservation status of these species on the International

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Union for Conservation of Nature red list is considered as of least concern, except for *S. vanzolinii* (black-headed squirrel monkey), which is listed as vulnerable and its entire population is confined to the Mamirauá Reserve [5].

Squirrel monkeys are widely used as animal models for a variety of research areas [6]. Such application can be explained by their small size and ease of handling in captivity [6–8]. Males of *S. sciureus* and *S. boliviensis* are the most studied intensely [6,9], and from which semen has been obtained by electroejaculation (EEJ) [10–15] and penile vibratory stimulation (PVS) [15] and characterized, showing variable data. For instance, mean seminal volume ranged from 50 to 160 μL for *S. sciureus* [10–14] and from 205 to 436 μL for *S. boliviensis* [15]. Sperm concentration varied within the same species, 0 to ~430 × 10^6 sperm/mL for *S. sciureus* [10–14] and from 2 to ~ 77 × 10^6 sperm/mL for *S. boliviensis* [15].

Paim et al. [2] investigated morphologic and ecological differences among *S. vanzolinii, S. cassiquiarensis,* and *S. macrodon* living in Mamirauá Sustainable Development Reserve, but specific reproductive aspects of these recent recognized species are still unknown. Studies in reproduction focused on the species can be more relevant than those focused on the genus because some parameters can be different among species of a single genus, such as body weight, hormonal response, behavior, growth, and development. These differences have to be taken under consideration in research projects, for they might introduce new biases in the results [6].

In the present work, we aim to describe seminal characteristics, testicular parameters, and their correlations for *S. vanzolinii, S. cassiquiarensis,* and *S. macrodon*, captured in wild, and for *S. collinsi*, maintained in captivity. Therefore, our main goal is to provide biologic information on these species, to support in the future, development of successful protocols for conservation of squirrel monkeys' sperm.

2. Materials and methods

2.1. Study site

We conducted our study at two different locations. The captive males (*S. collinsi*) were examined and maintained at National Primate Center, Ananindeua, Brazil (1°22′58″S and 48°22′51″W), where the climate is humid tropical, with an average annual temperature of 28 °C. The free-living males (*S. vanzolinii, S. cassiquiarensis*, and *S. macrodon*) were captured at Mamirauá Sustainable Development Reserve (Supplementary Fig. 2). The reserve is a protected area located at the confluence of the Solimões and Japurá rivers (03°02′22″S and 64°51′41″W), covering a total of 1,124,000 ha of floodplain ecosystems [2]. Monthly average precipitation is 131.1 mm, and average temperature is 27.5 °C (minimum average 23.02 °C and maximum average 31.86 °C) [16].

2.2. Study animals

All experimental protocols were approved by the Brazilian environmental authorities (SISBIO/ICMBio/MMA no 31542-2 for captive animals/no. 29906-3 for wild animals), by the Ethical Committee in Animal Research (no. 0010/2011/CEPAN/IEC/SVS/MS; for captive animals) and Ethics and Research Committee and the Animal Use Ethical Committee of the Mamirauá Institute for Sustainable Development (no. 002/2012; for wild animals). Only adult males (>5-year old) [17] from four species of the genus *Saimiri* were selected for our study: *S. collinsi* (n = 13), *S. vanzolinii* (n = 10), *S. cassiquiarensis* (n = 5), and *S. macrodon* (n = 9). The age of all animals was estimated on the basis of dentition considering tooth eruption, intraosseous tooth formation, and tooth wear [18].

Saimiri collinsi males were captive animals from National Primate Center, selected by their physical characteristics and clinical parameters such as complete hemogram, hepatic and renal function (Supplementary Tables 1 and 2). External genitalia were evaluated, and andrologic examination (i.e., inspection and palpation of the testes to verify size, consistency, symmetry, and mobility) was performed. Animals were collectively housed in mixed groups (males and females in a varied number of members), in cages of 4.74 × 1.45 × 2.26 m (length, width, and height, respectively), under natural photoperiod (i.e., 12 hours of light and 12 hours of dark). The diet consisted of fresh fruit, milk and commercial pellet chow (MEGAZOO P18, Protein 18%, Fiber Maxi. 6.5%, Betim, MG, Brazil), and cricket larvae (*Zophobas morio*). Vitamins, minerals, and eggs were supplied once a week, and water was available ad libitum. The physical restraint was done with netting and leather glove, by a trained animal caretaker. Semen was collected at the same period of the day, i.e., in the morning before feeding and throughout 2 months (October and November) of 2011 and 2012.

Saimiri vanzolinii, *S. cassiquiarensis*, and *S. macrodon* males were captured using a Tomahawk Live Trap (0.7 × 0.4 × 0.4 m; length, width, and height, respectively) in two field expeditions in November 2012 and October 2013. Traps were set up in the early morning and checked after four hours and at mid-afternoon. The animals caught were handled by a trained animal caretaker wearing leather gloves.

2.3. Chemical restraint and semen collection

After physical restraint, all studied animals were anesthetized with ketamine hydrochloride (Vetanarcol 15 mg/kg intramuscular; König S.A., Avellaneda, Argentina) and xylazine hydrochloride (Kensol 1 mg/kg intramuscular; König S.A.) by a veterinarian [19]. Achieved total anesthetic effect, the animals were weighed. Average body weights were 868 (705–1125), 818 (580–1055), 614 (555–675), and 777 (578–1005) grams for *S. collinsi*, *S. vanzolinii*, *S. cassiquiarensis*, and *S. macrodon*, respectively (Supplementary Table 3). Subsequently, the males were placed in dorsal recumbence; both testes were evaluated and measured: length (cranial-caudal), width (medial-lateral), and height (dorsal-ventral) with a caliper rule and circumference with a tape-measure. The testicular volume was calculated by the formula: length × width × height × 0.524. Genital region was then sanitized with a mild soap and distilled water (1:10) and gauze. The prepuce was retracted with the thumb and index fingers for a more efficient cleaning of the penis with saline solution.

Animals were stimulated by EEJ (Autojac-Neovet, Uberaba, Brazil) with a rectal probe as indicated by Bennett [10]: 0.6 cm diameter and 12.5 cm length with a rounded end, bearing two metal plates (2 cm in length and 0.8 cm wide) on opposite sides. The probe was smeared with a sterile lubricant jelly (KY Jelly, Johnson and Johnson Co., Arlington, TX, USA), introduced in the rectum (~2.5 cm deep), and electrical stimuli were delivered. The stimulation session consisted of three series (7–8 minutes), composed of 35 electrical stimuli (12.5–100 mA) within an interval of 30 seconds between series [19].

Ejaculate (liquid and coagulated fractions) was collected into microcentrifuge tubes (1.5 mL). If a male did not ejaculate after the session, no further attempts were made to collect semen in the case of wild animals. In the case of captive animals, another EEJ was attempted after intervals of at least 30 days.

In captivity, semen sampling was performed in a collection room. In the wild, it was performed near capture points, to avoid the removal of the caught animals from their place of origin. Rectal temperature was measured before the EEJ procedure. A veterinarian monitored the animals during EEJ and after recovering from anesthesia.

2.4. Seminal evaluation

Immediately after ejaculation in a graduated microtube, semen was placed in a water bath at 37 °C. Appearance and consistency were evaluated subjectively by a same measurer, i.e., color (colorless, yellowish or whitish), opacity (opaque or transparent), appearance (amorphous or filamentary seminal coagulum), and degree of coagulation (four-point scale) according to Dixson and Anderson [20]. The seminal liquid fraction was measured with the aid of a pipette. Coagulum volume was calculated as the total volume minus the liquid volume. Subsequently, the extender ACP-118 (powdered coconut water; ACP Biotecnologia, Fortaleza, Ceará, Brazil) was added to a microtube, which was maintained in the water bath (37 °C) for a period of 1 to 1.5 hour to allow coagulum liquefaction [19]. During incubation, periodically (every 15 minutes) gentle mixing of the samples with the help of a pipette tip was performed to improve sample homogeneity.

Sperm motility was expressed as the percentage of sperm actively moving in a forward direction. Sperm vibrating in a place were not considered to be motile. To measure sperm motility, 10 μL of semen was placed in a prewarmed (37 °C) glass slide with cover slip and evaluated. Sperm vigor was subjectively scored on a scale from 0 to 5 [19]. Sperm morphology and plasma membrane integrity were evaluated by a smear prepared adding 5 μL of eosin-nigrosine stain (Vetec, Rio de Janeiro, Brazil) to 5 μL of semen on a prewarmed (37 °C) glass slide. Morphologic defects detected in sperm were classified as major or minor [21]. Sperm concentration was determined in a Neubauer chamber after dilution of 1 μL semen in 99 μL formalin solution 10%. Seminal pH was measured with a pH strip (Merck Pharmaceuticals, Darmstadt, Germany). All evaluations were performed under a light microscope (Nikon, Tokyo, Japan), at a magnification × 100, by a same measurer.

2.5. Data analysis

All data are expressed as mean ± standard deviation (SD). Testicular biometry and seminal data were evaluated using one-way ANOVA followed by Tukey multiple comparisons posthoc test. The Spearman rank-order correlation coefficient was used to measure correlation between parameters. Statistical significance was obtained whenever $P < 0.05$. For all statistical analyses, software R, version 3.1.2, was used.

3. Results

Total time for anesthetic effect (approximately 4–5 minutes) and rectal temperature (38.6 ± 1.1 °C) were not species-specific. Animals selected for this study presented testes with normal consistency and mobility. Testicular biometry showed no differences between right and left testicles within the same species, neither among species, which presented all similar testicular biometry (Fig. 1). The testicular circumference was not measured in *S. cassiquiarensis*. Hence, there are no data available.

Penile erection began more frequently between the 13rd and 20th electrical stimuli of each series, and ejaculation occurred on average between 15th and 18th stimuli of each series, in all species. The collected semen was constituted by a liquid and coagulated fraction or only one (Table 1). Ejaculation was almost always initiated by the liquid fraction that often was partially or totally coagulated after about 10 seconds. Liquid and coagulated fractions were transparent or opaque and colorless, whitish or yellowish in all studied species. This wide variation in appearance and constitution of ejaculated among collections was regardless of species, and no pattern was observed within species. Coagulated fraction presented filamentary or amorphous appearance, and the most frequent coagulation degree observed was 3, where semen coagulates so that the ejaculate forms a whitish, nonfluid, nongelatinous mass but not a compact plug and was not spontaneously dissolved, according to Dixson and Anderson's scale [20] (Supplementary Fig. 3). The number of animals of each species in which EEJ was performed and number of ejaculations are listed in Table 1. Concerning volume of liquid and coagulated fractions, no significant difference was observed among species within each compared fraction or between fractions within the same species (Table 1).

There was no correlation between the testicular volume and seminal volume, when liquid ($R = 0.31$) and coagulated ($R = 0.32$) fractions from *S. collinsi* were evaluated. Likewise, liquid and coagulated fractions from *S. vanzolinii* were not correlated with testicular volume ($R = -0.69$ and $R = -0.37$, respectively). Correlations in *S. cassiquiarensis* and *S. macrodon* were not performed because of the insufficient number of seminal samples of both species (Fig. 2).

Sperm motility, vigor, and plasma membrane integrity were similar among species (Fig. 3). Owing to the limited number of seminal samples ($n = 1$) obtained from *S. macrodon*, sperm morphology was evaluated only on *S. collinsi, S. vanzolinii*, and *S. cassiquiarensis*, and no differences among these species was observed (Table 2). The most

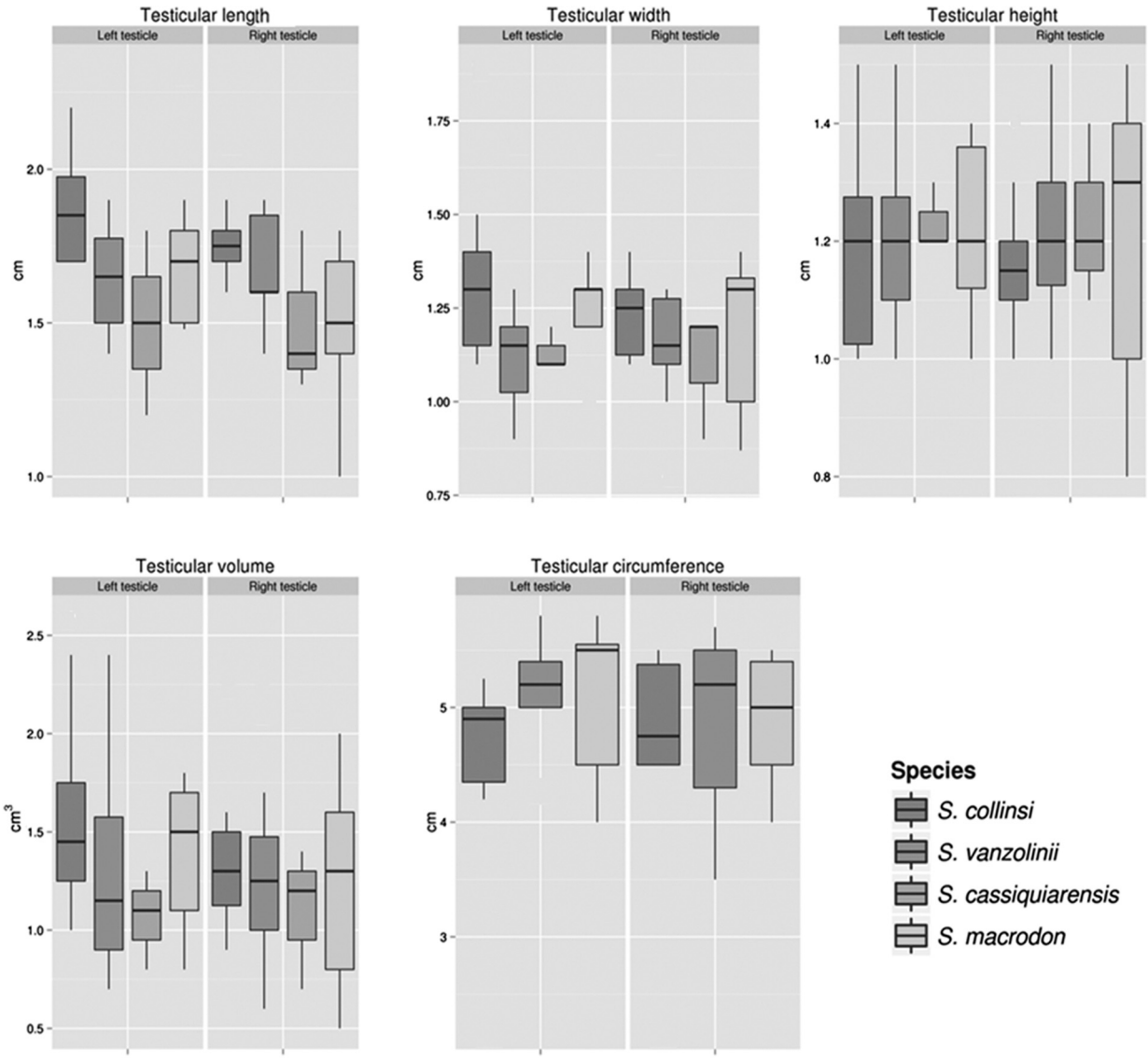

Fig. 1. Box plot of parameters of testicular biometry of *Saimiri collinsi* (n = 13), *Saimiri vanzolinii* (n = 10), *Saimiri cassiquiarensis* (n = 5), and *Saimiri macrodon* (n = 9) males. No significant differences were observed within and among species regarding testicular parameters.

common defects were observed in the tail with a predominance of secondary defects, i.e., coiled tail (see Fig. 4). Sperm concentration was measured in semen from captive animals, and the mean (± SD) value for liquid fraction of *S. collinsi* was 88.31 ± 36.64 × 10^6 sperm/mL. Mean (± SD) seminal pH of total ejaculate (liquid and coagulated fraction) was 7.43 ± 0.63. For none of these parameters, differences among species were observed.

Table 1
Number of animals submitted to electroejaculation (N), total number of trials (EEJ), trials with ejaculates (total, containing both liquid and coagulated fractions, only liquid, or only coagulated fraction), ejaculates presenting sperm, and mean (± standard deviation) seminal volume of liquid and coagulated fractions.

Species	N	EEJ	Trials with ejaculates (fractions)				Ejaculates with sperm (n)	Liquid fractions (μL)	Coagulated fractions (μL)
			Total	Liquid + coagulated	Liquid	Coagulated			
Saimiri collinsi	13	27	13	4	7	2	9	49.2 ± 68.9	65.4 ± 142.1
Saimiri vanzolinii	8	8	6	3	0	3	3	28.3 ± 59.8	125.8 ± 142.5
Saimiri cassiquiarensis	5	5	2	1	0	1	1	5 ± 7.1	175 ± 176.8
Saimiri macrodon	1	1	1	0	0	1	1	0[a]	500[a]

No significant differences were observed within and among species regarding seminal liquid and coagulated fractions.
[a] Only one sample obtained.

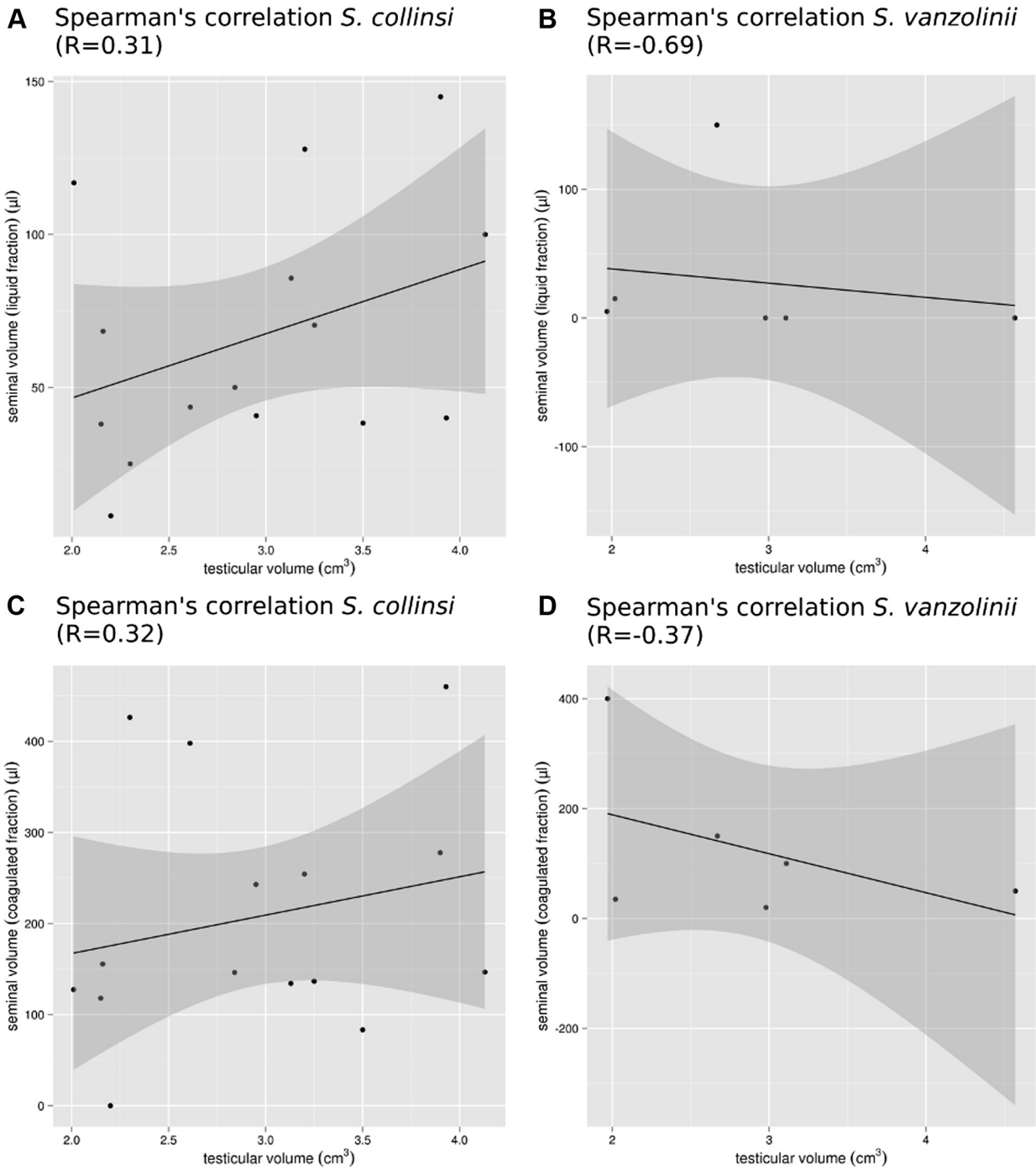

Fig. 2. Correlation found between testicular volume and seminal volume (liquid or coagulated fractions) in *Saimiri collinsi* (A and C; n = 13 males) and *Saimiri vanzolinii* (B and D; n = 6 males).

4. Discussion

This is the first report describing seminal and testicular parameters in free-living *S. vanzolinii, S. cassiquiarensis*, and *S. macrodon*. Previous studies on these themes were focused on *S. collinsi* [19], *S. sciureus* [22], and *S. boliviensis* [15]. Besides its substantial importance for the evaluation of male fertility, collection and characterization of semen from wild primates, in the field, is still scarce but necessary to evaluate reproduction from these free-living species [23].

Observed symmetry between right and left testicles within a same individual of all studied species was

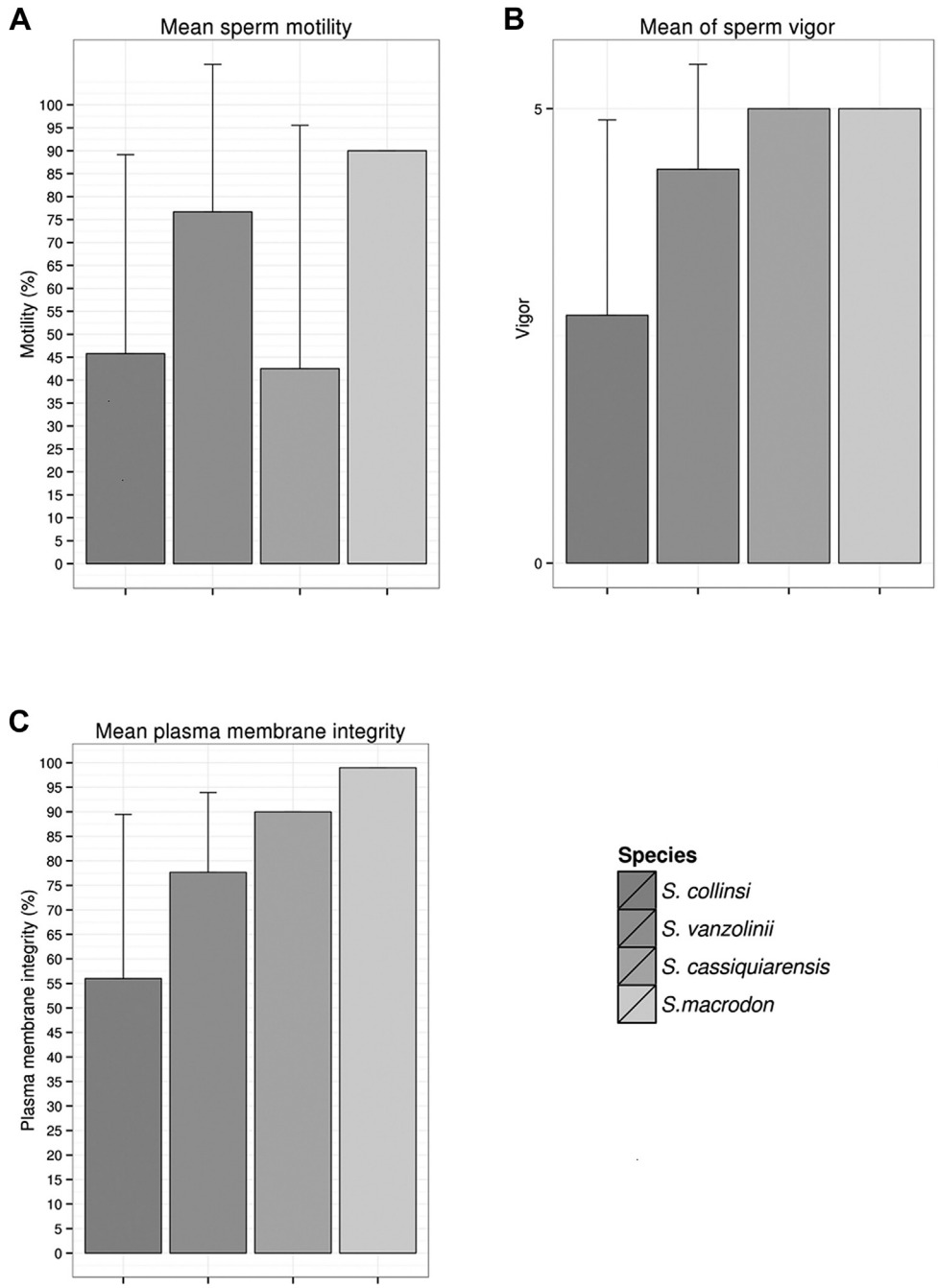

Fig. 3. Mean sperm motility (A), vigor (B), and plasma membrane integrity (C) of *Saimiri collinsi* (n = 13 males), *Saimiri vanzolinii* (n = 6 males), *Saimiri cassiquiarensis* (n = 2 males), and *Saimiri macrodon* (n = 1 male).

expected because the testicles are paired organs, and there are no major differences between them if organogenesis occurs without anomalies as monorchidism, cryptorchidism, or hypoplasia. Total testicular volume of *S. collinsi* (2934 ± 831 mm^3; mean ± SD), *S. vanzolinii* (2552 ± 900 mm^3), *S. cassiquiarensis* (2192 ± 619 mm^3), and *S. macrodon* (2608 ± 748 mm^3) presented greater measurements than those described in *S. sciureus* by Pasqualini et al. (951–1792 mm^3; minimum–maximum) [24] and Viana (1515 ± 87.18 mm^3; mean ± SD) [25] and were similar to the recent measurement reported for *S. collinsi* (2967 ± 635 mm^3; mean ± SD) [19]. Although wild monkeys may present different patterns of reproductive season and captive monkeys are usually able to mate and reproduce all year [22,25], no effect on testicular biometry was observed.

Table 2
Mean percentages (± standard deviation) of normal sperm and sperm with major and minor pathologic defects in fresh semen (liquid fraction) of *Saimiri collinsi, Saimiri vanzolinii*, and *Saimiri cassiquiarensis*.

Morphology	Species		
	S. collinsi	*S. vanzolinii*	*S. cassiquiarensis*[a]
Normal	69.06 ± 21.18	90 ± 9.19	81
Major pathologic defects			
Strongly coiled tail	8.14 ± 12.07	3.50 ± 3.54	0
Proximal droplet	0.24 ± 0.93	0.5 ± 0.70	0
Pseudo droplet	0.17 ± 0.48	0	0
Small abnormal head	0.02 ± 0.15	0	0
Narrow at base	0.02 ± 0.13	0	0
Pear-shaped defect	0.02 ± 0.15	0	0
Minor pathologic defects			
Terminally coiled tail	11.26 ± 8.72	4.50 ± 6.36	10
Simple bent tail	10.88 ± 8.87	1 ± 0	9
Distal droplet	0.19 ± 0.47	0	0
Forked tail	0	0.5 ± 0.70	0

It was not possible to evaluate sperm morphology of *Saimiri macrodon* because of inexistence of liquid fraction.
[a] Only one sample evaluated.

Positive correlation between ejaculate and testicular volumes observed in *S. collinsi* but not in *S. cassiquiarensis* was not significant, probably because of the wide variation in sperm quality between the individuals. Also, despite the large seminal volume, many ejaculates did not contain viable sperm or were oligospermic. This would have relation with the position of the stimulating probe within the rectum relative to adjacent prostate, loss of part of ejaculate into the bladder (retrograde ejaculation) due to anesthetic effect or electrical surge [15,26], or simply a possible masturbation or copula heretofore to EEJ.

Many attempts of semen collection were made during the present study. However, the number of ejaculates presenting good spermatic parameters was relatively small. This was previously observed in *S. boliviensis*, when males were rectally stimulated during EEJ and presented modest penile erection (frequently faded), and ejaculates were predominantly composed by a coagulated fraction [15]. Although these authors found that more than half of the ejaculates (60%) failed to contain motile sperm, and besides the risk of both equipment and stimulation procedure affecting seminal quality [27], semen collection with EEJ is still conventionally applied especially for Neotropical primates. Furthermore, EEJ was applied because other methods were not available, and conditioning free-living animals was not an option because it would affect their daily habits. Other successful semen collection methods are already described for essentially

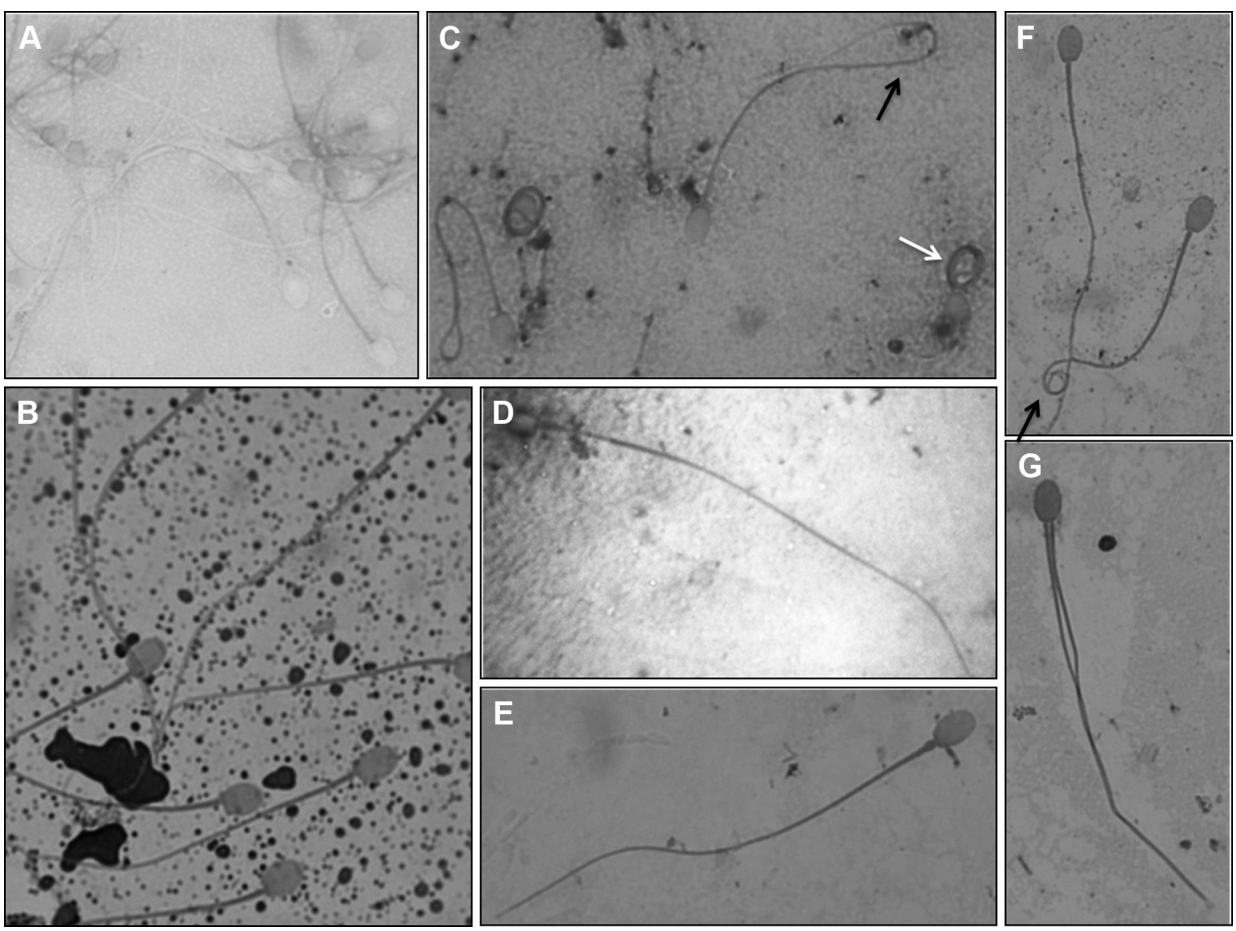

Fig. 4. Sperm morphology. (A) *Saimiri collinsi* sperm with intact (not stained) and injured (stained) plasma membrane; (B) abaxial insertion of *Saimiri vanzolinii*; (C) strongly coiled (white arrow) and simple bent tail (black arrow) of *S. collinsi*; (D) small abnormal head of *S. collinsi*; (E) proximal droplet of *S. vanzolinii*; (F) normal and terminally coiled tail (black arrow) of *S. vanzolinii*; (G) forked tail of *S. vanzolinii*.

terrestrial species, as *Macaca fuscata* [23], differently of *Saimiri sp.*, which is basically arboreal.

With some exceptions such as the *Alouatta caraya,* species, which does not present semen coagulum after ejaculation [28], semen from most primates coagulates during or after ejaculation, being difficult to evaluate seminal quality [10,13,15,26,27,29,30]. In the present study, one part of the ejaculate coagulated but another part remained liquid, without significant difference between the volumes of these two fractions. The seminal coagulum apparently has as main function to act as a reservoir of sperm and protects them against adverse vaginal conditions, e.g., acid pH acid [31]. The necessary time after ejaculation for spontaneous coagulum liquefaction is variable and species-specific [32]. In the present study, no spontaneous liquefaction occurred. Addition of proteolytic enzymes, e.g., trypsin, has been reported to be effective for liquefaction of seminal coagulum from several primate species [32]. However, some studies have shown that proteolytic enzymes can cause lesions in sperm membrane, compromising fertility capacity [33]. Therefore, we did not use proteolytic enzymes to accelerate liquefaction of seminal coagulum, but we diluted the coagulated in ACP-118 (powdered coconut water). This extender was previously tested for *S. collinsi* [19] and *Sapajus apella* [29], whose semen also does not liquefy spontaneously. ACP-118 was used in association with mechanical fragmentation and incubation in water bath at 37 °C. ACP-118 contains ascorbic acid and polyphenol oxidases, which are antioxidants that support sperm quality during and after incubation [29,30]. ACP-118 is also composed by different bioactive enzymes, e.g., phosphatase, catalase, and dehydrogenase, which may support coagulum liquefaction.

Collected semen was in general similar to that described in *S. sciureus* and *S. boliviensis* studies (employing EEJ or PVS) [10–15]. Mean seminal volume collected in this study was higher than that observed in the first seminal description of the genus *Saimiri* (in *S. sciureus*) [10]. In this former study, semen was also collected by EEJ, and Bennett [10] described two seminal fractions: a colorless sperm-free coagulum and a sperm-rich fluid. However, in our study, we observed sperm in both fractions. Ackerman and Roussel [12] described liquefaction of coagulum seminal by trypsin resulting in lower sperm motility and plasma membrane integrity than those obtained in the present and recent study with *S. collinsi* [19], maybe due a detrimental effect of trypsin on sperm quality. Other scientific reports on seminal characteristics of *Saimiri* species do not cite addition of proteolic enzymes for coagulum liquefaction [13,15,26].

The observed sperm motility was similar to that reported in other primate species when EEJ was applied. For instance, it was similar to the findings in fresh semen from *Papio anubis* (48%–92%) [34] or from *Ateles geoffroyi* (~56%) [32]. However, when the PVS method was applied, sperm motility was improved in semen collected from *Callithrix jacchus* [35] and *Macaca mulatta* [36], both with 76% and 71% of motility, respectively. Semen pH was similar to the one reported for *S. collinsi* (6.5–8.0) [19], *A. caraya* (8.1) [27], *A. geoffroyi* (8.0) [37], and *Callithrix jacchus* (7.6) [35]. Mean (± SD), sperm concentration of liquid fraction was similar to observed in ejaculates collected both by EEJ as by PVS, of *S. sciureus* [10,12] and *S. boliviensis* [15], despite the large variation between the ejaculates analyzed in this study (0–965 × 10^6 sperm/mL).

The percentage of normal sperm (69% *S. collinsi*, 90% *S. vanzolinii*, and 81% *S. cassiquiarensis*) was superior to that previously reported in *S. sciureus* (49%–65%) trypsin-digested semen [12] and similar to that described for some Neotropical primates in semen collected by EEJ: *S. collinsi* (74.7%) [19] and *Sapajus apella* (81.7%) [29] after semen liquefaction in ACP-118, *C. jacchus* (87.6%) [38], *A. geoffroyi* at dry season (73%) [39], *A. caraya* (78.7%) [27], and *Sapajus apella* after semen liquefaction in coconut water solution (78.2%) or TES-TRIS (83.7%) [30].

Spermatic pathologies observed in that study were classified according Blom [21], whose classification system is on the basis of relative importance of the sperm abnormality to fertility. Thus, although the major abnormalities have been correlated to impaired fertility, minor defects do not necessarily indicate a disturbance of spermatogenesis, but nevertheless, could cause a reduction in fertility if they are present in large proportions within the ejaculate [40]. The highest percentage of major pathologic defect was observed in *S. collinsi* (about 8.61%), being a relatively low value, which do not compromise the use of that semen in biotechnologies of reproduction. We observed the eccentric insertion (abaxial) of the middle piece in the posterior portion of the head in all species, as previously described for other species of this genus [14]. The number of tail defects was greater than head defects in this study, as described in *S. collinsi* (strongly coiled tail 8.3%, coiled tail 9.3%, and bent tail 7% vs. 0.33% pear-shaped defect) [19], *Sapajus apella* (strongly coiled tail 9%, bent tail 11%, and coiled tail 7% vs. 0 head defects) [29], and *C jacchus* (50% tail defects vs. 4.5% head defects) [41].

4.1. Conclusions

Despite the variation between ejaculates' quality and number of ejaculates, EEJ yielded satisfactory results, and these data expand the knowledge of reproductive biology in male squirrel monkeys. Furthermore, studies in free-living species during other periods of the year to meet the seasonal variations of the reproductive physiology are also necessary. Knowledge on semen characteristics will support the development of procedures for semen preservation [42] and further fundamental studies on seminal characteristics within free-living nonhuman primates, which is extremely important, especially for the conservation of endangered species like *S. vanzolinii.*

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.theriogenology.2016.03.009.

References

[1] Lavergne A, Ruiz-García M, Catzeflis F, Lacote S, Contamin H, Mercereau-Puijalon O, et al. Phylogeny and phylogeography of squirrel monkeys (genus *Saimiri*) based on cytochrome b genetic analysis. Am J Primatol 2010;72:242–53.

[2] Paim FP, Silva Júnior JS, Valsecchi J, Harada ML, Queiroz HL. Diversity, geographic distribution and conservation of squirrel monkeys, *Saimiri* (primates, Cebidae), in the floodplain forests of Central Amazon. Int J Primatol 2013;34:1055–76.

[3] Mittermeier RA, Rylands AB, Wilson DE. Handbook of the Mammals of the World, volume 3. Barcelona: Lynx Edicions; 2013.

[4] Mercês MP, Lynch Alfaro JW, Ferreira WAS, Harada ML, Silva Júnior JS. Morphology and mitochondrial phylogenetics reveal that the Amazon River separates two eastern squirrel monkey species: *Saimiri sciureus* and *S. collinsi*. Mol Phylogenet Evol 2015;82:426–35.

[5] Boubli JP, Rylands AB. *Saimiri vanzolinii*. The IUCN red list threatened species version. 2014.3. http://www.iucnredlist.org/details/19839/0; 2008 [accessed 10.02.15].

[6] Brady AG. Research techniques for the squirrel monkey (*Saimiri* sp.). Lab Anim Sci 2000;41:10–8.

[7] Baldwin JD. Reproductive synchronization in squirrel monkeys (*Saimiri*). Primates 1970;11:317–26.

[8] Chen JJ, Smith ER, Gray GD, Davidson JM. Seasonal changes in plasma testosterone and ejaculatory capacity in squirrel monkeys (*Saimiri sciureus*). Primates 1981;22:253–60.

[9] Abee CR. The squirrel monkey in biomedical research. Lab Anim Sci 1989;31:11–20.

[10] Bennett JP. Semen collection in the squirrel monkey. J Reprod Fertil 1967;13:353–5.

[11] Lang CM. A technique for the collection of semen from squirrel monkeys (*Saimiri sciureus*) by electroejaculation. Lab Anim Care 1967;17:218–21.

[12] Ackerman DR, Roussel JD. Fructose, lactic acid and citric acid content of the semen of eleven subhuman primate species and of man. J Reprod Fertil 1968;17:563–6.

[13] Denis LT, Poindexter AN, Ritter MB, Seager SW, Deter RL. Freeze preservation of squirrel monkey sperm for use in timed fertilization studies. Fertil Steril 1976;27:723–9.

[14] Dukelow WR. The squirrel monkey (*Saimiri sciureus*). In: Hearn JP, editor. Reproduction in New World Primates: new models in medical science. Lancaster: MTP Press Limited; 1983. p. 149–80.

[15] Yeoman RR, Sonksen J, Gibson SV, Rizk BM, Abee CR. Penile vibratory stimulation yields increased spermatozoa and accessory gland production compared with rectal electroejaculation in a neurologically intact primate (*Saimiri boliviensis*). Hum Reprod 1998;13:2527–31.

[16] Mamirauá Institute for Sustainable Development. Database on climatic parameters of Mamirauá Institute. http://mamiraua.org.br; 2014 [accessed 11.05.15].

[17] Stone AI. Is fatter sexier? Reproductive strategies of male squirrel monkeys (*Saimiri sciureus*). Int J Primatol 2014;35:628–42.

[18] Smith BH. Dental development as a measure of life history in primates. Evolution 1989;43:683–8.

[19] Oliveira KG, Leão DL, Almeida DVC, Santos RR, Domingues SFS. Seminal characteristics and cryopreservation of sperm from the squirrel monkey, *Saimiri collinsi*. Theriogenology 2015;84:743–9.

[20] Dixson AF, Anderson MJ. Sexual selection, seminal coagulation and copulatory plug formation in Primates. Folia Primatol 2002;73:63–9.

[21] Blom E. The ultrastructure of some characteristic sperm defects and a proposal for a new classification of bull spermogram. Nord Vet Med 1973;25:383–91.

[22] Trevino HS. Seasonality of reproduction in captive squirrel monkeys (*Saimiri sciureus*). Am J Primatol 2007;69:1001–12.

[23] Thomsen R. Non-invasive collection and analysis of semen in wild macaques. Primates 2013;55:231–7.

[24] Pasqualini T, Colillas O, Rivarola MA. Testicular and serum testosterone variations in squirrel monkeys during seasonal cyclicity. J Androl 1986;7:298–302.

[25] Viana CF. Semen characteristics, profile of testosterone concentration in fecal extract, change in body weight and testicular volume of squirrel monkey (*Saimiri sciureus*, Linnaeus, 1758) maintained in captivity under controlled environmental conditions [dissertation]. Campos dos Goytacazes (RJ): State University of North Fluminense Darcy Ribeiro; 2013.

[26] Yeoman RR, Ricker RB, Williams LE, Sonksen J, Abee CR. Vibrostimulation of ejaculation yields increased motile spermatozoa, compared with electroejaculation in squirrel monkeys (*Saimiri boliviensis*). Contemp Top Lab Anim Sci 1997;35:62–4.

[27] Valle RR, Guimarães MABV, Muniz JAPC, Barnabe RC, Vale WG. Collection and evaluation of semen from captive howler monkeys (*Alouatta caraya*). Theriogenology 2004;62:131–8.

[28] Moreland RB, Richardson ME, Lamberski N, Long JA. Characterizing the reproductive physiology of the male southern black howler monkey, *Alouatta caraya*. J Androl 2001;22:395–403.

[29] Leão DL, Miranda SA, Brito AB, Lima JS, Santos RR, Domingues SFS. Efficacious long-term cooling and freezing of *Sapajus apella* semen in ACP-118®. Anim Reprod Sci 2015;159:118–23.

[30] Oliveira KG, Miranda SA, Leão DL, Brito AB, Santos RR, Domingues SFS. Semen coagulum liquefaction, sperm activation and cryopreservation of capuchin monkey (*Cebus apella*) semen in coconut water solution (CWS) and TES-TRIS. Anim Reprod Sci 2011;123:75–80.

[31] García-Granados MD, Hernández-López LE, Córdoba-Aguilar A, Cerda-Molina AL, Pérez-Ramírez O, Mondragón-Ceballos R. Effect of photoperiod on characteristics of semen obtained by electroejaculation in stump-tailed macaques (*Macaca arctoides*). Primates 2014;55:393–401.

[32] Flores-Herrera H, Acuña-Hernández DG, Rivera-Rebolledo JA, González-Jiménez MA, Rodas-Martínez AZ, Swanson WF. Effect of increasing trypsin concentrations on seminal coagulum dissolution and sperm parameters in spider monkeys (*Ateles geoffroyi*). Theriogenology 2012;78:612–9.

[33] Morrell JM, Hodges JK. Germplasm cryopreservation of nonhuman primates. In: Watson PF, Holt VVV, editors. Cryobanking the Genetic resource: Wildlife conservation for the future? London: Taylor and Francis; 2001. p. 408–26.

[34] Nyachieo A, Spiessens C, Chai DC, Kiulia NM, Mwenda JM, D'Hooghe TM. Separate and combined effects of caffeine and dbcAMP on olive baboon (*Papio anubis*) sperm. J Med Primatol 2010;39:137–42.

[35] Valle RR, Valle CM, Nichi M, Muniz JAPC, Nayudu PL, Guimarães MABV. Semen characteristics of captive common marmoset (*Callithrix jacchus*): a comparison of a German with a Brazilian colony. J Med Primatol 2014;43:225–30.

[36] Yang S, Ping S, Ji S, Lu Y, Niu Y, Wang H, et al. The positive effects of seminal plasma during the freezing process on cryosurvival of sperm with poor freezability in the rhesus macaque (*Macaca mulatta*). J Reprod Dev 2011;57:737–43.

[37] Hernández-López L, Cerda-Molina AL, Páez-Ponce D, Mondragón-Ceballos R. The seminal coagulum favours passage of fast-moving sperm into the uterus in the black-handed spider monkey. Reproduction 2008;136:411–21.

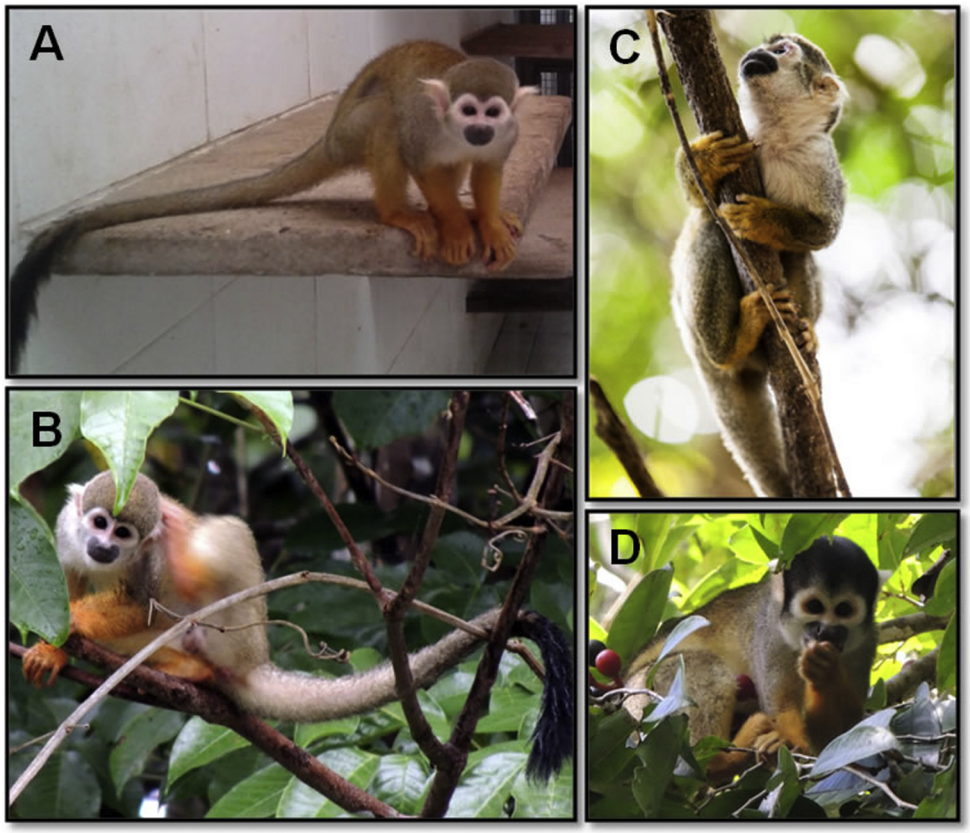
[38] Morrell JM, Küderling I, Hodges JK. Influence of semen collection method on ejaculate characteristics in the common marmoset, *Callithrix jacchus*. J Androl 1996;17:164–72.

[39] Hernández-López L, Cerezo-Parra G, Cerda-Molina AL, Pérez-Bolanõs SC, Díaz-Sánchez V, Mondragón-Ceballos R. Sperm quality differences between the rainy and dry seasons in captive black-handed spider monkeys (*Ateles geoffroyi*). Am J Primatol 2002;57:35–41.

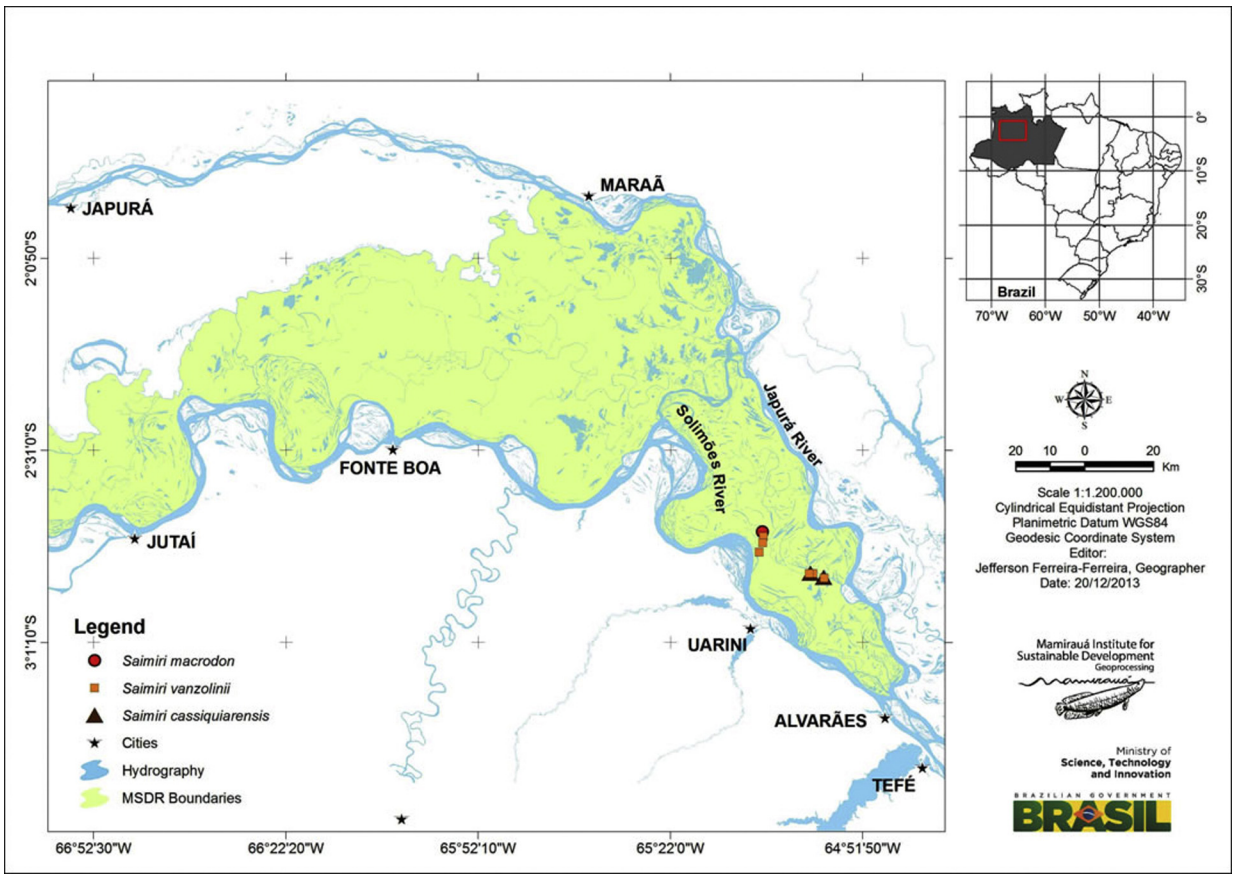
[40] Enciso M, Cisale H, Johnston SD, Sarasa J, Fernández JL, Gosálvez J. Major morphological sperm abnormalities in the bull are related to sperm DNA damage. Theriogenology 2011;76:23–32.

[41] Cui KH, Flaherty SP, Newble CD, Guerin MV, Napier AJ, Mathews CD. Collection and analysis of semen from the common marmoset (*Callithrix jacchus*). J Androl 1991;12:214–20.

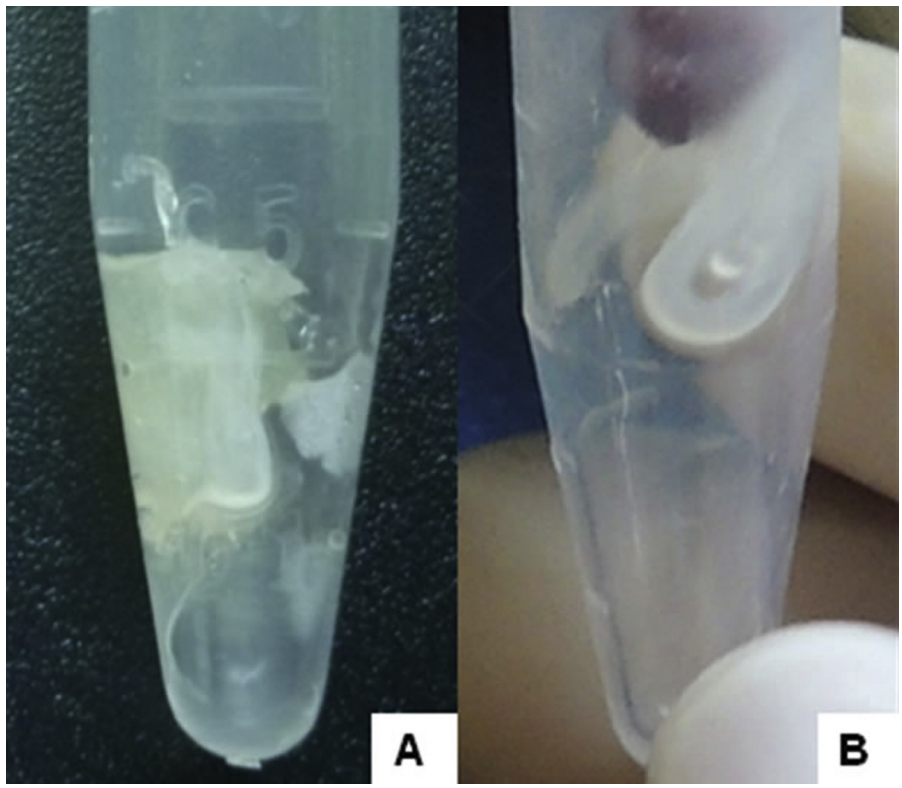
[42] Oliveira KG, Santos RR, Leão DL, Brito AB, Lima JS, Sampaio WV, et al. Cooling and freezing of sperm from captive, free-living and endangered squirrel monkey species [e-pub ahead of print]. Cryobiology 2016. doi: 10.1016/j.cryobiol.2016.03.004.



Supplementary Fig. 1. Pictures from *Saimiri collinsi* (A) by Tatyana Pinheiro; *Saimiri cassiquiarensis* (B) by Fernanda Paim; *Saimiri macrodon* (C) by Sônia Vill; and *Saimiri vanzolinii* (D) by Fernanda Paim.



Supplementary Fig. 2. Distribution of capture sites for *Saimiri vanzolinii*, *Saimiri cassiquiarensis*, and *Saimiri macrodon*. Source: Mamirauá Institute for Sustainable Development, Remote sensing and geoprocessing group, 2013.



Supplementary Fig. 3. Amorphous (A) and filamentous (B) coagulated fraction from *Saimiri macrodon* and *Saimiri vanzolinii*, respectively, collected by electroejaculation.

Supplementary Table 1
Hemogram results from *Saimiri collinsi* males (n = 10).

Parameter	Results	Reference values[a]
Hematocrit (%)	43.2 ± 3.6	44.00 ± 0.6
Red blood cells ($\times 10^6$/mL)	07.2 ± 0.4	07.1 ± 0.1
Hemoglobin (g/dL)	13.9 ± 1.2	13.8 ± 0.2
Mean corpuscular volume (fL)	59.9 ± 2.8	61.9 ± 0.6
Mean corpuscular hemoglobin (pg)	19.1 ± 1.1	19.4 ± 0.2
Mean corpuscular hemoglobin concentration (%)	31.8 ± 1.0	31.5 ± 0.2
White blood cells ($\times 10^3$/mL)	11.0 ± 4.1	10.5 ± 0.6
Platelets (million/mm^3)	268 ± 93	–
Basophils (%)	0.44 ± 0.7	0.0 ± 0.2
Eosinophils (%)	01.9 ± 0.9	01.0 ± 0.2
Neutrophils (%)	51.5 ± 11.7	35.0 ± 3.2
Lymphocytes (%)	44.1 ± 11.2	61.0 ± 3.1
Monocytes (%)	02.1 ± 1.6	02.0 ± 0.3

Values are expressed as mean ± standard deviation.
[a] Source of reference values: Kakoma I, James MA, Jackson W, Bennett G, Ristic M. 1985. Hematologic values of normal bolivian squirrel monkeys (*Saimiri sciureus*): a comparison between wild-caught and laboratory-bred male animals. Folia Primatologica 44:102 to 107.

Supplementary Table 2
Biochemical analysis of plasma from *Saimiri collinsi* males (n = 10).

Parameters	Results	Reference values[a]
Glucose (mg/dL)	108 ± 42.4	103 ± 30.3
Blood urea nitrogen (BUN; mg/dL)	48.8 ± 37.7	38.7 ± 10
Cholesterol (mg/dL)	188.3 ± 43.0	151 ± 64.7
Triglycerides (mg/dL)	72.5 ± 40.4	74.9 ± 32.7
Creatinine (mg/dL)	0.6 ± 0.1	0.9 ± 0.2
Total bilirubin (mg/dL)	<0.10	0.8 ± 0.6
Phosphatase (U/L)	249 ± 308	358 ± 175
Glutamic oxaloacetic transaminase (U/L)	190 ± 56.6	185 ± 95.3
Glutamic pyruvic transaminase (U/L)	201 ± 75.4	184 ± 110
Total protein (g/dL)	6.5 ± 0.7	6.9 ± 1.0
Calcium (mg/dL)	9.7 ± 0.9	9.6 ± 0.9
Albumina (g/dL)	3.7 ± 0.3	4.2 ± 0.6
Carbon dioxide	21	11.1 ± 3.9
Potassium	3.5	5.7 ± 1.0
Thyroxine-binding globulin	2.8 ± 0.5	–
Very low density lipoprotein	17.3 ± 7.6	–
Ammonia	46.5 ± 55.9	–
Phosphorus	5.3 ± 0.2	–
Magnesium	2.7 ± 0.3	–
Iron	154 ± 94.1	–

Values are expressed as mean ± standard deviation.
[a] Source reference values: KCCMR. Michale E. Keeling Center for Comparative Medicine and Research. [Internet]. Texas: The University of Texas MD. Anderson Cancer Center. [cited 2014 March 2]. Available from: http://www.mdanderson.org/education-and-research/departments-programs-and-labs/programs-centers-institutes/michale-e-keeling-center-for-comparative-medicine-and-research/animal-resources/squirrel-monkey-diagnostic-reference-values.html.

Supplementary Table 3
Body weight per animal, as well as mean (± standard deviation) and range values of studied males from *Saimiri collinsi* (n = 13), *Saimiri vanzolinii* (n = 10), *Saimiri cassiquiarensis* (n = 5), and *Saimiri macrodon* (n = 9) species.

Individual	Weight (grams)	Range
S collinsi	868 ± 104	705–1125
1	1055	
2	740	
3	717	
4	975	
5	915	
6	850	
7	878	
8	782	
9	748	
10	935	
11	845	
12	990	
13	852	
S vanzolinii	818 ± 136	580–1055
1	715	
2	835	
3	1055	
4	875	
5	860	
6	775	
7	675	
8	905	
9	906	
10	580	
S cassiquiarensis	614 ± 44	555–675
1	675	
2	605	
3	600	
4	555	
5	635	
S macrodon	777 ± 170	578–1005
1	720	
2	860	
3	1000	
4	905	
5	590	
6	615	
7	1005	
8	578	
9	723	