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A paradox of the ovulatory patterns in the living laboratory, African giant pouched rat (*Cricetomys gambianus*) from Tanzania

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Abstract

The African giant pouched rat (Cricetomys gambianus) has emerged as a valuable model animal deployed in biomedical and other researches with humanitarian application due to its intelligence, socio-behavior, and adaptability to captivity. This rodent lives in solitary and belongs to the family Cricetidae that synchronises sexual behavioral onset with subsequent maturation of their gonads and gametes just prior to the beginning of the short rains of Tanzania. The current study set out to investigate whether ovulation in this species is induced or spontaneous. The study involved five sexually mature intact tame females and five sexually matured vasectomized wild males. Females were monitored non-invasively for ovarian cyclicity by measuring urinary progesterone every 2 days over 84days. Females were subjected to three separate step-wise experiments with different treatments as follows: step one was singly housed, which served as the control (C), followed by Non-Physical Contact with a vasectomized male (NPC) and Physical Contact with the same vasectomized male (PC), respectively. The average urinary progesterone concentration was similar in all three treatments, with a urinary progesterone spike indicating ovulation occurring in all treatments, suggesting a spontaneous ovulating strategy. However, upon assessment of the male penis, it was revealed that part of the glans penis had small epidermal spines which somehow resembles to those reported in induced ovulators. The findings from this study on the African giant pouched rat leaves a paradox as profiles of progesterone from females strongly suggest a spontaneous pattern of ovulation although the penile morphology of males have features suggesting induced pattern of ovulation.

Keywords: Cricetomys, Creatinine, Progesterone, Ovulation, Copulation, Penile Epidermal Spines, Tanzania

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Background

For successful reproduction to occur, a fertile oocyte must be available for fertilisation by the sperm cells. These oocytes are released by the rupturing of the mature Graafian follicle during the process called ovulation. A surge of oestrogen by the mature Graafian follicle causes positive feedback on the gonadotrophin-releasing hormone (GnRH) neurons which in results in a surge of GnRH and luteinising hormone (LH), and subsequent rupture of the Graafian follicle and release of the oocyte, results in the formation of the corpus luteum [1]. Progesterone is released by the corpus luteum and aids in the implantation and maintenance of pregnancy, but in conjunction with oestrogen, it also reduces the release of GnRH and LH [1]. In previous studies focusing on ovulation, high progesterone concentrations have been used as indicators for ovulation [2]. Ovulation in mammals may occur by two methods, induced or spontaneous [3,4]. Spontaneous ovulators exhibit a continuous cycle of follicular development and release of an oocyte during each oestrous cycle, even in the absence of male stimuli [3,4]. This would result in the production of a progesterone surge at regular intervals representing the follicular and luteal phase of the cycle in the absence of pregnancy. Induced ovulators differ from spontaneous ovulators by only ovulating in the presence of male stimuli. The male stimulus may be visual, olfactory, tactile, auditory, vaginal, or perineal [5,6]. Often, ovulation occurs in response to a neurogenic stimulus caused by coitus (i.e., penile-vaginal penetration) [5-7]. The outer surface of the penis of induced ovulating mammals often possesses protruding structures (spines or ribbing on the shaft of the penis) [8]. During copulation, these structures cause a tactile stimulation of the vaginal wall and cervix, which is essential for initiating ovulation [9-11]. However, penile spines are not only indicative of induced ovulation, as several spontaneous ovulating species possess such spines [12,13]. In addition, these penile spines can often be used to help to establish a short copulatory lock [13]. Moreover, a correlation between the presence of penile spines and the mating system has been highlighted. Species high on the monogamy scale have relatively smooth penises, whereas species on the lower portion of the scale possess more apparent spines [13-15]. The African giant pouched rat, Cricetomys gambianus is a rodent with a body mass of up to 1.4kg [16]. Cricetomys gambianus is a seasonal breeder having a single breeding event per year tied to the short rainfall of Tanzania [17]. A comprehensive understanding of the ovulatory strategy of this species is needed in order to better understand the reproduction of the African giant pouched rat which is used as a source of meat in West Africa [18]. There is an ongoing debate on the ovulation strategy of the African giant pouched rat, with a study in the Democratic Republic of the Congo suggesting that it is an induced ovulator [19], while a more recent study from Nigeria suggests it is a spontaneous ovulator [20]. This study was undertaken using a well-established protocol for assessing ovulatory patterns. Adult females were either housed singly, or paired with non-related vasectomized males, urine was collected at regular intervals, and urinary progesterone was measured. If the African giant pouched rat is an induced ovulator, it was predicted that females would only show elevated urinary progesterone levels after some form of contact with a male. Whereas if this species is a spontaneous ovulator elevated urinary progesterone levels would be seen in females during all experimental set ups in the protocol including when the female was housed singly. These urinary progesterone spikes would also occur with some form of rhythmicity. In addition, we investigated if males had penile ornamentation in the form of spines. The aim of the study was to determine whether ovulation in the African giant pouched rat (Cricetomys gambianus) is induced or spontaneous by analyzing ovarian cyclicity through urinary progesterone levels and examining male penile morphology.

Methods

Study design

A prospective experimental design study was conducted among five sexually matured tamed females and five sexually matured wild vasectomized male African giant pouched rats were used in this study. Females were obtained from the SUA-APOPO project, while males were trapped around Morogoro municipal: Modeko S06.48044°E037.38035° and Mafiga S06.822412E037.651146°, Tanzania in June 2020. The males were maintained at the Small Animals Research Unit, College of Veterinary Medicine and Biomedical Sciences-Sokoine University of Agriculture, Tanzania, for a week before vasectomy. The animals were housed in separate cages.

Species characterization

Clade and species assignment were determined by (cytochrome oxidase subunit I (COI) gene amplification and nucleotide sequencing of 120 individuals obtained from the wild using previously described primers and thermal cycling conditions [21]. Full-length gene sequences submitted to GenBank under accession numbers (OQ259530) were complemented with homologous data from prior studies [21] and the best-fit model of sequence evolution identified under the AICC in Mega5 [22] was subsequently used for maximum likelihood inferences. Two haplotypes were recovered from the individuals with good PCR nucleotides amplification selected at random for genetic characterization. One of these had a 100% nucleotide sequence identity to GenBank sequences (MH989088.1 and MH988909.1), which correspond to Cricetomys gambianus [21]. The two haplotype sequences generated in this study cluster within the C. gambianus clade defined by Corti et al. in 2005 with 100% bootstrap support (not shown).

Experimental design

The five female African giant pouched rats used in this experiment were placed under three sequential experimental treatments, which differ in the degree of contact with males as follows; initially singly housed, control (C), in wire mesh cages (75cm x 55cm x 45cm). Subsequently in non-physical contact with a vasectomized male (NPC). A wire mesh separated male and female cages during the NPC treatment, allowing only olfactory, auditory or ophthalmic contact. Finally, females were in physical contact with the same vasectomized males (PC). The two cages were connected through an opening which facilitates escaping during courting if needed. Males were placed first for two minutes before females to allow them to mark both cages. This treatment allowed full physical and chemical contact. Each experimental treatment was conducted for 28 consecutive days.

Urine collection

In each treatment, urine was collected from the females every second day. Collection of urine was done early in the morning by putting the females in cages with trays at the bottom. Collected urine was immediately preserved in a freezer at −20◦C.

Creatinine determination

Progesterone concentrations had to be corrected for urine concentration. The correction was accomplished by analyzing each urine sample for creatinine concentration, as creatinine is excreted at a relatively constant rate. The creatinine concentration of each urine sample was determined using a modified Jaffe reaction [23]. Final standardized results are presented as nanograms of progesterone per milligram of creatinine (corrected progesterone – ng/mg creatinine).

Urine progesterone determination

Urine samples were analyzed for progesterone using a coat-account kit for progestogen determination (Diagnostic Products Corporation, Los Angeles, California, USA). The antiserum is highly specific for progesterone, with cross-reactivity to all naturally occurring steroids of <0.5%, except 17α-dihydroprogesterone (3.4%), 11-eoxycorticosterone (2.4%), 5β-pregnan-3,20-dione (3.2%), and 5α-pregnan-3,20-dione (9%). Standard concentrations ranged from 0.3 to 127.2 nmol/L. Steroids were neither purified nor separated by chromatography. A serial dilution of a high progesterone sample paralleled the standard curve (ANCOVA: $F(1,5) = 4.2$, $p > 0.05$). The intra- and inter-assay coefficient of variations were 7.8% and 12.0%, respectively.

Male vasectomy

The five males were vasectomised two months prior to the beginning of the experiment. The procedure involved surgical removal of part of the vas deferens from each testis to block the spermatozoa's passage, hence preventing pregnancy. Vasectomy was performed under general anaesthesia using xylazine 5mg/kg and ketamine 50mg/kg. Postoperative care was done using oxytetracycline wound spray once every two days for a week.

Assessment of penis morphology

Three males were sacrificed after the PC experimental phase by using an overdose of chloroform inhalation. Penises were dissected out and fixed in a 2.5% glutaraldehyde to formaldehyde solution. The samples were then exported to the University of Pretoria microscopy unit for scanning electron microscopy. The samples were rinsed in 1.5 mol/L phosphate buffer, dehydrated through a graded ethanol series, and dried in hexamethyldisilizane. The samples were then mounted onto aluminium stubs, sputter-coated with carbon (Polaron E5200C Carbon Coater; Quorum Technologies, Watford, UK) and examined with a field emission scanning electron microscope (Zeiss Ultra PLUS FEG SEM) operated at 5 kV.

Statistical analysis

The normality of corrected progesterone concentrations was determined using Shapiro–Wilk's tests. Log-transformation failed to obtain a normal distribution. A generalized linear mixed model (GLMM) fitted with a gamma distribution with a log-link function was conducted to compare corrected progesterone concentration between treatments for all five individuals. Individual was included as a random factor for all GLMMs. Post hoc comparisons were made following all models using least significant difference (LSD) pairwise comparisons. All statistical analyses were performed in R 3.5.2 and significance was assumed at $p \leq 0.05$. To determine the lengths of individual cycles and the presumed time of ovulation, we used a defined rise of urinary progesterone levels above a threshold to indicate the onset of the luteal (postovulatory) phase. For this, we evaluated individual baseline levels of urinary progesterone levels using an iterative process [24], where all values >the mean + 1.25 standard deviation (SD) of an individual's data set were removed, the average was then recalculated, and the procedure was repeated until no values exceeded the baseline level. Oestrous cycle length was calculated as the interval between the two consecutive luteal phases. The follicular phase was defined as the period in which the progestogen levels were below the threshold indicating ovulation.

Results

Urinary progesterone

Corrected urinary progesterone concentrations were not significantly different between the three treatments ($F_{[2,215]} = -1.72$, $p = 0.09$, Supplementary fig. 1 and fig 2). Overall mean cycle length, derived from the cycle lengths of the five females, is six days, with a mean length of the follicular phase of two days and a mean length for the luteal phase being four days (Table 1).

Penile morphology

Small epidemal spines were observed on the outer surface of the glans penis especially close to the external urethral openings (n= 3). These sharp structures are evenly distributed especially on the ventrolateral part of the glans penis (Supplementary fig. 3a and b). The spines on the penis of the the African giant pouched rat are small and sharp at the apex (Supplementary fig. 3b). The other parts of the glans close to the collum penis and the whole shaft were smooth (Supplementary Fig 3c and d).

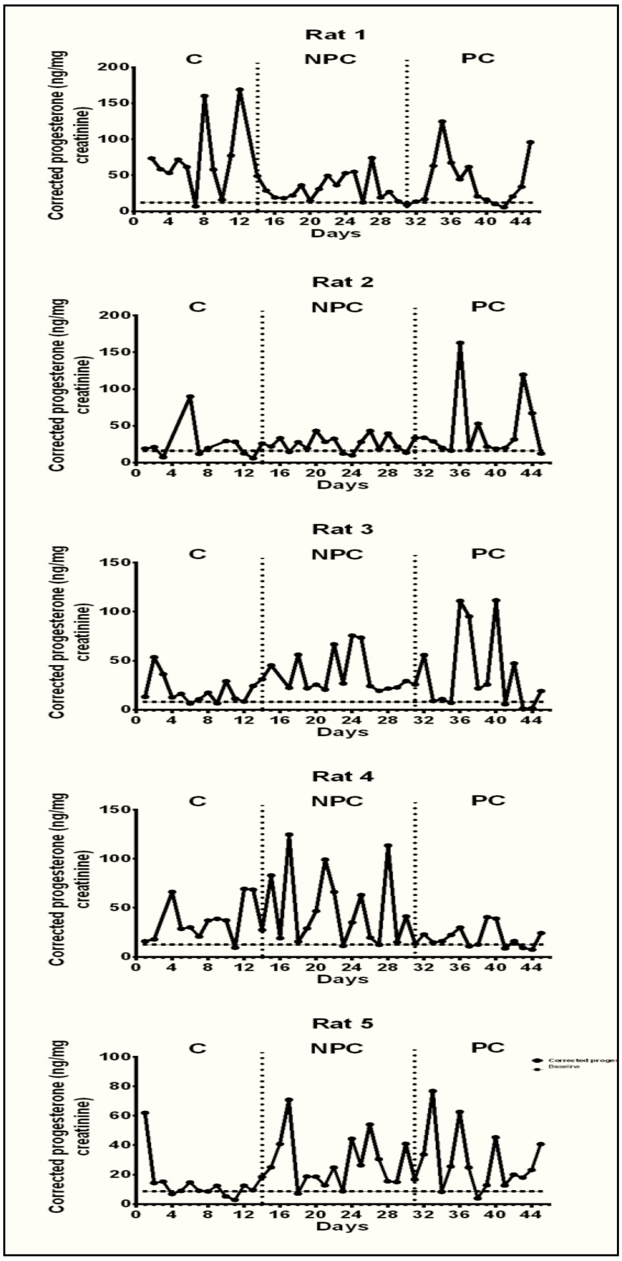


Figure 1: Urinary progesterone concentrations in nanogram per milligram of creatinine (ng/mg creatinine) of five females African giant pouched rats Cricetomys gambianus subjected to all three treatments: control (C), non-physical contact with a male (NPC), and physical contact with a male (PC). Horizontal dotted line indicates progesterone baseline.

Discussion

The findings from the progesterone profiles in this study strongly suggest that the African giant pouched rat is a spontaneous ovulator. Urinary progesterone levels were similar regardless of the presence or absence of a male stimulus. However, it should be noted that under our experimental conditions, it is likely that the analysed females were not at the same stage of the oestrous cycle, and therefore, the observation of urinary progesterone spikes occurred at random. This finding is in line with Akinloye and Oke [20] who worked on a population of C. gambianus from Nigeria, but in contrast to Malekani et al. [19] who worked on a population from the Democratic Republic of Congo. Yet, the mean cycle length proposed by this study (~6 days) was almost similar to that reported by Malekani et al. [19] (7.9days).

Table 1: Ovarian cycle length in five female African giant pouched rats.

Rat Cycle	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5
Cycle length (mean)	4-12 (8)	2-6 (4)	2-21 (12)	2-9 (6)	2-12 (6)
Follicular phase (mean)	1-2 (2)	1-2 (2)	1-2 (2)	1-2 (2)	1-2 (2)
Luteal phase (mean)	3-10 (6)	1-4 (2)	1-19 (5)	1-7 (3)	1-10 (4)

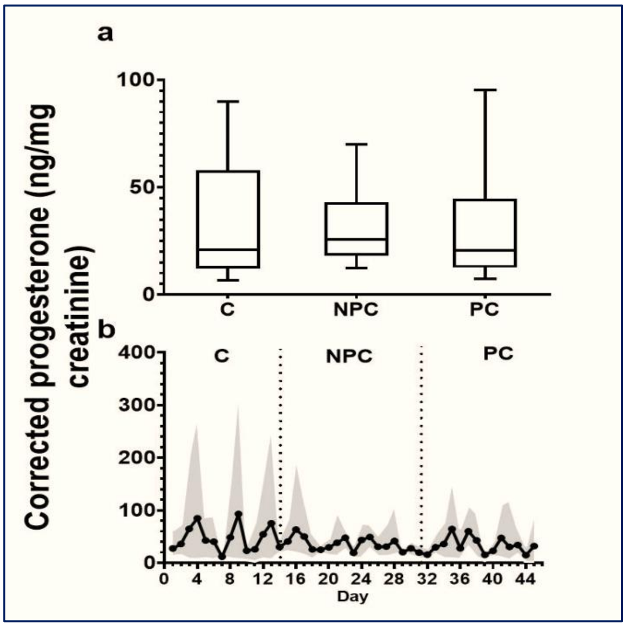
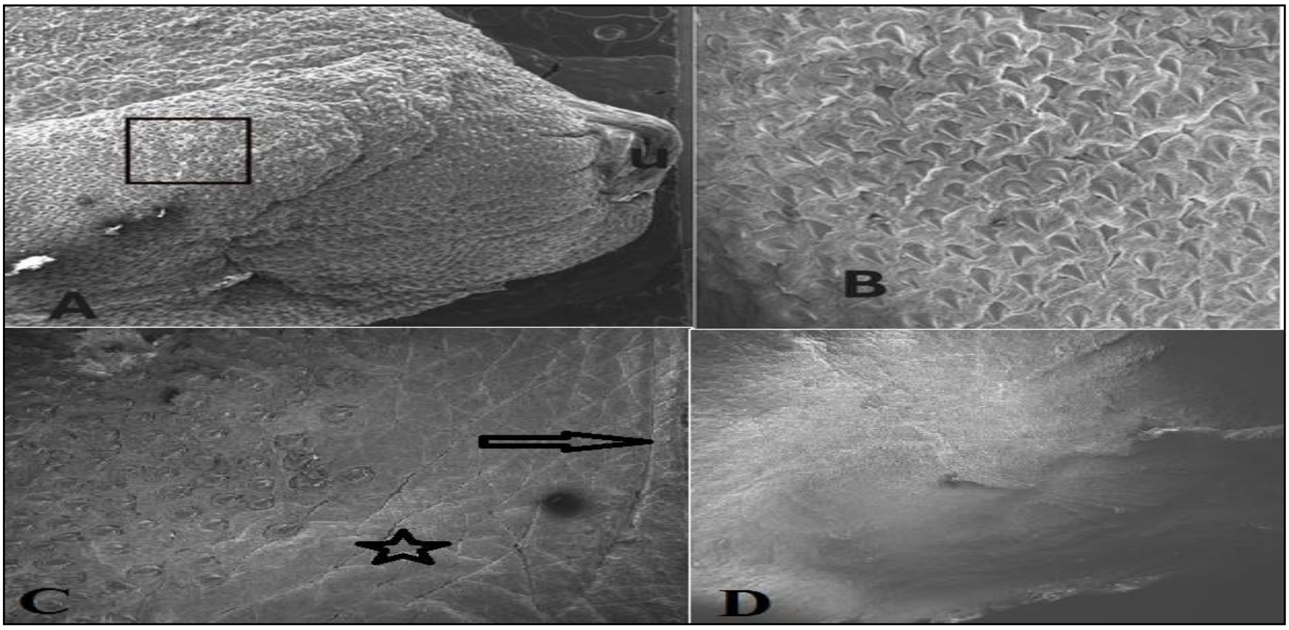


Figure 2: Average progesterone concentrations (ng/mg creatinine) for the five African giant pouched rats subjected to all three treatments: control (C), non-physical contact with a male (NPC), and physical contact with a male (PC). Horizontal dotted line indicates progesterone baseline.

African giant pouched rats were concluded as induced ovulators [19] due to the frequency and irregularity of oestrus observed and thus suggested that domestication caused extreme variation in the period of oestrus and the subsequent oestrous cycle duration, indicating that ovulation was induced [19]. Yet, in Malekani et al. [17] [19] own study, the authors observed that in sexually mature females not paired with a male, oestrus appeared 12 times, hence concluded that mating did not significantly affect oestrus frequency. Therefore, combining the findings of Malekani et al. [19], Akinloye and Oke [20] and this study suggest that the African giant pouched rat is a spontaneous ovulator. Interestingly the male African giant pouched rat has a penile morphology that somehow resembles that of an induced ovulator, namely small penile spines at the distal part of the glans penis. However the penile spines in the African giant pouched rat are only localised at a small part of the glans penis unlike in many induced ovulators whereby the spines cover the glans penis and in some species extend beyound the collum penis to cover part of the shaft [8]. Similar findings have been found in the Spiny mouse, Acomys spinosissimus; where females ovulate spontaneously, but males possess small penile [12]. Unlike the African giant pouched rat, the small penile spines found in A. spinosissimus aid in the female's stimulation and helped increase the chance of ovulation (shown by slight increases in progesterone in the PC treatment). This indicates that A. spinosissimus expresses some of the characteristics of an induced ovulator [12]. Also suggested that due to the monogamous breeding strategy likely employed by A. spinosissimus, the penile spines would be helpful in short copulatory lock with their mate. Furthermore de Bruin in [12] suggested the size of the penile spines would not be as pronounced as in species that are more promiscuous due to their monogamous breeding stragergy. This may be the case in African giant pouched rat which is has been suggested to be monogamous, but for which there is no definitive study [16]. A spontaneous breeding strategy is often beneficial in species that are characterised as being monogamous breeders, since access to good quality males is relaxed, and male competition is low [25]. In Malekani's study [17] it was suggested that rainfall does not affect ovulation and thus reproduction in the African giant pouched rat, is aseasonal. However, field based studies show evidence that the African giant pouched rat is a seasonal breeder, with breeding closely linked to rainfall [17,26]. The findings from this study on the African giant pouched rat leaves us with a paradox as profiles of progesterone from females imply a spontaneous pattern of ovulation, whereas the penile morphology of males have features similar to the induced ovulation species. The results from the female progesterone concentrations therefore suggest that females are spontaneous ovulators and that the penile surface morphology in males may be necessary for lock during copulation as has been reported in other spontaneous ovulators [12,13]. Recent reports show that the trained African giant pouched rats have helped to detect explosives in post-conflict countries including Cambodia, Angola and Mozambique where the rats have helped to clear over 23 million land mines [27] and also to detect tuberculosis in human sputum sample [28] and thus serve as addictive diagnostics in high TB-burden countries such as Tanzania, Mozambique and Ethiopia [27,28]. This study therefore has contributed to our understanding of the reproductive biology of the African giant pouched rat that can be utilized for the purpose of improving breeding in captivity as well as designing the strategy to maintain their population in the wild.

Conclusion

The findings of this study suggest that the African giant pouched rat is a spontaneous ovulator, as urinary progesterone concentrations showed no significant variation across treatments, regardless of male stimuli. The observed small penile spines on the males resemble features of induced ovulators but appear to serve a different function, possibly facilitating copulatory lock rather than inducing ovulation. The mean ovarian cycle length (~6 days) and progesterone patterns align with findings from previous studies in other populations of this species, further supporting spontaneous ovulation. These findings contribute to the understanding of the reproductive biology of African giant pouched rats, which may be valuable for enhancing captive breeding programs and conservation efforts. Additionally, the study underscores the importance of this species in biomedical research and humanitarian applications, including landmine detection and tuberculosis diagnosis, highlighting its broader significance beyond reproductive biology.



Supplementary figure.3: Scanning electron micrographs of the penis of the African giant pouched rat. A: A ventrolateral view showing the surface near to the urethral opening (u). B: Magnified part of A to show small spines. C: Glans penis showing part with spines and part without spines (asterisk), note the collum penis groove which separate glans penis and shaft (arrow). D: Smooth shaft (spineless).

Abbreviation

C: Control; NPC: Non-Physical Contact; PC: Physical contact; GnRH: Gonadotropin releasing hormone; LH: Luteinizing hormone; SUA: Sokoine University of Agriculture; APOPO: Anti-Persoonsmijnen Ontmijnende Product Ontwikkeling (Anti-Personnel Landmines Detection Product Development); COI: Cytochrome oxidase subunit one; USA: United states; ANCOVA: Analysis of covariance; UK: United Kingdom; GLMM: Generalized linear mixed model; LSD: Least significant difference; SD: Standard deviation

Declaration

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Availability of data and materials

Data will be available by emailing mungokisinza@sua.ac.tz

Authors' contributions

Mungo Kisinza Ngalameno (MKN) is the only author contributed to all this work including conceptualization, data curation, data analysis, writing of the original manuscript draft and revision. The author read and approved the final manuscript.

Ethics approval and consent to participate

We conducted the research following the declaration of Helsinki. Research and sample export permits were granted by the Ministry of Natural Resources and Tourism in Tanzania through Tanzania Wildlife Research Institute (TAWIRI) and Tanzania Commission for Science and Technology (COSTECH), permit number 2019-46-NA-2019-41. The project was approved by the Animal Ethics Committee of the University of Pretoria (NAS291/2021) and section 20 import permits from The Department of Forestry and Fisheries 12/11/1/1/8 (1816JD and veterinary import permits 2020/7/001725 and 202102004998 South Africa.

Consent for publication

Not applicable

Competing interest

The authors declare that they have no competing interests.

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