Zygote

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New insights in *Cercopithecinae* spermatozoa

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Summary

Sperm morphometric and morphologic data have been shown to represent useful tools for monitoring fertility, improving assisted reproduction techniques and conservation of genetic material as well as detecting inbreeding of endangered primates. We provide here for the first time sperm morphologic and morphometric data from *Cercopithecus neglectus*, *Cercopithecus cephus*, *Papio papio* and critically endangered *Cercopithecus roloway*, as well as comparative data from other *Cercopithecinae* species, i.e. *Allochrocebus lhoesti*, *Mandrillus sphinx* and *Papio anubis*. Following collection from the epididymis, spermatozoa were measured for each species for the following parameters: head length, head width, head perimeter, head area, midpiece length and total flagellum length, and the head volume, ellipticity, elongation, roughness and regularity were then calculated. Our data are consistent with both the general morphology and the morphometric proportions of *Cercopithecinae* sperm. Some specificities were observed, with *C. cephus* displaying a narrow head (width = 2.76 ± 0.26 µM) and *C. roloway* displaying a short midpiece (6.65 ± 0.61 µM). This data set represents an important contribution, especially for *Cercopithecus roloway*, one of the most endangered monkeys in the world, and further data on additional specimens coupled to data on mating systems and reproductive ecology should allow a better understanding of the mechanisms underlying these morphological differences across primate species.

Introduction

Primates, like other mammals, are subject to selection pressures (e.g. sperm competition, cryptic post-copulatory female choice, sexual coercion, climatological factors; see review in Martinez and Garcia, 2020) resulting in inter-specific and intra-specific variability in sperm shapes. Analyzing this variability with morphological sperm measurements is becoming increasingly relevant, especially in the context of animal health and fertility monitoring (García-Vázquez *et al*., 2016; Oliveira *et al*., 2016; Piña-Aguilar *et al*., 2016). Indeed, the worldwide decline of many primate populations (Estrada *et al*., 2017) could lead to an increase in the use of medically assisted reproduction techniques (ART) to conserve the genetic material, detect inbreeding and potentially address primate fertility issues (Andrabi and Maxwell, 2007) in the framework of *ex situ* conservation. For instance, evaluation of sperm morphology makes it possible to determine an individual sperm quality when standards exist, as morphology is closely linked to successful fertilization (García-Vázquez *et al*., 2016). Although most studies on primate semen parameters are primarily conducted on laboratory model species (de Villiers, 2018), there is a growing collection of published literature for endangered and critically endangered species (Martin *et al*., 1975; Thomson *et al*., 1992; Robson *et al*., 1997; Steinberg *et al*., 2009; Nakazato *et al*., 2015; Sampaio *et al*., 2017; Leão *et al*., 2020).

Here we provide, for the first time, sperm morphometric and morphologic data from De Brazza's monkey (*Cercopithecus neglectus*), moustached monkey (*Cercopithecus cephus*), Guinea baboon (*Papio papio*), and the critically endangered Roloway monkey (*Cercopithecus roloway*), as well as supplementary data for L'Hoest's monkey (*Allochrocebus lhoesti*), mandrill (*Mandrillus sphinx*) and olive baboon (*Papio anubis*). These data are highly valuable, especially for the Roloway monkey, which is listed as one of the 25 most endangered primate species in the world (Koné *et al*., 2019).

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Materials and methods

Animals

All samples were obtained from French zoos, EAZA (European Association of Zoos and Aquaria) members, assuring the best veterinary care practices for the animals. Testes from *A. lhoesti, C. roloway*, *C. cephus* and *C. neglectus* were collected from adult males of proven fertility (registered offspring) who died from accident or disease not affecting spermatogenesis. Testes from *M. sphinx*, *P. anubis* and *P. papio* were collected from sexually mature males at the time of castration. Testicles were sent to the laboratory for sperm cryoconservation and creation of a primate sperm biobank. Morphological analyses were carried out as part of the sperm evaluation before freezing.

Sperm collection and preparation

Testis were kept at 4°C and processed within 48 h after castration or death discovery. Sperm extraction was carried out by dissecting the tail of the epididymis using needles in a Petri dish with 2 ml Sperm Preparation Medium (SPM, Origio). Spermatozoa were allowed to swim for 10 min at 37°C, washed twice in SPM (by centrifugation at 500 *g*, 5 min), displayed over a slide, dried at room temperature, and then fixed in ethanol 70° for Harris–Schorr staining. Simple and easy to perform, this staining gives good results in routine human sperm evaluation (World Health Organization, 2010).

Sperm morphometric analysis

All cells measurements were realized on a Nikon Eclipse 80i microscope equipped with a Nikon DS-Ri1 camera with NIS-Elements D (version 3.1.) software. At least 100 cells were measured for each individual. Measured parameters were head length (L), head width (W), head perimeter (P), head area (A), midpiece length (MP) and total flagellum length (Flag). Calculated parameters were head volume ($V = 4/3\pi(L/2)^2(W/2)$), head ellipticity ($\xi = L/W$), head elongation ($\Sigma = (L - W)/(L + W)$), head roughness ($Ro = 4\pi(A/P^2)$) and head regularity ($Re = \pi((L*W)/(4*A))$. Given the small sample size within each species (only one individual), we were unable to statistically investigate differences in morphometric measurements between species.

Sperm morphologic analysis

Morphology was assessed by a trained biologist (GM) with a counting of at least 400 spermatozoa per slide using a magnification of ×1000. Analyses were performed according to World Health Organization (WHO) recommendations for human sperm analysis based on a modified Kruger classification (Cooper *et al.*, 2010).

Results

Morphometry

Our morphometric data are presented in Table 1 and alongside the literature in Table 2 with means (±SD) calculated for each genus. All individuals displayed an oval-shaped sperm head, with *C. cephus* displaying the most elongated and narrowest head within all known spermatozoa species (head width 2.76 ± 0.26, ellipticity 1.98 and elongation 0.32). Regarding total sperm length, *P. papio*, *P. anubis*, *M. sphinx* and *A. lhoesti* were measured at around 70 µM whereas *C. neglectus*, *C. cephus* and *C. roloway* lengths were around

Table 1. Sperm morphometric data in our sample of seven *Cercopithecinae* species

	Head								Flagellum	
Species	Length (µM)	Width (µM)	Perimeter (µM)	Area (µM²)	Volume (µM³)	Ellipticity (L/W)	Elongation (L−W)/(L+W)	Roughness (4π(A/P²))	Regularity (π(LW/4A))	Midpiece length (µM)	Total length (µM)	Total length (µM)
A. lhoesti	5.28 ± 0.16	3.59 ± 0.15	14.46 ± 0.42	14.87 ± 0.70	52.3	1.48	0.19	0.89	1.00	8.47 ± 0.78	66.22 ± 1.50	71.50 ± 1.51
C. neglectus	5.21 ± 0.17	3.65 ± 0.15	14.78 ± 0.46	16.21 ± 0.98	51.8	1.43	0.18	0.93	0.92	10.32 ± 0.57	37.35 ± 5.38	42.56 ± 5.43
C. cephus	5.39 ± 0.40	2.76 ± 0.26	13.51 ± 0.78	11.85 ± 1.19	42.0	1.98	0.32	0.82	0.99	11.3 ± 1.05	44.59 ± 2.49	49.98 ± 2.54
C. roloway	4.77 ± 0.20	3.17 ± 0.17	13.10 ± 0.55	11.57 ± 0.91	37.8	1.51	0.20	0.85	1.03	6.65 ± 0.61	47.66 ± 1.86	52.44 ± 1.89
M. sphinx	5.11 ± 0.15	3.68 ± 0.17	14.07 ± 0.60	15.62 ± 0.88	50.3	1.39	0.16	1.00	0.95	9.09 ± 0.51	65.23 ± 1.15	70.34 ± 1.17
P. Anubis	5.23 ± 0.17	3.33 ± 0.12	14.09 ± 0.32	14.48 ± 0.75	47.7	1.57	0.22	0.92	0.95	10.43 ± 0.21	69.11 ± 1.85	74.35 ± 1.93
P. papio	5.27 ± 0.15	3.49 ± 0.11	14.30 ± 0.37	14.90 ± 0.72	50.7	1.51	0.20	0.92	0.97	10.76 ± 0.89	69.32 ± 1.83	74.59 ± 1.87

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Table 2. Sperm morphometric data of *Cercopithecinae*

	Head									Flagellum					
Species	Length (μM)	Width (μM)	Perimeter (μM)	Area (μM^2)	Volume (μM^3)	Ellipticity (L/W)	Elongation (L−W)/(L+W)	Roughness ($4\pi(A/P^2)$)	Regularity ($\pi(LW/4A)$)	Midpiece length (μM)	Principal piece length (μM)	Terminal piece (μM)	Flagellum length (μM)	Total length (μM)	References
Allenopithecus genus															
A. nigroviridis	5.6	3.3	–	–	54.9	1.69	0.26	–	–	9.32	–	–	57.54	66.49	20
Allochrocebus genus															
A. lhoesti	4.64	3.04	14.46	14.87	52.3	1.52	0.21	0.89	0.75	8.73	–	–	53.61	62.75	20,21
Cercocebus genus															
C. galeritus	6.8	–	–	–	–	–	–	–	–	13.30	–	–	73.00	93.10	12
Cercopithecus genus															
C. cephus	5.39	2.76	13.51	11.85	42.0	1.98	0.32	0.82	0.99	11.35	–	–	44.59	49.98	21
C. neglectus	5.21	3.65	14.78	16.21	51.8	1.43	0.18	0.93	0.92	10.32	–	–	37.35	42.56	21
C. nictitans	5.3	–	–	–	46.2	–	–	–	–	12.30	–	–	40.50	–	16
C. petaurista	5.7	3.0	–	–	41.4	1.67	0.25	–	–	9.60	–	–	38.95	73.00	16,20
C. roloway	4.77	3.17	13.10	11.57	37.8	1.51	0.20	0.85	1.03	6.65	–	–	47.66	52.44	21
C. wolfi	5.0	4.0	–	–	52.4	1.25	0.11	–	–	–	–	–	68.00	73.00	20
Chlorocebus genus															
C. aethiops	5.8	3.3	14.0	13.3	44.0	1.60	0.23	0.86	1.13	11.40	52.26	2.87	65.10	77.32	2,4,7,9,16,17
Erythrocebus genus															
E. patas	7.7	–	–	–	63.2	–	–	–	–	13.55	–	–	69.90	89.50	12,16
Lophocebus genus															
L. albigena	5.8	–	–	–	37.2	–	–	–	–	11.10	–	–	17.30	–	16
L. aterrimus	5.2	3.8	15.2	16.6	53.4	1.36	0.15	0.90	0.93	8.51	4.54	–	–	–	19
Macaca genus															
M. arctoides	5.9	3.4	–	–	52.5	1.64	0.24			10.52	61.46	–	64.10	77.61	4,7,11,16
M. fascicularis	5.7	3.7	15.0	16.7	58.7	1.54	0.21	0.93	0.99	11.15	57.04	–	58.60	77.97	4,7,9,11,13,14,16
M. mulatta	5.3	3.3	15.9	17.1	51.7	1.71	0.26	0.85	0.80	11.19	57.88	2.97	62.58	75.86	1,4,7,10,11,16,17
M. nemestrina	7.4	–	–	–	48.7	–	–	–	–	13.50	–	–	69.25	90.40	12,16
M. radiata	5.5	–	–	–	–	–	–	–	–	10.50	50.00	–	75.00	–	3

(*Continued*)

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Table 2. (*Continued*)

	Head									Flagellum					
Species	Length (μM)	Width (μM)	Perimeter (μM)	Area (μM^2)	Volume (μM^3)	Ellipticity (L/W)	Elongation (L−W)/(L+W)	Roughness ($4\pi(A/P^2)$)	Regularity ($\pi(LW/4A)$)	Midpiece length (μM)	Principal piece length (μM)	Terminal piece (μM)	Flagellum length (μM)	Total length (μM)	References
M. silenus	5.7	3.2	–	–	49.7	1.50	0.20	–	–	10.83	–	–	29.93	60.04	16,20
M. sinica	–	–	–	–	–	–	–	–	–	–	–	–	–	69.10	9
M. sylvanus	5.5	–	–	–	49.5	–	–	–	–	12.80	–	–	62.10	–	16
M. thibetana	6.0	3.5	–	–	64.9	1.72	0.26	–	–	11.98	–	–	62.01	79.96	11
Mandrillus genus															
M. leucophaeus	4.5	3.0	–	–	31.3	1.33	0.14	–	–	12.10	–	–	36.30	70.00	16,20
M. sphinx	5.86	4.02	14.07	15.62	44.85	1.46	0.19	0.99	1.18	11.12	–	–	54.37	69.77	6,7,9,15,20, 21
Papio genus															
P. anubis	5.6	3.29	14.09	14.48	50.0	1.71	0.26	0.92	1.00	8.91	–	–	67.8	75.06	12,15,16,18, 21
P. cynocephalus	4.7	3.9	–	–	47.7	1.23	0.10	–	–	10.02	58.03	3.50	64.07	75.40	4,5,6,7,8,16
P. hamadryas	6.5	–	–	–	47.2	–	–	–	–	12.70	–	–	58.00	–	16
P. papio	5.27	3.49	14.30	14.90	50.7	1.51	0.20	0.92	0.97	10.76	–	–	69.32	74.59	21
P. ursinus	5.6	3.5	15.4	16.3	56.3	1.60	0.23	0.86	0.94	9.97	58.02	3.37	70.92	76.49	17
Theropithecus genus															
T. gelada	6.8	–	–	–	61.9	–	–	–	–	12.20	–	–	68.80	87.30	12,16

All measurements are presented uncritically and as mean when multiple sources.
References: [1]Mastroianni and Manson, 1963; [2]Bedford, 1967; [3]Flećhon and Hafez, 1975; [4]Martin *et al.*, 1975; [5]Flećhon *et al.*, 1976; [6]Gould and Martin, 1978; [7]Gould, 1980; [8]Carrick and Hughes, 1982; [9]Cummins and Woodall, 1985; [10]Bedford and Hoskins, 1990;[11]Shang *et al.*, 1993; [12]Gage, 1998; [13]Gago *et al.*, 1998; [14]Gago *et al.*, 1999; [15]Cseh *et al.*, 2000; [16]Anderson *et al.*, 2005; [17]2011 Maree, 2011; [18]Nyachieo *et al.*, 2012; [19]Gadea *et al.*, 2019; [20]San Diego Zoo, 2019; [21]This study.

Table 3. Sperm morphologic data

Species	*A. lhoesti*	*C. neglectus*	*C. cephus*	*C. roloway*	*M. sphinx*	*P. anubis*	*P. papio*
n individuals	1	1	1	1	2	3	2
n ejaculate	1	1	1	1	2	3	2
Abnormal base (%)	9.00	5.26	9.91	11.00	2.78	9.00	4.00
Abnormal or absent acrosome (%)	8.00	2.63	14.41	12.00	3.70	4.00	5.00
Macro head (%)	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Micro head (%)	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Tapered (%)	7.00	0.00	0.90	0.00	0.00	0.00	0.00
Elongated (%)	2.00	0.00	0.00	0.00	0.00	0.00	0.00
Multiple head (%)	0.00	0.00	0.90	0.00	0.00	0.00	0.00
Total head defects (%)	26.00	7.89	26.13	23.00	6.48	13.00	9.00
Midpiece defects (%)	0.00	1.75	0.00	0.00	1.85	1.00	0.00
Simple bent tail (%)	0.00	1.75	0.00	0.00	1.85	4.00	2.00
Coiled tail (%)	5.00	2.63	6.31	7.00	10.19	9.00	8.00
Multiple tail (%)	2.00	0.00	0.00	2.00	1.85	2.00	1.00
Short/hairpin tail (%)	0.00	1.75	1.80	4.00	4.63	1.00	3.00
Irregular calibre (%)	0.00	2.63	0.00	1.00	0.00	2.00	0.00
Destructured (%)	0.00	3.51	1.80	1.00	0.93	0.00	3.00
Total flagellum defects (%)	7.00	12.28	9.91	15.00	19.44	18.00	17.00
Cytoplasmic droplet (%)	0.00	0.00	0.00	0.00	0.00	1.00	0.00
Morphology (% normal)	67.00	78.07	63.96	62.00	72.22	67.00	74.00

50 µM (see Table 2 for further details). Moreover, *C. roloway* spermatozoa were characterized by a notable morphological particularity, i.e. the shortest intermediate piece among *Cercopithecinae* (6.65 ± 0.61 µM).

Morphology

Our morphologic data are presented in Table 3 and alongside the literature in Table 4. The proportion of morphologically normal spermatozoa in the ejaculate is homogeneous for the seven species examined (mean ± SD of 69.18 ± 5.77%, range 62.00–78.07%) and across the literature (72.19 ± 12.17%, range 50–96.95%). Conversely, the localization of the morphological abnormalities is heterogeneous from one species to another and mainly concentrated on the tail (15.70 ± 6.52%, range 7–29.32%) or head (10.53 ± 9.83%, range 0.4–26.13%) but few on the midpiece (1.78 ± 2.80%, range 0–8%). At the species level, the anomalies are mostly present on the tail, with the exception of *A. lhoesti*, *C. cephus* and *C. roloway*, for which mostly present were head anomalies (26.00, 26.13 and 23.00% respectively). The presence of cytoplasmic droplets should be noted for *P. anubis* and *C. neglectus* but in very low proportion (2.00 and 0.08%, respectively).

Discussion

This study contributes to increase our knowledge of primate sperm morphometry and morphology by providing original data for four primate species scarcely studied and supplementary data for three other species. Our data are consistent with the general morphometric and morphologic proportions reported in the literature for primates (Tables 2 and 4).

All the species measured here had an oval-shaped sperm head, like most primates, the only exceptions being *Lorisidae* and *Lemuroidea* taxa (Gould and Martin, 1978). It should be noted that *C. cephus* displays a specific head pattern with a narrow head. Some species of the *Cercopithecus* genus tend to have shorter spermatozoa with an average length of 58.20 ± 13.99 µM (*Macaca*: 75.85 ± 9.41; *Papio:* 69.89 ± 0.33), with *C. neglectus*, *C. cephus* and *C. roloway* being the shortest representatives of all *Cercopithecinae*. Yet, due to the small sample sizes inherent in such studies, we report only suggestive trends that may illuminate further studies with more individuals in order to determine if our current findings can be generalized to these different species. Therefore, these morphometric differences between species deserve more attention and future studies combining sperm morphometrics on additional specimens with data on mating systems and reproductive ecology are needed.

This study brings to 48 the number of primate species for which we have sperm morphological data. Our normal-form sperm rates (mean 69.18%, range 62.00–78.07%) are consistent with those observed in the literature for *Cercopithecinae* (mean 73.44%, range 49.50–96.95%). Although preliminary, these data could help to assess the reproductive potential of individuals, as an abnormal decrease in the number of normal-form sperm is often associated with infertility (Gould *et al.*, 1993; Piña-Aguilar *et al.*, 2016) when it occurs during a reproductive period (Seier *et al.*, 1993; Hernández-López *et al.*, 2002).

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Table 4. Sperm morphologic data of *Cercopithecinae*

Species	IUCN red list status	Sperm collection method	n		Normal morphology (%)	% defects				Reference
			Individuals	Samples		Head	Midpiece	Tail	Cytoplasmic droplet	
Allochrocebus genus										
A. lhoesti	Vulnerable	EE	1	1	67	26	0	7	0	This study
Cercocebus genus										
C. galeritus	Endangered	ES	4	26	83.8	4.67	0	10.54	0	Kyaligonza, 1998
Cercopithecus genus										
C. neglectus	Least concern	ES	3	17	71	10.1	1.36	16.26	0.16	Kyaligonza, 1998
C. neglectus	Least concern	EE	1	1	78.07	7.89	1.75	12.28	0	This study
C. cephus	Least concern	EE	1	1	63.96	26.13	0	9.91	0	This study
C. roloway	Critically endangered	EE	1	1	62	23	0	15	0	This study
Chlorocebus genus										
C. aethiops	Least concern	ES	2	6	67	–	–	–	–	Ackerman *et al.*, 1968
C. aethiops	Least concern	ES	20	20	61.04	1.64	8	29.32	–	Mdhluli *et al.*, 2004
Erythrocebus genus										
E. patas	Least concern	ES	5	19	59.5	–	–	–	–	Ackerman *et al.*, 1968
Macaca genus										
M. arctoides	Vulnerable	ES	5	10	70	–	–	–	–	Ackerman *et al.*, 1968
M. fascicularis	Least concern	ES	2	7	67	–	–	–	–	Ackerman *et al.*, 1968
M. fascicularis	Least concern	–	14	–	82.5	0.4	6.3	12.1	–	Gago *et al.*, 1999
M. fuscata	Least concern	M	32	48	94.4	–	–	–	–	Thomsen *et al.*, 2014
M. mulatta	Least concern	ES	7	23	63.5	–	–	–	–	Ackerman *et al.*, 1968
M. mulatta	Least concern	ES	11	–	58.37	–	–	–	–	Wickings and Nieschlag, 1980
M. nemestrina	Vulnerable	ES	4	22	70.57	–	–	–	–	Zainuddin *et al.*, 2019
M. nigra	Critically endangered	ES	4	40	96.95	1.25	0	14	–	Thomson *et al.*, 1992
M. radiata	Least concern	ES	7	121	87.2	–	–	–	–	Kholkute *et al.*, 2000
M. silenus	Endangered	ES	4	12	82	–	–	–	–	Cranfield *et al.*, 1988
M. silenus	Endangered	ES	7	7	18	–	–	–	–	Schaffer *et al.*, 1989
Mandrillus genus										
M. sphinx	Vulnerable	EE	3	3	72.22	6.48	1.85	19.44	0	This study

Table 4. (*Continued*)

Papio genus											
P. anubis	Least concern	ES	1	1	53.25	8	3.5	33.5	2.5	Chan *et al.*, 1999
P. anubis	Least concern	ES	1	–	49.5	8	0	41	2.5	Cseh *et al.*, 2000
P. anubis	Least concern	ES	9	27	89.22	3.88	3.11	3.77	–	Nyachieo *et al.*, 2012
P. anubis	Least concern	ES	3	3	67	13	1	18	1	This study
P. papio	Least concern	ES	2	2	74	9	0	17	0	This study
Theropithecus genus										
T. gelada	Least concern	ES	1	1	81	–	–	–	–	Ackerman *et al.*, 1968

All measurements are presented uncritically and as mean when multiple sources. EE: epididymal extraction, ES: electrostimulation, M: masturbation.

Interestingly, the distribution of morphological abnormalities appears heterogeneous, with some species mainly presenting head anomalies (*A. lhoesti*, *C. cephus*, *C. roloway*) and others flagellum anomalies (*C. neglectus*, *M. sphinx*, *P. anubis*, *P. papio*) with sometimes strong ratios (3.7 times more head abnormalities than flagellum for *A. lhoesti* and 3 times more flagellum than head abnormalities for *M. sphinx*). The causes of this differential positioning are not known and non-human primate spermocytograms in the literature are rarely detailed, making further analysis impossible without additional data collection.

Sperm morphological abnormalities increasing with the inbreeding degree (Gomendio *et al.*, 2000), the data on sperm parameters presented here can also be of major importance for ART as valuable indicators of inbreeding depression. The increase in matings between genetically related individuals due to shrinking habitats and declining populations in the wild or space and management constrains in captivity can lead to a reduction in genetic diversity. The negative impact of inbreeding on reproductive fitness is also well known and the avoidance of inbreeding is one of the priorities in the conservation and captive breeding of endangered species (Hedrick and Kalinowski, 2000). Therefore, assisted breeding techniques may be useful in the future to ensure assisted selective genetic propagation, avoid inbreeding and thus prevent the decline of the general reproductive ability of the captive species (Pukazhenthi *et al.*, 2005).

In conclusion, all data on primate reproduction are critical in the current context of global population declines, especially for endangered species. Beyond the improvement of our general knowledge on primate sperm, we provide here essential data for potential future implementation of *ex situ* conservation actions and monitoring of animal health and fertility.

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