Host specificity and basic ecology of *Mammomonogamus* (Nematoda, Syngamidae) from lowland gorillas and forest elephants in Central African Republic

BARBORA ČERVENÁ[1]*, PETER VALLO[2,3], BARBORA PAFČO[1], KATEŘINA JIRKŮ[4], MILOSLAV JIRKŮ[4], KLÁRA JUDITA PETRŽELKOVÁ[2,4,5], ANGELIQUE TODD[6], ANDREA K. TURKALO[7] *and* DAVID MODRÝ[1,4,8]

[1] *Department of Pathology and Parasitology, Faculty of Veterinary Medicine, University of Veterinary and Pharmaceutical Sciences Brno, Palackého tř. 1946/1, 61242, Brno, Czech Republic*
[2] *Institute of Vertebrate Biology, Czech Academy of Sciences, Květná 8, 603 65, Brno, Czech Republic*
[3] *Evolutionary Ecology and Conservation Genomics, University of Ulm, Albert Einstein Allee 11, 89069, Ulm, Germany*
[4] *Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, Branišovská 31, 370 05, České Budějovice, Czech Republic*
[5] *Liberec Zoo, Lidové sady 425/1, 460 01 Liberec, Czech Republic*
[6] *WWF, Dzanga-Sangha Protected Areas, BP 1053 Bangui, Central African Republic*
[7] *The Wildlife Conservation Society, BP 1053 Bangui, Central African Republic*
[8] *Central European Institute for Technology (CEITEC), University of Veterinary and Pharmaceutical Sciences, Palackého tř. 1946/1, 612 42 Brno, Czech Republic*

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SUMMARY

Syngamid strongylids of the genus *Mammomonogamus* undoubtedly belong among the least known nematodes with apparent zoonotic potential and the real diversity of the genus remains hard to evaluate without extensive molecular data. Eggs of *Mammomonogamus* sp. are frequently found in feces of African forest elephants (*Loxodonta cyclotis*) and western lowland gorillas (*Gorilla gorilla gorilla*) in Dzanga-Sangha Protected Areas. Using sedimentation-based coproscopic techniques, we found the eggs of *Mammomonogamus* in 19·7% elephant and 54·1% gorilla fecal samples with 8–55 and 1–24 eggs per gram of fecal sediment for elephants and gorillas, respectively. We used a combination of light microscopy, scanning electron microscopy and analysis of cytochrome *c* oxidase subunit I (*cox1*) and a partial sequence of 18S rDNA isolated from single eggs to test the hypothesis of possible *Mammomonogamus* conspecificity in gorillas and elephants. Whereas 18S rDNA sequences were identical in both gorillas and elephants, we distinguished seven different haplotypes within the *cox1*. Two haplotypes were found in both gorillas and elephants suggesting sharing of *Mammomonogamus*. Assignment of the parasite to *M. loxodontis* is proposed. Provided sequences represent the first genomic data on *Mammomonogamus* spp.

Key words: 18S rDNA, *cox1*, Dzanga-Sangha Protected Areas, haplotype, interspecies transmission, *Loxodonta cyclotis*, *Gorilla gorilla gorilla*.

INTRODUCTION

Nematodes of the genus *Mammomonogamus* Ryzhikov, 1948 are parasitic strongyles predominantly occurring in the respiratory tract of mammals, predominantly in tropical and subtropical (locally also temperate) areas of the world. Until the revision by Ryzhikov (1948), *Mammomonogamus* was recognized as a subgroup within the genus *Syngamus* Montagu, 1811. Similarly to the well-known *Syngamus trachea*, a common bird parasite, adults of *Mammomonogamus* are joined in permanent copulation firmly attached to mucosa by the buccal capsule (Graber *et al.* 1971). *Mammomonogamus* species are described from several mammalian groups; however, this list might include several synonymies. Felid carnivores are hosts of *M. dispar* (Diesing, 1857), *M. felis* (Cameron, 1931), *M. ierei* (Buckley, 1934), *M. auris* (Faust and Tang, 1934) and *M. mcgaughei* (Seneviratne, 1954). Artiodactyls host *M. laryngeus* (Railliet, 1899), *M. nasicola* (von Linstow, 1899), *M. hippopotami* (Gedoelst, 1924) and *M. okapiae* (van den Berghe, 1937). Two further species, namely *M. indicus* (Mönnig, 1932) and *M. loxodontis* (Vuylsteke, 1935), are found in Asian elephants and African forest elephants, respectively.

Compared with other species of Strongylida, some *Mammomonogamus* spp. exhibit surprisingly low host specificity. At least two species originally described from ruminants (*M. laryngeus* and *M. nasicola*) are considered as zoonotic (Magdeleine *et al.* 1974; Nosanchuk *et al.* 1995) and *M. laryngeus*

* Corresponding author: Department of Pathology and Parasitology, University of Veterinary and Pharmaceutical Sciences Brno, Palackého tř. 1946/1, 612 42 Brno, Czech Republic. E-mail: bara.cervena@gmail.com

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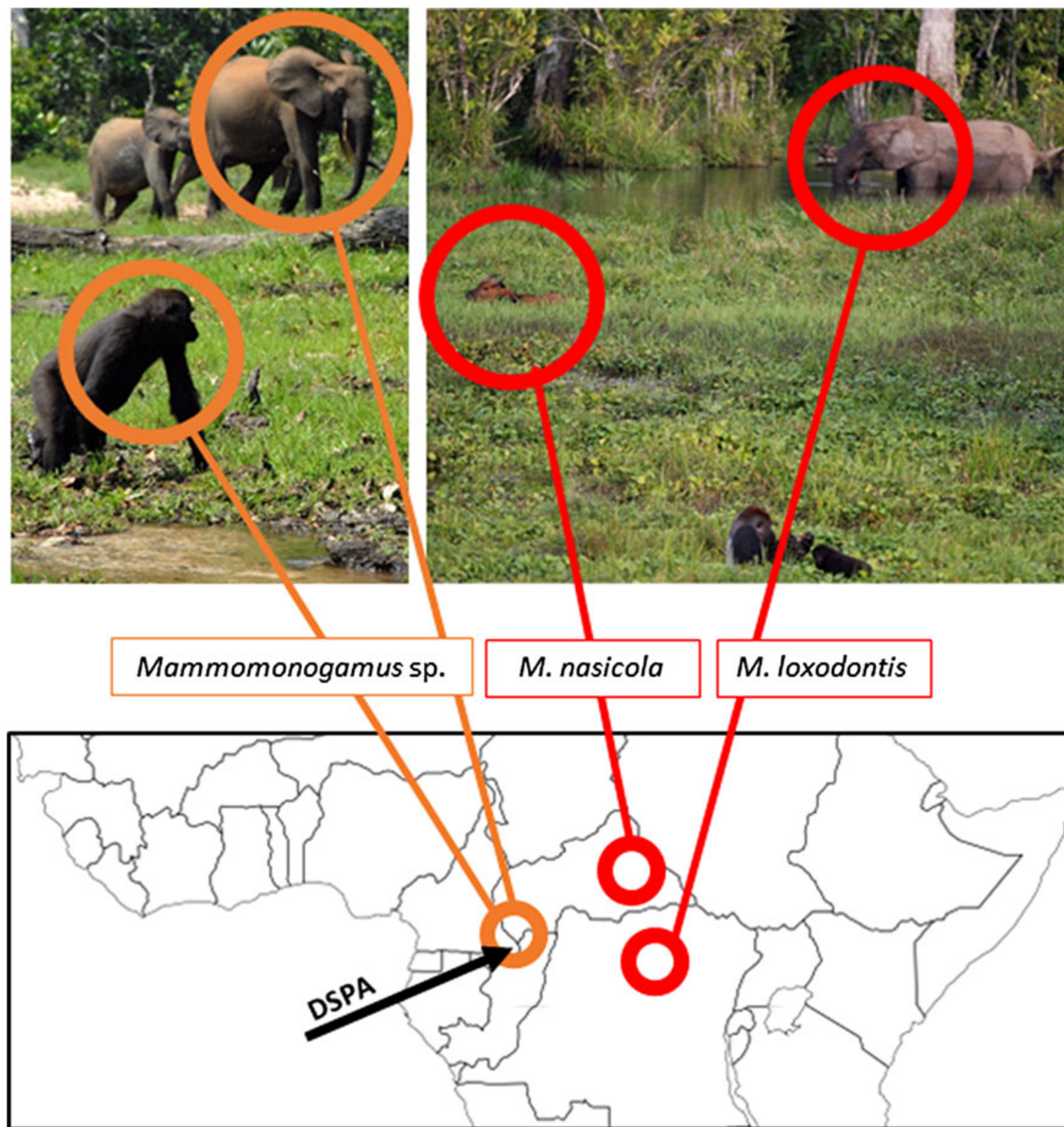

Fig. 1. Spatial contact of western lowland gorillas (*Gorilla gorilla gorilla*), African forest elephants (*Loxodonta cyclotis*) and African forest buffaloes (*Syncerus caffer nanus*) at Central African localities provides an environment for possible transmission of *Mammomonogamus* infection. However, taxonomic classification of findings of eggs (orange) or adults (red) of *Mammomonogamus* in different hosts at different sites varies. Photo credit A. Todd, WWF (left); D. Schulz, Mbeli Bai Study, WCS Congo Program (right).

is also known as a pathogen of Sumatran orangutans (Foitová *et al*. 2008). However, as this assumption is based on taxonomic assignment to insufficiently defined species, real diversity, as well as its level of host specificity remains unclear. Experimental and molecular approaches are usually applied to investigate host specificity as a fundamental property of parasitic organisms (Poulin and Keeney, 2008). However, in the case of *Mammomonogamus* infecting endangered species of large herbivores, experiments are hardly feasible and molecular methods remain the only tools for host specificity assessment.

Due to their thicker shell with typically striated surface, the eggs of *Mammomonogamus* spp. can be easily distinguished from those of gastrointestinal strongyles (Freeman *et al*. 2004). Unfortunately, the egg morphology is not sufficient for further determination to species level. Eggs of *Mammomonogamus* sp. are frequently detected in feces of free ranging western lowland gorillas in Central African Republic (Freeman *et al*. 2004; Masi *et al*. 2012; Kalousová, 2013; Shutt, 2014; Fig. 1). Since adult worms have never been obtained from gorillas and morphologically examined, their identity remains unknown. Throughout their range in Congolian forest, western lowland gorillas share habitat with forest elephants. As *M. loxodontis* has been described in African forest elephants in the Congo Basin (Vuylsteke, 1935; Fig. 1), and *Mammomonogamus* spp. tend to have rather low host specificity, we hypothesize that elephants and gorillas share identical species. To test this

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Table 1. Basic characteristic of individuals in Makumba group and distribuiton of *Mammomonogamus*-positive and *Mammomonogamus*-negative samples throughout the sample set (F = female, M = male; J = juvenile, S = subadult, A = adult, I = infant)

Individual	Sex	Age
Bokata	F	J
Essekerende	M	J
Kunga	M	S
Makumba	M	A
Malui	F	A
Mobangui	M	J
Mopambi	F	A
Mossoko	F	J
Sopo	F	I
Tembo	M	J

2010: XI, XII; 2011: I, II, III, IV, V, VI, VII, VIII, IX, X, XI

Mammomonogamus-positive sample; *Mammomonogamus*-negative sample; no sample in the period

hypothesis, we combined examination of egg shell morphology with sequence analysis of most commonly used nuclear and mitochondrial markers isolated from *Mammomonogamus* eggs infecting gorillas and elephants living symptarically in Dzanga Sangha Protected Areas (DSPA) in south-western Central African Republic. Syngamid strongylids of the genus *Mammomonogamus* undoubtedly belong among the least known nematodes with apparent zoonotic potential and the real diversity of the genus remains hard to evaluate without extensive molecular data. Provided sequences represent the first genomic data on *Mammomonogamus* spp. and we believe they will stimulate general interest in these zoonotic worms.

MATERIAL AND METHODS

Sampling

This study is a part of a long-term health-monitoring programme of primates followed by the Primate Habituation Programme (PHP, launched in 1997). Sampling was carried out at the Bai Hokou study site (2°50′N, 16°28′E), within the northern Dzanga sector of the Dzanga-Sangha Protected Area Complex in Central African Republic. The area is known for dense populations of large forest mammals including western lowland gorillas (*Gorilla gorilla gorilla*), African forest elephants (*Loxodonta cyclotis*) and a range of other herbivores such as forest buffalo (*Syncerus caffes nanus*), red river hog (*Potamochoerus porcus*), giant forest hog (*Hylochoerus mainertzhageni*), bongo (*Tragelaphus spekei*), seven species of duikers and 14 species of non-human primates, including the central chimpanzee (*Pan troglodytes troglodytes*) (Goldsmith, 1996). Local lowland gorillas' density was estimated as 1·6 individuals km^{-2} (Blom *et al*. 2001) and the area is inhabited/regularly visited by approximately 1700 individuals of forest elephants (Turkalo *et al*. 2013). Vegetation in the Dzanga sector is a patchwork of primary and secondary forests intermixed with natural forest clearings (so-called bais, Blom *et al*. 2004). Bais are characterized by a very low tree density and high mineral abundance in seep-hole water, most concentrated during dry periods (Turkalo and Fay, 1996; Blake, 2002). For these reasons, bais are sites of frequent contact between large mammals, including elephants and gorillas (Blom *et al*. 2004; Turkalo *et al*. 2013; Fig. 1).

For the present analyses, we used a sub-set of samples collected from a habituated group of western lowland gorillas (named 'Makumba' after its silverback). During sampling, the group was comprised of three adults, one sub-adult, five juveniles and one infant (Table 1). When possible, three samples per month were collected from each individual during daily follows from November 2010 to December 2011; 9–35 samples were collected from each gorilla individual (Table 1). Elephant fecal samples were collected during the same period ($n = 29$) and additionally from August to October 2012 ($n = 32$); all from unidentified individuals (Table 2). All samples were immediately in the field preserved in 10% formalin and 96% ethanol and stored in 10–50 mL tubes.

Coproscopic analysis

Fecal samples fixed in formalin were mixed with water using pestle and mortar, sieved to a 50 mL Falcon tube of known weight and centrifuged for 10 min at 2000 rpm. The supernatant was strained and the tube with the sediment was weighed. Then, the sediment of known weight was resuspended in 10% formalin to the final volume 10 mL. Two millilitres of homogenized formalin suspension were processed by merthiolate–iodine–formaldehyde concentration (MIFC) (Jirků-Pomajbíková and Hůzová, 2015) and examined by light

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Table 2. Detection of *Mammomonogamus* eggs in fecal samples of African forest elephants and western lowland gorillas by MIFC sedimentation technique

Host	Samples collected	*Mammomonogamus*-positive samples	EPG minimum	EPG maximum	EPG median
L. cyclotis	61	12	8	55	25
G. g. gorilla	257	139	1·1	24·4	3·7

EPG = eggs per gram of fecal sediment.

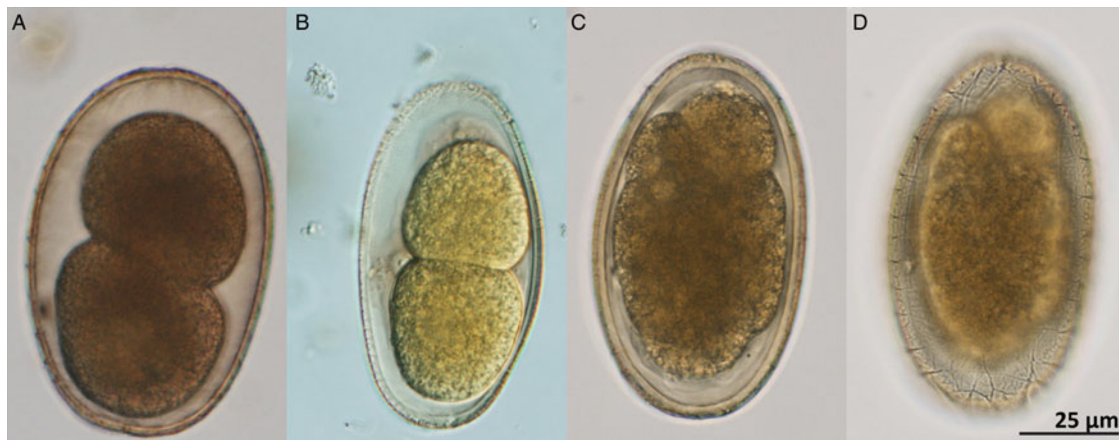

Fig. 2. Eggs of *Mammomonogamus* sp. isolated from feces of western lowland gorillas (A, B) and African forest elephants (C, D) from DSPA. (A) egg with typical two big blastomeres and thick shell; (B) egg observed in NIC showing the striation of the egg shell (photo credit: I. Pšenková); (C) egg divided into eight blastomeres; (D) egg shell surface typically divided by striations into irregular rectangular fields.

microscopy under 100–400× magnification to detect *Mammomonogamus* sp. eggs. These were distinguished from eggs of gastrointestinal nematodes (GIN) based on a 2–3 μm-thick striated shell and typically two big blastomeres (Fig. 2). Detailed structures of the eggs were observed and photographed using an Olympus AX70 microscope equipped with Nomarski interference contrast (NIC) and a DP 70 digital camera. The eggs were quantified using a sedimentation-based technique (Kalousová, 2013) and their quantity was recalculated per gram of the sediment.

Electron microscopy

For scanning electron microscopy (SEM), eggs of *Mammomonogamus* were obtained from filtrated gorilla and elephant fecal sediments preserved in 10% formalin, following the protocol by Jirků *et al.* (2009) and observed using a JEOL JSM-7401F – FE SEM (JEOL Ltd., Tokyo, Japan) capable of high resolution of up to 1 nm.

DNA extraction, PCR and sequencing

Individual eggs of *Mammomonogamus* sp. were isolated from fecal samples preserved in 96% ethanol using a fine glass capillary. Each egg was transferred to a drop of distilled water, its shell was mechanically disrupted by the pipette under a microscope and the crushed egg was transferred into a 200 μL PCR microtube containing 20 μL of deionized water. DNA was extracted by Genomic DNA Mini Kit GT300 (Tissue) (Geneaid, New Taipei City, Taiwan). DNA was eluted to 50 μL of elution buffer provided with the DNA extraction kit. PCR was carried out in 25-μL reactions containing 12·5 μL of PPP Master Mix (Top-Bio, Prague, Czech Republic), 2 μL of each primer (10 μM), 3·5 μL PCR H_2O and 5 μL of template DNA.

Amplification of the partial sequences of 18S rDNA and cytochrome *c* oxidase subunit I (*cox1*) gene were attempted with primers previously used for different strongylid nematodes (Folmer *et al.* 1994; Chilton *et al.* 2006) and additionally designed ones (Table 3).

PCR conditions for all amplifications were identical: initial denaturation at 94 °C for 1 min, followed by 35 cycles of 94 °C denaturation for 40 s, 50 °C annealing for 40 s, 65 °C elongation for 40 s, followed by a 5 min post-amplification extension at 65 °C. The PCR products were mixed with GoodView™ Nucleic Acid Stain (Ecoli s.r.o., Bratislava, Slovakia), electrophoresed in a 2%

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Table 3. Primers used for amplification and sequencing of 18S rDNA and *cox1* gene of *Mammomonogamus* sp. from eggs isolated from fecal samples of African forest elephants and western lowland gorillas from DSPA

Gene	Name		Sequence 5′→3′	Product size	Reference
18S rDNA	NC18SF1	F	AAAGATTAAGCCATGCA	≈1800 bp	Chilton *et al*. (2006)
	NC5BR	R	GCAGGTTCACCTACAGAT		
	18SmamF	F	TGCGAATAGACCCTGACTTTC	≈700 bp	This study
	18SmamR	R	CTGCGGTCCACGAATTTCAC		
	18Smam_F3	F	ATTAGAGTGCTCAGAACAGG	≈700 bp	This study
	18Smam_R3	R	CTGATTCTTCCATTGTAGCG		
cox1	LCO1490	F	GGTCAACAAATCATAAAGATATTGG	≈700 bp	Folmer *et al*. (1994)
	HCO2198	R	TAAACTTCAGGGTGACCAAAAAATCA		
	coxmamF	F	ATTCTATTATTACAGCTCATG	≈450 bp	This study
	coxmamR	R	GAAGTATTGAAATTACGATCTG		

agarose gel plate and detected using a UV illuminator. DNA was purified directly from the PCR products or from the bands cut from the gel after electrophoresis, using Gel/PCR DNA Fragments Extraction Kit (Geneaid, New Taipei City, Taiwan). Products were commercially sequenced in both directions by Macrogen (Amsterdam, Netherlands). The nucleotide sequences determined in this study were registered in the GenBank under the numbers KX980402–KX980409.

DNA sequence alignment and phylogenetic analysis

DNA sequences of both 18S rDNA and *cox1* were trimmed, assembled and manually edited in Sequencher (Gene Codes) and BioEdit (Hall, 1999) and aligned using ClustalW (Larkin *et al*. 2007) implemented in BioEdit 7·2·3 (Hall, 1999). Phylogenetic analysis of 18S sequences was carried out using Bayesian analysis (BA) in MrBayes 3·2·2 (Hastings, 1970; Ronquist and Huelsenbeck, 2003). GenBank sequences of selected strongylids (*Syngamus trachea, Chabertia ovina, Stephanurus dentatus, Cylicocyclus insignis, Necator americanus, Ancylostoma caninum, Ostertagia leptospicularis, Trichostrongylus colubriformis, Metastrongylus elongatus* and *Protostrongylus rufescencs*) were added into the alignment for comparison. BA was done in two simultaneous runs of four Metropolis-couple Monte Carlo Markov chains of one million generations sampled each 100 generations and 25% generations discarded as burn-in. The GTR + I model of sequence evolution used in BA was chosen with Modeltest 3·7 (Posada and Crandall, 1998).

Sequences of *cox1* were analysed in Network 5 (Fluxus Technology Ltd, Suffolk, UK) and a resulting median-joining network was used for graphical presentation of relationships among haplotypes. Pairwise uncorrected genetic distances among haplotypes were calculated in PAUP 4b10 (Sinauer Associates, Sunderland, MA, USA) and compared with available *cox1* variations in other strongylid nematodes.

RESULTS

The eggs of *Mammomonogamus* sp. were found in 12 of 61 elephant fecal samples (19·7%) and in 139 of 257 gorilla fecal samples (54·1%). Repeated examination of all gorillas of Makumba group revealed 100% cumulative prevalence of *Mammomonogamus* eggs. Not one individual gorilla was positive in all examined samples; the samples determined as negative or positive were randomly distributed throughout the dataset (Table 1). The highest number of the samples from a single individual needed to detect *Mammomonogamus* was six (adult gorilla female Malui, Table 1). The quantification results expressed as eggs per gram of fecal sediment (EPG) are showed in Table 2.

Mammomonogamus eggs from gorillas and elephants were morphologically identical, 90–100 μm long, brownish, thick-shelled, containing usually two, but sometimes more blastomeres (Fig. 2A–C). In gorillas, four blastomeres were observed only rarely, while in elephants eggs containing more blastomeres were more frequent. Microscopic examination of the outer surface of the egg shell revealed a complex pattern of very fine grooves dividing the surface into irregular angular fields (Fig. 2D). The same structures were observed with SEM, demonstrating the irregularity in depths and widths of the surface grooves (Fig. 3). No differences in the fine external shell pattern were observed between the eggs originating from gorillas and elephants.

We extracted, amplified and sequenced 18S rDNA from eggs isolated from three gorilla and six elephant fecal samples and mitochondrial *cox1* from seven gorilla and seven elephant fecal samples. As previously published primers (Folmer *et al*. 1994; Chilton *et al*. 2006) did not amplify DNA in all our samples, we designed our own primers (Table 3). In total, we obtained 12 sequences of 18S rDNA, which were trimmed to 1216 bp for analysis. All of them were identical, represented in computation of the phylogenetic tree by one unique haplotype. The phylogenetic tree constructed by BA showed *Mammomonogamus*

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clustering with *Syngamus trachea* (Fig. 4A). This clade, corresponding taxonomically to the family Syngamidae, clustered together with other mammalian strongylids from Ancylostomatidae, Chabertiidae and Strongylidae families. Twenty-two sequences of mitochondrial *cox1* trimmed to 386 bp could be narrowed down to seven haplotypes (Fig. 4B). The haplotypes were rather closely related, differing mostly by 1–5 substitutions from each other, with H6 being the most divergent. Differences between haplotypes expressed as pairwise uncorrected distances varied between 0·3 and 1·3%. The most frequent haplotype, H1, representing 14 *cox1* sequences was found in both gorillas ($n = 8$) and elephants ($n = 6$). Another haplotype, H7, was also found in both gorilla and elephant. Haplotypes H2, H5 and H6 were found only in elephants, each represented by a single sequence; haplotypes H3 and H4 were obtained only from gorillas and are represented by two and one sequences, respectively. In seven samples, we isolated and successfully amplified the DNA from more than one egg and proved the co-occurrence of two (in three elephants, two gorillas) or three (in one gorilla) *Mammomonogamus cox1* haplotypes, while in two gorilla samples the haplotypes from different eggs were identical.

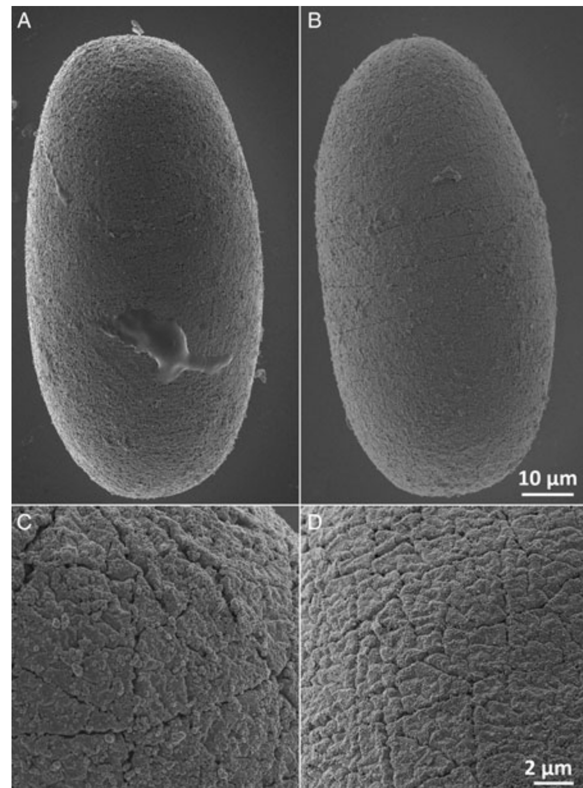

Fig. 3. SEM of *Mammomonogamus* sp. eggs isolated from western lowland gorilla (A, C) and African forest elephant (B, D).

DISCUSSION

Occurrence of *Mammomonogamus* nematodes in sympatric lowland gorillas and forest elephants in DSPA posed a question of their possible transmission (Freeman *et al.* 2004). To investigate assumed conspecificity, we combined phylogenetic analysis of nuclear and mitochondrial markers with examinations of egg shell morphology by light microscopy and SEM.

The fine structure of nematode eggs with rough or micro-ornamented outer walls is considered an important taxonomic criterion, used to determine them to species level, e.g. in *Capillaria* or *Syphacia* spp. (Baruš *et al.* 1979; Magi *et al.* 2012). However, neither NIC microscopy nor SEM revealed any differences in the morphology of the outer surface of *Mammomonogamus* eggs originating from gorillas and elephants. This finding is consistent with the results of Freeman *et al.* (2004), who found no microscopic differences among the *Mammomonogamus* eggs from gorilla and elephant feces and eggs dissected from the only voucher specimen of *M. loxodontis*.

Analysis of both mitochondrial and nuclear markers confirmed the conspecificity of *Mammomonogamus* species infecting gorillas and elephants in DSPA. While the sequence of 18S rDNA was identical in both host species, seven different haplotypes were recognized within the *cox1* sequences. Two haplotypes were found in both gorillas and elephants, three and two haplotypes were found only in elephants and gorillas respectively, suggesting a degree of intraspecific mitochondrial DNA variability, as also known from other strongylids (Miranda *et al.* 2008; Ngui *et al.* 2013; Hasegawa *et al.* 2014). Co-occurrence of two different haplotypes was detected in three elephant and two gorilla fecal samples, most probably as a result of plural *Mammomonogamus* couples in these host animals.

Obtaining *Mammomonogamus* sequences does not explicitly contribute to its taxonomy as there are no other sequences of *Mammomonogamus* available and the adults were not accessible to us. Sequencing DNA from the voucher specimen of *M. loxodontis* and comparing it to our sequences would be the ideal solution. Unfortunately, this option proved unfeasible. Alternatively, a field mission to obtain and consequently sequence the topotypic material from elephants from the type locality of *M. loxodontis* (= Yangambi, Democratic Republic of the Congo, Vuylsteke, 1935) could be proposed, but this option is rather unrealistic too. In our opinion, suggested conspecificity of *Mammomonogamus* in gorillas and elephants, together with the fact that there are no other species of *Mammomonogamus* named from African elephants and gorillas, sufficiently justifies the assignment of the observed nematodes to *M. loxodontis*, at least until the full revision of African *Mammomonogamus* taxa proves otherwise.

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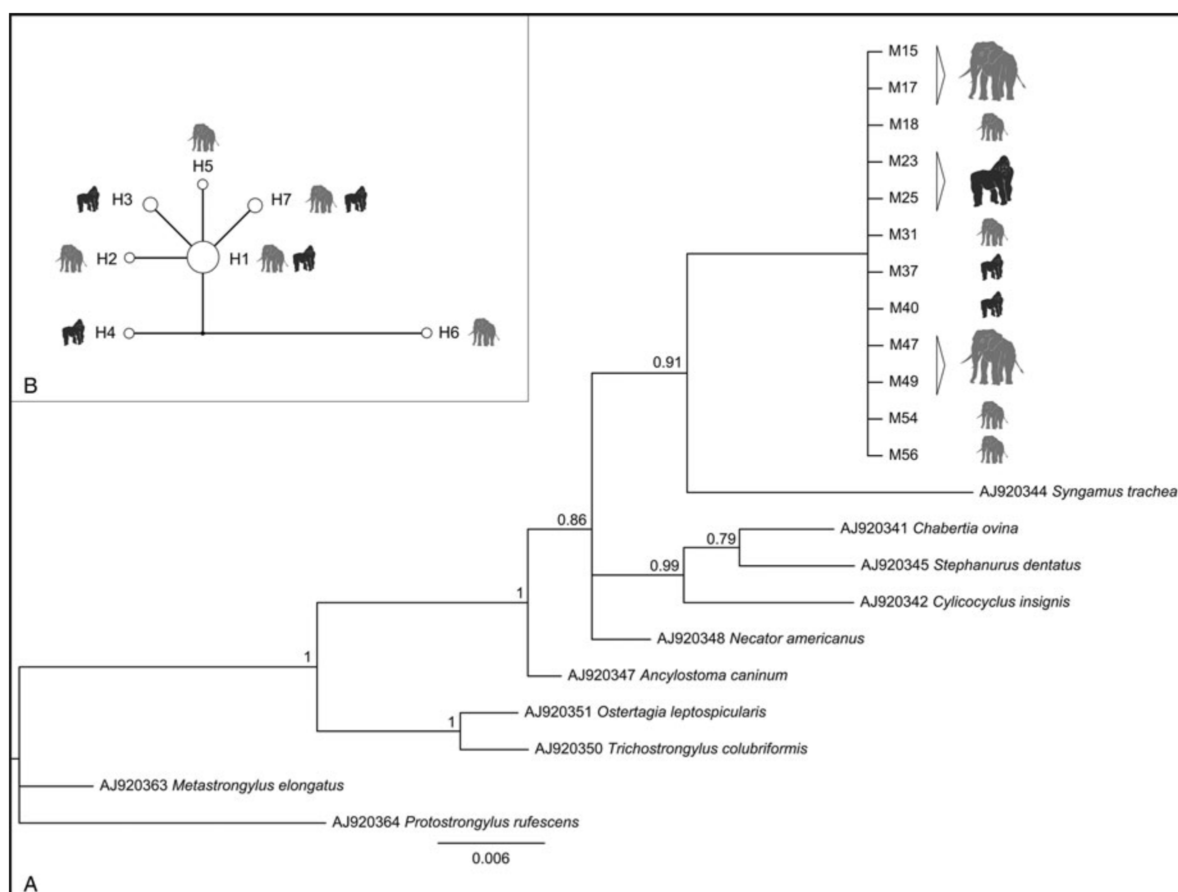

Fig. 4. Phylogenetic analysis of the 18S rDNA (A) and *cox1* (B) of *Mammomonogamus* sp. from gorillas and elephants from DSPA. (A) Phylogenetic tree constructed by Bayesian analysis of 1216 bp of 18S rDNA depicting conspecificity of all sequences of *Mammomonogamus* isolated from gorillas and elephants in DSPA and their clustering with *Syngamus trachea*. Sequences were obtained from three gorilla and six elephant fecal samples. Triangles indicate sequences originating from the same fecal sample. Bootstrap values over 70% are shown. (B) Haplotype network inferred from 386 bp *cox1* sequences of *Mammomonogamus* created in Network 5. Twenty-two sequences were narrowed down to seven haplotypes. Size of the circle indicates number of sequences belonging to the haplotype. Hosts of each haplotype are depicted.

Almost all reports of *Mammomonogamus* nematodes in large African herbivores are based on occasional findings (Gedoelst, 1924; Mönnig, 1932; Vuylsteke, 1935; van den Berghe, 1937; Graber *et al*. 1971; Freeman *et al*. 2004; Kinsella *et al*. 2004). Examination of a large sample set allowed us to investigate at least the basic features of *Mammomonogamus* infection in both host species, namely the prevalence and egg shedding intensity. Prevalence of infection, one of basic variables in epidemiological studies, is defined as the number of individuals infected with a particular parasite species (or taxonomic group) divided by the number of hosts examined (Bush *et al*. 1997). Due to biological and methodological reasons, prevalence of certain parasites in a group of hosts is positively correlated with the number of samples examined from each individual (Huffman *et al*. 1997; Muehlenbein, 2005; Pomajbíková *et al*. 2012). Recent studies on parasites of endangered free-ranging animals are hampered by their dependence on non-invasive sampling. Apparently, coproscopic examination is a fairly suboptimal method to investigate the finer aspects of nematode infections, mainly due to the intermittent nature of egg shedding and sensitivity bias leading to false negative results. Thus, even though great ape habituation can have certain negative impacts on the animals (Blom *et al*. 2004; Sak *et al*. 2013; Shutt *et al*. 2014), it also brings, among other advantages, the possibility of repeated sampling of identified individuals. This allows the inclusion of additional parameters, potentially influencing parasite infections (e.g. sex, age or behaviour), into analyses and also to assess infection dynamics (Huffman *et al*. 1997; Masi *et al*. 2012; Kalousová, 2013; Ghai *et al*. 2015).

With two exceptions, approximately half of the samples of each sampled gorilla were *Mammomonogamus*-positive; after examination of six samples from an individual, we detected *Mammomonogamus* eggs in all studied gorillas within a relatively short period, strongly suggesting that all the animals are permanently or almost permanently infected. The intermittent negativity can have several explanations: (i) the adult females may not be present in the host at the given time, (ii) the eggs are laid at irregular intervals and/or (iii) the

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quantity of shed eggs is below the threshold level of examination method used.

In related *Syngamus trachea,* egg production is variable and associated with worm longevity. Baruš (1966) has demonstrated maximum egg excretion shortly after the infection (100–350 EPG of feces) and confirmed that 75–85% of eggs were laid during the first 35 days of the patency; later, egg counts decreased below 40 per gram. Our EPG results ranging from 1·1 to 24·4 in gorillas and from 8 to 55 in elephants are lower or comparable with the latter egg counts in *S. trachea*, but lower than the fecal egg counts of GIN (Condy, 1974; Lilly *et al*. 2002; Obanda *et al*. 2011). Low *Mammomonogamus* EPG values suggest either very low abundance of adult females or their relatively low fecundity. Unknown amounts of *Mammomonogamus* eggs can get coughed or sneezed out, so those eggs do not enter the gastrointestinal tract, which further complicates the estimation of the actual number of adult worms per host based on EPG values.

Since every diagnostic method has its sensitivity limits, low EPG values imply the chance of false negatives. In our case, the theoretical threshold to classify the sample as *Mammomonogamus*-positive is 1·1 EPG (Table 2). If we theoretically presume, that (i) female of *Mammomonogamus* lays 1000 of eggs per day (based on values for *S. trachea* from Baruš, 1966), (ii) African forest elephant produces 60 kg of feces per day (Lehnhardt, 2006), (iii) 3 g of feces are needed to obtain 1 g of fecal sediment (our experience from laboratory), and (iv) conditions are ideal, i.e. eggs are evenly dispersed in the feces, there would be one egg per 20 g of fecal sediment. Considering our detection threshold, the infections caused by less than 19 females in one elephant could easily evade detection. Study of mammomonogamiasis of zebus in Central African Republic (Vercruysse, 1978) revealed a 17–44% prevalence when examining cadavers (with an average 2–3 pairs of adult worms per host) compared with 1% prevalence assessed by coproscopic examination, confirming that low intensity infections can remain undetected by coproscopy. Considering the high prevalence in gorillas after serial examinations and the high probability of false negativity, we can presume that the real prevalence of *Mammomonogamus* in elephants in DSPA is probably much higher than we detected.

The identity or close similarity of 18S and *cox1* sequences suggests the transmission of *Mammomonogamus* between elephants and gorillas. Presence of the same parasite in these two phylogenetically distant hosts indicates relatively low host specificity (Poulin and Mouillot, 2003), which raises further questions about the real/potential host spectrum of *M. loxodontis* and its zoonotic potential. Cases of human mammomonogamiasis are known from different parts of the world. Most cases are reported from the Caribbean (Magdeleine *et al*. 1974; Nosanchuk *et al*. 1995), South America (da Costa *et al*. 2005; Castaño *et al*. 2006) and South-East Asia (Limawongpranee *et al*. 2004) but no cases have ever been reported from Africa. People cohabiting DSPA were never found to be *Mammomonogamus*-positive, even though sharing of intestinal strongylids between humans and gorillas occurs frequently in the studied area (Hasegawa *et al*. 2014). The absence of *M. loxodontis* in humans does not necessarily mean its inability to infect human hosts. Beside parasite–host compatibility, it is also the encounter filter impacting the observed host specificity of parasites (Combes, 1991). Gorillas and elephants extensively share their habitat, coming into close spatial contact especially in bais (Turkalo and Fay, 1996). Thanks to the high concentration of potential hosts and suitable conditions for larval development, we presume that bais could play important role in transmission of *M. loxodontis* between elephants and gorillas. The life cycle of *Mammomonogamus* spp. is unknown (Buckley, 1934; Magdeleine *et al*. 1974; Nosanchuk *et al*. 1995), however, direct oral infection by L3 larvae (either in eggs or free) is most plausible. Even in situations, when people (usually BaAka hunters or trackers) visit bais, the probability of oral ingestion of strongylid larvae is relatively low, as they do not harvest any ground vegetation. This situation contrasts with transmission of *Necator* spp. between gorillas and humans in the same habitat, probably facilitated by percutaneous penetration of L3 larvae of *Necator* (Hasegawa *et al*. 2014; Kalousová *et al*. 2016). DSPA hosts good population of central chimpanzees *P. t. troglodytes* but these animals only rarely visit the bais. In limited set of samples (~10) from previous years (data not shown), the *Mammomonogamus* eggs were not observed; however, possible occurrence in these apes deserves future attention.

This is the first study on the host specificity of *Mammomonogamus* using a combination of egg morphology and analysis of molecular markers to strongly suggest interspecies transmission between gorillas and elephants. It provides a solid foundation for further investigation of *Mammomonogamus* strongyles in other host species and areas. Although the eggs are an adequate source of DNA and the molecular approach can be easily applied to confirm or reject the conspecificity of *Mammomonogamus* from various hosts, the adult worms will be eventually required for taxonomic assignment of analysed material.

SUPPLEMENTARY MATERIAL

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REFERENCES

Baruš, V. (1966). The longevity of the parasitic stages and the dynamics of eggs production of the nematode *Syngamus trachea* (Montagu, 1811) in chicken and turkeys. *Folia Parasitologica* **13**, 274–277.

Baruš, V., Tenora, F. and Wiger, R. (1979). Scanning electron microscopy of eggs of European species of the genus *Syphacia* Seurat, 1916 (Nematoda). *Folia Parasitologica* **26**, 21–26.

Blake, S. (2002). The ecology of forest elephant distribution and its implication for conservation. PhD Thesis, University of Edinburgh, Scotland. 319 pp. https://www.researchgate.net/profile/Stephen_Blake2/publication/265032366_The_Ecology_of_Forest_Elephant_Distribution_and_Its_Implications_for_Conservation/links/54ad7c6b0cf24aca1c6f5d95/The-Ecology-of-Forest-Elephant-Distribution-and-Its-Implications-for-Conservation.pdf

Blom, A., Almaši, A., Heitkönig, I. M. A., Kpanou, J.-B. and Prins, H. H. T. (2001). A survey of the apes in the Dzanga-Ndoki National Park, Central African Republic: a comparison between the census and survey methods of estimating the gorilla (*Gorilla gorilla gorilla*) and chimpanzee (*Pan troglodytes*) nest group density. *African Journal of Ecology* **39**, 98–105.

Blom, A., Cipolletta, C., Brunsting, A. M. H. and Prins, H. H. T. (2004). Behavioral responses of gorillas to habituation in the Dzanga-Ndoki National Park, Central African Republic. *International Journal of Primatology* **25**, 179–196.

Buckley, J. J. C. (1934). On *Syngamus ierei* sp. nov. from domestic cats, with some observations on its life-cycle. *Journal of Helminthology* **9**, 89–98.

Bush, A. O., Lafferty, K. D., Lotz, J. M. and Shostak, A. W. (1997). Parasitology meets ecology on its own terms: Margolis *et al*. revisited. *Journal of Parasitology* **83**, 575–583.

Cameron, W. M. (1931). On some lungworms of the Malay tiger. *Journal of Helminthology* **9**, 147–152.

Castaño, J. C., Núñez, F. Á., González, M. M., Téllez, G. and Giraldo, M. I. (2006). Reporte del primer caso humano de infección parasitaria por *Mammomonogamus laryngeus* en Colombia. *Biomédica* **26**, 337–341.

Chilton, N. B., Huby-Chilton, F., Gasser, R. B. and Beveridge, I. (2006). The evolutionary origins of nematodes within the order Strongylida are related to predilection sites within hosts. *Molecular Phylogenetics and Evolution* **40**, 118–128.

Combes, C. (1991). Evolution of parasite life cycles. In *Parasite–host Associations: Coexistence or Conflict?* (ed. Toft, C. A., Aeschlimann, A. and Bolis, L.), pp. 62–82. Oxford University Press, Oxford, UK.

Condy, J. B. (1974). Observations on internal parasites in Rhodesian elephant, *Loxodonta africana*. *Proceedings and Transactions of the Rhodesian Scientific Association* **55**, 67–99.

da Costa, J. C., Delgado, M. L., Vieira, P., Afonso, A., Conde, B. and Cross, J. H. (2005). Syngamoniasis in tourist. *Emerging Infectious Diseases* **11**, 1976–1977.

Diesing, C. M. (1857). Sechzehn Arten von Nematoideen (Mit IV Tafeln). *Bande der Denkschriften der mathematisch-naturwissenschaftlichen Classe der Kaiserlichen Akademie der Wissenschaften* **13**, 6–26.

Faust, E. C. and Tang, C.-C. (1934). A new species of *Syngamus* (*S. auris*) from the middle ear of the cat in Foochow, China. *Parasitology* **26**, 455–459.

Foitová, I., Koubková, B., Baruš, V. and Nurcahyo, W. (2008). Presence and species identification of the gapeworm *Mammomonogamus laryngeus* (Railliet, 1899) (Syngamidae: Nematoda) in a semi-wild population of Sumatran orangutan (*Pongo abelii*) in Indonesia. *Research in Veterinary Science* **84**, 232–236.

Folmer, O., Black, M., Hoeh, W., Lutz, R. and Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* **3**, 294–299.

Freeman, A. S., Kinsella, J. M., Cipoletta, C., Deem, S. L. and Karesh, W. B. (2004). Endoparasites of western lowland gorillas (*Gorilla gorilla gorilla*) at Bai Hokou, Central African Republic. *Journal of Wildlife Diseases* **40**, 775–781.

Gedoelst, L. (1924). Un syngame parasite de l'hippopotame. *Annales de Parasitologie* **2**, 307–311.

Ghai, R. R., Fugère, V., Chapman, C. A., Goldberg, T. L. and Davies, T. J. (2015). Sickness behaviour associated with non-lethal infections in wild primates. *Proceedings of the Royal Society of London B: Biological Sciences* **282**, 20151436.

Goldsmith, M. (1996). *Ecological influences on the ranging and grouping behavior of western lowland gorillas at Bai Hokou, Central African Republic*. Ph.D. thesis. State University of New York, Stony Brook, New York.

Graber, M., Euzeby, J., Gevrey, J., Troncy, P. M. and Thal, J. (1971). La mammomonogamose des ruminants domestiques et sauvages. *Revue d'Élevage et de Médecine Vétérinaire des Pays Tropicaux* **24**, 525–541.

Hall, T. A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* **41**, 95–98.

Hasegawa, H., Modrý, D., Kitagawa, M., Shutt, K. A., Todd, A., Kalousová, B., Profousová, I. and Petrželková, K. J. (2014). Humans and great apes cohabiting the forest ecosystem in Central African Republic harbour the same hookworms. *PLoS Neglected Tropical Diseases* **8**, e2715.

Hastings, W. K. (1970). Monte Carlo sampling methods using Markov chanis and their applications. *Biometrika* **57**, 97–109.

Huffman, M. A., Gotoh, S., Turner, L. A., Hamai, M. and Yoshida, K. (1997). Seasonal trends in intestinal nematode infection and medicinal plant use among chimpanzees in the Mahale Mountains, Tanzania. *Primates* **38**, 111–125.

Jirků, M., Jirků, M., Oborník, M., Lukeš, J. and Modrý, D. (2009). *Goussia Labbé*, 1896 (Apicomplexa, Eimeriorina) in Amphibia: diversity, biology, molecular phylogeny and comments on the status of the genus. *Protist* **160**, 123–136.

Jirků-Pomajbíková, K. and Hůzová, Z. (2015). Coproscopic techniques. In *Parasites of African Great Apes Atlas of Coproscopic Diagnostics* (ed. Modrý, D., Petrželková, K. J., Kalousová, B. and Hasegawa, H.), pp. 14–24. HPI-lab, Department of Pathology and Parasitology, University of Veterinary and Pharmaceutical Sciences Brno, Brno, Czech Republic.

Kalousová, B. (2013). Gastrointestinal parasites of wild western lowland gorillas (Gorilla gorilla gorilla) in Dzanga Sector of Dzanga-Ndoki National Park, Central African Republic. Master thesis. Masaryk University, Brno, Czech Republic, 109 pp. https://is.muni.cz/th/269851/prif_m/.

Kalousová, B., Hasegawa, H., Petrželková, K. J., Sakamaki, T., Kooriyma, T. and Modrý, D. (2016). Adult hookworms (*Necator* spp.) collected from researchers working with wild western lowland gorillas. *Parasites & Vectors* **9**, 75.

Kinsella, J. M., Deem, S. L., Blake, S. and Freeman, A. S. (2004). Endoparasites of African forest elephant (*Loxodonta africana cyclotis*) from the Republic of Congo and Central African Republic. *Comparative Parasitology* **71**, 104–110.

Larkin, M. A., Blackshields, G., Brown, N. P., Chenna, R., McGettigan, P. A., McWilliam, H., Valentin, F., Wallace, I. M., Wilm, A., Lopez, R., Thompson, J. D., Gibson, T. J. and Higgins, D. G. (2007). Clustal W and ClustalX version 2.0. *Bioinformatics* (*Oxford, England*) **23**, 2947–2948.

Lehnhardt, J. (2006). Husbandry. In *Biology, medicine and surgery of elephants* (ed. Fowler, M. E. and Mikota, S. K.), pp. 45–56. Blackwell Publishing, Ames, Iowa, USA.

Lilly, A. A., Mehlman, P. T. and Doran, D. (2002). Intestinal parasites in gorillas, chimpanzees and humans at Mondika Research Site, Dzanga-Ndoki National Park, Central African Republic. *International Journal of Primatology* **23**, 555–573.

Limawongpranee, S., Samanthai, S. and Yoolek, A. (2004). Human *Mammomonogamus laryngeus* infection (Syngamosis): the second case report in Thailand. *Siriraj Hospital Gazette* **56**, 82–86.

https://doi.org/10.1017/S0031182017000221 Published online by Cambridge University Press

Magdeleine, J., Magnaval, J.-F., Brossard, B., Michel, M. and Turiaf, J. (1974). La syngamose humaine à la Martinique. *Médecine d'Afrique Noire* **21**, 651–655.

Magi, M., Guardone, L., Prati, M. C., Torracca, B. and Macchioni, F. (2012). First report of *Eucoleus boehmi* (syn. *Capillaria boehmi*) in dogs in north-western Italy, with scanning electron microscopy of the eggs. *Parasite* **19**, 433–435.

Masi, S., Chauffour, S., Bain, O., Todd, A., Guillot, J. and Krief, S. (2012). Seasonal effects on great ape health: a case study of wild chimpanzees and western gorillas. *PLoS ONE* **7**, e49805.

Miranda, R. R., Tennessen, J. A., Blouin, M. S. and Rabelo, É. M. (2008). Mitochondrial DNA variation of the dog hookworm *Ancylostoma caninum* in Brazilian populations. *Veterinary Parasitology* **151**, 61–67.

Mönnig, H. O. (1932). *Syngamus indicus. 18th Report of Director of Veterinary Services and Animal Industry, PLACE, Union of South Africa*, p. 173.

Muehlenbein, M. P. (2005). Parasitological analyses of the male chimpanzees (*Pan troglodytes schweinfurthii*) at Ngogo, Kibale National Park, Uganda. *American Journal of Primatology* **65**, 167–179.

Ngui, R., Mahdy, M. A. K., Chua, K. H., Traub, R. and Lim, Y. A. L. (2013). Genetic characterization of the partial mitochondrial cytochrome oxidase c subunit I (*cox* 1) gene of the zoonotic parasitic nematode, *Ancylostoma ceylanicum* from humans, dogs and cats. *Acta Tropica* **128**, 154–157.

Nosanchuk, J., Wade, S. and Landolf, M. (1995). Case report of and description of parasite in *Mammomonogamus laryngeus* (human syngamosis) infection. *Journal of Clinical Microbiology* **33**, 998–1000.

Obanda, V., Iwaki, T., Mutinda, N. M. and Gakuya, F. (2011). Gastrointestinal parasites and associated lesions in starving free-ranging African elephants. *South African Journal of Wildlife Research* **41**, 167–172.

Pomajbíková, K., Petrželková, K. J., Petrášová, J., Profousová, I., Kalousová, B., Jirků, M., Sá, R. M. and Modrý, D. (2012). Distribution of the entodiniomorphid ciliate *Troglocorys cava* Tokiwa, Modrý, Ito, Pomajbíková, Petrželková & Imai, 2010, (Entodiniomorphida: Blepharocorythidae) in wild and captive chimpanzees. *Journal of Eukaryotic Microbiology* **59**, 97–99.

Posada, D. and Crandall, K. A. (1998). Modeltest: testing the model of DNA substitution. *Bioinformatics* **14**, 817–818.

Poulin, R. and Keeney, D. B. (2008). Host specificity under molecular and experimental scrutiny. *Trends in Parasitology* **24**, 24–28.

Poulin, R. and Mouillot, D. (2003). Parasite specialization from a phylogenetic perspective: a new index of host specificity. *Parasitology* **126**, 473–480.

Railliet, M. A. (1899). Syngame laryngien du boeuf. *Comptes Rendu des Séances de la Société de Biologie et des ses Filiales* **6**, 174–176.

Ronquist, F. and Huelsenbeck, J. P. (2003). MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**, 1572–1574.

Ryzhikov, K. M. (1948). Phylogenetic interrelationships of nematodes of the family Syngamidae and an attempt to reconstruct their systematics. *Doklady Akademii Nauk SSSR* **62**, 733–736.

Sak, B., Petrzelkova, K. J., Kvetonova, D., Mynarova, A., Shutt, K. A., Pomajbikova, K., Kalousová, B., Modrý, D., Benavides, J., Todd, A. and Kváč, M. (2013). Long-term monitoring of microsporidia, *Cryptosporidium* and *Giardia* infections in western lowland gorillas (*Gorilla gorilla gorilla*) at different stages of habituation in Dzanga Sangha Protected Areas, Central African Republic. *PLoS ONE* **8**, e71840.

Seneviratne, P. (1954). *Syngamus mcgaughei* sp. nov. in domestic cats in Ceylon. *Ceylon Veterinary Journal* **2**, 55–60.

Shutt, K. A. (2014). Wildlife tourism and conservation: an interdisciplinary evaluation of gorilla ecotourism in Dzanga-Sangha, Central African Republic. Ph.D. thesis. Durham University, Durham, UK, 252 pp. Available at Durham E-Theses Online: http://etheses.dur.ac.uk/10586/.

Shutt, K., Heistermann, M., Kasim, A., Todd, A., Kalousová, B., Profousová, I., Petželková, K., Fuh, T., Dicky, J.-F., Bopalanzognako, J.-B. and Setchell, J. M. (2014). Effects of habituation, research and ecotourism on feacal glucocorticoid metabolites in wild western lowland gorillas: implications for conservation management. *Biological Conservation* **172**, 72–79.

Turkalo, A. and Fay, J. M. (1996). Studying forest elephants by direct observation: preliminary results from the Dzanga Clearing, Central African Republic. *Pachyderm* **20**, 45–54.

Turkalo, A. K., Wrege, P. H. and Wittemyer, G. (2013). Long-term monitoring of Dzanga Bai forest elephants: forest clearing use patterns. *PLoS ONE* **8**, e85154.

van den Berghe, L. (1937). Contribution à l'etude des parasites de l'okapi. *Revue de Zoologie et de Botanique Africaines* **29**, 141–150.

Vercruysse, J. (1978). La mammomonogamose des zébus en empire centrafrican. *Revue d'Élevage et de Médecine Vétérinaire des Pays Tropicaux* **31**, 427–430.

von Linstow, O. (1899). Nematoden aus der Berliner Zoologischen Sammlung. *Mitteilungen aus der Zoologischen Sammlung des Museums für Naturkunde in Berlin* **1**, 1–28.

Vuylsteke, C. (1935). Etude de quelques nématodes parasites de l'éléphant. *Revue de Zoologie et de Botanique Africaines* **27**, 319–325.

https://doi.org/10.1017/S0031182017000221 Published online by Cambridge University Press