PREVALENCE AND MOLECULAR CHARACTERISATION OF INTESTINAL PARASITES INFECTING NON-HUMAN PRIMATES IN NATURAL AND CAPTIVE CONDITIONS

A thesis submitted by

SILVIA RONDÓN

Advisors
PROF. STEFANO D'AMELIO
Dr. SERENA CAVALLERO
Sapienza University of Rome. Rome, Italy

Co-advisors
PROF. CAMILA GONZÁLEZ
PROF. ANDRÉS LINK
Universidad de Los Andes. Bogotá, Colombia

Sapienza University of Rome
Department of Public Health and Infectious Diseases
Academic year 2022/2023
Cycle XXXV

INDEX Page

ABSTRACT

Introduction: Non-human primates (NHPs) have been found infected with a diversity of intestinal zoonotic protozoan and metazoan parasites of public health concern. Ecosystem transformation increases the contact between human and NHPs, incrementing the chances of zoonotic parasite transmission. Likewise, contact with captive NHPs can represent a risk for humans, leading to parasitic infections. This study aimed to assess the prevalence of intestinal parasites in NHPs living in two scenarios, fragmented forests in Colombia and captive conditions in Italy (two wildlife recovery centres and a zoological garden),to molecularly characterise selected parasite species of zoonotic interest (*Blastocystis* sp., *Giardia* sp., *Ascaris* sp., *Trichuris* sp.), and to provide a preliminary description of the distribution of parasite taxa in different ecological contexts.

Methodology: Faecal samples were collected from free-ranging *Alouatta seniculus, Ateles hybridus, Aotus griseimembra, Cebus versicolor, Saimiri cassiquiarensis*, and *Sapajus apella* in Colombia, as well as from captive *Macaca tonkeana, Macaca fascicularis,* and *Sapajus apella* living at the wildlife recovery centre Parco Faunistico Piano dell'Abatino, in Rieti (Lazio region). Two hundred twelve and 33 faecal samples were collected from free-ranging and captive NHPs, respectively. Flotation and faecal smears were performed in order to identify parasites based on morphology. Samples microscopically classified as positive for *Blastocystis* sp, *Giardia* sp. and *Ascaris* sp. were processed for molecular characterisation. Additionally, one carcass of *M. fuscata* from Fondazione Bioparco di Roma (Rome, Lazio) and one of *M.fascicularis* from Centro Recupero Animali Selvatici della Maremma (Semproniano, Tuscany) were necropsied and intestinal

adult nematodes were collected, morphologically identified, and molecularly characterised.

Results: About 93% of the samples from free-ranging NHPs were positive for intestinal parasites, including: protozoans (*Blastocystis* sp., *Balantidium* sp., *Dientamoeba fragilis*-like, Entamoebidae, *Giardia* sp.), cestodes (*Hymenolepis* sp.), trematodes (*Controrchis* sp.), nematodes (*Ascaris* sp., Strongyliform larvae, *Trypanoxyuris* sp., Ancylostomatidae), and acanthocephalans. *Ascaris lumbricoides, Giardia intestinalis* (Assemblages A and B) and *Blastocystis hominis* (Subtype 8) were identified through molecular techniques, from samples of free-ranging NHP. Captive primates were found infected with protozoans (*Entamoeba coli*, *Dientamoeba fragilis*-like, *Iodamoeba bütschlii*, *Balantidium* sp.) and nematodes (*Oesophagostomum* sp., strongyliform larvae, *Trichuris* sp.). The collected adult nematodes were morphologically identified as whipworms (genus *Trichuris*) and after molecular characterisation, the phylogenetic analyses grouped *Trichuris* specimens from *M. fuscata* into a host- specific branch of the *Trichuris trichiura* complex of species, while whipworms from *M. fascicularis* clustered within a less specific clade formed by *Trichuris* infecting several primate species, including humans.

Conclusions: This study provides new information of intestinal parasites infecting wild NHPs exposed to anthropogenic disturbance and captive conditions. The finding of parasites with zoonotic potential suggests epidemiological implications in NHP conservation and human health, at the human-NHP interface, in transformed ecosystems. Additionally, the results of this study could be useful in the design of public health policies, and within NHP conservation programs.

1. INTRODUCTION

Non-human primates (NHPs), both captive and free-ranging, have been found infected with a diversity of intestinal zoonotic protozoan and metazoan parasites [1,2]. Some of those parasites are of public health concern, as in the case of the protozoans *Blastocystis* and *Giardia*, and the helminths *Trichuris* and *Ascaris*.

Trichuris trichiura and *Ascaris lumbricoides* are nematodes included into the main species causing soil-transmitted helminth infections, which are neglected tropical diseases with a global burden of a 1.5 billion people worldwide infected, according to the World Health Organization [3]. Moreover, *Giardia* has a global importance, infecting a wide range of hosts and causing ≈28.2 million cases of diarrhoea each year [4], while *Blastocystis* is the most commonly found eukaryote in the gut of humans and other animals [5].

Parasite surveys in NHPs are important in situations such as designing of conservation strategies, reintroduction programs, and in the NHP acquisition for research laboratories or zoos. Determining the composition of parasite communities in captive NHPs allows the identification of parasites of concern, regarding the introduction of novel parasites to potentially susceptible wildlife populations during reintroduction programs, and also lead to a better understanding of parasite ecology. For instance, it has been observed that vector-borne parasites are more likely found in free-ranging NHPs, while parasites transmitted through either close and non-close contact, including the faecal-oral transmission, are more likely detected in captive NHPs [6].

Parasite identification and surveillance in NHPs are frequently based on morphological approaches. However, the use of molecular techniques can provide

important contributions to the field of NHP parasitology by precisely identifying the species/variants of parasites, which is highly relevant for the understanding of their epidemiology and for the inference of zoonotic potential [7], especially in conditions of ecosystem transformation which increase the contact between humans and NHPs incrementing the chances of zoonotic parasite transmission. Likewise, parasite surveys on NHP populations under human care are relevant as part of the evaluation of NHPs welfare, the zoonotic disease risk assessment, and in the exploration of parasite transmission pathways.

In the last decades, the use of molecular markers for diagnostics or molecular systematics purposes have greatly improved our understanding of parasite biology and biodiversity, assisting the discovery of new species or their proper identification, and allowing the study of processes such as transmission, the evolution of host specificity, patterns of speciation, and the evolution and control of drug resistance [8].

In the case of *Ascaris*, there were described *Ascaris lumbricoides* (Linnaeus, 1758) and *Ascaris suum* (Goeze, 1782) infecting humans and pigs, respectively. Different molecular methods like restriction-fragment-length polymorphism (PCR-RFLP), single-strand conformation polymorphism (PCR-SSCP), random amplified polymorphic DNA (RAPD), DNA fingerprinting (AFLP), real-time PCR, and microsatellite markers allow the study of the population structure of infections in pigs and people around the world, the identification of adult worms collected from occasional autochthonous human infections, the determination of the maternal source of larvae recovered from a host, the verification of the development of eggs after inactivation processes, and for the identification of *Ascaris* eggs in human

coprolites [9]. In this way, it has been possible to confirm the identity of *A. lumbricoides* and *A. suum*, as well as to detect their hybrids [10], likewise, there have been reported interesting findings such as cross-infections and hybridization in non-endemic countries like Italy [11], and high number of infections with *A. suum* in humans in China [12].

Similarly, within the *Trichuris* genus (Roederer, 1761), morphological differentiation of species is very difficult as the ranges of most characters are overlapped in some species [13]. For instance, in early studies based on morphology of adult worms, *Trichuris suis* and *Trichuris trichiura,* parasites of pigs and humans, respectively, were considered as the same species. However, further morphological, biometrical, and molecular studies had confirmed that *T. trichiura* and *T. suis* are morphologically very similar but genetically different species that can be differentiated by digestion of DNA with endonucleases [14]. In addition, the use of molecular techniques has made it possible to identify cross-infections of humans with *T. suis*, and of pigs with *T. trichiura* [15,16], as well as infections of humans with *Trichuris vulpis*, the whipworm of dogs [17].

Regarding *Blastocystis*, some studies consider it as a commensal because most infected humans are asymptomatic, although, in other cases, the pathogenicity of *Blastocystis* has been showed [18]. Through the use of molecular tools it has been possible to identify a remarkable degree of genetic variability within the small subunit ribosomal RNA gene of *Blastocystis*, and subsequently there have been proposed 29 genetic groupings called subtypes, which are important when trying to explain the pathogenicity and tracing the infection routes, and zoonotic potential within the genus [19].

Furthermore, there have been described eight species of *Giardia* including *Giardia duodenalis*, a species complex with eight assemblages (A-H) based on genetic differences and host specificity [4]. Assemblages A and B are zoonotic, and have been furtherly subdivided in sub-assemblages (AI, AII, BIII,BIV) based on multi-locus genotyping of the 18SrRNA, triose phosphate isomerase, beta-giardin, and analyses of single nucleotide polymorphisms [20]. Assemblages C and D have been largely reported in dogs, assemblage E in hoofed animals, assemblage F in cats, assemblage G in rodents, and assemblage H in pinnipeds [21].

Taking into account the importance of the use of molecular techniques for the study of parasites, this study aimed to assess the prevalence of intestinal parasites in NHPs living in two scenarios, native forest fragments in Colombia and captive conditions in Italy (two wildlife recovery centres and a zoological garden in central Italy), to molecularly characterise selected parasite species of zoonotic interest (*Blastocystis* sp., *Giardia* sp., *Ascaris* sp., *Trichuris* sp.), and to provide a preliminary description of the distribution of parasite taxa in different ecological contexts.

2. METHODOLOGY

2.1 Study sites, primate species, and sample collection

2.1.1 Free-ranging primates

Fieldwork was carried out between December 2019 and February 2022, in seven locations (San Juan, Cumaral, Cabuyaro, Guacavía, Villavicencio, Maní, Yopal) in Santander, Meta and Casanare Departments, in Colombia (Figure 1).

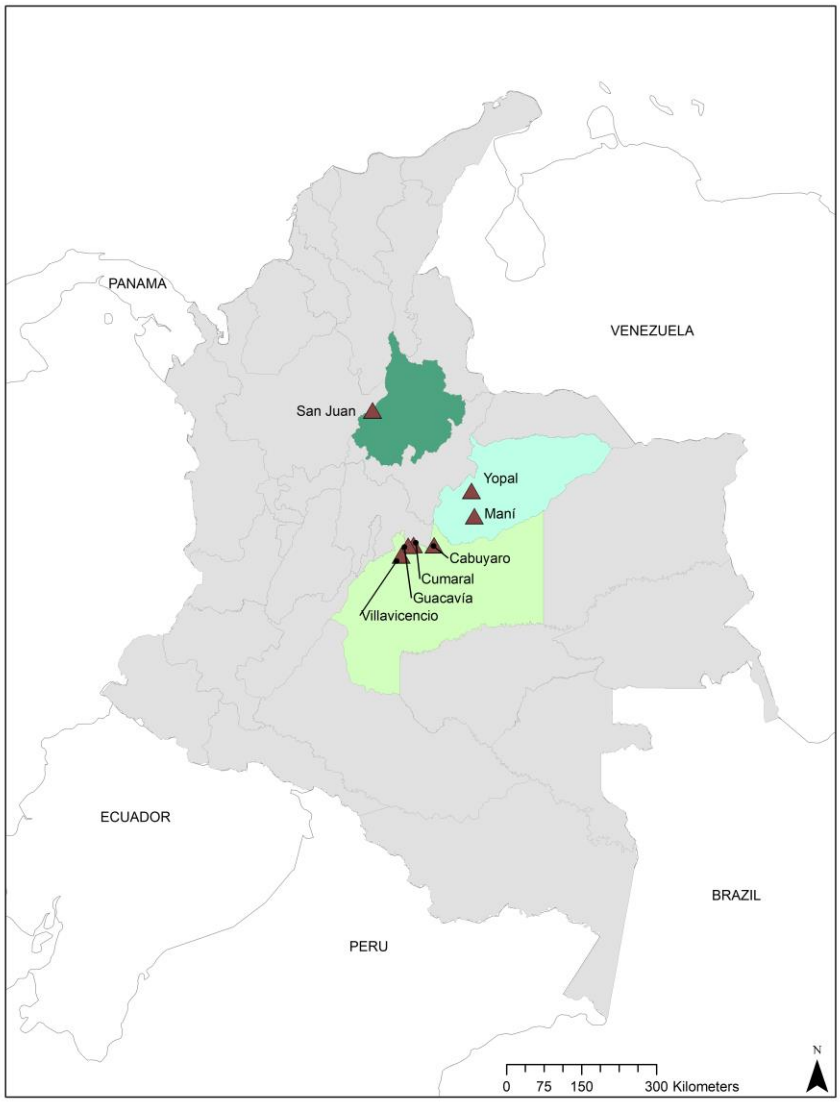


Figure 1. Study sites in Colombia. Santander Department (San Juan), Casanare Department (Yopal, Maní), Meta Department (Cabuyaro, Cumaral, Guacavía, Villavicencio).

Primates were followed from dawn to dusk, and one faecal sample per individual was collected from the soil immediately after defecation. For each sample, one aliquot was stored in 10% formalin solution, and another aliquot in 96% ethanol solution. Overall, 212 samples were collected from six primate species, including free-ranging *Ateles hybridus*, *Cebus versicolor*, *Alouatta seniculus*, *Aotus griseimembra*, *Sapajus apella* and *Saimiri cassiquiarensis* (Table 1).

Table 1. Study sites and number of samples collected per primate species in Colombia.

Study site	Characteristics of the forest	Coordinates	Department	Primate species	N samples
San Juan	Flooded, surrounded by cattle ranching	06°43′ N 74°09′ W	Santander	*Alouatta seniculus*	28
				Cebus versicolor	20
				Ateles hybridus	13
				Aotus griseimembra	5
Cumaral	*Terra firme,* surrounded by cattle ranching and palm oil plantations	04°17′ N 73°24′ W	Meta	*Saimiri cassiquiarensis*	42
Villavicencio	*Terra firme,* surrounded by urbanization	04°06′ N 73°38′ W	Meta	*Saimiri cassiquiarensis*	24
Guacavía	*Terra firme,* surrounded by cattle ranching	04°17′ N 73°30′ W	Meta	*Saimiri cassiquiarensis*	30
Cabuyaro	*Terra firme,* surrounded by palm oil plantations	04°17′ N 73°02′ W	Meta	*Sapajus apella*	3
Maní	*Terra firme,* surrounded by cattle ranching and palm oil plantations	04°48′ N 72°19′ W	Casanare	*Alouatta seniculus*	18
Yopal	*Terra firme,* surrounded by cattle ranching	05°16′ N 72°22′ W	Casanare	*Sapajus apella*	18
				Alouatta seniculus	11

These primate species are grouped into three families: Atelidae (*A. hybridus* and *A. seniculus*), Aotidae (*A. griseimembra*), and Cebidae (*C. versicolor*, *S. apella* and *S. cassiquiarensis*).

The brown spider monkey (*A. hybridus*) is found in north-eastern Colombia and across the Andes into western Venezuela. This species forages and spends most of the time in the upper levels of the forest, is mainly frugivorous, and lives in multi-

male/multi-female groups. It is listed as Critically Endangered with a decreasing population trend, according to the International Union for Conservation of Nature (IUCN) [22].

The red howler monkey (*A. seniculus*) has a wide geographical distribution, mainly in Colombia and Ecuador, but is also found in Venezuela, Brazil, Peru, and Trinidad and Tobago. Its diet includes a large proportion of leaves and mature fruits, as well as flowers, moss, stems, termitaria, and seeds as secondary items. The species lives in small groups (2-13 animals) with usually a dominant male, sub-adults, juveniles, and 2-5 females. This species is listed as Least Concern, however, the population has a decreasing trend [23].

The grey-handed night monkey (*A. griseimembra*) is found in the lowland forests of the Magdalena River and the valleys of the Cauca and San Jorge Rivers in northern Colombia, as well as in the north-west of Venezuela. This species is nocturnal, socially monogamous living in small groups including an adult couple and offspring, and its diet includes nectar, flowers, leaves, and insects. This species is listed as Vulnerable with a decreasing population trend according to the IUCN [24].

The varied white-fronted capuchin (*C. versicolor*) is distributed into the lowland forest of the Magdalena River in northern Colombia between the Eastern and Central Andes Mountains. This species lives in multi-male multi-female groups, is typically found in the lower to mid-canopy and understory, and has a wide diet including fruits, vegetative parts of plants, eggs, invertebrates, and vertebrates. This species is assessed as Endangered with a decreasing population trend [25].

The black-capped capuchin (*S. apella*) is listed as Least Concern with a decreasing population trend. It is widely distributed mainly in Colombia and

Ecuador, but it is also found in Bolivia, Brazil, French Guiana, Guyana, Peru, Suriname, and Venezuela. The species is frugivorous-insectivorous, including fruits, seeds, frogs, arthropods, flowers, leaves, and stems in its diet. The socials groups are composed of an average of 18 individuals, with numbers of females exceeding the numbers of males [26].

The squirrel monkey (*S. cassiquiarensis*) occurs in the Colombian Llanos, and in the Amazon region, including Ecuador, Peru, Colombia, Venezuela, and Brazil. The social groups include several males, several females, juveniles, and infants. This species is listed as Least Concern given its widen range and its adaptability to some degree of forest disturbance, however, the population trend is unknown [27].

2.1.2 Captive primates

Faecal samples and adult nematodes were collected during 2020-2021 from NHPs living in confined environments in Italy.

Thirty-two faecal samples from *Macaca tonkeana* (n=4), *Macaca fascicularis* (n=9) and *Sapajus apella* (n=19) were collected into the wildlife recovery centre Parco Faunistico Piano dell'Abatino (Rieti, Lazio), in the framework of a routine parasitological survey. In this centre, the animals are hosted in different premises. There is one premise for *M. tonkeana* with six individuals, one premise for *M. fascicularis* with seven individuals, and three premises for *S. apella* with eleven, twelve, and fourteen individuals, respectively. Samples were collected from the soil inside the premises and were not directly related to a specific individual. For each sample, one aliquot was stored in 10% formalin solution, and one aliquot in 70% ethanol solution.

Additionally, two dead macaques were inspected during necropsies carried out at the Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "Mariano Aleandri" to depict the cause of death. Ten entire adult nematodes (three males and seven females) and few disrupted nematode body portions were collected from the caecum of one dead *M. fascicularis* hosted at the Maremma Center for the Recovery of Exotic Wild Animals (Semproniano, Tuscany), and from one dead *Macaca fuscata* hosted at the Bioparco Zoological Garden of Rome (Rome, Lazio) eight adult nematodes (all females - not well preserved) were collected from the caecum.

The captive primate species here sampled included three catarrhine species (*M. tonkeana*, *M. fascicularis,* and *M. fuscata*), and one platyrrhini species (*S. apella*).

The Tonkean macaque (*M. tonkeana*) is found in Sulawesi (Indonesia), inhabiting primary and secondary tropical rainforest, and forming multi-male multi-female groups. This species has a diverse diet, including fruits, plants, insects, and cultivated crops when available. The species is considered as Vulnerable with a decreasing population trend [28].

The long-tailed macaque (*M. fascicularis*) has a patchy distribution across south and southeast Asia (Cambodia, India, Indonesia, Malaysia, Myanmar, Philippines, Singapore, Timor-Leste, Vietnam, Brunei Darussalam), living in a wide range of habitats (forests, hills, mountains, and coasts), in group sizes of 10 to 40 individuals. This species is listed as Endangered with a decreasing population trend according to the IUCN [29].

The Japanese macaque (*M. fuscata*) is endemic to Japan, with an expanding population and therefore, listed as Least Concern according to the IUCN. This

species feeds on fruits, leaves, seeds, insects, cultivated crops, and small animals, and occurs in evergreen and deciduous forests [30].

The black-capped capuchin (*S. apella*) features have been already provided in the "2.1.1 Free-ranging primates" section.

2.2 Morphological analyses

2.2.1 Faecal samples

Faecal samples stored in 10% formalin solution were used to perform smears with 1% iodine solution and 0.85% saline solution [31]. For each sample, two microscope slides were mounted and systematically examined under a microscope using magnifications at 100x, 400x and 1000x.

Additionally, flotation with a salt-sugar solution was performed. Each sample was placed in a 15ml Falcon tube with flotation solution, followed by centrifugation at 1800g for 5 minutes. Flotation solution was added until a slight positive meniscus was formed, and a coverslip was placed on the top of the tube. After 10 minutes, the coverslip was removed and placed into a microscope slide. Thereafter, the meniscus was taken and placed into a new 15ml Falcon tube and flotation solution was added until the formation of a slight positive meniscus. A coverslip was placed on the top of the tube and after 10 minutes the coverslip was removed and placed into a microscope slide with a drop of iodine solution. For each faecal sample, one microscope slide was examined using the objectives 10x, 40x and 100x after the flotation procedure.

Morphological identification of protozoan and helminth parasites was performed after direct faecal smears and flotation. Photos and measures of parasites

(larvae, cysts, oocysts) were taken for later identification according to identification keys [32,33].

2.2.2 Adult nematodes

Collected adult nematodes were repeatedly washed with saline solution and then clarification in lactophenol and morphological observation were carried out. The general gross morphology of the nematodes corresponded to the *Trichuris* genus (whipworms).

From specimens collected from *M. fascicularis,* three male and four female were used for biometric measurements of the main available diagnostic morphological features, according to Rivero et al. [34], as following: for males (=M): M1 = total body length; M2 = Length of esophageal region of body; LP = Length of posterior region of body; M4 = Maximum width of posterior region of body, thickness; M5 = Body width in the place of junction of esophagus and the intestine; M8 = Length of spicule; M10 = Width of proximal end of spicule; M12=Maximum width of spicule sheath; for females (=F): F1 = Total body length of adult worm; F2 = Length of oesophageal region of body; LP = Length of posterior region of body; F3 = Width of esophageal region of body; F4 = Maximum width of posterior region of body, thickness; F5 = Body width in the place of junction of oesophagus and the intestine).

2.3 Molecular analyses

2.3.1 Faecal samples

- DNA extraction

For faecal samples microscopically classified as positive for *Blastocystis* sp., *Giardia* sp., and *Ascaris* sp. the respective aliquots stored in 96% ethanol solution were individually subjected to DNA extraction, using the Isolate II Fecal DNA Kit (Meridian Bioscience) according to the manufacturer's protocol.

- PCR, RFLP, and electrophoresis

For *Blastocystis*, two conventional PCR were performed using the pair of primers BhRDr - RD5 and Blast505-532 - Blast998-1017, in order to amplify two different regions of the small subunit ribosomal RNA (SSU rRNA or 18S) gene, following published methods [35,36]. The amplified fragments contain variable regions suitable for *Blastocystis* subtyping [37].

For *Giardia*, a conventional PCR was carried out using the forward primer RH11 and the reverse primer RH4, in order to amplify a 292 bp fragment of the SSU rDNA gene, following published methods [38]. As different *Giardia* loci differ in total, synonymous, and nonsynonymous substitution rates, their applications vary according to the aim of the study, from species differentiation, genotyping (assemblage differentiation), to subtyping. The conserved SSU rRNA gene of *Giardia,* with 0.01 substitutions per nucleotide, is traditionally used for species and assemblage differentiation (mostly genotyping) [20].

For both *Blastocystis* and *Giardia*, all PCR products were visualized on a 1% agarose gel stained with SYBR Safe and positive samples were purified using Sure Clean Plus (Bioline, UK) and shipped to an external company for sequencing (Eurofins Genomics). Sequences were manually edited using Trace implemented in MEGA7 [39] for quality check and inference of consensus sequences and thereafter were used as input for BLAST search and strain/allele assignment using the PubMLST.org

website and reference datasets for each genus, according to the most recent available literature (Tables 2 and 3).

For *Ascaris*, a conventional PCR was carried out in order to amplify the entire ITS nuclear ribosomal region, using the forward primer NC5 and the reverse primer NC2. PCR products were visualized on a 1% agarose gel stained with SYBR Safe. Then, positive amplicons were digested with the *Hae*III restriction endonuclease, followed by an electrophoresis in a 2% agarose gel stained with SYBR Safe. PCR-RPLF of the ITS region allow to differentiate *Ascaris lumbricoides* and *Ascaris suum* [40], thus it was here conducted according to published methods [11].

- Phylogenetic analyses

For *Blastocystis* and *Giardia* obtained sequences, alignments were carried out per each partial region of the studied genes, using ClustalW implemented in MEGA7. A first comparison aimed to confirm the strain/assemblage identity and to explore the phylogenetic relationships was performed including reference sequences of all available *Blastocystis* strains (Table 2), and *Giardia* assemblages A-G (Table 3). Assemblage H was not included in the analyses since homologous sequences are missing in public repositories. Verified sequences here obtained were submitted to GenBank and the accession numbers are listed in the tables.

Table 2. Material used for strain assignment according to *Blastocystis hominis* 18S polymorphisms. *samples analysed in the present study; a: primers Blast 505-532 - Blast 998-1017 from Santin et al. 2011[36]; b: primers BhRDr-RD5 from Scicluna et al. 2006 [35]. • Free-ranging, ♂ Captive.

Subtype code	Host	Accession number	Country
ST1_USA	Human	U51151	USA
ST1_Thai	Human	AY618266	Thailand
ST2_Jap1	Human	AB070987	Japan
ST2_Jap2	*Macaca fuscata*	AB070997	Japan
ST3_Sen	Human	JX132219	Senegal
ST3_Jap	Cattle	AB107965	Japan

ST4_Ger	Human	AY244620	Germany
ST4_Jap	Rat	AB071000	Japan
ST5_Jap	Cattle	AB107966	Japan
ST5_Ger	Pig	MK801369	Germany
ST6	Human	AB091236	Unknown
ST6_Bra	Chicken	MW538478	Brazil
ST7_Chi	Human	DQ366343	China
ST7_Bra	Chicken	MW538475	Brazil
ST8_Jap1	*Varecia variegata*[ϕ]	AB107970	Japan
ST8_Jap2	Pheasant	AB107971	Japan
512*a	Red howler monkey[•]	OP328758	Colombia
603*a	Red howler monkey[•]	OP328759	Colombia
615*a	Red howler monkey[•]	OP328760	Colombia
616*a	Red howler monkey[•]	OP328761	Colombia
61*a	Red howler monkey[•]	OP328762	Colombia
63*a	Red howler monkey[•]	OP328763	Colombia
79*a	Red howler monkey[•]	OP328764	Colombia
81*a	Red howler monkey[•]	OP328765	Colombia
548*b	Red howler monkey[•]	OP329405	Colombia
603*b	Red howler monkey[•]	OP329406	Colombia
608*b	Red howler monkey[•]	OP329407	Colombia
615*b	Red howler monkey[•]	OP329408	Colombia
616*b	Red howler monkey[•]	OP329409	Colombia
617*b	Red howler monkey[•]	OP329410	Colombia
61*b	Red howler monkey[•]	OP329411	Colombia
63*b	Red howler monkey[•]	OP329412	Colombia
79*b	Red howler monkey[•]	OP329413	Colombia
81*b	Red howler monkey[•]	OP329414	Colombia
ST9_Jap	Human	AF408425	Japan
ST9_Den	Human	KC138681	Denmark
ST10_USA	Cattle	MT898456	USA
ST10_Lib	Dromedary	KC148207	Libya
ST11_USA	Elephant	MT898454	USA
ST12_unk	Wallaby	EU427515	Unknown
ST13_UK	Mousedeer	KC148209	UK
ST14_USA1	Cattle	MT898458	USA
ST14_USA2	Cattle	MT898459	USA
ST15_UK	Gibbon[ϕ]	KC148211	UK
ST16_unk1	Red kangaroo	EU427512	Unknown
ST16_unk2	Red kangaroo	EU427514	Unknown
ST17_Lib	Gundi	KC148208	Libya
ST21_USA	White-tailed deer	MW887929	USA
ST23_USA	Cattle	MW887931	USA
ST24_USA	White-tailed deer	MW887928	USA
ST25_USA	Cattle	MW887933	USA
ST26_USA	Cattle	MW887932	USA
ST27_Bra	Indian peafowl	MW887934	Brazil
ST28_Bra	Indian peafowl	MW887935	Brazil

ST29_Bra	Chicken	MW538473	Brazil

Table 3. Material used for assemblage assignment according to *Giardia duodenalis* 18S polymorphisms.

Assemblage code	Host	Accession number	Country
LC437354_assA	Dog	LC437354	Japan
MK487706_assB	Goat	MK487706	Nigeria
LC437359_assC	Dog	LC437359	Japan
LC437362_assD	Dog	LC437362	Japan
LN875383_assE	Chamois	LN875383	Italy
LC341258_assF	Cat	LC437365	Japan
AF199450_assG	Rat	AF199450	Australia
71_c	*Sapajus apella*	Pending	Colombia
73_c	*Sapajus apella*	Pending	Colombia
77c	*Alouatta seniculus*	Pending	Colombia
79c	*Alouatta seniculus*	Pending	Colombia
81c	*Alouatta seniculus*	Pending	Colombia
82c	*Alouatta seniculus*	Pending	Colombia
564c	*Alouatta seniculus*	Pending	Colombia
603f	*Alouatta seniculus*	Pending	Colombia

The best evolutionary models were obtained using ModelTest implemented in MEGA7. The Maximum Likelihood (ML) method was used to infer phylogenies with the aim to confirm the relationships among *Blastocystis* and *Giardia* here identified, respectively, with the other strains/assemblages. Statistical support at nodes were obtained using the bootstrap pseudoreplication method.

Moreover, evolutionary relationships among *Blastocystis* sequences belonging to the same strain circulating in NHPs in Mexico and South America (Table 4) were explored in a smaller scale using phylogenetic network analysis, in particular selecting the Median Joining Network [41] carried out with PopART [42].

Table 4. Material used for partial 18S sequencing comparison of *Blastocystis hominis* ST8 infecting platyrrhines, using Median Joining Network. a: primers Blast 505-532 - Blast 998-1017 from Santin et al. 2011 [36]; b: primers BhRDr-RD5 from Scicluna et al. 2006 [35]. • Free-ranging, $^{\phi}$Captive.

Subtype code	Host	Accession number	Country
NHPJap1	*Varecia variegata*$^{\phi}$	AB107970	Japan
Jap2	Pheasant	AB107971	Japan

AtBr1a	*Ateles* sp.$^{\phi}$	MG280768	Brazil
LlBr1a	*Lagothrix lagotricha*$^{\phi}$	MG280770	Brazil
ApMe1a	*Alouatta palliata*$^{\bullet}$	KT591854	Mexico
ApMe3a	*Alouatta pigra*$^{\bullet}$	KT591853	Mexico
AlBr1a	*Alouatta* sp.$^{\phi}$	MG280771	Brazil
AoBr1a	*Aotus* sp.$^{\phi}$	MG280767	Brazil
ApEc1b	*Alouatta palliata aequatorialis*$^{\bullet}$	KM374608	Ecuador
ApEc2b	*Alouatta palliata aequatorialis*$^{\bullet}$	KM374609	Ecuador
ApEc3b	*Alouatta palliata aequatorialis*$^{\bullet}$	KM374610	Ecuador
AfBr1b	*Ateles fusciceps*$^{\phi}$	MH784453	Brazil
AnPe1b	*Aotus nigriceps*$^{\bullet}$	MT509449	Peru
AnPe2b	*Aotus nigriceps*$^{\bullet}$	MT509450	Peru
AnPe3b	*Aotus nigriceps*$^{\bullet}$	MT509451	Peru

2.3.2 Adult nematodes

- DNA extraction

From each collected specimen, except one from *M. fascicularis* that was fixed and mounted in a microscope slide, a body portion not useful for detailed morphological observations was used for DNA extraction, using the Isolate II Genomic DNA Kit (Meridian Bioscience) according to the manufacturer's protocol.

- PCR and electrophoresis

Conventional PCR for molecular characterisation based on sequence analyses of the two partial mitochondrial regions *cox*1 and *rrn*L, informative for phylogenetic assignment, were conducted according to published methods [34,43]. All PCR products were visualized on a 1% agarose gel stained with SYBR Safe and amplicons were purified using Sure Clean Plus (Bioline, UK) and shipped to an external company for sequencing (Eurofins Genomics).

- Phylogenetic analyses

The obtained sequences were compared to homologous GenBank retrieved data, and used for phylogenetic inferences with the maximum likelihood (ML) method, after

testing for best evolutionary models explaining the data [39]. Sequences of *Trichinella spiralis* and *Trichinella britovi* were used as outgroups (AF293969, KM357413). Tables 5 and 6 show the material used for comparative analyses, as well as the accession numbers of the verified sequences here obtained and submitted to GenBank.

Table 5: Material used for *Trichuris* phylogenetic inferences based on the partial mitochondrial rrnL region.

Code	Host species	GenBank accession number	Reference
TMM5	*Macaca sylvanus*	MW448471	Rivero et al. (2021) [34]
TRMF4	*Macaca fuscata*	MW403712	Cavallero et al. (2021) [44]
TRMF34	*Macaca fuscata*	MW403713	Cavallero et al. (2021) [44]
TRM48	*Macaca fuscata*	MW403714	Cavallero et al. (2021) [44]
TRMF61	*Macaca fuscata*	MW403715	Cavallero et al. (2021) [44]
TRMF72	*Macaca fuscata*	MW403716	Cavallero et al. (2021) [44]
MFA3	*Macaca fuscata*	MN088542	Cavallero et al. (2019) [43]
MFA5	*Macaca fuscata*	MN088543	Cavallero et al. (2019) [43]
TF1	*Trachypithecus francoisi*	KC481232	Liu et al. (2013) [45]
TF2	*Trachypithecus francoisi*	KC481233	Liu et al. (2013) [45]
TPM1	*Papio papio*	MW448472	Rivero et al. (2021) [34]
TMF31	*Macaca sylvanus*	MW448470	Rivero et al. (2021) [34]
TRMF87	*Macaca fuscata*	Pending	Present study
RIS1	*Macaca fascicularis*	Pending	Present study
RIS2	*Macaca fascicularis*	Pending	Present study
RIS3	*Macaca fascicularis*	Pending	Present study
RIS5	*Macaca fascicularis*	Pending	Present study
RIS6	*Macaca fascicularis*	Pending	Present study
RIS7	*Macaca fascicularis*	Pending	Present study
RIS8	*Macaca fascicularis*	Pending	Present study
RIS9	*Macaca fascicularis*	Pending	Present study
RIS10	*Macaca fascicularis*	Pending	Present study
H1	*Homo sapiens*	GU385218	Liu et al. (2012) [46]
H2	*Homo sapiens*	AM993017	Liu et al. (2012) [46]
H9	*Homo sapiens*	KP781899	Meekums et al. (2015) [47]
H10	*Homo sapiens*	KP781900	Meekums et al. (2015) [47]
H22	*Homo sapiens*	KU524541	Hawash et al. (2016) [48]
H23	*Homo sapiens*	KU524542	Hawash et al. (2016) [48]
CA1	*Chlorocebus aethiops*	MN088565	Cavallero et al. (2019) [43]
CA2	*Chlorocebus aethiops*	MN088566	Cavallero et al. (2019) [43]
CS1	*Chlorocebus sabaeus*	MN088559	Cavallero et al. (2019) [43]
CS2	*Chlorocebus sabaeus*	MN088560	Cavallero et al. (2019) [43]
C2	*Chlorocebus sabaeus*	KU524595	Hawash et al. (2016) [48]
C3	*Chlorocebus sabaeus*	KU524596	Hawash et al. (2016) [48]
P1	*Papio* sp.	KU524558	Hawash et al. (2016) [48]
P2	*Papio* sp.	KU524559	Hawash et al. (2016) [48]
PH92	*Papio hamadryas*	MN088578	Cavallero et al. (2019) [43]
PH93	*Papio hamadryas*	MN088579	Cavallero et al. (2019) [43]

S1	*Sus scrofa*	KP781894	Hawash et al. (2016) [48]
S2	*Sus scrofa*	KP781895	Hawash et al. (2016) [48]
CG1	*Colobus guereza*	MN088583	Cavallero et al. (2019) [43]
CG2	*Colobus guereza*	MN088584	Cavallero et al. (2019) [43]

Table 6: Material used for *Trichuris* phylogenetic inferences based on the partial mitochondrial cox1 region.

Code	**Host species**	**GenBank accession number**	**Reference**
JPT6_co1	*Macaca fascicularis*	JF690967	Petrasova et al. (2016) (unpublished)
RIS1	*Macaca fascicularis*	OP108821	Present study
RIS3	*Macaca fascicularis*	OP108822	Present study
RIS5	*Macaca fascicularis*	OP108823	Present study
RIS6	*Macaca fascicularis*	OP108824	Present study
TMM5_co1	*Macaca sylvanus*	MW448471	Rivero et al. (2021) [34]
TMF31_co1	*Macaca sylvanus*	MW448470	Rivero et al. (2021) [34]
TM18_co1	*Macaca sylvanus*	LR130784	Cutillas et al. (2020) (unpublished)
TPM1_co1	*Papio papio*	MW448472	Rivero et al. (2021) [34]
H1_co1	*Homo sapiens*	GU385218	Liu et al. (2012) [46]
H2_co1	*Homo sapiens*	AP017704	Kikuchi et al. (2019) (unpublished)
H3_co1	*Homo sapiens*	KT449826	Hawash et al. (2015) [49]
H4_co1	*Homo sapiens*	JF690962	Petrasova et al. (2016) (unpublished)
C6_co1	*Colobus* sp.	FR846241	Callejón et al. (2016) (unpublished)
C1_co1	*Colobus guereza*	HE653116	Callejón et al. (2013) [50]
C2_co1	*Colobus guereza*	HE653117	Callejón et al. (2013) [50]
Cg1_co1	*Colobus guereza*	MK762948	Cavallero et al. (2019) [43]
Cg2_co1	*Colobus guereza*	MK762949	Cavallero et al. (2019) [43]
Ca3_co1	*Chlorocebus aethiops*	MK762931	Cavallero et al. (2019) [43]
Ca4_co1	*Chlorocebus aethiops*	MK762932	Cavallero et al. (2019) [43]
Ca14_co1	*Chlorocebus aethiops*	MK762941	Cavallero et al. (2019) [43]
Ca15_co1	*Chlorocebus aethiops*	MK762942	Cavallero et al. (2019) [43]
Cs1_co1	*Chlorocebus sabaeus*	MK762923	Cavallero et al. (2019) [43]
Cs2_co1	*Chlorocebus sabaeus*	MK762924	Cavallero et al. (2019) [43]
Cs3_co1	*Chlorocebus sabaeus*	MK762925	Cavallero et al. (2019) [43]
Mfa1_co1	*Macaca fuscata*	MK762905	Cavallero et al. (2019) [43]
Mfa5_co1	*Macaca fuscata*	MK762906	Cavallero et al. (2019) [43]
Mfa6_co1	*Macaca fuscata*	MK762907	Cavallero et al. (2019) [43]
Mfb2_co1	*Macaca fuscata*	MK762908	Cavallero et al. (2019) [43]
Mfb3_co1	*Macaca fuscata*	MK762909	Cavallero et al. (2019) [43]
Mfb4_co1	*Macaca fuscata*	MK762910	Cavallero et al. (2019) [43]
PU1_co1	*Papio ursinus*	LT627353	Callejón and Cutillas (2018) (unpublished)
Ph1_co1	*Papio hamadryas*	JF690963	Petrasova et al. (2016) (unpublished)
Ph91_co1	*Papio hamadryas*	MK762943	Cavallero et al. (2019) [43]
Ph92_co1	*Papio hamadryas*	MK762944	Cavallero et al. (2019) [43]

2.4 Ecological analyses

2.4.1 Parasite prevalence and richness

For the sampling of free-ranging NHPs in Colombia, the Quantitative Parasitology Software [51] was used to calculate the prevalence of parasites per study site and per primate species with more than 30 samples (*A. seniculus* and *S. cassiquiarensis*), with 95% confidence intervals. For captive NHPs it was not possible to calculate the parasite prevalence, as the faecal samples were not related to a specific individual.

The parasite richness was estimated as the number of taxa recovered from the host's faecal samples. For selected parasites of interest infecting free-ranging primates, namely *Blastocystis* sp., *Giardia* sp., strongyliform larvae, and *Entamoeba* sp., additional significance tests based on Fisher test were performed to compare prevalence values on i) the same primate species from different study sites and ii) on different primate species from the same study site.

The RStudio integrated development environment for the R Software was used to perform a two-way analysis of variance (ANOVA) in order to evaluate the effect of primate species and study site on the prevalence and richness of parasites, using a dataset that included the primate species for which more than 30 samples were collected (*A. seniculus* and *S. cassiquiarensis*).

2.4.2 Richness accumulation curves and nestedness

The Vegan package within the RStudio integrated development environment for the R Software was used to build parasite richness accumulation curves for the whole sampling of free-ranging NHPs and for each study site separately, using the specaccum function and the rarefaction method [52].

Additionally, the Vegan package was used to generate a parasite nestedness plot for each study site and for each primate species, and to calculate nested temperature and nodf values on a presence/absence matrix, using the nestedtemp and nestednodft functions respectively [53]. Finally, the significance of nestedness temperature was determined using the quasiswap method and the oecosimu function.

3. RESULTS

3.1 Free-ranging primates

3.1.1 Morphological analyses

About 96% (N=203) of the faecal samples were positive for intestinal parasites. Co-infections with 2-5 parasite taxa were observed in 139 samples. Overall, fifteen parasite taxa were found (Figure 2), including:

- Protozoans (*Blastocystis* sp., *Balantidium* sp., *Dientamoeba fragilis**, *Entamoeba* sp., *Giardia* sp., *Eimeria* sp.).

  **Dientamoeba fragilis* is suspected, confirmation is needed.
- Cestodes (*Hymenolepis* sp., Cestoda).
- Trematodes (*Controrchis* sp., Trematoda).
- Nematodes (*Ascaris* sp., strongyliform larvae**, *Trypanoxyuris* sp., Ancylostomatidae).

  **Based on morphology it was not possible to identify the genus/species.
- Acanthocephalans.

Regarding the selected parasite taxa of zoonotic interest:

- 64 samples were classified as positive for *Blastocystis* infecting all NHP species except *A. griseimembra* from five of the seven study locations.

- *Giardia* sp. was found in 35 samples infecting NHPs from six of the seven study locations, except Cabuyaro, and including all NHP species.

-*Ascaris* sp. was only found in two different samples of *S. cassiquiarensis* from Cumaral.

- No positive samples for *Trichuris* sp. were observed.

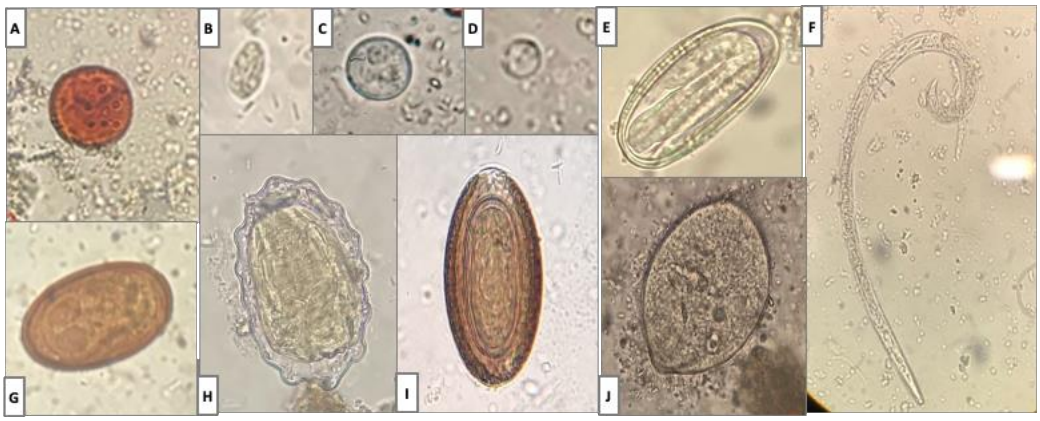


Figure 2. Representative images from the parasite taxa observed by microscopy. A. *Entamoeba* sp. (30 µm), B. *Giardia* sp. (12x8 µm), C. *Dientamoeba fragilis*-like (10 µm), D. *Blastocystis* sp. (8 µm), E. *Trypanoxyuris* sp. (45x20 µm), F. Strongyliform larvae (40x), G. *Controrchis* sp. (35x20 µm), H. *Ascaris* sp. (40x75 µm), I. Acanthocephala (40x70 µm), J. *Balantidium* sp. (50x70 µm).

3.1.2 Molecular analyses

- Blastocystis

For *Blastocystis*, ten high-quality sequences from the PCR carried out with primers BhRDr - RD5 (all collected from *A. seniculus*: five from San Juan, one from Maní, and four from Yopal), and eight high-quality sequences with primers Blast505-532 - Blast998-1017 (all from *A. seniculus*: four from San Juan and four from Yopal) were obtained. For seven samples, sequences were obtained with both pairs of primers. According to the best match in BLAST all sequences were identified as *Blastocystis hominis* ST8, with 97.6 - 99.8% identity to the reference strain sequences.

Strain and allele assignment supported by the use of the PubMLST website confirmed the ST8 identity for the barcoding region and the assignment to allele 21, with the exception of two sequences assigned to alleles 156 (OP329405) and 157 (OP329407).

Results obtained by ML phylogenetic inferences with the T92+G+I model (G=0.63 I=0.56) described the cluster affiliation of material here analysed with reference

sequences of ST8 available for birds from Japan and captive NHPs, for both partial 18S datasets, with 99% and 100% of bootstrap support. The best ML consensus tree built with the dataset of primers Scicluna et al. 2006 [35] is shown in Figure 3, while the best consensus tree built using the dataset of primers Santin et al. 2011 [36] is shown in Supplementary material 1, as the same topology was obtained.

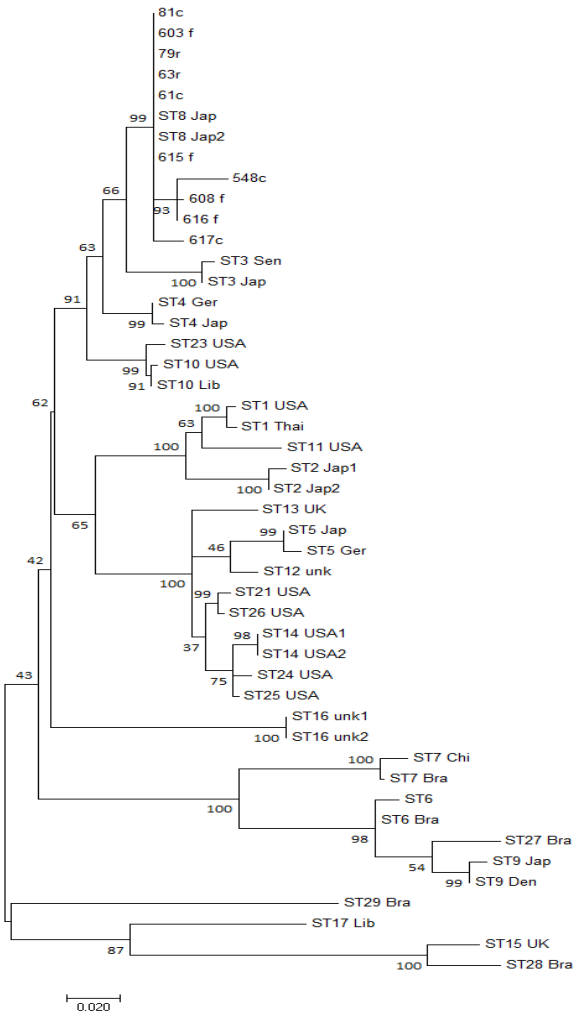


Figure 3. Best ML consensus tree of partial 18S *Blastocystis hominis* reference strains and samples here analysed collected from NHPs in Colombia (dataset primers Scicluna et al. 2006 [35]).

The *Blastocystis hominis* ST8 Median Joining Network built with the dataset of sequences obtained with primers of Scicluna et al. 2006 [35] (Supplementary material 2) showed five haplotypes, with a main haplotype shared by most of the sequences of *A. seniculus* from this study (blue color, Fig 4A) and Peruvian samples of *Aotus nigriceps* (pink color, Fig 4A), separated by three or more SNPs from haplotypes circulating in *Alouatta palliata equatorialis* in Ecuador (red color, Fig 4A). For the dataset of sequences obtained with primers of Santin et al 2011 [36] (Supplementary material 3), four haplotypes were obtained, each one separated from another by one SNP (Figure 4B). Two haplotypes were characterised by Colombian samples circulating in *A. seniculus* from Yopal and San Juan regions (Blue color, Fig4B), separated by a central haplotype shared by Brazilian and Mexican specimens reported in several NHP species (*Ateles* sp., *L. lagotricha*, *A. palliata*, *A. pigra*, *A. fusciceps*) (Pink and green colors, Fig 4B).

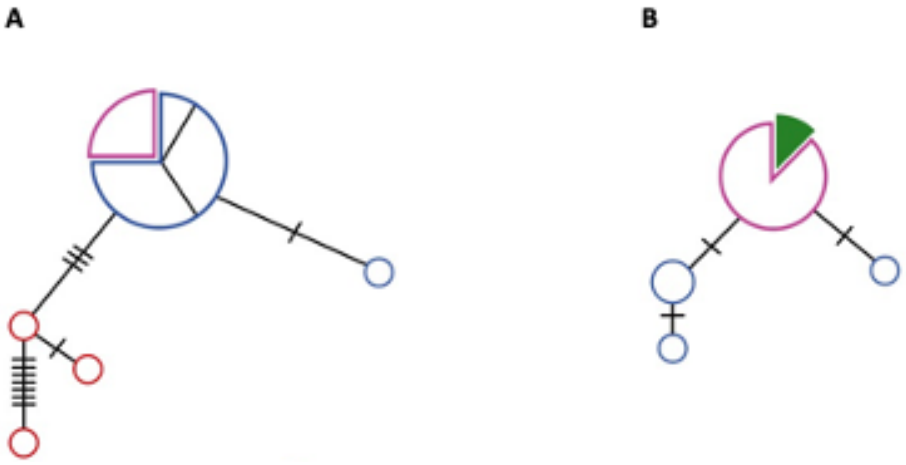


Figure 4. Median Joining Network of *Blastocystis hominis* ST8 partial 18S built with **A.** the dataset of sequences obtained with primers of Scicluna et al. 2006 [35], **B.** the dataset of sequences obtained with primers of Santin et al. 2011 [36], circulating in platyrrhines. The size of each circle is proportional to the frequency of the haplotype and the transversal lines indicate a SNP.

- Giardia

Overall, there were obtained eight high-quality sequences, including one of *A. seniculus* from Maní, two of *S. apella* and four of *A. seniculus* from Yopal, and one of *A. seniculus* from San Juan.

According to the best match in BLAST all sequences were identified as *Giardia duodenalis*, with 95.2 - 100% identity. Sequences are still under evaluation before being deposited in GenBank.

Assemblage assignment was obtained using the PubMLST website and a 297bp alignment with 19 variable positions (Supplementary material 4). Results obtained for the partial 18S dataset by ML phylogenetic inferences with the T92 model described a cluster affiliation of material here analysed with a reference sequence of assemblage B (goat host), and a cluster including two sequences from NHPs from Colombia and reference sequences of assemblages A (dog host) and F (cat host) (Figure 5).

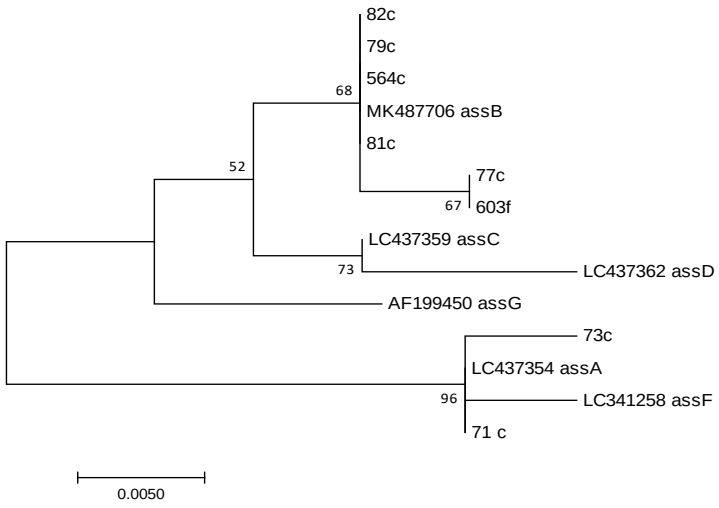


Figure 5. Best ML consensus tree of partial 18S *Giardia duodenalis* reference assemblages and samples here analysed collected from NHPs in Colombia. Assemblage E was removed for length diversity of input sequences.

- Ascaris

For both faecal samples microscopically classified as positive for *Ascaris* sp., a PCR product of around 1000 bp was obtained. After the digestion with the *Hae*III restriction enzyme there were obtained banding pattern belonging to the genus *Ascaris* and the "*lumbricoides"* genotype, displaying two bands of about 610 bp and 370 bp (Figure 6).

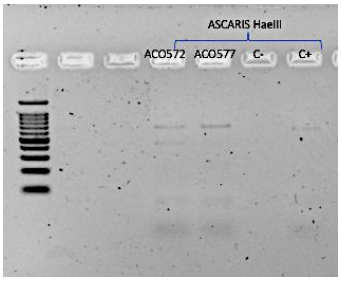


Figure 6. Results of molecular characterisation of *Ascaris* by PCR-RFLP.

3.2 Ecological analyses

3.2.1 Parasite prevalence and richness

- Free-ranging primates

The prevalence of each parasite taxa according to primate species and study sites is shown in tables 7A, 7B, and 7C.

Table 7A. Parasite prevalence (%) in faecal samples, per NHP species, for the two study sites at Casanare department. Confidence limits for the prevalence are indicated between square brackets.

Parasite taxa	**Maní**	**Yopal**	
	Alouatta seniculus (n=18)	*Alouatta seniculus* (n=11)	*Sapajus apella* (n=18)
Blastocystis sp.	8 (44.4%) [21.5%-69.2%]	5 (50.0%) [18.7%-81.3%]	5 (27.8%) [9.7%-53.5%]
Dientamoeba fragilis*	12 (66.7%) [41.0%-86.7%]	2 (18.2%) [2.3%-51.8%]	
***Entamoeba* sp.**		3 (27.3%) [6.0%-61.0%]	

***Giardia* sp.**	4 (22.2%) [6.4%-47.6%]	6 (54.5%) [23.4%-83.3%]	6 (33.3%) [13.3%-59.0%]
Strongyliform larvae**		5 (45.5%) [16.7%-76.6%]	18 (100%) [81.5%-100%]
***Trypanoxyuris* sp.**	2 (11.1%) [1.4%-34.7%]	2 (18.2%) [2.3%-51.8%]	
***Hymenolepis* sp.**			2 (11.1%) [1.4%-34.7%]

**Dientamoeba fragilis* is suspected, confirmation is needed.
** Based on morphology it was not possible to identify the genus/species.

Table 7B. Parasite prevalence (%) in faecal samples, per NHP species, at Santander department. Confidence limits for the prevalence are indicated between square brackets.

Parasite taxa	**San Juan**			
	Alouatta seniculus (n=28)	*Aotus griseimembra* (n=5)	*Ateles hybridus* (n=13)	*Cebus versicolor* (n=20)
Blastocystis sp.	21 (75.0%) [55.1%-89.3%]		7 (53.8%) [25.1%-80.8%]	6 (30.0%) [11.9%-54.3%]
***Balantidium* sp.**	2 (7.1%) [0.9%-23.5%]		10 (76.9%) [46.2%-95.0%]	
Dientamoeba fragilis*	11 (39.3) [21.5%-59.4%]		1 (7.7%) [0.2%-36.0%]	
***Entamoeba* sp.**	5 (17.9%) [6.1%-36.9%]	1 (20.0%) [0.5%-71.6%]	1 (7.7%) [0.2%-36.0%]	3 (15.0%) [3.2%-37.9%]
***Giardia* sp.**	5 (17.9%) [6.1%-36.9%]	1 (20.0%) [0.5%-71.6%]	1 (7.7%) [0.2%-36.0%]	2 (10.0%) [1.2%-31.7%]
***Eimeria* sp.**	1 (3.6%) [0.1%-18.3%]	1 (20.0%) [0.5%-71.6%]		1 (5.0%) [0.1%-24.9%]
Ancylostomatidae				1 (5.0%) [0.1%-24.9%]
Strongyliform larvae**	4 (14.3%) [4.0%-32.7%]	2 (40.0%) [5.3%-85.3%]	7 (53.8%) [25.1%-80.8%]	19 (95.0%) [75.1%-99.9%]
***Trypanoxyuris* sp.**	2 (7.1%) [0.9%-23.5%]			
***Controrchis* sp.**				10 (50.0%) [27.2%-72.8%]
Trematoda				3 (15.0%) [3.2%-37.9%]
Acanthocephala				4 (20.0%) [5.7%-43.7%]

**Dientamoeba fragilis* is suspected, confirmation is needed.
** Based on morphology it was not possible to identify the genus/species.

Table 7C. Parasite prevalence (%) in faecal samples, per NHP species, for the four study sites at Meta department. Confidence limits for the prevalence are indicated between square brackets.

	Cabuyaro	**Cumaral**	**Guacavía**	**Villavicencio**
Parasite taxa	*Sapajus apella* (n=3)	*Saimiri cassiquiarensis* (n=42)	*Saimiri cassiquiarensis* (n=30)	*Saimiri cassiquiarensis* (n=24)
Blastocystis sp.	1 (33.3%) [0.8%-90.6%]	11 (26.2%) [13.9%-42.0%]		
Dientamoeba fragilis*		10 (23.8%) [12.1%-39.5%]	1 (3.3%) [0.1%-17.2%]	
***Entamoeba* sp.**		4 (9.5%) [2.7%-22.6%]	3 (10.0%) [2.1%-26.5%]	2 (8.3%) [1.0%-27.0%]
***Giardia* sp.**		3 (7.1%) [1.5%-19.5%]	3 (10.0%) [2.1%-26.5%]	4 (16.7%) [4.7%-37.4%]
***Eimeria* sp.**			1 (3.3%) [0.1%-17.2%]	
Ancylostomatidae	1 (33.3%) [0.8%-90.6%]			
***Ascaris* sp.**		2 (4.8%) [0.6%-16.2%]		
Strongyliform larvae**	2 (66.7%) [9.4%-99.2%]	38 (90.5) [77.4%-97.3%]	25 (83.3%) [65.3%-94.4%]	23 (95.8%) [78.9%-99.9%]
***Trypanoxyuris* sp.**		1 (2.4%) [0.1%-12.6%]	3 (10.0%) [2.1%-26.5%]	1 (4.2%) [0.1%-21.1%]
***Controrchis* sp.**	1 (33.3%) [0.8%-90.6%]	4 (9.5%) [2.7%-22.6%]	1 (3.3%) [0.1%-17.2%]	
Trematoda		4 (9.5%) [2.7%-22.6%]		1 (4.2%) [0.1%-21.1%]
***Hymenolepis* sp.**		1 (2.4%) [0.1%-12.6%]	1 (3.3%) [0.1%-17.2%]	
Cestoda		1 (2.4%) [0.1%-12.6%]	2 (6.7%) [0.8%-22.1%]	
Acanthocephala		29 (69.0%) [52.9%-82.4%]	19 (63.3%) [43.9%-80.1%]	9 (37.5%) [18.8%-59.4%]

**Dientamoeba fragilis* is suspected, confirmation is needed.
** Based on morphology it was not possible to identify the genus/species.

The prevalence of *Blastocystis* was 30.3%. According to NHP species the prevalence was 53.8% [25.1%-80.8%] for *A. hybridus*, 30.0% [11.9%-54.3%] for *C. versicolor*, 60.7% [46.8%-73.5%] for *A. seniculus*, 28.6% [11.3%-52.2%] for *S. apella*, and 11.3% [5.8%-19.4%] *S. cassiquiarensis*.

For *Giardia*, the prevalence was 16.5%. According to NHP species the prevalence was 10.4% [5.1%-18.3%] for *S. cassiquiarensis*, 26.3% [15.5%-39.7%] for *A. seniculus*, 28.6% [11.3%-52.2%] for *S. apella*, 20.0% [0.5%-71.6%] for *A. griseimembra*, 7.7% [0.2%-36.0%] for *A. hybridus*, 10.0% [1.2%-31.7%] for *C. versicolor*.

Parasite prevalence values obtained from the same primate species from different study sites and from different primate species from the same study site revealed significant differences on *Blastocystis* sp. prevalence for *S. cassiquiarensis* from Cumaral, Guacavía and Villavicencio (p=0.0002) and for *A. hybridus, C. versicolor* and *A. seniculus* from San Juan (p=0.001). Similarly, *Entamoeba* sp. prevalence values were significantly different in *A. seniculus* from San Juan, Maní and Yopal (p=0.05). Prevalence values for strongyliform larvae were significantly different in *A. seniculus* from San Juan, Maní and Yopal (p=0.003), and in different primate species (*A. hybridus, A. griseimembra, C. versicolor* and *A. seniculus*) from San Juan (p<0.001), and (*A. seniculus* and *S. apella*) from Yopal (p=0.001). Comparisons performed for *Giardia* sp. prevalence were not significant.

Anova and Tukey's tests for the dataset of *A. seniculus* and *S. cassiquiarensis* showed that parasite prevalence was not related to primate species (Anova: p-value= 0.718, F-value= 0.131, Df= 1), nor to study site (Anova: p-value=0.935, F-value= 0.205, Df= 4). While parasite richness was related to primate species (Anova: p-value=0.014, F-value=6.063, Df=1), as well as to the study site (Anova: p-value:0.001, F-value=4.506, Df=4), (Tukey's test: *S. cassiquiarensis* vs. *A. seniculus* (0.014), Villavicencio vs. Cumaral (p-value:0.005)) (Supplementary material 5).

3.2.2 Richness accumulation curves and nestedness

The obtained richness accumulation curves for intestinal parasites found at each study site and for the whole sampling on free-ranging primates are shown in Figure 7.

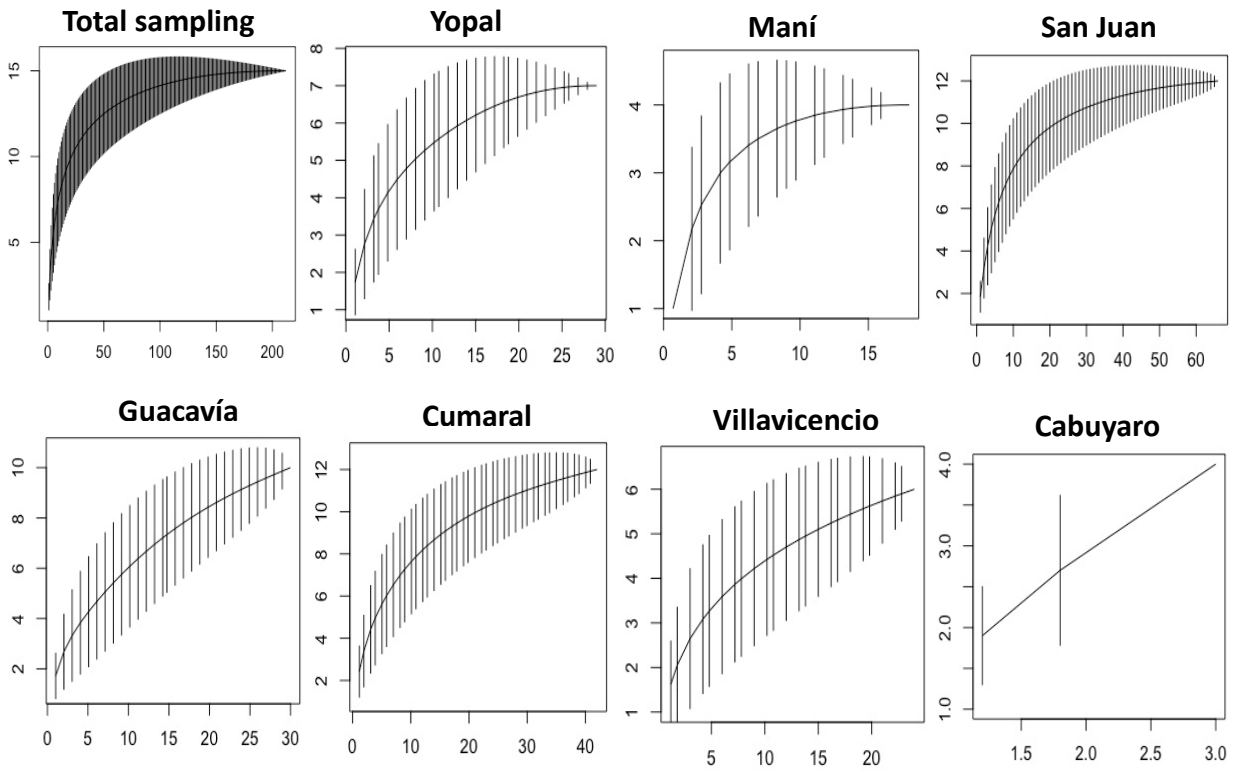


Figure 7. Richness accumulation curves for the whole sampling of free-ranging primates, and for each study site. The Y axes indicate the parasite richness (number of parasite taxa) and the X axes the number of samples from NHPs.

For nestedness, the obtained plots are shown in Figure 8. According to the nested temperature values, the nodf values, and the nestedness temperature significance values, there is no evidence of a perfect nestedness achieved for the parasite taxa datasets from the study sites where free-ranging primates were sampled: Guacavía (nestedness temperature: 2.352, nodf: 48.545, P: 0.204), Villavicencio (nestedness temperature: 4.768, nodf: 45.377, P:0.642), Cumaral (nestedness temperature: 12.912, nodf: 44.118, P: 0.872), Cabuyaro (nestedness temperature: 3.627, nodf: 1.851, P: 0.520), Yopal (nestedness temperature: 3.257, nodf: 41.311, P: 0.110), Maní (nestedness temperature: 1.131, nodf: 22.335, P: 0.816), San Juan (nestedness temperature: 7.534, nodf: 30.008, P: 0.320). A perfect nestedness is given by a nested temperature value=0 or a nodf value= 100. Additionally, a perfect nestedness would be achieved if the occurrence of parasite taxa were entirely to the left of the curved black lines shown in Figure 8.

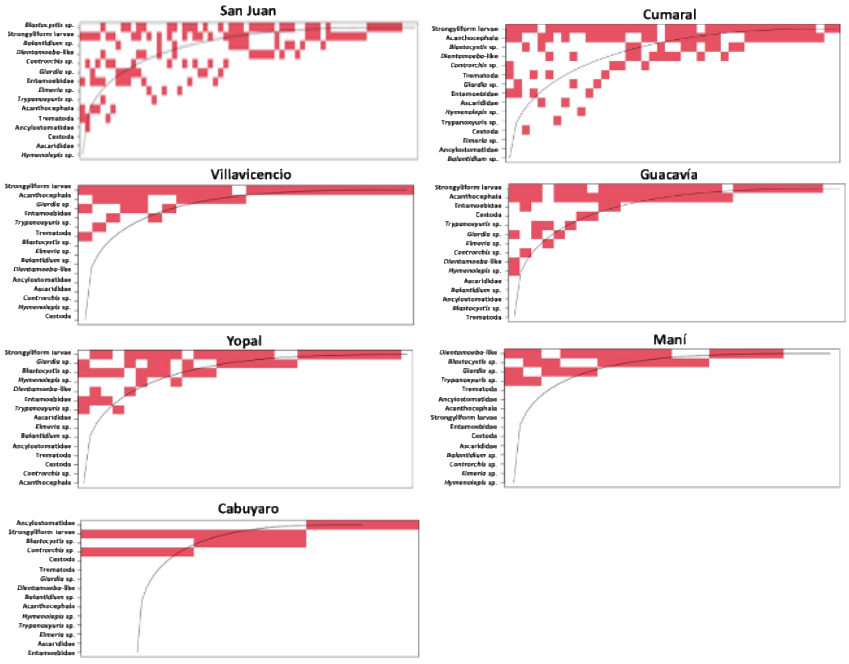


Figure 8. Nestedness plot of intestinal parasites infecting free-ranging primates, per each study site.

Similarly, a perfect nestedness for the parasite taxa datasets from each primate species was not observed. *A. seniculus* (nestedness temperature: 3.556, nodf: 30.351, P: 0.198), *S. cassiquiarensis* (nestedness temperature: 5.304, nodf: 56.308, P: 0.210), *A. hybridus* (nestedness temperature: 6.799, nodf: 26.190, P: 0.778), *C. versicolor* (nestedness temperature: 7.361, nodf: 43.954, P: 0.620), *S. apella* (nestedness temperature: 5.197, nodf: 39.047, P: 0.222), *A. griseimembra* (nestedness temperature: 1.148, nodf: 1.739, P: 1).

3.3 Captive primates

3.3.1 Morphological analyses

- Faecal samples

Four taxa of protozoans and three of helminths were identified in the faecal samples from NHPs living at the Parco Faunistico Piano dell'Abatino (Table 8).

In particular, all NHP species were positive for the potentially zoonotic ciliate *Balantidium* sp. and all samples from *M. tonkeana* showed the presence of *Entamoeba coli*, observed in less extent in samples from *M. fascicularis* and not reported in *S. apella*. *Dientamoeba fragilis*- like and *Trichuris* sp. were observed only in samples from *M. fascicularis*. All parasite taxa were found both by faecal smears and flotation, except *Oesophagostomum* sp. observed only in faecal smears. Representative images from microscopic analyses are available in the Figure 9.

Table 8. Number of positive samples for each parasite taxa, per primate species.

Parasite taxa	***Macaca tonkeana*** (n=4)	***Macaca fascicularis*** (n=9)	***Sapajus apella*** (n=19)
Entamoeba coli	4	3	0
Balantidium sp.	4	1	1
*Dientamoeba fragilis**	0	6	0

Iodamoeba bütschlii	0	5	4
Trichuris sp.	0	2	0
Strongyliform larvae**	0	0	5
Oesophagostomum sp.	1	0	0

**Dientamoeba fragilis* is suspected, confirmation is needed.
** Based on morphology it was not possible to identify the genus/species.

Figure 9. A. *Oesophagostomum* sp. (50x85 µm), B. *Balantidium* sp. (90µm), C. *Trichuris* sp. (25x55 µm), D. Strongyliform larva (40x), E. *Entamoeba coli* (20 µm), F. *Iodamoeba bütschlii* (10x12 µm), G. *Dientamoeba fragilis*-like (10 µm).

- Adult nematodes

Nematodes collected from *M. fascicularis* and *M. fuscata* intestinal caeca were roughly identified as whipworms (genus *Trichuris*), because of the presence of a filiform long anterior part and a broad and handle-like posterior part, typical of whipworms. Most of the measurements were taken from specimens from *M. fascicularis,* given the absence of male specimens and well-preserved females from *M. fuscata*.

The cuticle shows transversal striation and the anterior portion of the body shows bacillary band. Males and females showed similar morphological features described for *Trichuris* sp. in *Macaca sylvanus* [54] and *Papio ursinus* [55].

Males showed the intestine and ejaculatory duct close to the term of testis and a wide proximal cloacal tube followed by the distal cloacal tube and cloaca (Figure 10A). The latter includes the only one spicule, that may be projected or not, always surrounded by a spicular tube covered by spines; the spicule has a clear distal tip (Figure 10B). The ratio between the anterior and posterior body part is 1.44:1 and the thin anterior part is 1:1.69 of the entire length of the body.

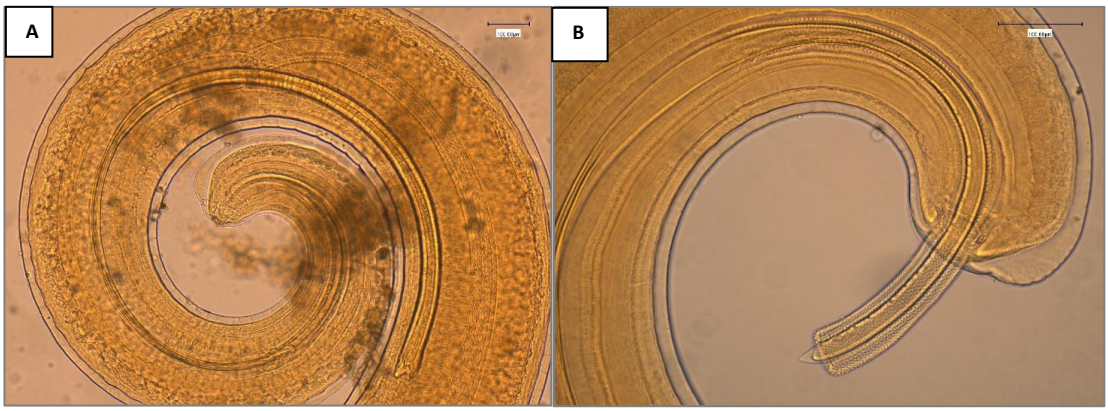


Figure 10. Morphology of male *Trichuris* sp. from *Macaca fascicularis*. **A.** Posterior end showing the arrowed and invaginated spicule, with distal and proximal cloacal tube and ejaculatory duct. **B.** Posterior end with evaginated spicule and spicule sheath with spines.

Females were slightly smaller than males in the esophagus-intestinal junction area corresponding to the vulva (Figure 11A), with an average 370µm for *Trichuris* sp. from both macaques; vulvas were non-protrusive and covered inside with spines followed by a relatively short and slightly circumvoluted vagina (Figure 11B). The distal end showed a subterminal anus (Figure 11C). The eggs measurements ranged from 25.50-27.90 x 54.30-56.80 µm in *Trichuris* from *M. fascicularis* and from 30-35 x 53-61,6 µm in *Trichuris* from *M. fuscata*. The ratio between the anterior and posterior body part is 1.47:1 and the thin anterior part is 1:2.47 of the entire length of the body.

Comparing measurements and gross morphological features to the other *Trichuris spp* included in Table 9, allowed to infer that individual and ratio values are in near complete agreement with those reported for *T. trichiura* in Rivero et al. (2021) [34] and *Trichuris* sp. from *M. sylvanus* [54,56].

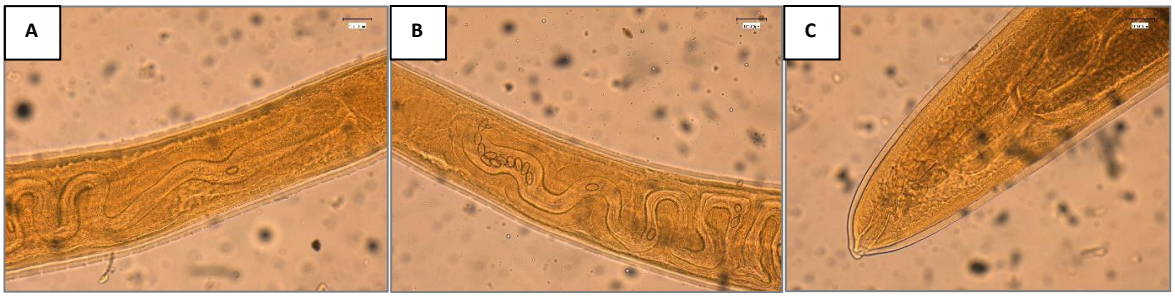


Figure 11. Morphology of female *Trichuris* sp. from *Macaca fascicularis*. **A.** Vulva region with visible tegument covered by spines, and **B.** circumvoluted vagina with eggs. **C.** Posterior end showing the end of uterus and cloaca.

Table 9: Biometric data of adult males and females of *Trichuris* from *Macaca fascicularis* here analysed, together with data available from the literature regarding other *Trichuris* spp. infecting primates (Males: M1 = total body length; M2 = Length of esophageal region of body; LP = Length of posterior region of body; M4 = Maximum width of posterior region of body, thickness; M5 = Body width in the place of junction of esophagus and the intestine; M8 = Length of spicule; M10 = Width of proximal end of spicule; M12=Maximum width of spicule sheath. Females: F1 = Total body length of adult worm; F2 = Length of oesophageal region of body; LP = Length of posterior region of body; F3 = Width of esophageal region of body; F4 = Maximum width of posterior region of body, thickness; F5 = Body width in the place of junction of oesophagus and the intestine). All measurements are indicated in mm.

	Trichuris* from *Macaca fascicularis (present study)	***Trichuris* sp. from *Macaca sylvanus*** (García-Sánchez et al 2019; Rivero et al 2020) [54,56]	***T. trichiura* redescription** (Rivero et al 2021) [34]	***T. trichiura* from *Pan troglodytes*** (Cutillas et al., 2009) [14]	***T. colobae* from *Colobus guereza kikuyensis*** (Cutillas et al., 2014) [57]	***T. ursinus* from *Papio ursinus*** (Callejón et al. 2017) [55]
M1	35.7	34.5	37.15	33.5	34.5	36.5
M2	24.7	21.9	23.45	20.5	26.4	24.3
LP	11	12.5	13.5	13	8.1	12.7
M4	0.45	0.61	0.56	0.5	0.4	0.62
M5	0.35	0.37	0.3	0.2	0.2	0.38
M8	2.77	2.65	2.71	1.9	1.64	2.1
M10	0.02	0.06	0.04	0.02	0.04	0.08
M12	0.06	0.07	0.08	0.09	0.05	0.07
F1	28	34.1	34.3	33.4	46	38

F2	19	21.9	23	25.3	33.8	26
LP	8.7	12.1	10.8	8.1	12.2	12.1
F3	0.15	0.15	0.14	0.14	0.11	0.17
F4	0.63	0.72	0.64	0.45	0.6	0.68
F5	0.37	0.42	0.3	0.17	0.23	0.4

3.3.2 Molecular analyses

- Adult nematodes

Ten high quality *rrn*L sequences (nine from *M. fascicularis* and one from *M. fuscata*) and four *cox*1 sequences (all from *M. fascicularis*) were obtained from the collected nematodes and used for phylogenetic inferences in comparison to GenBank retrieved data, with final datasets of 43 input and 460bp and of 32 input and 341bp, respectively. Both phylogenetic trees identified the presence of two main clades, namely "Clade 1 and Clade 2" [34]. The *rrn*L ML consensus tree in Figure 12 described Clade 1 named as the *T. suis* clade, including *Trichuris colobae* as sister clade of *T. suis* + *Trichuris sp*. from *Chlorocebus*. The *Trichuris* specimens from *M. fascicularis* here analysed were placed into the "subclade c" of the so-called Clade 2 or *T. trichiura* clade branch [34], with high statistical support (99%-100%). The specimen from *M. fuscata* here collected was grouped in the subclade defined MF in previous reports from the same host species living in the Bioparco Zoological Garden of Rome [44,58]. The specific "subclade c" branch included *T. trichiura* with a broad host range for primates, shared by several species such as the Japanese macaque, the Barbary macaque, the green monkey, the baboon, and humans from Africa and Europe.

Figure 12. Maximum likelihood consensus tree of partial mitochondrial rrnL of *Trichuris* spp. analysed in the present study (for specimen codes information, see Table 5). Numbers at nodes indicate the bootstrap statistical support and red arrows indicate specimens from the present study.

The same topology was obtained for *cox*1 ML consensus tree, for which two repeated analyses were carried out including or not including the only available sequence from public repositories of *Trichuris* from *M. fascicularis* for comparative purposes. In fact, the specimen JPT6 (Accession number JF690967) showed a high number of mismatches compared to the remaining specimens placed in Clade 2, creating a very long branch in the tree (Figure 13). A BLAST run analysis on this sample was performed using different stringent parameters and results showed 100% identity

with itself, and no other *Trichuris spp*. listed among the best matches. The second best match with 84% of identity was the fungal species *Parengyodontium album* (accession number KX061492). For this reason, a second ML inference was obtained removing the mentioned sample (Figure 14).

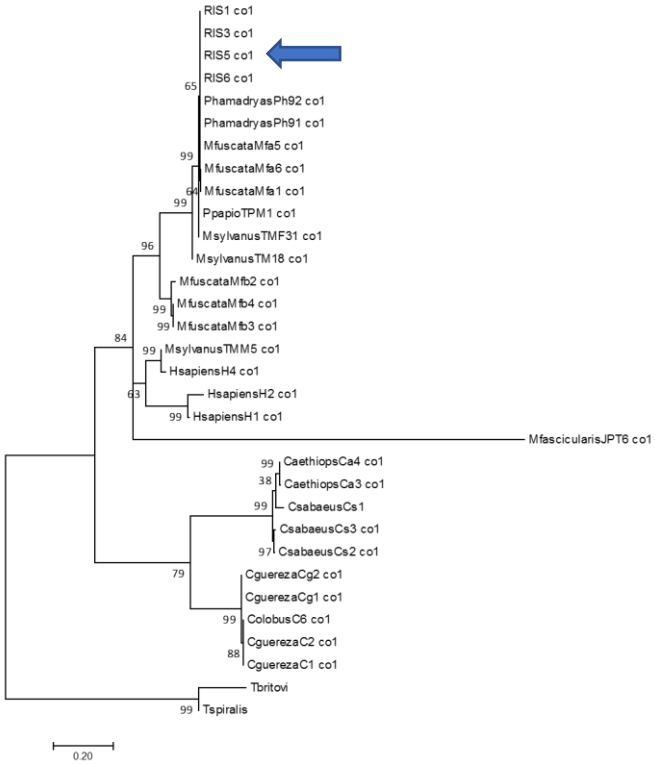


Figure 13: ML consensus tree of partial mitochondrial cox1 of *Trichuris* spp. analysed in the present study (for specimen codes information, see Table 6). Numbers at nodes indicate the bootstrap statistical support.

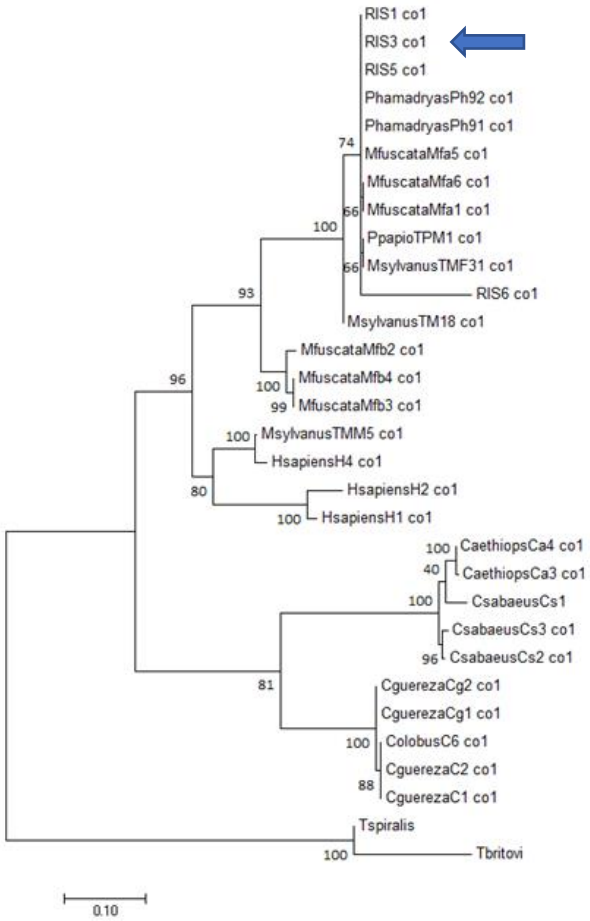


Figure 14: ML consensus tree of partial mitochondrial cox1 of *Trichuris* spp. analysed in the present study (for specimen codes information, see Table 6). Numbers at nodes indicate the bootstrap statistical support.

Such evidence confirmed that specimens infecting *M. fascicularis* here analysed can be identified as *T. trichiura,* given the similarity with this taxon reported also in other primates, including humans.

4. DISCUSSION

Parasites are a diverse and challenging group of eukaryotes, including zoonotic pathogens naturally occurring in the environment, already significantly changed by globalization and anthropogenic impact. Climate changes can further modify fundamental features and transmission dynamics of zoonosis (e.g., parasites' host preference, infectivity, geographical distribution). The proximity of humans and animals in several settings (e.g., rural landscapes, fragmented sylvatic habitats, natural environments close to urban areas), as well as the companion relationship between humans and other animals may represent additional risk factors. In this scenario, parasitological surveys on primates living in impacted areas or in captivity is of high importance for animal health, public health and for conservation strategies, involving the One-Health concept.

4.1 Free-ranging primates

Through the morphological analyses it was possible to identify intestinal parasites that have already been reported for the sampled primate species, as well as new host records.

For the brown spider monkey (*Ateles hybridus)*, *Balantidium* sp.*,* *Entamoeba* sp.*,* and Strongyloididae have been previously reported [2]*,* while *Blastocystis* sp.*, Giardia* sp.*,* and *Dientamoeba fragilis*-like are new records. Similarly, for the varied white-fronted capuchin (*Cebus versicolor)* there are also new records: *Blastocystis* sp.*, Giardia* sp.*, Eimeria* sp.*,* and *Controrchis* sp., while *Ancylostomatidae, Entamoeba* sp., Strongyloididae*,* and Acanthocephala have been previously reported [59]. For the tufted capuchin (*Sapajus apella)*, the new records

include *Hymenolepis* sp., Ancylostomatidae, and *Controrchis* sp., while *Blastocystis* sp. and *Giardia* sp. are previous records [2].

For the gray-handed night monkey (*Aotus griseimembra*), all parasites found (*Entamoeba* sp., *Giardia* sp., *Eimeria* sp., and strongyliform larvae) are new reports for this primate species. Also, for the Humboldt's squirrel monkey (*Saimiri cassiquiarensis*) all parasites observed are new records: *Blastocystis* sp., *Dientamoeba fragilis*-like, *Entamoeba* sp., *Giardia* sp., *Eimeria* sp., *Ascaris* sp., strongyliform larvae, *Trypanoxyuris* sp., *Controrchis* sp., *Hymenolepis* sp., and Acanthocephala. On the contrary, for the Colombian Red Howler (*Alouatta seniculus*) almost all parasites here observed have been previously reported: *Blastocystis* sp., *Entamoeba* sp., *Giardia* sp., *Trypanoxyuris* sp., and *Balantidium* sp. [2,7], while only *Eimeria* sp. and *Dientamoeba fragilis*-like are new records.

Overall, considering the parasite species of zoonotic interest selected for this study, three of them were found by morphological analysis on the samples from free-ranging NHPs. *Giardia* sp. was observed in all primate species, *Blastocystis* sp. was found infecting all NHP species but *A. griseimembra*, and *Ascaris* sp. was present only in samples of *S. cassiquiarensis*. *Trichuris* sp. was not observed.

Regarding the results obtained for one single primate species when sampled in different locations, *S. cassiquiarensis* was found infected by 14 parasite taxa. Interestingly, some parasites as *Blastocystis* and *Ascaris* were present only in Cumaral, the location where primates are in greater contact with humans and domestic animals, and are often fed by people, while parasites such as *Giardia* sp., *Entamoeba* sp., *Trypanoxyuris* sp., and acanthocephalans were found in primates from all the three sampled locations. In the case of *A. seniculus,* a higher number of

parasite taxa was observed in a flooded forest (i.e. San Juan) rather than in *terra firme* forests (i.e. Maní and Yopal).

For *S. apella*, the number of parasite taxa observed was equal in both sampling sites, Yopal surrounded by cattle ranching and Cabuyaro surrounded by palm oil plantations. However, *Giardia* sp. and *Hymenolepis* sp. were present only in samples from Yopal, while Ancylostomatidae and *Controrchis* sp. were present only in Cabuyaro. The presence of palm oil plantations is an ecological factor that might have an effect on parasite richness/prevalence by affecting the development of the eggs/larvae, as it is known that palm oil has different impacts, for instance in the local climate tending to be hotter, drier and brighter than forests due to the less dense canopy [60].

For San Juan, the study site with four sympatric primate species, differences in the parasite taxa according to the NHP species were observed. For instance, for *C. versicolor* we found infection with the higher number of parasite taxa, which could be explained by factors as its omnivorous diet and the use of different strata of the forest, including ground contact, which could pose an infection risk with soil-transmitted helminths, supported by the finding of Ancylostomatidae and strongyliform larvae. Taking into account the parasites of zoonotic interest selected for this study, we found *Giardia* sp. infecting the four primate species present in San Juan, and *Blastocystis* sp. infecting *A. seniculus, A. hybridus* and *C. versicolor*. In a previous survey on parasites carried out at the same study site between 2010 and 2015, *Blastocystis hominis* was reported only in one sample of *A. seniculus*, while *Giardia* sp. was not detected by microscopy or by molecular analysis [59].

Besides, it is worth highlighting the finding of strongyliform larvae in all sampling primate species, and in almost all study sites except Maní. Morphological characters for the larvae identification were not clearly visible, and since the presence of *Strongyloides* could be suspected, we made an attempt at molecular identification by amplifying the 18S rDNA, with prior DNA extraction carried out from faecal samples and not from worms tissues, as performed in other studies [61,62]. According to the best matches in BLAST the obtained sequences were similar to sequences of nematodes, including *Molineus patens, Ancylostoma, Trichostrongylus, Travassostrongylus orloffi,* and *Oesophagostomum muntiacum*. As strongyloidiasis is a neglected tropical disease [63], future molecular studies on *Strongyloides* from platyrrhini are encouraged to elucidate the species circulating, especially in free-ranging populations living in fragmented habitats. For Latin America there are reports of *Strongyloides* sp., *Strongyloides cebus,* and *Strongyloides fuelleborni* infecting free-ranging primates, while *Strongyloides stercoralis* and *Strongyloides venezuelensis* have been reported only for captive primates. Nevertheless, those records rely mainly only in coproparasitological studies, and to a lesser extent in morphological identification of adult parasites obtained during necropsy, while molecular studies on *Strongyloides* are almost null, resulting in only one available partial 18D rDNA sequence of *S. cebus* isolated from a fecal sample of *Saimiri boliviensis* hosted in a zoo in Japan [64]. The distribution of Strongyloidiasis was poorly know in Latin America, however, in 2015 a review article summarized the literature on the subject, recovering information of *Strongyloides* studies confirming the presence of the parasite in humans in different countries, including Colombia [65]. Furthermore, the morphology of larval stages

recovered corresponded to rhabditoid L2, not allowing speculation about possible species identification.

Besides, the presence of suspected forms of being *Dientamoeba fragilis* should be furtherly studied to understand if it is actually this parasite species, taking into account its potential for zoonotic transmission. Dientamoebiasis is worldwide reported causing human gastrointestinal symptoms, equaling or exceeding the incidence of giardiasis [66], and in addition to humans, *D. fragilis* has been reported in livestock (pigs), pets (cat and dog), and NHPs [67]. For NHPs in the Americas, *Dientamoeba* sp. was found infecting *Alouatta palliata* in areas with low percentage of trees, in Ecuador [68]. Specifically in Colombia, *D. fragilis* has been recently found circulating in pigs raised in a farm in the Andean region [69].

Concerning the molecular analyses, there were found samples positive for *Blastocystis* ST8, providing new information from free-ranging NHPs. Until this study, in Colombia, *Blastocystis* ST4 was the only reported ST infecting NHPs [70], while ST8 had been only found circulating in marsupials [70]. Accordingly, this is the first report of *Blastocystis* ST8 from Colombian free-ranging NHPs, confirmed also by phylogenetic analyses.

Blastocystis ST8 has been already detected circulating in NHPs from other countries in South America, in particular in free-ranging mantled howler monkeys from Ecuador [71] and in captive *A. seniculus*, *A. caraya*, *A. fusciceps* and *L. lagotricha* in Brazil [72]. Moreover, ST8 has been found in captive gibbons, and in lemurs from a zoo in Spain [73,74]. Human ST8 infections are rarely reported, however, it has been observed a high prevalence of ST8 among primate handlers in the United Kingdom [75], and there are some other cases in symptomatic patients in

Italy and Australia, among others [76,77]. In Latin America, it has been identified in Brazil, in patients with diabetes mellitus and in asymptomatic patients [78,79].

In this study, the ST8 allele assignment was prevalently to the allele 21, that has been previously reported in captive NHPs in Brazil [72], from sequences obtained with primers of Scicluna et al [35], and in Colombia this allele has been found in samples from *Didelphis marsupialis* [70]. Network analyses allowed to explore *Blastocystis* haplotype lineages and relationships among ST8 circulating in primates in Central and South America suggesting a general low level of variation, and only slight differences in the 18S haplotypes were revealed in the Colombian specimens according to the sampling site (San Juan and Yopal).

In surveys on *Blastocystis* infecting NHPs, it is relevant to take into account the host specificity and ecological factors such as the patterns of forest strata use. In fact, as from a *Blastocystis* sequence data including 30 genera of free-ranging and captive NHPs it was observed a cryptic host specificity for some STs, and likewise, ST8 was primarily seen in arboreal NPH native to South America and Asia [74]. In this study, ST8 was identified in samples of *A. seniculus*, a species of which a preference for the upper strata of the forest has been observed [80]. It is worth to underline that the animals here analysed did not show any symptoms related to gastrointestinal infections.

Furthermore, the identification of *Giardia duodenalis* contributes new information from free-ranging NHPs. In the Americas, *G. duodenalis* assemblages A-H had been reported infecting humans and/or other animals [81]. Reports from 2017 to 2021 regarding molecular identification of *Giardia* spp. in Latin America include investigations conducted in Brazil, Cuba, Ecuador, Mexico, Venezuela and

Colombia, most of them were cross-sectional studies performed in school-age children, and assemblages A and B were the most frequently found [82]. Also in the Amazon region cosmopolitan assemblages A and B prevail within domestic and wild animals [83].

In Colombia, reports of infections with the zoonotic assemblages A and B included asymptomatic children from a rural area in central Colombia [84], children from the Amazon region [85], soil and water samples from the Quindío River basin [86], and samples of horses from four different regions, in molecular studies using molecular markers such as the 18SrRNA, triose phosphate isomerase, beta-giardin, and glutamate dehydrogenase genes [87]. Additionally, assemblage D was reported in children from southwest Colombia [88], assemblage H in raw and treated water samples from Nariño department [89], assemblage B in a dog in Cauca [88], assemblages C and D in dogs from Tolima [90], assemblage F in cats in Bogota [91], and assemblage G in human populations in Montería, Caribbean region [92]. According to the most recent systematic review on epidemiology of human *G. duodenalis* infection in Colombia [93], between January 2010 and September 2022 there were no retrieved records for departments such as Santander and Meta, and within the sampled departments the most frequent assemblages were A and B.

Giardia duodenalis has been previously reported infecting free-ranging NHPs in the Americas: *Alouatta pigra* in Mexico [94], *Saimiri oerstedi* and *Cebus capucinus* in Costa Rica [95,96], and *Alouatta caraya* in Argentina [97,98]. For captive primates there are reports of *G. duodenalis* infecting *Callithrix jacchus*, *Saimiri sciureus,* and *A. caraya* in the USA, China, and Argentina, respectively [99–

101], as well as *Alouatta caraya, Alouatta guariba* and *A. seniculus* in Brazil [102,103], and *Alouatta* sp. in Mexico [104].

Additionally, there are records of *Giardia* sp. infecting free-ranging *Alouatta caraya* and *Aotus azarae* in Argentina [105,106], *Alouatta palliata* in Costa Rica and Panama [107,108], *Cebus capucinus* in Costa Rica [109], *Alouatta pigra* in Belize [110] and Mexico [111], and *Brachyteles hypoxanthus* in Brazil [112]. While in captivity it has been reported *Giardia* sp. infecting *Saimiri sciureus* in Colombia [113], and several primate species in Brazil including *Sapajus apella, Callithrix jacchus, Alouatta caraya, Alouatta guariba, Aotus trivirgatus, Ateles* sp., *Brachyteles arachnoides, Callithrix penicillata, Cebus kaapori, Lagothrix lagothricha,* and *Leontopithecus rosalia* [114,115].

In general, molecular studies on *G. duodenalis* infecting primates from Latin America are scarce. However molecular approaches were used in some of the above mentioned studies on NHPs [97], but only few of them had performed genotyping of the parasite, for instance, assemblage A was found in captive *Alouatta clamitans* from Brazil [103]. In Colombia, this is the first report of *G. duodenalis* assemblages A and B circulating in free-ranging *Sapajus apella* and *Alouatta seniculus*, respectively. Some of the samples here genotyped as assemblage B and one sample classified as assemblage A were collected in a forest fragment in Yopal, a city within the Casanare department. A previous study including human samples from the Casanare department reported assemblages A and B circulating in Poré city, and assemblage A circulating in Yopal city [92].

The genotyping of *G. duodenalis* allows the assessment of infection sources and public health potential, improving the understanding of the transmission and

epidemiology of giardiasis [116], particularly in zoonotic scenarios, allowing a better disease regulation and helping to identify sources of exposure in giardiasis outbreaks; especially in areas where wildlife-human interactions are common [117]. The use of several genotyping molecular markers is highly recommended to provide more robust information and to avoid inaccuracy of the genotype assignment, as different *Giardia* loci differ in total, synonymous, and nonsynonymous substitution rates [20]. In this study it was possible to amplify only the conserved SSU rRNA gene, which is traditionally used for species and gross assemblage differentiation, however, taxonomic inference obtained from a single locus typing must remain cautious [117], and further amplification of extra molecular markers is expected to be carried out to confirm such evidence. Since assemblages A and F differ by only one nucleotide, the obtained cluster including assemblages A and F, and sequences of NHPs is not very reliable. In this case the sequences from NHPs presumably belong to assemblage A that has been previously reported infecting NHPs, while assemblage F has only been reported infecting cats.

The finding of *Ascaris lumbricoides* infecting NHPs in Colombia is of high interest, as the observation of this parasite infecting free-ranging primates is sporadic. Infection with *A. lumbricoides* has been reported in free-ranging *Alouatta caraya* and *A. seniculus* [118,119], *Alouatta palliata* in Costa Rica [120], and *Ateles fusciceps* in Panama [121], as well as in captive *Lagothrix lagothricha* and *A. seniculus* in Peru and Colombia, respectively [122,123]. In addition, there are records of *Ascaris* sp. infecting free-ranging NHPs in Latin America, including *Alouatta guariba* in Brazil [124], *Sapajus nigritus* in Argentina [125], *Saguinus leucopus* in Colombia [126], *Alouatta palliata* in Mexico [127], *Alouatta pigra* in Belize [128],

Alouatta seniculus in French Guiana [129], and *Aotus vociferans* and *Sapajus apella* in Peru [130]. Those previous records of *A. lumbricoides* and *Ascaris* sp. were based on morphology of the parasite, so are unable to distinguish between *A. lumbricoides* or *Ascaris suum* infection, as it is impossible to distinguish between species by their eggs [131]. Thus, this is the first study using a molecular approach for identification of *Ascaris* at the species level from samples of American NHPs, and it is also the first record for *S. cassiquiarensis*.

In Colombia, the presence of *A. lumbricoides* circulating in pigs and human populations has been confirmed, as indicated by a recent study [10]. In the present study, NHPs that were found infected with *A. lumbricoides* are in close contact with human populations, so this finding may suggest cross contamination between humans and NHPs.

Ascariasis is still considered a neglected tropical disease [132], therefore, additional studies on the molecular epidemiology of *Ascaris* sp. are required to increase our knowledge on the species and genotypes circulating in different epidemiological scenarios. Additionally, molecular studies including new vertebrate hosts could contribute to the debate on *Ascaris* taxonomy, as some authors suggest that *A. lumbricoides* and *A. suum* are different species [133,134], while some others consider that the species could be considered as a single species [131,135].

Regarding the ecological analyses performed, the richness accumulation curves revealed that the number of samples for the total sampling collected was sufficient to estimate the number of parasite taxa on free-ranging primates in the study areas. However, when evaluating each study site separately, for Yopal, Maní and San Juan it is observed that the curves approached an asymptote with the x axis,

while for Guacavía, Cumaral, Villavicencio, and much more for Cabuyaro, the curves did not reach the asymptote, indicating that more samples would be necessary to reasonably detect the total parasite taxa infecting primates in these study sites.

Nestedness provides a measure of the heterogeneous distributions of links among species [136]. In a highly nested network, specialist parasites infect a subset of the host species infected by generalist parasites, whereas host species with few parasite taxa harbour parasite species that form subsets of those infecting hosts with richer parasite faunas. The obtained nestedness values with two different metrics do not statistically support evidence of a perfect nestedness in the parasite taxa datasets according to study sites or primate species. However, the plots show that for Guacavía, Villavicencio, Maní, and Yopal, the occurrence of parasite taxa is mostly to the left of the curved black lines, indicating proximity to be nested, while on the contrary, San Juan and Cumaral present several occurrences of parasite taxa to the right of the curved black lines and higher values of nestedness temperature. While for San Juan this could be related to the number of different host species sampled in the area (four species), probably reflecting the occurrence of some specialist parasite taxa, such explanation does not apply to Cumaral, where only *Saimiri cassiquiarensis* has been sampled. In this case, the not-nested condition may be related to the high number of parasite taxa detected, not homogeneously distributed in single individual hosts.

It would be interesting, in the future, to compare the nestedness data obtained from sampling in fragmented forest with those from conserved forests, since differences could be found in the rate of connecting interactions between individuals or species. For instance, a recent study found differences on nestedness when

evaluating arbuscular mycorrhizal fungi communities in remnant vs. restored forests [53]; restored forest communities showed strong evidence of nestedness, while remnant forest communities did not. Additionally, it must be taken into account that analyses here performed were on a presence/absence matrix, but it has been observed that nestedness which was strongly apparent in binary structures, disappeared when quantitative data were analysed [137]. Thus, considering parasite abundances, as well as the evaluation of nestedness along time since it might increase in habitat fragments, could provide a better ecological picture of the interactions that occur in different ecosystems.

Moreover, for future research projects several considerations could be recommended, as the inclusion of environmental variables (e.g. temperature, humidity, forest coverage, forest fragmentation degree) and the performing of longitudinal samplings. These will allow more complete analyses, to achieve a better understanding of the ecological dynamics of intestinal parasites infecting free-ranging primates, as well as of the anthropic effects in fragmented forests.

4.2 Captive primates

Based on microscopy both helminths and protozoans were identified, almost all of them presenting direct life cycles. Captive NHPs may be more susceptible to parasites with direct life cycles, which are more prevalent and prone to disseminate in confined conditions where the animals might be more stressed, showing clinical signs as diarrhea and dehydration, requiring veterinary care [138,139]. Parasite transmission mainly occurs through the faecal-oral route via direct contact with infected hosts (or their faecal material), or indirectly through the ingestion of contaminated water or food [140]. Captive NHPs may act as reservoirs for zoonotic

parasite transmission and for parasitic evasion of pharmacological treatments [141,142], thus, confined environments are of great interest for parasitological studies, involving the One-Health concept.

Parasitological investigations have been carried out in some European zoological parks housing NHPs, such as the Dublin Zoological Garden (Ireland), the Belgrade Zoo (Serbia), the Kiev Zoo (Ukraine), the Antwerpen Zoo (Belgium), the Brno Zoological Garden (Czech Republic), the Sofia Zoo (Bulgaria), the Wroclaw Zoo (Poland), among others. It has been observed that most often NHPs are the host animals, and that nematodes (e.g. *Ascaris* sp., *Trichuris* sp., *Strongyloides* sp.) are the most common parasites, followed by cestodes and trematodes [143]. Furthermore, *Giardia duodenalis*, *Cryptosporidium hominis, Blastocystis* sp., and *Entamoeba dispar* circulation between NHPs and their zookeepers has been identified in European zoological gardens, with the confirmation of zoonotic transmission events involving *Blastocystis* sp., and a partial demonstration of *C. hominis* zoonotic transmission [140].

In Italy, surveys on intestinal parasites infecting NHPs living in zoological gardens have been conducted. In central Italy, at the Giardino Zoologico of Pistoia *Cryptosporidium* sp. and *Trichuris* sp. have been reported in *Lemur catta* [144], and at the Bioparco Zoological Garden of Rome it has been reported *G. duodenalis* infecting *L. catta*, as well as *Entamoeba* spp. infecting *Cercocebus torquatus*, *Chlorocebus aethiops*, *Macaca fuscata*, *Mandrillus sphinx*, *Pan troglodytes*, *L. catta*, and *Pongo pygmaeus* [145]. In southern Italy, there have been found *Trichuris* sp., *Strongyloides fűelleborni*, and *Cryptosporidium* sp. infecting *Papio cynocephalus* at the Fasano Zoo Safari, while *G. duodenalis* was found infecting *L.*

catta, *Cercopithecus mona*, *Alouatta caraya*, *Nomascus concolor*, *Colobus Guereza*, and *Semnopithecus entellus* in a zoological garden in the Benevento province [146]. Moreover, *Cyclospora* was detected in *P. troglodytes* from a Wildlife Animal Rescue Center, and in *Macaca fascicularis* from an Experimental Primate Research Center [147], while eight taxa of intestinal parasites (*Trichuris* sp., *Oesophagostomum* sp., *Entamoeba coli*, *Endolimax nana*, *Iodamoeba bütschlii*, *Chilomastix mesnili*, *Balantidium coli*, and *Blastocystis* sp.) were recorded infecting *M. fascicularis* in a biomedical research center [148].

In this study, almost all the parasites found have been previously reported in captive NHPs in Europe, as is the case of *Trichuris* sp., *Oesophagostomum* sp., *Balantidium* sp., *E. coli,* and *I. bütschlii*. However, other parasites of zoonotic interest such as *Blastocystis* sp., *Giardia* sp., and *Ascaris* sp. were not found in this survey.

Balantidium sp. was found infecting the three captive NHP species sampled. Pigs are the main reservoir host of *Balantidium*, while rodents and NHPs may function as its alternative reservoir host [149]. Wild boars are present at the study site (wildlife recovery centre Parco Faunistico Piano dell'Abatino) but in small quantity and within a separate facility from the NHPs. Thus, in this case swine are unlikely to participate in the transmission cycle, while wild rodents are very common within the primate enclosures. For future studies it is highly recommended the use of morphological characteristics combined with molecular-phylogenetic data, as it has been demonstrated how misleading the cyst morphology-based diagnostics of *Balantidium* and *Buxtonella* can be, leading to ambiguity in the epidemiology of these infections [150]. In Italy, both *Buxtonella* and *Balantidium* have been

reported, for instance *Buxtonella sulcata* infecting cattle in central Italy [151], and *Balantidium coli* infecting swine in the south of the country [152].

Molecular testing should be also recommended for the optimal identification of *Dientamoeba fragilis,* given the increased sensitivity and specificity of the molecular assays [153]. In our survey, *D. fragilis*-like was found in samples from *M. fascicularis*, which is a parasite recently reported infecting free-ranging *M. fascicularis* in Indonesia [154]. Additionally, future molecular studies to determine the species of the strongyliform larvae found infecting *S. apella* are required; peculiarly to confirm the presence of *Strongyloides*, as it is a zoonotic parasite and canine and human strongyloidiasis have been reported in Italy [155].

Diet can affect parasite prevalence and richness; in this case it must be considered that captive primates have different diets and habits than free-ranging primates. This fact could explain the absence of parasites such as acanthocephalans (e.g. *Oncicola machadoi, Prosthenorchis elegans*), trematodes (e.g. *Athesmia heterolecithoides*), and cestodes (e.g. *Bertiella mucronata*), that have been reported in free-ranging *S. apella* [2]. Additionally, ecological factors such as temperature, rainfall, land surface temperature and soil humidity are important for the development of some parasites, for example the hookworm larvae which require warm and moist conditions to survive [156]. In our study, samples were only taken during the winter season, thus, is would be interesting the conduction of new surveys including all seasons, in order to better understand parasite dynamics at the wildlife recovery centre Parco Faunistico Piano dell'Abatino.

Regarding *Trichuris* spp., there have been carried out analyses for the morphological and/or molecular characterisation of the parasite, from samples of

different *Macaca* species: the Japanese macaque *M. fuscata* [43,44,58], the Barbary macaque *M. sylvanus* [54,56] and the long-tailed macaque *M. fascicularis* [148]; the latter performed only in terms of eggs presence in stool samples without any molecular characterisation. Such studies revealed the presence of two separated taxonomic entities able to infect the Japanese macaque living in a confined environment as the zoological garden of Rome, one observed only in this host and one shared also by other primates, demonstrating the ability of this taxa to be highly or less specific for a host [43,44,58]. Analogous molecular results were obtained also regarding the Barbary macaque hosted in the Zoo Castellar (Spain), infected by two genotypes within the *T. trichiura* lineage, supported also by morphological data [54].

Here we confirm that Japanese macaques hosted at the Bioparco Zoological Garden of Rome are still infected with the species-specific *T. trichiura* of *M. fuscata*. Moreover, we provide for the first time morphological and molecular data from *T. trichiura* from *M. fascicularis* to be shared with the scientific community for comparative purposes, showing that nematodes from this species cluster with many *T. trichiura* sequences from other primates including humans. Although limits of species designation are not always easy to define with morphology, given the high frequency of convergence, the high rate of variation and the low number of specimens collected, evidences here collected are suggestive of *T. trichiura* circulation in captive conditions. The long-tailed macaque analysed in the present study lived in a colony of around 30 macaques in a wildlife recovery center [157], and this condition may be of high risk for the other macaques of the colony, also considering that *M. fascicularis* has been recently listed as a vulnerable species according to IUCN (2021) [29].

Moreover, there is a significant risk for handlers and visitors, in terms of zoonotic transmission. Captive primates have more opportunities to reach humans than wild primates have, thus, confined environments should be constantly monitored to trace the presence of eventual parasitic species of zoonotic interest, taking into account the One-Health concept.

5. CONCLUSIONS

The present survey could be of support to elaborate a more accurate epidemiological picture of intestinal parasites infecting NHP species. Differences on parasite prevalence and richness were found for some parasite taxa, primate species, and study sites, as well as between captive and free-ranging primates. Factors as the diet, habits, environmental conditions, interaction between humans and NHP influence the data obtained.

Parasitological studies including other NHP species are strongly encouraged using both microscopy and molecular analyses, especially for NHP species listed as critically endangered, endangered or vulnerable according to the IUCN Red List, as in the case of *A. hybridus* and *C. versicolor*, both included in the present survey.

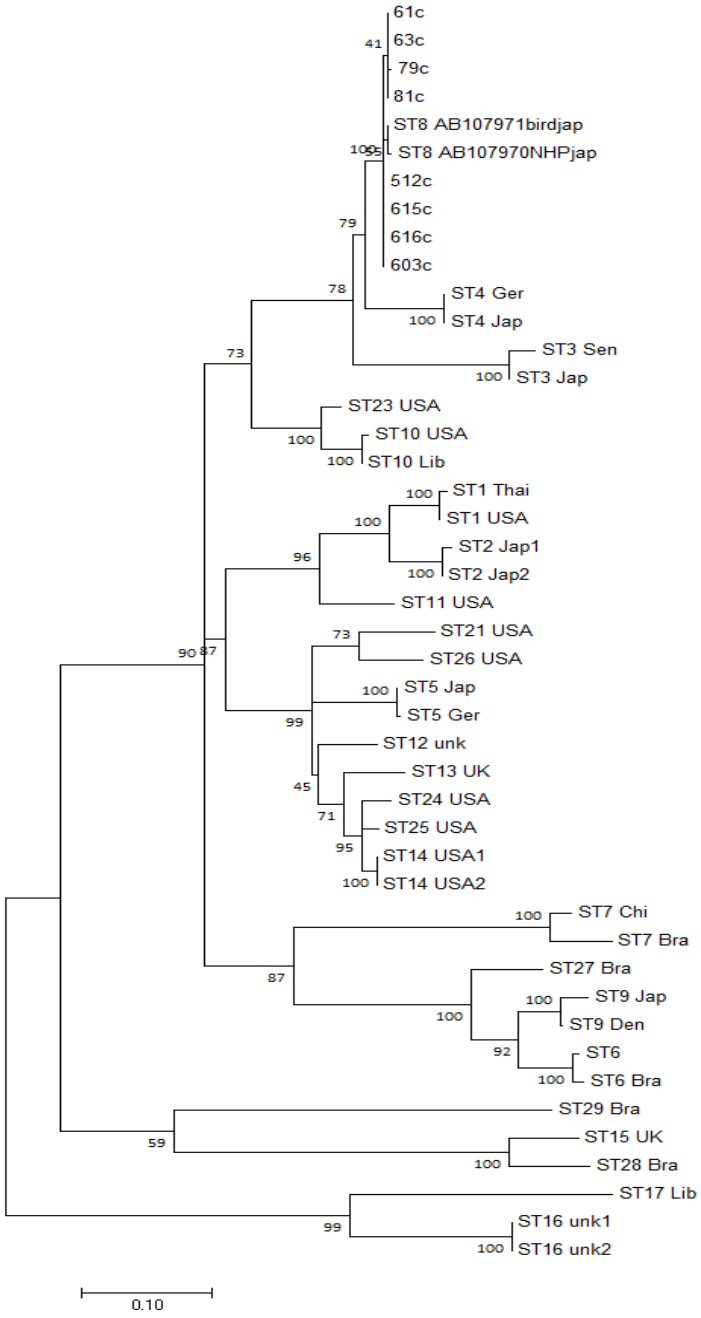
In order to properly determine the zoonotic potential of intestinal parasites infecting NHPs, it is necessary the use of molecular approaches. In this survey, the finding of intestinal parasites with zoonotic potential (e.g. *Ascaris lumbricoides*, *Trichuris*) suggests epidemiological implications, especially because NHPs that are in close contact with humans are involved.

For a better understanding of the molecular epidemiology of intestinal parasites, new surveys including humans and other NHP species, aiming to explore the distribution, genetic variation and host specificity are strongly encouraged.

We recommend conducting regular parasite surveys in NHPs in order to monitor the potential zoonotic transmission risk. Additionally, educational activities with the exposed local communities should be encouraged to increase the awareness regarding the potential risk of zoonotic transmissions, and the importance of avoiding food provisioning and physical contact with NHP.

6. SUPPLEMENTARY MATERIAL

Supplementary material 1. Best ML consensus tree of partial 18S *Blastocystis hominis* reference strains and samples here analysed collected from NHPs in Colombia (dataset Santin et al. 2011 [36]).



Supplementary material 2. Dataset of sequences obtained with primers of Scicluna et al. 2006 [35] used to build a *Blastocystis hominis* ST8 Median Joining Network.

```
MEGA
!Title Phylogenetic Analysis;
!Format
   DataType=Nucleotide
   NSeqs=18 NSites=621
   Identical=. Missing=? Indel=-; 52variable sites

ST8_Jap2    ---GCTTATC TGG--TTGAT CCTGCCAGTA GTCATACGCT CGTCTCAAAG ATTAAGCCAT GCATGTGTAA  [ 70]
ST8_Jap     ---....... ...--..... .......... .......... .......... .......... ..........  [ 70]
617c        ---------- -CTGG..... .......... .......... .......... .......... ..........  [ 70]
548c        -----..... ...-T.-... .......... .......... .......... .......... ..........  [ 70]
61c         -----..T.T .T.GGG.TTG T......... .......... .......... .......... ..........  [ 70]
63r         ---------- --------.. .......... .......... .......... .......... ..........  [ 70]
79r         ---------- --------.. .......... .......... .......... .......... ..........  [ 70]
81c         ---------- --------.. .......... .......... .......... .......... ..........  [ 70]
603_f       -----G.... ...--..... .......... .......... .......... .......... ..........  [ 70]
615_f       CCT.T..... ...GT..... .......... .......... .......... .......... ..........  [ 70]
608_f       -----..... ...-T.-... .......... .......... .......... .......... ..........  [ 70]
616_f       ----T..... ...-T..... .......... .......... .......... .......... ..........  [ 70]
ApEc1       ---------- ---------- ---------- ---------- ---------- ---------- ..........  [ 70]
ApEc2       ---------- ---------- ---------- ---------- ---------- ---------- ----......  [ 70]
ApEc3       ---------- ---------- -----..... .......... .......... .......... ..........  [ 70]
AnPe1       ---------- ---------- ---------- ---------- ---------- ---------- ----------  [ 70]
AnPe2       ---------- ---------- ---------- ---------- ---------- ---------- ----------  [ 70]
AnPe3       ---------- -..--..... .......... .......... .......... .......... ..........  [ 70]

ST8_Jap2    GTATAAATAG TTAACTTTAA AACTGCGAAT GGCTCATTAT ATCAGTTATA GTTTATTTGA TGCAATCTAC  [140]
ST8_Jap     .......... .......... .......... .......... .......... .......... ..........  [140]
617c        .GTA...... ..C......G .......... .......... .......... .......... ..........  [140]
548c        .......... .......... .......... .......... .......... .......... ..........  [140]
61c         .......... .......... .......... .......... .......... .......... ..........  [140]
63r         .......... .......... .......... .......... .......... .......... ..........  [140]
79r         .......... .......... .......... .......... .......... .......... ..........  [140]
81c         .......... .......... .......... .......... .......... .......... ..........  [140]
603_f       .......... .......... .......... .......... .......... .......... ..........  [140]
615_f       .......... .......... .......... .......... .......... .......... ..........  [140]
608_f       .......... .......... .......... .......... .......... .......... ..........  [140]
616_f       .......... .......... .......... .......... .......... .......... ..........  [140]
ApEc1       .......... .......... .......... .......... .......... .......... ..........  [140]
ApEc2       .......... .........GG .......... .......... ...G...... .......... ..........  [140]
ApEc3       .......... .......... .......... .......... .......... .......... ..........  [140]
AnPe1       ---------- --------.. .......... .......... .......... .......... ..........  [140]
AnPe2       ---------- --------.. .......... .......... .......... .......... ..........  [140]
AnPe3       .......... .......... .......... .......... .......... .......... ..........  [140]

ST8_Jap2    TACTTGGATA ACCGTAGTAA TTCTAGAGCT AATACATGGT AAAGTCCTCA ACTTGTGAAG AGGTGTATTT  [210]
ST8_Jap     .......... .......... .......... .......... .......... .......... ..........  [210]
617c        .......... .......... .......... .......... .......... .......... ..........  [210]
548c        .......... .......... .......... .......... .......... .......... ..........  [210]
61c         .......... .......... .......... .......... .......... .......... ..........  [210]
63r         .......... .......... .......... .......... .......... .......... ..........  [210]
79r         .......... .......... .......... .......... .......... .......... ..........  [210]
81c         .......... .......... .......... .......... .......... .......... ..........  [210]
603_f       .......... .......... .......... .......... .......... .......... ..........  [210]
615_f       .......... .......... .......... .......... .......... .......... ..........  [210]
608_f       .......... .......... .......... .......... .......... .......... ..........  [210]
616_f       .......... .......... .......... .......... .......... .......... ..........  [210]
ApEc1       .......... .......... .......... .......... .......... ....AG.... ..........  [210]
ApEc2       .......... .......... .......... .....G...G .G........ ....AG.... ..........  [210]
ApEc3       .......... .......... .......... .......... .......... G...AG.... ..........  [210]
AnPe1       .......... .......... .......... .......... .......... .......... ..........  [210]
AnPe2       .......... .......... .......... .......... .......... .......... ..........  [210]
AnPe3       .......... .......... .......... .......... .......... .......... ..........  [210]

ST8_Jap2    ATTAGAATGA AAACCAGTAG ACTTAGTCTA TTCGTGAGTA ATAATAACTT ATCATATCGC ATGCTTAGGT  [280]
ST8_Jap     .......... .......... .......... .......... .......... .......... ..........  [280]
617c        .......... .......... .......... .......... .......... .......... ..........  [280]
548c        .......... .......... .......... .......... .......... .......... ..........  [280]
61c         .......... .......... .......... .......... .......... .......... ..........  [280]
63r         .......... .......... .......... .......... .......... .......... ..........  [280]
79r         .......... .......... .......... .......... .......... .......... ..........  [280]
81c         .......... .......... .......... .......... .......... .......... ..........  [280]
603_f       .......... .......... .......... .......... .......... .......... ..........  [280]
615_f       .......... .......... .......... .......... .......... .......... ..........  [280]
608_f       .......... .......... .......... .......... .......... .......... ..........  [280]
616_f       .......... .......... .......... .......... .......... .......... ..........  [280]
```

```
ApEc1       .......... .......... ..A....... .......... .......... .......... ..........  [280]
ApEc2       .......... .......... ..A-...... .......... .......... .......... ..........  [280]
ApEc3       .......... .......... ..A....... .......... .......... .......... ..........  [280]
AnPe1       .......... .......... .......... .......... .......... .......... ..........  [280]
AnPe2       .......... .......... .......... .......... .......... .......... ..........  [280]
AnPe3       .......... .......... .......... .......... .......... .......... ..........  [280]

ST8_Jap2    AGCGATATGT CTTTCAAGTT TCTGCCCTAT CAGCTTTCGA TGGTAGTGTA TTGGACTACC ATGGCAGTAA  [350]
ST8_Jap     .......... .......... .......... .......... .......... .......... ..........  [350]
617c        .......... .......... .......... .......... .......... .......... ..........  [350]
548c        .......... .......... .......... .......... .......... .......... ..........  [350]
61c         .......... .......... .......... .......... .......... .......... ..........  [350]
63r         .......... .......... .......... .......... .......... .......... ..........  [350]
79r         .......... .......... .......... .......... .......... .......... ..........  [350]
81c         .......... .......... .......... .......... .......... .......... ..........  [350]
603_f       .......... .......... .......... .......... .......... .......... ..........  [350]
615_f       .......... .......... .......... .......... .......... .......... ..........  [350]
608_f       .......... .......... .......... .......... .......... .......... ..........  [350]
616_f       .......... .......... .......... .......... .......... .......... ..........  [350]
ApEc1       .......... .......... .......... .......... .......... .......... ..........  [350]
ApEc2       .......... .......... .......... ......G--- ---------- ---------- ----------  [350]
ApEc3       .......... .......... .......... .......... .......... .......... ..........  [350]
AnPe1       .......... .......... .......... .......... .......... .......... ..........  [350]
AnPe2       .......... .......... .......... .......... .......... .......... ..........  [350]
AnPe3       .......... .......... .......... .......... .......... .......... ..........  [350]

ST8_Jap2    CGGGTAACGA AGAATTTGGG TTCGATTTCG GAGAGGGAGC CTGAGAGATG GCTACCACAT CCAAGGAAGG  [420]
ST8_Jap     .......... .......... .......... .......... .......... .......... ..........  [420]
617c        .......... .......... .......... .......... .......... .......... ..........  [420]
548c        .......... .......... .........T .......... .......... .......... A.........  [420]
61c         .......... .......... .......... .......... .......... .......... ..........  [420]
63r         .......... .......... .......... .......... .......... .......... ..........  [420]
79r         .......... .......... .......... .......... .......... .......... ..........  [420]
81c         .......... .......... .......... .......... .......... .......... ..........  [420]
603_f       .......... .......... .......... .......... .......... .......... ..........  [420]
615_f       .......... .......... .......... .......... .......... .......... ..........  [420]
608_f       .......... ...T...... .......... .......... .......... .......... ..........  [420]
616_f       .......... .......... .......... .......... .......... .......... ..........  [420]
ApEc1       .......... .......... .......... .......... .......... .......... ..........  [420]
ApEc2       ---------- ---------- ---------- ---------- ---------- ---------- ----------  [420]
ApEc3       .......... .......... .......... .......... .......... .......... ..........  [420]
AnPe1       .......... .......... .......... .......... .......... .......... ..........  [420]
AnPe2       .......... .......... .......... .......... .......... .......... ..........  [420]
AnPe3       .......... .......... .......... .......... .......... .......... ..........  [420]

ST8_Jap2    CAGCAGGCGC GTAAATTACC CAATCCTGAC ACAGGGAGGT AGTGACAATA AATCACAATG CGGAACTATT  [490]
ST8_Jap     .......... .......... .......... .......... .......... .......... ..........  [490]
617c        .......... .......... .......... .......... .......... .......... ..........  [490]
548c        ....C..... ....T..... ..C....... .......... .......... .......... ....T.....  [490]
61c         .......... .......... .......... .......... .......... .......... ..........  [490]
63r         .......... .......... .......... .......... .......... .......... ..........  [490]
79r         .......... .......... .......... .......... .......... .......... ..........  [490]
81c         .......... .......... .......... .......... .......... .......... ..........  [490]
603_f       .......... .......... .......... .......... .......... .......... ..........  [490]
615_f       .......... .......... .......... .......... .......... .......... ..........  [490]
608_f       .......... .......... .......... .......... .......... .......... .....-....  [490]
616_f       .......... .......... .......... .......... .......... .......... ..........  [490]
ApEc1       .......... .......... .......... .......... .......... .......... ..........  [490]
ApEc2       ---------- ---------- ---------- ---------- ---------- ---------- ----------  [490]
ApEc3       .......... .......... .......... .......... .......... .......... ..........  [490]
AnPe1       .......... .......... .......... .......... .......... .......... ..........  [490]
AnPe2       .......... .......... .......... .......... .......... .......... ..........  [490]
AnPe3       .......... .......... .......... .......... .......... .......... ..........  [490]

ST8_Jap2    AGTTTTGCAA TTGGATTGAG AACAACGTAC AAACCTTATC GATAAACAAT TGGAGGGCAA GTCTGGTGCC  [560]
ST8_Jap     .......... .......... .......... .......... .......... .......... ..........  [560]
617c        .......... .......... .......... .......... .......... .......... ..........  [560]
548c        .......... .......... .......... ..T....... .......... ....T..... ....------  [560]
61c         .......... .......... .......... .......... .......... .......... ..........  [560]
63r         .......... .......... .......... .......... .......... .......... ..........  [560]
79r         .......... .......... .......... .......... .......... .......... ..........  [560]
81c         .......... .......... .......... .......... .......... .......... ..........  [560]
603_f       .......... .......... .......... .......... .......... .......... ..........  [560]
615_f       .......... .......... .......... .......... .......... .......... ..........  [560]
608_f       .......... ......---- ---------- ---------- ---------- ---------- ----------  [560]
616_f       .......... .......... .......... .......... .......... .......... ..........  [560]
ApEc1       .......... .......... .......... .......... .......... .......... ..........  [560]
ApEc2       ---------- ---------- ---------- ---------- ---------- ---------- ----------  [560]
ApEc3       .......... .......... .......... .......... .......... .......... ..........  [560]
AnPe1       .......... .......... .......... .......... .........- ---------- ----------  [560]
AnPe2       .......... .......... .......... .......... .......... .......... ..........  [560]
AnPe3       .......... .......... .......... .......... ........-- ---------- ----------  [560]
```

```
ST8_Jap2    AGCAGCCGCG GTAATTCCAG CTCCAATAGC GTATATTAAC GTTGTTGCAG TTAAAAAGCT C [621]
ST8_Jap     .......... .......... .......... .......... .......... .......... . [621]
617c        .......... .......... .......... .......... .......... .......... . [621]
548c        ---------- ---------- ---------- ---------- ---------- ---------- - [621]
61c         .......... .......... .......... .......... .......... .---------- - [621]
63r         .......... .......... .......... .......... .........T A--------- - [621]
79r         .......... .......... .......... .......... .......... .A--------- - [621]
81c         .......... .......... .......... .......... .........T A--------- - [621]
603_f       .......... .......... .......... ..-------- ---------- ---------- - [621]
615_f       .......... .......... .......... ...------- ---------- ---------- - [621]
608_f       ---------- ---------- ---------- ---------- ---------- ---------- - [621]
616_f       .......... .......... ....C.A..G A.T...---- ---------- ---------- - [621]
ApEc1       .......... .......... .......... .......... .......... .......... - [621]
ApEc2       ---------- ---------- ---------- ---------- ---------- ---------- - [621]
ApEc3       .......... .......... .......... .......... ......---- ---------- - [621]
AnPe1       ---------- ---------- ---------- ---------- ---------- ---------- - [621]
AnPe2       .......... .......... .......... .......... .......... ......GCTC A [621]
AnPe3       ---------- ---------- ---------- ---------- ---------- ---------- - [621]
```

Supplementary material 3. Dataset of sequences obtained with primers of Santin et al 2011 [36] used to build a *Blastocystis hominis* ST8 Median Joining Network.

```
#MEGA
!Title Phylogenetic Analysis;
!Format
   DataType=Nucleotide
   NSeqs=16 NSites=483
   Identical=. Missing=? Indel=-; 34 VARIABLE SITES
```

```
ST8_AB107971birdjap    GGAGGTAGTG ACAATAAATC ACAATGCGGA ACTATTAGTT TTGCAATTGG ATTGAGAACA ACGTACAAAC  [ 70]
ST8_AB107970NHPjap     .......... .......... .......... .......... .......... .......... ..........  [ 70]
AlBr1                  ---------- ---------- ---------- ---------- ---------- ----...... ..........  [ 70]
ApMe3                  --------.. .......... .......... .......... .......... .......... ..........  [ 70]
ApMe1                  ---------- ---------- ---------- ---------- ---------- ---------- ----------  [ 70]
AoBr1                  ---------- ---------- ---------- ---------- ---------- ----...... ..........  [ 70]
512c                   .......... .......... .......... .......... .......... .......... ..........  [ 70]
615c                   .......... .......... .......... .......... .......... .......... ..........  [ 70]
616c                   .......... .......... .......... .......... .......... .......... ..........  [ 70]
AtBr1                  ---------- ---------- ---------- ---------- ---------- ----...... ..........  [ 70]
LlBr1                  ---------- ---------- ---------- ---------- ---------- ----...... ..........  [ 70]
603c                   .......... .......... .......... .......... .......... .......... ..........  [ 70]
61c                    .......... .......... .......... .......... .......... .......... ..........  [ 70]
63c                    .......... .......... .......... .......... .......... .......... ..........  [ 70]
79c                    .......... .......... .......... .......... .......... .......... ..........  [ 70]
81c                    .......... .......... .......... .......... .......... .......... ..........  [ 70]

ST8_AB107971birdjap    CTTATCGATA AACAATTGGA GGGCAAGTCT GGTGCCAGCA GCCGCGGTAA TTCCAGCTCC AATAGCGTAT  [140]
ST8_AB107970NHPjap     .......... .......... .......... .......... .......... .......... ..........  [140]
AlBr1                  .......... .......... .......... .......... .......... .......... ..........  [140]
ApMe3                  .......... .......... .......... .......... .......... .......... ..........  [140]
ApMe1                  ---------- ---------- ---------- ---------- --------.. .......... ..........  [140]
AoBr1                  .......... .......... .......... .......... .......... .......... ..........  [140]
512c                   .......... .......... .......... .......... .......... .......... ..........  [140]
615c                   .......... .......... .......... .......... .......... .......... ..........  [140]
616c                   .......... .......... .......... .......... .......... .......... ..........  [140]
AtBr1                  .......... .......... .......... .......... .......... .......... ..........  [140]
LlBr1                  .......... .......... .......... .......... .......... .......... ..........  [140]
603c                   .......... .......... .......... .......... .......... .......... ..........  [140]
61c                    .......... .......... .......... .......... .......... .......... ..........  [140]
63c                    .......... .......... .......... .......... .......... .......... ..........  [140]
79c                    .......... .......... .......... .......... .......... .......... ..........  [140]
81c                    .......... .......... .......... .......... .......... .......... ..........  [140]

ST8_AB107971birdjap    ATTAACGTTG TTGCAGTTAA AAAGCTCGTA GTTGAAGTGA AGTGAACAAA GAAGAGAAGA ATCTTCGGAT  [210]
ST8_AB107970NHPjap     .......... .......... .......... .......... .......... .......... ..........  [210]
AlBr1                  .......... .......... .......... .......... .......... .......... ..........  [210]
ApMe3                  .......... .......... .......... .........T G.A..... .......... ..GG......  [210]
ApMe1                  .......... .......... .......... .......... .......... .......... ..........  [210]
AoBr1                  .......... .......... .......... .......... .......... .......... ..........  [210]
512c                   .......... .......... .......... .......... .......... .......... ..........  [210]
615c                   .......... .......... .......... .......... .......... .......... ..........  [210]
616c                   .......... .......... .......... .......... .......... .......... ..........  [210]
AtBr1                  .......... .......... .......... .......... .......... .......... ..........  [210]
LlBr1                  .......... .......... .......... .......... .......... .......... ..........  [210]
603c                   .......... .......... .......... .......... .......... .......... ..........  [210]
61c                    .......... .......... .......... .......... .......... .......... ..........  [210]
63c                    .......... .......... .......... .......... .......... .......... ..........  [210]
79c                    .......... .......... .......... .......... .......... .......... ..........  [210]
81c                    .......... .......... .......... .......... .......... .......... ..........  [210]
```

```
ST8_AB107971birdjap    TCGGATCTAA TGCGTTGACT CTTTCCAAAG TCAATGTATG GGTATTCATT TATCTATATA TTGTGTTTGG  [280]
ST8_AB107970NHPjap     .......... .......... .......... .......... .......... .......... ..........  [280]
AlBr1                  .......... .......... .......... .......... .......... .......... ..........  [280]
ApMe3                  .......... ....G..... ..GGA....C ....A....C ..C....... .....GG... ..T.......  [280]
ApMe1                  .......... .......... .......... .......... .......... .......... ..........  [280]
AoBr1                  .......... .......... .......... .......... .......... .......... ..........  [280]
512c                   .......... .......... .......... .......... .......... .......... ..........  [280]
615c                   .......... .......... .......... .......... .......... .......... ..........  [280]
616c                   .......... .......... .......... .......... .......... .......... ..........  [280]
AtBr1                  .......... .......... .......... .......... .......... .......... ..........  [280]
LlBr1                  .......... .......... .......... .......... .......... .......... ..........  [280]
603c                   .......... .......... .......... .......... .......... .......... ..........  [280]
61c                    .......... .......... .......... .......... .......... .......... ..........  [280]
63c                    .......... .......... .......... .......... .......... .......... ..........  [280]
79c                    .......... .......... .......... .......... .......... .......... ..........  [280]
81c                    .......... .......... .......... .......... .......... .......... ..........  [280]

ST8_AB107971birdjap    TCGTTTACTG TGAGAAAATT AGAGTGTTCA AAGCAGACGT TTGTTTGAAT ACATTAGCAT GGAATAATCA  [350]
ST8_AB107970NHPjap     .......... .......... .......... .......... .......... .......... ..........  [350]
AlBr1                  .......... .......... .......... .......... .......... .......... ..........  [350]
ApMe3                  .T..CAG... .......... .......... ....C..... ........GG ..CA...... T.........  [350]
ApMe1                  .......... .......... .......... .......... .......... .......... ..........  [350]
AoBr1                  .......... .......... .......... .......... .......... .......... ..........  [350]
512c                   .......... .......... .......... .......... .......... .......... ..........  [350]
615c                   .......... .......... .......... .......... .......... .......... ..........  [350]
616c                   .......... .......... .......... .......... .......... .......... ..........  [350]
AtBr1                  .......... .......... .......... .......... .......... .......... ..........  [350]
LlBr1                  .......... .......... .......... .......... .......... .......... ..........  [350]
603c                   .......... .......... .......... .......... .......... .......... ..........  [350]
61c                    .......... .......... .......... .......... .......... .......... ..........  [350]
63c                    .......... .......... .......... .......... .......... .......... ..........  [350]
79c                    .......... ..........T. .......... .......... .......... .......... ..........  [350]
81c                    .......... .......... .......... .......... .......... .......... ..........  [350]

ST8_AB107971birdjap    TATAAGGCTT TCATGTGTAT TTGATTGGTT TGGATATGAA AGCAAGGTTA ATAGGGACAG TTGGGGGTAT  [420]
ST8_AB107970NHPjap     G......... .......... .......... .......... .......... .......... ..........  [420]
AlBr1                  .......... .......... .......... .......... .......... .......... ..........  [420]
ApMe3                  .....T.T.. ......C.-- ---------- ---------- ---------- ---------- ----------  [420]
ApMe1                  .......... .......... .......... .......... -......... .......... ..........  [420]
AoBr1                  .......... .......... .......... .......... .......... .......... ..........  [420]
512c                   ....G..... .......... .......... .......... .......... .......... ..........  [420]
615c                   ....G..... .......... .......... .......... .......... .......... ..........  [420]
616c                   ....G..... .......... .......... .......... .......... .......... ..........  [420]
AtBr1                  .......... .......... .......... .......... .......... .......... ..........  [420]
LlBr1                  .......... .......... .......... .......... .......... .......... ..........  [420]
603c                   ....G..... .......... .......... .......... .......... .......... ..........  [420]
61c                    G......... .......... .......... .......... .......... .......... ..........  [420]
63c                    G......... .......... .......... .......... .......... .......... ..........  [420]
79c                    G......... .......... .......... .......... .......... .......... ..........  [420]
81c                    G......... .......... .......... .......... .......... .......... ..........  [420]

ST8_AB107971birdjap    TCATATTCAA TAGTCAGAGG TGAAATTCTC GGATTTATGG AAGATGAACA AATGCGAAAG CA-  [483]
ST8_AB107970NHPjap     .......... .......... .......... .......... .......... .......... ..-  [483]
AlBr1                  ......---- ---------- ---------- ---------- ---------- ---------- ---  [483]
ApMe3                  ---------- ---------- ---------- ---------- ---------- ---------- ---  [483]
ApMe1                  .......... .......... .......... .......... .......... .G........ ..A  [483]
AoBr1                  ......---- ---------- ---------- ---------- ---------- ---------- ---  [483]
512c                   .......... .......... .......... .......... .......... .G........ ..-  [483]
615c                   .......... .......... .......... .......... .......... .G........ ..-  [483]
616c                   .......... .......... .......... .......... .......... .G........ ..-  [483]
AtBr1                  ......---- ---------- ---------- ---------- ---------- ---------- ---  [483]
LlBr1                  ......---- ---------- ---------- ---------- ---------- ---------- ---  [483]
603c                   .......... .......... .......... .......... .......... .G........ ..-  [483]
61c                    .......... .......... .......... .......... .......... .G........ ..-  [483]
63c                    .......... .......... .......... .......... .......... .G........ ..A  [483]
79c                    .......... .......... .......... .......... .......... .G........ ..A  [483]
81c                    .......... .......... .......... .......... .......... .G........ ..A  [483]
```

Supplementary material 4. 18S gene sequences alignment of *Giardia duodenalis* isolates.

```
#LC437354_assA    TCATCCGGTC GATCCTGCCG GAGCGCGACG CTCTCCCCAA GGACG-AAGC CATGCATGCC CGCTCACCCG  [ 70]
#LC341258_assF    .......... .......... .......... ........C. .....-.... ..........  ..........  [ 70]
#71_c             .......... .......... .......... .......... .....-.... ..........  ..........  [ 70]
#73c              .......... .......... .......... .......... .....-.... ..........  ..........  [ 70]
#MK487706_assB    .......... .......... ..ATC..... .......... ....AC.... ..........  ...G......  [ 70]
#LC437359_assC    .......... .......... ..ATC..... .......... ....AC.... ..........  ...A......  [ 70]
#LN875383_assE    ---------- ---------- ------.... .......... .....-.... ..........  ..........  [ 70]
#AF199450_assG    -......... .......... ..ATC..... .......... .....-.... ..........  ...A......  [ 70]
#LC437362_assD    .......... .......... ..ATC..... .......... ....ACC... ..........  ...A......  [ 70]
#564c             .......... .......... ..ATC..... .......... ....AC.... ..........  ...G......  [ 70]
```

```
#82c             .......... .......... ..ATC..... .......... ....AC.... .......... ...G......  [ 70]
#79c             .......... .......... ..ATC..... .......... ....AC.... .......... ...G......  [ 70]
#81c             .......... .......... ..ATC..... .......... ....AC.... .......... ...G......  [ 70]
#77c             .......... .......... ..ATC..... .......... ....AC.... .......... ...G......  [ 70]
#603f            .......... .......... ..ATC..... .......... ....AC.... .......... ...G......  [ 70]

#LC437354_assA   GGACGCGGCG GACGGCTCAG GACAACGGTT GCACCCCCCG CGGCGGTCCC TGCTAGCCGG ACACCGCTGG  [140]
#LC341258_assF   .......... .......... .......... .......... .......... .......... ..........  [140]
#71_c            .......... .......... .......... .......... .......... .......... ..........  [140]
#73c             .......... .......... .......... .......... .......... .......... ..........  [140]
#MK487706_assB   ...G...... .......... .......... .......... .......... .......... ..........  [140]
#LC437359_assC   ...G...... .......... .......... .......... .......... .......... ..........  [140]
#LN875383_assE   .......... .......... ...G...... .......... .......... .......... ..........  [140]
#AF199450_assG   ...G...... .......... .......... .......... .......... .......... ..........  [140]
#LC437362_assD   ...A...... .......... .......... .......... .......... .......... ..........  [140]
#564c            ...G...... .....----- ---------- ---------- ---------- ---------- -----.....  [140]
#82c             ...G...... .......... .......... .......... .......... .......... ..........  [140]
#79c             ...G...... .......... .......... .......... .......... .......... ..........  [140]
#81c             ...G...... .......... .......... .......... .......... .......... ..........  [140]
#77c             ...G...... .......... .......... .......... .......... .......... ..........  [140]
#603f            ...G...... .......... .......... .......... .......... .......... ..........  [140]

#LC437354_assA   CAACCCGGCG CCAAGACGTG CGCGCAAGGG CGGGCGCCCG CGGGCGAGCA GCGTGACGCA GCGACGGCCC  [210]
#LC341258_assF   .......... .......... .......... .......... .......... .......... ..........  [210]
#71_c            .......... .......... .......... .......... .......... .......... ..........  [210]
#73c             .......... .......... .......... .......... .......... .......... ..........  [210]
#MK487706_assB   .......... .......... .......... .......... .......... .......... ..........  [210]
#LC437359_assC   .......... .......... ........T. .......... .......... .......... ..........  [210]
#LN875383_assE   .......... .......... .......... .......... .......... .......... ..........  [210]
#AF199450_assG   .......... .......... .......... ...A...... .......... .......... ..........  [210]
#LC437362_assD   .......... .......... ........T. ...R...... .......... .......... ..........  [210]
#564c            .......... .......... .......... .......... .......... .......... ..........  [210]
#82c             .......... .......... .......... .......... .......... .......... ..........  [210]
#79c             .......... .......... .......... .......... .......... .......... ..........  [210]
#81c             .......... .......... .......... .......... .......... .......... ..........  [210]
#77c             .......... .......... .......... .......... .......... .......... ..........  [210]
#603f            .......... .......... .......... .......... .......... .......... ..........  [210]

#LC437354_assA   GCCCGGGCTT CCGGGGCATC ACCCGGTCGG CGCGGTCGCG GCGCGCCGAG GGCCCGACGC CTGG-CGGAG  [280]
#LC341258_assF   .......... .......... .......... .......... .......... .......... ....-.....  [280]
#71_c            .......... .......... .......... .......... .......... .......... ....-.....  [280]
#73c             .......... .......... .......... .......... .......... .......... ....-.....  [280]
#MK487706_assB   .......... .......... .......... .......... .......... .......... ....G.....  [280]
#LC437359_assC   .......... .....R.... .......... .......... .......... .......... ....-.....  [280]
#LN875383_assE   .--------- ---------- ---------- ---------- ---------- ---------- ----------  [280]
#AF199450_assG   .......... .......... ...T...... .......... .......... .......... ...A-.....  [280]
#LC437362_assD   .......... .......... .......... .......... .......... .......... ....-.....  [280]
#564c            .......... .......... .......... .......... .......... .......... ....-.....  [280]
#82c             .......... .......... .......... .......... .......... .......... ....-.....  [280]
#79c             .......... .......... .......... .......... .......... .......... ....-.....  [280]
#81c             .......... .......... .......... .......... .......... .......... ....-.....  [280]
#77c             .......... .......... .......... .......... .......... .......... ....-.....  [280]
#603f            .......... .......... .......... .......... .......... .......... ....-.....  [280]

#LC437354_assA   AATCAGGGTT CGACTA- [297]
#LC341258_assF   .......... ......- [297]
#71_c            .......... ......- [297]
#73c             .......... ....A.A [297]
#MK487706_assB   .......... .....-- [297]
#LC437359_assC   .......... ......- [297]
#LN875383_assE   ---------- ------- [297]
#AF199450_assG   .......... .....CC [297]
#LC437362_assD   .......... ......- [297]
#564c            .......... ......- [297]
#82c             .......... ......A [297]
#79c             .......... ......A [297]
#81c             .......... ......A [297]
#77c             .......... ....A.A [297]
#603f            .......... ....ACA [297]
```

Supplementary material 5. Parasite richness calculations carried out using RStudio, with a dataset including only *A. seniculus* and *S. cassiquiarensis*

```
> richnessAloSai<-read.table("Desktop/Calculos
R/Alouatta&Saimiri_Riqueza.csv",sep=";",header=T)

> res.aov2<-aov(N_Taxa ~ Primate_species + Study.site, data= richnessAloSai)
> summary(res.aov2)
                Df Sum Sq Mean Sq F value  Pr(>F)
Primate_species   1   5.78   5.776   6.063 0.01496 *
Study.site        4  17.17   4.293   4.506 0.00184 **
Residuals       147 140.05   0.953
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
1048363 observations deleted due to missingness

> res.aov3<-aov(N_Taxa ~ Primate_species*Study.site, data= richnessAloSai)
> summary(res.aov3)
                Df Sum Sq Mean Sq F value  Pr(>F)
Primate_species   1   5.78   5.776   6.063 0.01496 *
Study.site        4  17.17   4.293   4.506 0.00184 **
Residuals       147 140.05   0.953
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
1048363 observations deleted due to missingness

> TukeyHSD(res.aov3)
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = N_Taxa ~ Primate_species * Study.site, data = richnessAloSai)

$Primate_species
                                      diff       lwr
Saimiri_cassiquiarensis-Alouatta_seniculus 0.401864 0.07932062
                                       upr     p adj
Saimiri_cassiquiarensis-Alouatta_seniculus 0.7244075 0.0149631

$Study.site
                                    diff          lwr          upr
Guacavía-Cumaral          -0.60476190 -1.2785238  0.06899998
Maní-Cumaral              -0.72512009 -1.5191561  0.06891590
San_Juan-Cumaral          -0.34813596 -1.0357913  0.33951938
Villavicencio-Cumaral     -0.90476190 -1.6259809 -0.18354290
```

Yopal-Cumaral	-0.07865545	-1.0333016	0.87599067
Maní-Guacavía	-0.12035819	-0.9606869	0.71997052
San_Juan-Guacavía	0.25662594	-0.4840015	0.99725341
Villavicencio-Guacavía	-0.30000000	-1.0718912	0.47189121
Yopal-Guacavía	0.52610646	-0.4673765	1.51958942
San_Juan-Maní	0.37698413	-0.4745246	1.22849287
Villavicencio-Maní	-0.17964181	-1.0584786	0.69919499
Yopal-Maní	0.64646465	-0.4322135	1.72514276
Villavicencio-San_Juan	-0.55662594	-1.3406737	0.22742178
Yopal-San_Juan	0.26948052	-0.7334767	1.27243774
Yopal-Villavicencio	0.82610646	-0.2001538	1.85236674

	p adj
Guacavía-Cumaral	0.1057467
Maní-Cumaral	0.0949281
San_Juan-Cumaral	0.6889136
Villavicencio-Cumaral	0.0052829
Yopal-Cumaral	0.9998940
Maní-Guacavía	0.9984236
San_Juan-Guacavía	0.9171016
Villavicencio-Guacavía	0.8714431
Yopal-Guacavía	0.6460678
San_Juan-Maní	0.7963283
Villavicencio-Maní	0.9915530
Yopal-Maní	0.5139228
Villavicencio-San_Juan	0.3195273
Yopal-San_Juan	0.9711889
Yopal-Villavicencio	0.1909602

7. REFERENCES

1. Li, M.; Zhao, B.; Li, B.; Wang, Q.; Niu, L.; Deng, J.; Gu, X.; Peng, X.; Wang, T.; Yang, G. Prevalence of gastrointestinal parasites in captive non-human primates of twenty-four zoological gardens in China. *J. Med. Primatol.* **2015**, *44*, 168–173, doi:10.1111/jmp.12170.
2. Solórzano-García, B.; Pérez-Ponce de León, G. Parasites of Neotropical Primates: A Review. *Int. J. Primatol.* **2018**, *39*, 155–182, doi:10.1007/s10764-018-0031-0.
3. World Health Organization Neglected Tropical Diseases. Soil-trasmitted helminth infections Available online: https://www.who.int/news-room/fact-sheets/detail/soil-transmitted-helminth-infections (accessed on May 25, 2022).
4. Ryan, U.; Hijjawi, N.; Feng, Y.; Xiao, L. *Giardia*: an under-reported foodborne parasite. *Int. J. Parasitol.* **2019**, *49*, 1–11, doi:10.1016/j.ijpara.2018.07.003.
5. Jinatham, V.; Maxamhud, S.; Popluechai, S.; Tsaousis, A.D.; Gentekaki, E. *Blastocystis* One Health approach in a rural community of northern Thailand: Prevalence, subtypes and novel transmission routes. *Front. Microbiol.* **2021**, *12*, 1–12, doi:10.3389/fmicb.2021.746340.
6. Herrera, J.P.; Chakraborty, D.; Rushmore, J.; Altizer, S.; Nunn, C. The changing ecology of primate parasites: Insights from wild-captive comparisons. *Am. J. Primatol.* **2019**, *81*, 1–12, doi:10.1002/ajp.22991.
7. Rondón, S.; Cavallero, S.; Renzi, E.; Link, A.; González, C.; D'amelio, S. Parasites of free-ranging and captive american primates: A systematic review. *Microorganisms* **2021**, *9*, doi:10.3390/microorganisms9122546.
8. Criscione, C.D.; Poulin, R.; Blouin, M.S. Molecular ecology of parasites: Elucidating ecological and microevolutionary processes. *Mol. Ecol.* **2005**, *14*, 2247–2257, doi:10.1111/j.1365-294X.2005.02587.x.
9. Bowman, D.; Liotta, J. *Ascaris*. In *Molecular detection of human parasitic pathogens*; Liu, D., Ed.; CRC Press: Boca Ratojn, 2013; pp. 501–512.
10. Cavallero, S.; Rondón, S.; Monterrosa, I.A.; Šnábel, V.; Papajová, I.; Goldová, M.; Štrkolcová, G.; Caraballo, L.; Acevedo, N.; D'Amelio, S. Genotyping of *Ascaris* spp. infecting humans and pigs in Italy, Slovakia and Colombia. *Infect. Genet. Evol.* **2021**, *94*, doi:10.1016/j.meegid.2021.104997.
11. Cavallero, S.; Snabel, V.; Pacella, F.; Perrone, V.; Amelio, S.D. Phylogeographical Studies of *Ascaris* spp. based on Ribosomal and Mitochondrial DNA Sequences. *PLoS Negl. Trop. Dis.* **2013**, *7*, doi:10.1371/journal.pntd.0002170.
12. Zhou, C.; Li, M.; Yuan, K.; Deng, S.; Peng, W. Pig *Ascaris*: An important source of human ascariasis in China. *Infect. Genet. Evol.* **2012**, *12*, 1172–1177, doi:10.1016/j.meegid.2012.04.016.
13. Cutillas, C.; de Rojas, M.; Callejón, R. *Trichuris*. In *Molecular Detection of Human Parasitic Pathogens*; Liu, D., Ed.; CRC Press: Boca Raton, 2013; pp. 711–722.
14. Cutillas, C.; Callejón, R.; de Rojas, M.; Tewes, B.; Ubeda, J.M.; Ariza, C.; Guevara, D.C. *Trichuris suis* and *Trichuris trichiura* are different nematode species. *Acta Trop.* **2009**, *111*, 299–307, doi:10.1016/j.actatropica.2009.05.011.
15. Nissen, S.; Al-Jubury, A.; Hansen, T.V.A.; Olsen, A.; Christensen, H.; Thamsborg, S.M.; Nejsum, P. Genetic analysis of *Trichuris suis* and *Trichuris trichiura* recovered from humans and pigs in a sympatric setting in Uganda. *Vet. Parasitol.* **2012**, *188*, 68–77, doi:10.1016/j.vetpar.2012.03.004.
16. Phosuk, I.; Sanpool, O.; Thanchomnang, T.; Sadaow, L.; Rodpai, R.; Anamnart, W.; Janwan, P.; Wijit, A.; Laymanivong, S.; Aung, W.; et al. Molecular identification of *Trichuris suis* and *Trichuris Trichiura* eggs in human populations from Thailand, Lao PDR, and Myanmar. *Am. J. Trop. Med. Hyg.* **2018**, *98*, 39–44, doi:10.4269/ajtmh.17-0651.
17. Mohd-Shaharuddin, N.; Lim, Y.A.L.; Hassan, N.A.; Nathan, S.; Ngui, R. Molecular characterization of *Trichuris* species isolated from humans, dogs and cats in rural community Peninsular Malaysia. *Acta Trop.* **2019**, *190*, 269–272, doi:10.1016/j.actatropica.2018.11.026.
18. Sylla, K.; Sow, D.; Lelo, S.; Dieng, T.; Tine, R.C.; Faye, B. *Blastocystis* sp. infection: Prevalence and clinical aspects among patients attending to the laboratory of parasitology–mycology of Fann University Hospital, Dakar, Senegal. *Parasitologia* **2022**, *2*, 292–301,

doi:10.3390/parasitologia2040024.
19. Maloney, J.G.; Santin, M. Mind the Gap: New Full-Length Sequences of *Blastocystis* Subtypes Generated via Oxford Nanopore Minion Sequencing Allow for Comparisons between Full-Length and Partial Sequences of the Small Subunit of the Ribosomal RNA Gene. *Microorganisms* **2021**, *9*, 997, doi:10.3390/microorganisms9050997.
20. Feng, Y.; Xiao, L. *Giardia*. In *Molecular detection of human parasitic pathogens*; Liu, D., Ed.; CRC Press: Boca Raton, 2013; pp. 77–87.
21. Cacciò, S.M.; Lalle, M.; Svärd, S.G. Host specificity in the *Giardia duodenalis* species complex. *Infect. Genet. Evol.* **2018**, *66*, 335–345, doi:10.1016/j.meegid.2017.12.001.
22. Link, A.; Urbani, B.; Stevenson, P..; Mittermeier, R.. Ateles hybridus. The IUCN Red List of Threatened Species 2020 Available online: https://dx.doi.org/10.2305/IUCN.UK.2020-2.RLTS.T39961A17929680.en.
23. Link, A.; Palacios, E.; Cortés-Ortiz, L.; Stevenson, P.R.; Cornejo, F.M.; Mittermeier, R.A.; Shanee, S.; de la Torre, S.; Boubli, J.P.; Guzmán-Caro, D.C.; et al. *Alouatta seniculus*. The IUCN Red List of Threatened Species 2021 Available online: https://dx.doi.org/10.2305/IUCN.UK.2021-.
24. Link, A.; Urbani, B.; Mittermeier, R.. *Aotus griseimembra* (amended version of 2019 assessment). The IUCN Red List of Threatened Species 2021 Available online: https://www.iucnredlist.org/species/1807/190452803.
25. Link, A.; Boubli, J..; Lynch Alfaro, J.. *Cebus versicolor*. The IUCN Red List of Threatened Species 2021.
26. Boubli, J.P.; Stevenson, P.R.; Palacios, E.; de la Torre, S.; Ravetta, A.L.; Messias, M.R.; Carvalho, A.S.; Mittermeier, R.. *Sapajus apella* (amended version of 2020 assessment). The IUCN Red List of Threatened Species 2021.
27. Paim, F..; De La Torre, A.; Carretero, X.; Guzmán-Caro, D..; Stevenson, P..; Lynch Alfaro, J..; Boubli, J..; Urbani, B.; Palacios, E.; Silva Júnior, J..; et al. *Saimiri cassiquiarensis* (amended version of 2020 assessment). The IUCN Red List of Threatened Species 2021 Available online: https://dx.doi.org/10.2305/IUCN.UK.2021-1.RLTS.T160940148A192585552.en%0ADisclaimer.
28. Riley, E.; Lee, R.; Sangermano, F.; Cannon, C.; Shekelle, M. Macaca tonkeana. The IUCN Red List of Threatened Species 2020.
29. Hansen, M.F.; Ang, A.; Trinh, T.; Sy, E.; Paramasiwam, S.; Ahmed, T.; Dimalibot, J.; Jones-Engel, L.; Ruppert, N.; Griffioen, C.; et al. *Macaca fascicularis*. The IUCN Red List of Threatened Species 2022.
30. Watanabe, K.; Tokita, K. *Macaca fuscata* (errata version published in 2021). The IUCN Red List of Threatened Species 2020 Available online: https://dx.doi.org/10.2305/IUCN.UK.2020-2.RLTS.T12552A195347803.en.
31. Botero, D & Restrepo, M. *Parasitosis humanas*; Quinta Edición.; Corporación para Investigaciones Biológicas, 2012; ISBN 9789589076774.
32. Genchi, M.; Traldi, G.; Genchi, C. *Manuale di parassitologia veterinaria*; Casa Editrice Ambrosiana: Milano, 2010;
33. World Health Organization *Bench aids for the diagnosis of intestinal parasites*; Second.; Geneve, 2019;
34. Rivero, J.; Callejón, R.; Cutillas, C. Complete mitochondrial genome of *Trichuris trichiura* from *Macaca sylvanus* and *Papio papio*. *Life* **2021**, *11*, 1–15, doi:10.3390/life11020126.
35. Scicluna, S.M.; Tawari, B.; Clark, C.G. DNA barcoding of *Blastocystis*. *Protist* **2006**, *157*, 77–85, doi:10.1016/j.protis.2005.12.001.
36. Santín, M.; Gómez-Muñoz, M.T.; Solano-Aguilar, G.; Fayer, R. Development of a new PCR protocol to detect and subtype *Blastocystis* spp. from humans and animals. *Parasitol. Res.* **2011**, *109*, 205–212, doi:10.1007/s00436-010-2244-9.
37. Maloney, J.G.; Molokin, A.; da Cunha, M.J.R.; Cury, M.C.; Santin, M. *Blastocystis* subtype distribution in domestic and captive wild bird species from Brazil using next generation amplicon sequencing. *Parasite Epidemiol. Control* **2020**, *9*, doi:10.1016/j.parepi.2020.e00138.
38. Hopkins, R.M.; Meloni, B.P.; Groth, D.M.; Wetherall, J.D.; Reynoldson, J.A.; Thompson, R.C.A. Ribosomal RNA sequencing reveals differences between the genotypes of *Giardia* isolates recovered from humans and dogs living in the same locality. *J. Parasitol.* **1997**, *83*,

44–51, doi:10.2307/3284315.
39. Kumar, S.; Stecher, G.; Tamura, K. MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol. Biol. Evol.* **2016**, *33*, 1870–1874, doi:10.1093/MOLBEV/MSW054.
40. Nejsum, P.; Parker, D.; Frydenberg, J.; Roepstorff, A.; Boes, J.; Haque, R.; Astrup, I.; Prag, J.; Skov, U. Ascariasis is a Zoonosis in Denmark. *J. Clin. Microbiol.* **2005**, *43*, 1142–1148, doi:10.1128/JCM.43.3.1142.
41. Bandelt, H.J.; Forster, P.; Röhl, A. Median-joining networks for inferring intraspecific phylogenies. *Mol. Biol. Evol.* **1999**, *16*, 37–48, doi:10.1093/oxfordjournals.molbev.a026036.
42. Leigh, J.W.; Bryant, D. POPART: Full-feature software for haplotype network construction. *Methods Ecol. Evol.* **2015**, *6*, 1110–1116, doi:10.1111/2041-210X.12410.
43. Cavallero, S.; Nejsum, P.; Cutillas, C.; Callejón, R.; Dole, J.; Modrý, D.; Amelio, S.D. Insights into the molecular systematics of *Trichuris* infecting captive primates based on mitochondrial DNA analysis. *Vet. Parasitol.* **2019**, *272*, 23–30, doi:10.1016/j.vetpar.2019.06.019.
44. Cavallero, S.; Di Filippo, M.M.; Rondón, S.; De Liberato, C.; D'amelio, S.; Friedrich, K.G.; Berrilli, F. Nuclear and mitochondrial data on *Trichuris* from *Macaca fuscata* support evidence of host specificity. *Life* **2021**, *11*, 1–9, doi:10.3390/life11010018.
45. Liu, G.H.; Gasser, R.B.; Nejsum, P.; Wang, Y.; Chen, Q.; Song, H.Q.; Zhu, X.Q. Mitochondrial and nuclear ribosomal DNA evidence supports the existence of a new *Trichuris* species in the endangered François' leaf-monkey. *PLoS One* **2013**, *8*, doi:10.1371/journal.pone.0066249.
46. Liu, G.H.; Gasser, R.B.; Su, A.; Nejsum, P.; Peng, L.; Lin, R.Q.; Li, M.W.; Xu, M.J.; Zhu, X.Q. Clear genetic distinctiveness between human- and pig-derived *Trichuris* based on analyses of mitochondrial datasets. *PLoS Negl. Trop. Dis.* **2012**, *6*, 1–10, doi:10.1371/journal.pntd.0001539.
47. Meekums, H.; Hawash, M.B.; Sparks, A.M.; Oviedo, Y.; Sandoval, C.; Chico, M.E.; Stothard, J.R.; Cooper, P.J.; Nejsum, P.; Betson, M. A genetic analysis of *Trichuris trichiura* and *Trichuris suis* from Ecuador. *Parasites and Vectors* **2015**, *8*, 1–5, doi:10.1186/s13071-015-0782-9.
48. Hawash, M.B.F.; Betson, M.; Al-Jubury, A.; Ketzis, J.; Leewillingham, A.; Bertelsen, M.F.; Cooper, P.J.; Littlewood, D.T.J.; Zhu, X.Q.; Nejsum, P. Whipworms in humans and pigs: Origins and demography. *Parasites and Vectors* **2016**, *9*, 1–11, doi:10.1186/s13071-016-1325-8.
49. Hawash, M.B.F.; Andersen, L.O.; Gasser, R.B.; Stensvold, C.R.; Nejsum, P. Mitochondrial Genome Analyses Suggest Multiple *Trichuris* Species in Humans, Baboons, and Pigs from Different Geographical Regions. *PLoS Negl. Trop. Dis.* **2015**, *9*, 1–16, doi:10.1371/journal.pntd.0004059.
50. Callejón, R.; Nadler, S.; De Rojas, M.; Zurita, A.; Petrášová, J.; Cutillas, C. Molecular characterization and phylogeny of whipworm nematodes inferred from DNA sequences of cox1 mtDNA and 18S rDNA. *Parasitol. Res.* **2013**, *112*, 3933–3949, doi:10.1007/s00436-013-3584-z.
51. Reiczigel, J.; Marozzi, M.; Fábián, I.; Rózsa, L. Biostatistics for Parasitologists – A Primer to Quantitative Parasitology. *Trends Parasitol.* **2019**, *35*, 277–281, doi:10.1016/j.pt.2019.01.003.
52. Dp970970 Vegan R package tutorial Available online: https://www.youtube.com/watch?v=zzoO8f4nkv4.
53. Kajihara, K.; Egan, C.; Swift, S.; Wall, C.; Muir, C.; Hynson, N. Core arbuscular mycorrhizal fungi are predicted by their high abundance occupancy. *New Phytol.* **2022**, *234*, 1464–1476.
54. Rivero, J.; García-Sánchez, Á.M.; Zurita, A.; Cutillas, C.; Callejón, R. *Trichuris trichiura* isolated from *Macaca sylvanus*: morphological, biometrical, and molecular study. *BMC Vet. Res.* **2020**, *16*, 1–19, doi:10.1186/s12917-020-02661-4.
55. Callejón, R.; Halajian, A.; Cutillas, C. Description of a new species, *Trichuris ursinus* n. sp. (Nematoda: Trichuridae) from *Papio ursinus* Keer, 1792 from South Africa. *Infect. Genet. Evol.* **2017**, *51*, 182–193, doi:10.1016/j.meegid.2017.04.002.
56. García-Sánchez, A.M.; Rivero, J.; Callejón, R.; Zurita, A.; Reguera-Gomez, M.; Valero, M.A.; Cutillas, C. Differentiation of *Trichuris* species using a morphometric approach. *Int. J.*

Parasitol. Parasites Wildl. **2019**, *9*, 218–223, doi:10.1016/j.ijppaw.2019.05.012.
57. Cutillas, C.; De Rojas, M.; Zurita, A.; Oliveros, R.; Callejón, R. *Trichuris colobae* n. sp. (Nematoda: Trichuridae), a new species of *Trichuris* from *Colobus guereza kikuyensis*. *Parasitol. Res.* **2014**, *113*, 2725–2732, doi:10.1007/s00436-014-3933-6.
58. Cavallero, S.; De Liberato, C.; Friedrich, K.G.; Di Cave, D.; Masella, V.; D'Amelio, S.; Berrilli, F. Genetic heterogeneity and phylogeny of *Trichuris* spp. from captive non-human primates based on ribosomal DNA sequence data. *Infect. Genet. Evol.* **2015**, *34*, 450–456, doi:10.1016/j.meegid.2015.06.009.
59. Rondón, S.; Ortiz, M.; León, C.; Galvis, N.; Link, A.; González, C. Seasonality, richness and prevalence of intestinal parasites of three neotropical primates (*Alouatta seniculus, Ateles hybridus* and *Cebus versicolor*) in a fragmented forest in Colombia. *Int. J. Parasitol. Parasites Wildl.* **2017**, *6*, 202–208, doi:10.1016/j.ijppaw.2017.07.006.
60. IUCN Oil Palm Task *Oil palm and biodiversity: a situation analysis by the IUCN Oil Palm Task Force*; 2018;
61. Hasegawa, H.; Hayashida, S.; Ikeda, Y.; Sato, H. Hyper-variable regions in 18S rDNA of *Strongyloides* spp. as markers for species-specific diagnosis. *Parasitol. Res.* **2009**, *104*, 869–874, doi:10.1007/s00436-008-1269-9.
62. Jaleta, T.G.; Zhou, S.; Bemm, F.M.; Schär, F.; Khieu, V.; Muth, S.; Odermatt, P.; Lok, J.B.; Streit, A. Different but overlapping populations of *Strongyloides stercoralis* in dogs and humans—Dogs as a possible source for zoonotic strongyloidiasis. *PLoS Negl. Trop. Dis.* **2017**, *11*, 1–21, doi:10.1371/journal.pntd.0005752.
63. Krolewiecki, A.; Nutman, T. Strongyloidiasis: a neglected Neglected Tropical Disease (NTD). *Infect. Dis. Clin. North Am.* **2019**, *33*, 135–151, doi:10.1016/j.idc.2018.10.006.Strongyloidiasis.
64. Ko, P.P.; Haraguchi, M.; Hara, T.; Hieu, D.D.; Ito, A.; Tanaka, R.; Tanaka, M.; Suzumura, T.; Ueda, M.; Yoshida, A.; et al. Population genetics study of *Strongyloides fuelleborni* and phylogenetic considerations on primate-infecting species of *Strongyloides* based on their mitochondrial genome sequences. *Parasitol. Int.* **2023**, *92*, 102663, doi:10.1016/j.parint.2022.102663.
65. Buonfrate, D.; Mena, M.A.; Angheben, A.; Requena-Mendez, A.; Munõz, J.; Gobbi, F.; Albonico, M.; Gotuzzo, E.; Bisoffi, Z.; Bonati, M.; et al. Prevalence of strongyloidiasis in Latin America: A systematic review of the literature. *Epidemiol. Infect.* **2015**, *143*, 452–460, doi:10.1017/S0950268814001563.
66. Garcia, L.S. Dientamoeba fragilis, one of the neglected intestinal protozoa. *J. Clin. Microbiol.* **2016**, *54*, 2243–2250, doi:10.1128/JCM.00400-16.
67. Cacciò, S.M. Molecular epidemiology of *Dientamoeba fragilis*. *Acta Trop.* **2018**, *184*, 73–77, doi:10.1016/j.actatropica.2017.06.029.
68. Helenbrook, W.D.; Stehman, S. V.; Shields, W.M.; Whipps, C.M. Association of anthropogenic disturbances and intestinal parasitism in Ecuadorian Mantled Howler Monkeys, *Alouatta palliata aequatorialis*. *Folia Primatol.* **2017**, *88*, 307–322, doi:10.1159/000479687.
69. Suárez, K.; García, M.; Restrepo, E.; Campo, L.; Galván-Díaz, A. Prevalencia de *Dientamoeba fragilis* y otros protozoarios intestinales en porcinos de una granja en la región Andina de Colombia. *Rev. Med. Vet. y Zootec.* **2022**, *69*, 129–142.
70. Ramírez, J.D.; Sánchez, L.V.; Bautista, D.C.; Corredor, A.F.; Flórez, A.C.; Stensvold, C.R. *Blastocystis* subtypes detected in humans and animals from Colombia. *Infect. Genet. Evol.* **2014**, *22*, 223–228, doi:10.1016/j.meegid.2013.07.020.
71. Helenbrook, W.D.; Shields, W.M.; Whipps, C.M. Characterization of *Blastocystis* species infection in humans and mantled howler monkeys, *Alouatta palliata aequatorialis*, living in close proximity to one another. *Parasitol. Res.* **2015**, *114*, 2517–2525, doi:10.1007/s00436-015-4451-x.
72. Oliveira-Arbex, A.P.; David, É.B.; Tenório, M. da S.; Cicchi, P.J.P.; Patti, M.; Coradi, S.T.; Lucheis, S.B.; Jim, J.; Guimarães, S. Diversity of *Blastocystis* subtypes in wild mammals from a zoo and two conservation units in southeastern Brazil. *Infect. Genet. Evol.* **2020**, *78*, doi:10.1016/j.meegid.2019.104053.
73. Köster, P.C.; Dashti, A.; Bailo, B.; Muadica, A.S.; Maloney, J.G.; Chicharro, C.; Miguel, S.; Nieto, F.J.; Cano-terriza, D.; Garc, I.; et al. Occurrence and genetic diversity of protist

parasites in captive non-human primates, zookeepers, and free-living sympatric rats in the Córdoba Zoo Conservation Centre, southern Spain. *Animals* **2021**, *11*, 700.
74. Alfellani, M.A.; Jacob, A.S.; Perea, N.O.; Krecek, R.C.; Taner-Mulla, D.; Verweij, J.J.; Levecke, B.; Tannich, E.; Clark, C.G.; Stensvold, C.R. Diversity and distribution of *Blastocystis* sp. subtypes in non-human primates. *Parasitology* **2013**, *140*, 966–971, doi:10.1017/S0031182013000255.
75. Stensvold, C.R.; Alfellani, M.A.; Nørskov-Lauritsen, S.; Prip, K.; Victory, E.L.; Maddox, C.; Nielsen, H. V.; Clark, C.G. Subtype distribution of *Blastocystis* isolates from synanthropic and zoo animals and identification of a new subtype. *Int. J. Parasitol.* **2009**, *39*, 473–479, doi:10.1016/j.ijpara.2008.07.006.
76. Meloni, D.; Sanciu, G.; Poirier, P.; El Alaoui, H.; Chabé, M.; Delhaes, L.; Dei-Cas, E.; Delbac, F.; Luigi Fiori, P.; Di Cave, D.; et al. Molecular subtyping of *Blastocystis* sp. isolates from symptomatic patients in Italy. *Parasitol. Res.* **2011**, *109*, 613–619, doi:10.1007/s00436-011-2294-7.
77. Roberts, T.; Stark, D.; Harkness, J.; Ellis, J. Subtype distribution of *Blastocystis* isolates identified in a Sydney population and pathogenic potential of Blastocystis. *Eur. J. Clin. Microbiol. Infect. Dis.* **2013**, *32*, 335–343, doi:10.1007/s10096-012-1746-z.
78. Melo, G.B.; Mazzaro, M.C.; Gomes-Gouvêa, M.S.; Dos Santos, É.A.; de Souza, L.V.; Elias-Oliveira, J.; Gryschek, R.C.B.; Rodrigues, R.M.; de Paula, F.M. *Blastocystis* subtypes in patients with diabetes mellitus from the midwest region of Brazil. *Rev. Inst. Med. Trop. Sao Paulo* **2021**, *63*, 1–9, doi:10.1590/s1678-9946202163032.
79. Ramírez, J.D.; Sánchez, A.; Hernández, C.; Flórez, C.; Bernal, M.C.; Giraldo, J.C.; Reyes, P.; López, M.C.; García, L.; Cooper, P.J.; et al. Geographic distribution of human *Blastocystis* subtypes in South America. *Infect. Genet. Evol.* **2016**, *41*, 32–35, doi:10.1016/j.meegid.2016.03.017.
80. Zuñiga, S.; Defler, T. Sympatric distribution of two species of *Alouatta* (*A. seniculus* and *A. palliata*: Primates) in Chocó, Colombia. *Neotrop. Primates* **2013**, *20*, 1–11.
81. Garcia–R, J.C.; French, N.; Pita, A.; Velathanthiri, N.; Shrestha, R.; Hayman, D. Local and global genetic diversity of protozoan parasites: Spatial distribution of *Cryptosporidium* and *Giardia* genotypes. *PLoS Negl. Trop. Dis.* **2017**, *11*, 1–20, doi:10.1371/journal.pntd.0005736.
82. Sarria-Guzmán, Y.; Chávez-Romero, Y.; Bernal, J.; González-Jiménez, F.; Serrano-Silva, N.; Fusaro, C. Molecular identification of *Giardia* spp. in Latin America: An updated systematic review on reports from 2017 to 2021. *J. Infect. Dev. Ctries.* **2022**, *16*, 392–401, doi:10.3855/jidc.15806.
83. dos Reis, L.; Lima, D.; da Silva, T.; Braga, F.; Nava, A.; Vicente, A. Circulation of *Giardia duodenalis* in domestic and wild animals from Amazon region: A systematic review. *Acta Trop.* **2023**, *237*, doi:10.1016/j.actatropica.2022.106708.
84. Ramírez, J.D.; Heredia, R.D.; Hernández, C.; León, C.M.; Moncada, L.I.; Reyes, P.; Pinilla, A.E.; Lopez, M.C. Molecular diagnosis and genotype analysis of *Giardia duodenalis* in asymptomatic children from a rural area in central Colombia. *Infect. Genet. Evol.* **2015**, *32*, 208–213, doi:10.1016/j.meegid.2015.03.015.
85. Sánchez, A.; Munoz, M.; Gómez, N.; Tabares, J.; Segura, L.; Salazar, Á.; Restrepo, C.; Ruíz, M.; Reyes, P.; Qian, Y.; et al. Molecular epidemiology of *Giardia, Blastocystis* and *Cryptosporidium* among Indigenous children from the Colombian Amazon basin. *Front. Microbiol.* **2017**, *8*, 1–14, doi:10.3389/fmicb.2017.00248.
86. Pinto-Duarte, V.A.; Hérnandez-Arango, N.M.; Marin-Gallego, B.J.; Toloza-Beltrán, P.A.; Lora-Suarez, F.M.; Gómez-Marín, J.E. Detection of *Giardia duodenalis* and *Toxoplasma gondii* in soil and water samples in the Quindío River basin, Colombia. *Food Waterborne Parasitol.* **2022**, *28*, doi:10.1016/j.fawpar.2022.e00175.
87. Santín, M.; Cortés Vecino, J.A.; Fayer, R. A large scale molecular study of *Giardia duodenalis* in horses from Colombia. *Vet. Parasitol.* **2013**, *196*, 31–36, doi:10.1016/j.vetpar.2013.02.006.
88. Villamizar, X.; Higuera, A.; Herrera, G.; Vasquez-A, L.; Buitron, L.; Muñoz, L.; Gonzalez-C, F.; Lopez, M.; Giraldo, J.; Ramírez, J. Molecular and descriptive epidemiology of intestinal protozoan parasites of children and their pets in Cauca, Colombia: A cross-sectional study. *BMC Infect. Dis.* **2019**, *19*, 1–11, doi:10.1186/s12879-019-3810-0.

89. Sánchez, C.; López, M.C.; Galeano, L.A.; Qvarnstrom, Y.; Houghton, K.; Ramírez, J.D. Molecular detection and genotyping of pathogenic protozoan parasites in raw and treated water samples from southwest Colombia. *Parasites and Vectors* **2018**, *11*, 1–11, doi:10.1186/s13071-018-3147-3.
90. Rodríguez, V.; Espinosa, O.; Carranza, J.; Duque, S.; Arévalo, A.; Clavijo, J.; Urrea, D.; Vallejo, G. Genotipos de *Giardia duodenalis* en muestras de niños de las guarderías del Instituto Colombiano de Bienestar Familiar y de perros en Ibagué, Colombia. *Biomedica* **2014**, *34*, 271–281, doi:10.7705/biomedica.v34i2.1713.
91. Santín, M.; Trout, J.M.; Vecino, J.A.C.; Dubey, J.P.; Fayer, R. *Cryptosporidium, Giardia* and *Enterocytozoon bieneusi* in cats from Bogota (Colombia) and genotyping of isolates. *Vet. Parasitol.* **2006**, *141*, 334–339, doi:10.1016/j.vetpar.2006.06.004.
92. Higuera, A.; Villamizar, X.; Herrera, G.; Giraldo, J.C.; Vasquez-A, L.R.; Urbano, P.; Villalobos, O.; Tovar, C.; Ramírez, J.D. Molecular detection and genotyping of intestinal protozoa from different biogeographical regions of Colombia. *PeerJ* **2020**, *2020*, 1–26, doi:10.7717/peerj.8554.
93. Fusaro, C.; Chávez-Romero, Y.A.; Prada, S.L.G.; Serrano-Silva, N.; Bernal, J.E.; González-Jiménez, F.E.; Sarria-Guzmán, Y. Burden and epidemiology of human intestinal *Giardia duodenalis* infection in Colombia: A systematic review. *Trop. Med. Infect. Dis.* **2022**, *7*, doi:10.3390/tropicalmed7100325.
94. Bonilla-Moheno, M. Prevalencia de parásitos gastroentéricos en primates (*Alouatta pigra* y *Ateles geoffroyi yucatanensis*) localizados en hábitat conservado y fragmentado de Quintana Roo, México, Universidad Nacional Autónoma de México, 2002.
95. Appleton, C.; Boinski, S. A preliminary parasitological analysis of fecal samples from a wild population of Costa Rican squirrel-monkeys (*Saimiri oerstedi*). *J. Med. Primatol.* **1991**, *20*, 402–403.
96. Parr, N.A.; Fedigan, L.M.; Kutz, S.J. Predictors of Parasitism in Wild White-Faced Capuchins (*Cebus capucinus*). *Int. J. Primatol.* **2013**, *34*, 1137–1152, doi:10.1007/s10764-013-9728-2.
97. Kuthyar, S.; Kowalewski, M.M.; Roellig, D.M.; Mallott, E.K.; Zeng, Y.; Gillespie, T.R.; Amato, K.R. Effects of anthropogenic habitat disturbance and *Giardia duodenalis* infection on a sentinel species' gut bacteria. *Ecol. Evol.* **2021**, *11*, 45–57, doi:10.1002/ece3.6910.
98. Milozzi, C.; Bruno, G.; Cundom, E.; Mudry, M.D.; Navone, G.T. Intestinal parasites of *Alouatta caraya* (Primates, Ceboidea): preliminary study in semi-captivity and in the wild in Argentina. *Mastozoología Neotrop.* **2012**, *19*, 271–278.
99. Kramer, J.A.; Hachey, A.M.; Wachtman, L.M.; Mansfield, K.G. Treatment of giardiasis in common marmosets (*Callithrix jacchus*) with tinidazole. *Comp. Med.* **2009**, *59*, 174–179.
100. Du, S.; Zhao, G.; Shao, J.; Fang, Y.; Tian, G.; Zhang, L. *Cryptosporidium* spp., *Giardia intestinalis*, and *Enterocytozoon bieneusi* in captive non-human primates in Qinling mountains. *Korean J. Parasitol.* **2015**, *53*, 395–402.
101. Servián, A.; Zonta, M.L.; Cociancic, P.; Falcone, A.; Ruybal, P.; Capasso, S.; Navone, G.T. Morphological and molecular characterization of *Bertiella* sp. (Cestoda, Anoplocephalidae) infection in a human and howler monkeys in Argentina. *Parasitol. Res.* **2020**, *119*, 1291–1300, doi:10.1007/s00436-020-06615-5.
102. David, É.; Patti, M.; Coradi, S.; Oliveira-Sequeira, T.; Ribolla, P.; Guimarães, S. Molecular typing of *Giardia duodenalis* isolates from nonhuman primates housed in a Brazilian zoo. *Rev. Inst. Med. Trop. Sao Paulo* **2014**, *56*, 49–54, doi:10.1590/S0036-46652014000100007.
103. Volotão, A.; Souza Júnior, J.; Grassini, C.; Peralta, J.; Fernandes, O. Genotyping of *Giardia duodenalis* from Southern Brown Howler Monkeys (*Alouatta clamitans*) from Brazil. *Vet. Parasitol.* **2008**, *158*, 133–137, doi:10.1016/j.vetpar.2008.07.003.
104. Gual, F.; Guerrero, C.; Quiroz, H. Determinación de parásitos gastroentéricos en primates del Zoológico de Chapultepec, Universidad Nacional Autónoma de México, 1990.
105. Kowalewski, M.M.; Salzer, J.S.; Deutsch, J.C.; Raño, M.; Kuhlenschmidt, M.S.; Gillespie, T.R. Black and gold howler monkeys (*Alouatta caraya*) as sentinels of ecosystem health: Patterns of zoonotic protozoa infection relative to degree of human-primate contact. *Am. J. Primatol.* **2011**, *73*, 75–83, doi:10.1002/ajp.20803.
106. Perea-Rodriguez, J.P.; Milano, A.M.; Osherov, B.E.; Fernandez-Duque, E. Gastrointestinal parasites of owl monkeys (*Aotus azarai azarai*) in the Argentinean Chaco. *Neotrop.*

Primates **2010**, *17*, 7–11, doi:10.1896/044.017.0105.
107. Chinchilla, M.; Guerrero, O.; Gutiérrez-Espeleta, G.; Porras, R.; Ortiz, B. Parásitos intestinales en monos congo *Alouatta palliata* (Primates: Cebidae) de Costa Rica. *Rev. Biol. Trop.* **2005**, *53*, 437–445.
108. Hegner, R. Intestinal protozoa from Panama monkeys. *J. Parasitol.* **1935**, *21*, 60–61.
109. Parr, N.A.; Fedigan, L.M.; Kutz, S.J. A coprological survey of parasites in white-faced capuchins (*cebus capucinus*) from Sector Santa Rosa, ACG, Costa Rica. *Folia Primatol.* **2013**, *84*, 102–114, doi:10.1159/000348287.
110. Vitazkova, S.K.; Wade, S.E. Effects of ecology on the gastrointestinal parasites of *Alouatta pigra*. *Int. J. Primatol.* **2007**, *28*, 1327–1343, doi:10.1007/s10764-007-9229-2.
111. Trejo-Macías, G.; Estrada, A.; Ángel, M.; Cabrera, M. Survey of helminth parasites in populations of *Alouatta palliata mexicana* and *A. pigra* in continuous and in fragmented habitat in southern Mexico. *Int. J. Primatol.* **2007**, *28*, 931–945, doi:10.1007/s10764-007-9137-5.
112. Angonesi, P.; Almeida-Silva, B.; Mendes, S.; Pyrrho, A. Endoparasitos em Muriquis-Do-Norte, *Brachyteles hypoxanthus*, Isolados em Pequeno Fragmento de Mata Atlântica. *Neotrop. Primates* **2009**, *16*.
113. Botero, L.; Fernández, A.; Forero, A.; Rosas, S.; Soler-Tovar, S. Análisis retrospectivo de las enfermedades parasitarias del mono ardilla (*Saimiri sciureus*) en dos condiciones ex situ en el noroccidente de los Andes suramericanos. *Rev. Med. Vet. (Bogota).* **2011**, *22*, 85–93.
114. Da Silva, A.S.; Coradini, G.P.; Gressler, L.T.; Soares, J.F.; Lara, V.M.; Carregaro, A.B.; Monteiro, S.G. Ocorrência de protozoários gastrintestinais em primatas mantidos em cativeiro na região sul do Brasil. *Cienc. Rural* **2008**, *38*, 2658–2661, doi:10.1590/S0103-84782008000900044.
115. Chagas, C.R.F.; Gonzalez, I.H.L.; Salgado, P.A.B.; Rodrigues, B.; Ramos, P.L. *Giardia* spp., ten years of parasitological data in the biggest zoo of Latin America. *Ann. Parasitol.* **2019**, *65*, 35–51, doi:10.17420/ap6501.181.
116. Xiao, L.; Feng, Y. Molecular epidemiologic tools for waterborne pathogens *Cryptosporidium* spp. and *Giardia duodenalis*. *Food Waterborne Parasitol.* **2017**, *8–9*, 14–32, doi:10.1016/j.fawpar.2017.09.002.
117. Zajaczkowski, P.; Lee, R.; Fletcher-Lartey, S.M.; Alexander, K.; Mahimbo, A.; Stark, D.; Ellis, J.T. The controversies surrounding *Giardia intestinalis* assemblages A and B. *Curr. Res. Parasitol. Vector-Borne Dis.* **2021**, *1*, 100055, doi:10.1016/j.crpvbd.2021.100055.
118. Boero, J.; Mayer, H.; Prosen, A. *Ascaris lumbricoides* en el mono aullador, *Alouatta caraya*. Reflexiones sobre su hallazgo. *Rev. Fac. Ciencias Vet. La Plata* **1968**, *10*, 331–335.
119. Stuart, M.; Pendergast, V.; Rumfelt, S.; Pierberg, S.; Greenspan, L.; Glander, K.; Clarke, M. Parasites of wild howlers (*Alouatta* spp.). *Int. J. Primatol.* **1998**, *19*, 493–512.
120. Stuart, M.D.; Greenspan, L.L.; Glander, K.E.; Clarke, M.R. A coprological survey of parasites of wild mantled howling monkeys, *Alouatta palliata palliata*. *J. Wildl. Dis.* **1990**, *26*, 547–549, doi:10.7589/0090-3558-26.4.547.
121. Stephens, P.; Pappalardo, P.; Huang, S.; Byers, J.; Farrell, M.; Gehman, A.; Ghai, R.; Haas, S.; Han, B.; Park, A.; et al. Global mammal parasite database version 2.0. *Ecology* **2017**, *98*, 1476, doi:10.1002/ecy.1799.
122. Michaud, C.; Tantalean, M.; Ique, C.; Montoya, E.; Gozalo, A. A survey for helminth parasites in feral New World non-human primate populations and its comparison with parasitological data from man in the region. *J. Med. Primatol.* **2003**, *32*, 341–345, doi:10.1046/j.1600-0684.2003.00037.x.
123. de la Hoz, D.M.E.; Cañate González, A.S.; Vergel, E.F.; Payares Ramírez, K.J.; Morales López, S.E. Parasitic and fungal agents in *Aotus* sp., *Alouatta seniculus* and *Cebus albifrons* in the Colombian Caribbean. *Rev. Investig. Vet. del Peru* **2020**, *31*, doi:10.15381/RIVEP.V31I4.17632.
124. Schott, D.; Ribeiro, P.R.; de Souza, V.K.; Surita, L.E.; de Amorim, D.B.; Bianchi, M.V.; Anicet, M.Z.; Alievi, M.M.; Pavarini, S.P.; de Carvalho, R.W.; et al. Clinical and pathological aspects of first report of *Tunga penetrans* infestation on southern brown howler monkey (*Alouatta guariba clamitans*) in Rio Grande do Sul, Brazil. *J. Med. Primatol.* **2020**, *49*, 315–321, doi:10.1111/jmp.12491.
125. Agostini, I.; Vanderhoeven, E.; Beldomenico, P.M.; Pfoh, R.; Notarnicola, J. First

coprological survey of helminths in a wild population of black capuchin monkeys (*Sapajus nigritus*) in northeastern Argentina. *Mastozool. Neotrop.* **2018**, *25*, 269–281, doi:10.31687/SAREMMN.18.25.2.0.11.
126. Acevedo-Garcés, Y.; Álvarez-Cardona, J.; Vargas-Valencia, V.; Hernández-Castro, C.; García-Montoya, G.; Soto-Calderón, I. Clinical and parasitological evaluation of White-footed Tamarins (Primates- Cebidae- *Saguinus leucopus*) from two free-range populations located in San Carlos and San Rafael (Antioquia, Colombia). *Rev. CES Med. Vet. y Zootec.* **2014**, *9*.
127. Cristóbal-Azkarate, J.; Hervier, B.; Vegas-Carrillo, S.; Osorio-Sarabia, D.; Rodríguez-Luna, E.; Veà, J.J. Parasitic infections of three Mexican howler monkey groups (*Alouatta palliata mexicana*) living in forest fragments in Mexico. *Primates* **2010**, *51*, 231–239, doi:10.1007/s10329-010-0193-7.
128. Behie, A.; Kutz, S.; Pavelka, M. Cascading Effects of Climate Change : Do Hurricane-damaged Forests Increase Risk of Exposure to Parasites ? *Biotropica* **2014**, *46*, 25–31.
129. De Thoisy, B.; Vogel, I.; Reynes, J.M.; Pouliquen, J.F.; Carme, B.; Kazanji, M.; Vié, J.C. Health evaluation of translocated free-ranging primates in French Guiana. *Am. J. Primatol.* **2001**, *54*, 1–16, doi:10.1002/ajp.1008.
130. Phillips, K.A.; Haas, M.E.; Grafton, B.W.; Yrivarren, M. Survey of the gastrointestinal parasites of the primate community at Tambopata National Reserve, Peru. *J. Zool.* **2004**, *264*, 149–151, doi:10.1017/S0952836904005680.
131. Leles, D.; Gardner, S.L.; Reinhard, K.; Iñiguez, A.; Leles, D.; Gardner, S.L.; Reinhard, K.; Iñiguez, A.; Araujo, A. Are *Ascaris lumbricoides* and *Ascaris suum* a single species? *Parasit. Vectors* **2012**, *5*.
132. World Health Organization Neglected tropical diseases Available online: https://www.who.int/neglected_diseases/diseases/en/ (accessed on Feb 3, 2020).
133. Betson, M.; Nejsum, P.; Bendall, R.P.; Deb, R.M.; Stothard, J.R. Molecular epidemiology of ascariasis: A global perspective on the transmission dynamics of *Ascaris* in people and pigs. *J. Infect. Dis.* **2014**, *210*, 932–941, doi:10.1093/infdis/jiu193.
134. Jensen, M.; Kapel, C.M.O.; Nejsum, P. *Ascaris* from humans and pigs appear to be reproductively isolated species. **2016**, 4–7, doi:10.1371/journal.pntd.0004855.
135. Shao, C.C.; Xu, M.J.; Alasaad, S.; Song, H.Q.; Peng, L.; Tao, J.P.; Zhu, X.Q. Comparative analysis of microRNA profiles between adult *Ascaris lumbricoides* and *Ascaris suum*. *BMC Vet. Res.* **2014**, *10*, 1–6, doi:10.1186/1746-6148-10-99.
136. Poulin, R.; McDougall, C. Fish-parasite interaction networks reveal latitudinal and taxonomic trends in the structure of host-parasite associations. *Parasitology* **2022**, *149*, 1815–1821, doi:10.1017/S0031182022000944.
137. Staniczenko, P.; Kopp, J.; Allesina, S. The ghost of nestedness in ecological networks. *Nat. Commun.* **2013**, *4*, 1–6, doi:10.1038/ncomms2422.
138. Vonfeld, I.; Prenant, T.; Polack, B.; Guillot, J.; Quintard, B. Gastrointestinal parasites in non-human primates in zoological institutions in France. *Parasite* **2022**, *29*, 43, doi:10.1051/parasite/2022040.
139. Levecke, B.; Dorny, P.; Geurden, T.; Vercammen, F.; Vercruysse, J. Gastrointestinal protozoa in non-human primates of four zoological gardens in Belgium. *Vet. Parasitol.* **2007**, *148*, 236–246, doi:10.1016/j.vetpar.2007.06.020.
140. Köster, P.C.; Martínez-Nevado, E.; González, A.; Abelló-Poveda, M.T.; Fernández-Bellon, H.; de la Riva-Fraga, M.; Marquet, B.; Guéry, J.P.; Knauf-Witzens, T.; Weigold, A.; et al. Intestinal protists in captive non-human primates and their handlers in six European zoological gardens. Molecular evidence of zoonotic transmission. *Front. Vet. Sci.* **2022**, *8*, doi:10.3389/fvets.2021.819887.
141. Mir, A.Q.; Dua, K.; Singla, L.D.; Sharma, S.; Singh, M.P. Prevalence of parasitic infection in captive wild animals in Bir Moti Bagh mini zoo (Deer Park), Patiala, Punjab. *Vet. World* **2016**, *9*, 540–543, doi:10.14202/vetworld.2016.540-543.
142. Kvapil, P.; Kastelic, M.; Dovc, A.; Bártová, E.; Cížek, P.; Lima, N.; Štrus, Š. An eight-year survey of the intestinal parasites of carnivores, hoofed mammals, primates, ratites and reptiles in the Ljubljana zoo in Slovenia. *Folia Parasitol. (Praha).* **2017**, *64*, 1–6, doi:10.14411/fp.2017.013.
143. Panayotova-Pencheva, M.S. Parasites in captive animals: A review of studies in some European zoos. *Der Zool. Garten* **2013**, *82*, 60–71, doi:10.1016/j.zoolgart.2013.04.005.

144. Fagiolini, M.; Lia, R.; Laricchiuta, P.; Cavicchio, P.; Mannella, R.; Cafarchia, C.; Otranto, D.; Finotello, R.; Perrucci, S. Gastrointestinal parasites in mammals of two Italian zoological gardens. *J. Zoo Wildl. Med.* **2010**, *41*, 662–670, doi:10.1638/2010-0049.1.
145. Berrilli, F.; Prisco, C.; Friedrich, K.G.; Cerbo, P. Di; Cave, D. Di; De Liberato, C. *Giardia duodenalis* assemblages and *Entamoeba* species infecting non-human primates in an Italian zoological garden: zoonotic potential and management traits. *Parasit. Vectors* **2011**, *4*, 199, doi:10.1186/1756-3305-4-199.
146. Capasso, M.; Ciuca, L.; Procesi, I.G.; Zinno, F.; Berrilli, F.; Cringoli, G.; Rinaldi, L. Single and synergistic effects of fenbendazole and metronidazole against subclinical infection by *Giardia duodenalis* in non-human primates in a zoological garden in southern Italy. *Front. Vet. Sci.* **2022**, *9*, doi:10.3389/fvets.2022.929443.
147. Marangi, M.; Koehler, A. V.; Zanzani, S.A.; Manfredi, M.T.; Brianti, E.; Giangaspero, A.; Gasser, R.B. Detection of *Cyclospora* in captive chimpanzees and macaques by a quantitative PCR-based mutation scanning approach. *Parasites and Vectors* **2015**, *8*, doi:10.1186/s13071-015-0872-8.
148. Zanzani, S.A.; Gazzonis, A.L.; Epis, S.; Manfredi, M.T. Study of the gastrointestinal parasitic fauna of captive non-human primates (*Macaca fascicularis*). *Parasitol. Res.* **2016**, *115*, 307–312, doi:10.1007/s00436-015-4748-9.
149. Liu, D. Balantidium. In *Molecular Detection of Human Parasitic Pathogens*; Liu, D., Ed.; CRC Press: Boca Raton, 2013; pp. 161–166.
150. Pomajbíková, K.; Oborník, M.; Horák, A.; Petrželková, K.J.; Grim, J.N.; Levecke, B.; Todd, A.; Mulama, M.; Kiyang, J.; Modrý, D. Novel insights into the genetic diversity of *Balantidium* and *Balantidium*-like Cyst-forming ciliates. *PLoS Negl. Trop. Dis.* **2013**, *7*, doi:10.1371/journal.pntd.0002140.
151. Secchioni, E.; Sgorbini, M.; Perrucci, S. Gastrointestinal parasites, liver flukes and lungworms in domestic ruminants from central Italy. *Large Anim. Rev.* **2016**, *22*, 195–201.
152. Giarratana, F.; Nalbone, L.; Napoli, E.; Lanzo, V.; Panebianco, A. Prevalence of *Balantidium coli* (Malmsten, 1857) infection in swine reared in South Italy: A widespread neglected zoonosis. *Vet. World* **2021**, *14*, 1044–1049, doi:10.14202/vetworld.2021.1044-1049.
153. Stark, D.; Barratt, J.; Chan, D.; Ellis, J.T. *Dientamoeba fragilis*, the neglected trichomonad of the human bowel. *Clin. Microbiol. Rev.* **2016**, *29*, 553–580, doi:10.1128/CMR.00076-15.
154. Junaidi; Cahyaningsih, U.; Purnawarman, T.; Latif, H.; Sudarnika, E.; Farida; Maryatun The distribution of intestinal Amoebae in wild long-tailed macaques (*Macaca fascicularis*) in Sabang city, Aceh Province, Indonesia. *Trends Sci.* **2022**, *19*, 1–8, doi:10.48048/tis.2022.1717.
155. Ottino, L.; Buonfrate, D.; Paradies, P.; Bisoffi, Z.; Antonelli, A.; Rossolini, G.M.; Gabrielli, S.; Bartoloni, A.; Zammarchi, L. Autochthonous human and canine *Strongyloides stercoralis* infection in Europe: Report of a human case in an italian teen and systematic review of the literature. *Pathogens* **2020**, *9*, 1–25, doi:10.3390/pathogens9060439.
156. Riess, H.; Clowes, P.; Kroidl, I.; Kowuor, D.O.; Nsojo, A.; Mangu, C.; Schüle, S.A.; Mansmann, U.; Geldmacher, C.; Mhina, S.; et al. Hookworm Infection and environmental factors in Mbeya region, Tanzania: A cross-Sectional, population-based study. *PLoS Negl. Trop. Dis.* **2013**, *7*, doi:10.1371/journal.pntd.0002408.
157. Albanese, V.; Kuan, M.; Accorsi, P.A.; Berardi, R.; Marliani, G. Evaluation of an enrichment programme for a colony of long-tailed macaques (*Macaca fascicularis*) in a rescue centre. *Primates* **2021**, *62*, 585–593, doi:10.1007/s10329-021-00908-8.

8. APPENDIX

Peer- reviewed publications related to the PhD thesis project here presented.

- **Rondón S**, Cavallero S, Link A, González C, D'Amelio S. Prevalence and molecular characterisation of *Blastocystis* sp. infecting free-ranging primates in Colombia. Pathogens. 2023 Apr 6;12(4):569. doi: 10.3390/pathogens12040569. PMID: 37111455; PMCID: PMC10143058.

pathogens

MDPI

Article

Prevalence and Molecular Characterisation of *Blastocystis* sp. Infecting Free-Ranging Primates in Colombia

Silvia Rondón [1,*], Serena Cavallero [1], Andrés Link [2], Camila González [3] and Stefano D'Amelio [1]

[1] Department of Public Health and Infectious Diseases, Sapienza University of Rome, Piazzale Aldo Moro 5, 00185 Rome, Italy
[2] Laboratorio de Ecología de Bosques Tropicales y Primatología (LEBTYP), Departamento de Ciencias Biológicas, Universidad de los Andes, Cra. 1 N° 18a-12, Bogotá 111711, Colombia
[3] Centro de Investigaciones en Microbiología y Parasitología Tropical (CIMPAT), Departamento de Ciencias Biológicas, Universidad de los Andes, Cra. 1 N° 18a-12, Bogotá 111711, Colombia
* Correspondence: silvia.rondon@uniroma1.it

Abstract: Infection with *Blastocystis* sp. has been reported in free-living and captive non-human primates (NHPs); however, surveys on *Blastocystis* sp. from north-western South America are scarce. This study aimed to identify *Blastocystis* sp. in free-ranging NHPs living in Colombia. A total of 212 faecal samples were collected from *Ateles hybridus*, *Cebus versicolor*, *Alouatta seniculus*, *Aotus griseimembra*, *Sapajus apella*, and *Saimiri cassiquiarensis*. Smears and flotation were used for morphological identification. For samples microscopically classified as positive for *Blastocystis* sp., we used conventional PCR to amplify and sequence two regions of the SSU rRNA gene and used Maximum Likelihood methods and Median Joining Network analyses for phylogenetic analyses. Via microscopy, 64 samples were *Blastocystis* sp. positive. Through molecular analyses, 18 sequences of *Blastocystis* sp. subtype 8 (ST8) were obtained. Strain and allele assignment together with a comparative phylogenetic approach confirmed that the sequences were ST8. Alleles 21, 156, and 157 were detected. Median Joining network analyses showed one highly frequent haplotype shared by specimens from Colombia and Peru and close relationships between haplotypes circulating in NHPs from Colombia, Ecuador, Brazil, and Mexico. This survey could support the elaboration of a more accurate epidemiological picture of the *Blastocystis* sp. infecting NHPs.

Keywords: *Blastocystis* sp.; free-ranging primates; Colombia; molecular characterisation

check for updates

Citation: Rondón, S.; Cavallero, S.; Link, A.; González, C.; D'Amelio, S. Prevalence and Molecular Characterisation of *Blastocystis* sp. Infecting Free-Ranging Primates in Colombia. *Pathogens* **2023**, *12*, 569. https://doi.org/10.3390/pathogens12040569

Academic Editor: Stefania Perrucci

Received: 15 February 2023
Revised: 4 April 2023
Accepted: 5 April 2023
Published: 6 April 2023

1. Introduction

Non-human primates (NHPs) have been found infected with a diverse array of intestinal parasites, including many protozoans and protists. *Blastocystis* is one of the most widespread enteric protists infecting animals such as reptiles, birds, and mammals (including humans). Its high occurrence in animal and human caeca and large intestine has raised a debate regarding its pathogenic role [1], as is it frequently found in asymptomatic individuals [2]. However, *Blastocystis* sp. may cause clinical signs including abdominal pain, constipation, and flatulence with diarrhoea [3]. *Blastocystis* has been found infecting NHPs, both free-living and captive platyrrhines and catarrhines, in areas where different subtypes (STs) have been identified: ST1-5, ST8, ST13, ST15, and ST39 [4–7]. Detailed information about *Blastocystis* sp. prevalence and STs occurrence in free-ranging and captive primates around the world has been recently reviewed by Hublin et al. [2].

In NHPs native to Central America and South America, studies on *Blastocystis* sp. have been mainly conducted on captive individuals and resulted in the identification of the following strains: ST1 in *Lagothrix* sp. in the United Kingdom [8], *Aotus* sp. in Brazil [5], *Leontopithecus chrysomelas* and *Pithecia pithecia* in France [4,9], and *Ateles paniscus* and *Saguinus labiatus* in the Netherlands [4]. ST2 was reported in *Alouatta seniculus*, *Ateles fusciceps*, and *Ateles belzebuth* in Brazil [6]; *Pithecia pithecia* and *Ateles hybridus* in France [4,9];

- **Rondón S**, Cavallero S, Renzi E, Link A, González C, D'Amelio S. Parasites of free-ranging and captive American primates: A systematic review. Microorganisms. 2021 Dec 9;9(12):2546. doi: 10.3390/microorganisms9122546. PMID: 34946149; PMCID: PMC8706906.

microorganisms

MDPI

Systematic Review

Parasites of Free-Ranging and Captive American Primates: A Systematic Review

Silvia Rondón [1,*], Serena Cavallero [1], Erika Renzi [1], Andrés Link [2], Camila González [3] and Stefano D'Amelio [1]

[1] Department of Public Health and Infectious Diseases, Sapienza University of Rome, Piazzale Aldo Moro 5, 00185 Rome, Italy; serena.cavallero@uniroma1.it (S.C.); erika.renzi@uniroma1.it (E.R.); stefano.damelio@uniroma1.it (S.D.)
[2] Laboratorio de Ecología de Bosques Tropicales y Primatología, Departamento de Ciencias Biológicas, Universidad de Los Andes, Cra. 1 N° 18a-12, Bogotá 111711, Colombia; a.link74@uniandes.edu.co
[3] Centro de Investigaciones en Microbiología y Parasitología Tropical, CIMPAT, Departamento de Ciencias Biológicas, Universidad de los Andes, Cra. 1 N° 18a-12, Bogotá 111711, Colombia; c.gonzalez2592@uniandes.edu.co
* Correspondence: silvia.rondon@uniroma1.it

Abstract: The diversity, spread, and evolution of parasites in non-human primates (NHPs) is a relevant issue for human public health as well as for NHPs conservation. Although previous reviews have recorded information on parasites in NHPs (Platyrrhines) in the Americas, the increasing number of recent studies has made these inventories far from complete. Here, we summarize information about parasites recently reported in Platyrrhines, attempting to build on earlier reviews and identify information gaps. A systematic literature search was conducted in PubMed, ISI Web of Science, and Latin American and Caribbean Health Sciences Literature (LILACS), and following the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines. Ninety-three studies were included after the screening process. Records for 20 genera of NHPs, including 90 species were found. Most of the studies were conducted on captive individuals (54.1%), and morphological approaches were the most used for parasite identification. The most commonly collected biological samples were blood and stool, and Protozoa was the most frequent parasite group found. There is still scarce (if any) information on the parasites associated to several Platyrrhine species, especially for free-ranging populations. The use of molecular identification methods can provide important contributions to the field of NHPs parasitology in the near future. Finally, the identification of parasites in NHPs populations will continue to provide relevant information in the context of pervasive habitat loss and fragmentation that should influence both human public health and wildlife conservation strategies.

Keywords: American non-human primates; parasites; zoonosis; diagnostic methods

Citation: Rondón, S.; Cavallero, S.; Renzi, E.; Link, A.; González, C.; D'Amelio, S. Parasites of Free-Ranging and Captive American Primates: A Systematic Review. *Microorganisms* **2021**, *9*, 2546. https://doi.org/10.3390/microorganisms9122546

Academic Editor: Nancy Guillén

Received: 14 October 2021
Accepted: 6 December 2021
Published: 9 December 2021

1. Introduction

Public health, animal welfare, and pathogen transfer to and from wild populations are among the current primary issues of concern in the framework of the One-Health concept. Such aspects are even more relevant in areas of the world such as South America, where biodiversity is declining at high rates and the rate of deforestation is growing. There is compelling evidence on how habitat loss and fragmentation may favor contact between humans and other animals, representing a potential threat for both [1]. In this scenario, non-human primates (NHPs) are of particular interest because of their close phylogenetic relationship with humans and their known role as reservoirs of zoonotic agents [2].

So far, six major groups of organisms have been found infecting NHPs: viruses, bacteria, fungi, protozoa, helminths, and arthropods [3]. For a series of multiple issues

- Cavallero S, Montalbano Di Filippo M, **Rondón S**, Liberato C, D'Amelio S, Friedrich KG, Berrilli F. Nuclear and mitochondrial data on *Trichuris* from *Macaca fuscata* support evidence of host specificity. Life (Basel). 2020 Dec 31;11(1):18. doi: 10.3390/life11010018. PMID: 33396199; PMCID: PMC7823418.

life

MDPI

Communication

Nuclear and Mitochondrial Data on *Trichuris* from *Macaca fuscata* Support Evidence of Host Specificity

Serena Cavallero [1,*], Margherita Montalbano Di Filippo [2,3], Silvia Rondón [1], Claudio De Liberato [4], Stefano D'Amelio [1], Klaus G. Friedrich [5] and Federica Berrilli [2]

[1] Department of Public Health and Infectious Diseases, Sapienza University of Rome, Piazzale Aldo Moro 5, 00185 Rome, Italy; silvia.rondon@uniroma1.it (S.R.); stefano.damelio@uniroma1.it (S.D.)
[2] Department of Clinical Sciences and Translational Medicine, University of Rome Tor Vergata, Via Montpellier 1, 00133 Rome, Italy; montalbano.margherita89@gmail.com (M.M.D.F.); berrilli@uniroma2.it (F.B.)
[3] Istituto Superiore di Sanità—Viale Regina Elena 299, 00161 Rome, Italy
[4] Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri", Via Appia Nuova 1411, 00178 Rome, Italy; claudio.deliberato@izslt.it
[5] Fondazione Bioparco di Roma, Piazzale del Giardino Zoologico 1, 00197 Rome, Italy; klaus.friedrich@bioparco.it
* Correspondence: serena.cavallero@uniroma1.it

Abstract: Whipworms are parasitic intestinal nematodes infecting mammals, and traditionally humans and other primates that have so far been considered infected by *Trichuris trichiura*. Recent molecular studies report a more complex scenario suggesting the presence of a species complex with several *Trichuris* taxa specifically infecting only one primate species as well as taxa able to infect a range of primate species. The systematics of the group is important for taxonomic inference, to estimate the relative zoonotic potential, and for conservation purposes. In fact, captive animals living in zoological gardens are usually infected by persistent monoxenous intestinal parasites. Here, two Japanese macaques living in the Bioparco Zoological Garden of Rome were found infected by *Trichuris* sp. Nematodes were characterized at the molecular level using nuclear (*btub* and 18S) and mitochondrial (16S and *cytb*) markers and then compared to *Trichuris* collected previously in the same location, and to other *Trichuris* infecting primates. Evidences from mitochondrial and nuclear markers allowed for the identification of *Trichuris* sp. specific to *Macaca fuscata*. Results obtained here also described a uniform taxonomic unit of *Trichuris*, separated but closely related to *Trichuris trichiura*, thus, emphasizing its zoonotic potential for workers and visitors.

Keywords: *Trichuris*; *Macaca fuscata*; captive animals; zoonotic risk

Citation: Cavallero, S.; Montalbano Di Filippo, M.; Rondón, S.; Liberato, C.D.; D'Amelio, S.; Friedrich, K.G.; Berrilli, F. Nuclear and Mitochondrial Data on *Trichuris* from *Macaca fuscata* Support Evidence of Host Specificity. *Life* **2021**, *11*, 18. https://doi.org/10.3390/life11010018

Received: 27 November 2020
Accepted: 25 December 2020
Published: 31 December 2020

1. Introduction

Gastrointestinal parasites infecting animals in captivity include zoonotic species and may raise public health concerns. In addition, monoxenous gastrointestinal protozoa and nematodes may cause diarrhea as a least concern or endanger non-human primate (NHP) species [1], contributing to morbidity and mortality [2]. Among the others, *Trichuris* spp. infect captive animals worldwide [3,4] and they are reported as the most prevalent species in primates living in zoological gardens in China [5].

Nematodes of the genus *Trichuris* are intestinal parasites infecting mammals including humans, with a significant degree of host affiliation [6]. Human trichuriasis, caused by the species *Trichuris trichiura*, is one of three major soil-transmitted helminthiases, affecting around 800 million people worldwide [7]. *Trichuris trichiura* was proposed to be a complex of cryptic species able to infect human and NHPs living in the wild and in captivity [8].

Trichuris sp. worms were found also in Italy, infecting colonies of the crab-eating macaque (*Macaca fascicularis*) used for research [9], as well as the Japanese macaque (*Macaca fuscata*) living in the Bioparco Zoological Garden of Rome [10,11]. These infections can

ACKNOWLEDGEMENTS

To Prof. Stefano D'Amelio and Dr. Serena Cavallero for having welcomed me in your laboratory and giving me the opportunity to continue my research on the primate parasitology topic. Thank you for involving me in other research topics and for showing me alternative ways to manage research (and life!...the Italian way). Thanks for the support, the discussions from different points of view, and for the infinite patience you have had with me.

To my beloved "academic parents" Prof. Camila González and Prof. Andrés Link for the unconditional support both academically and personally, I have always felt you by my side despite the distance. Thanks for the encouragement and for having inspired me to follow the path of research and to get to this point even with the difficulties.

To Fundación Proyecto Primates, El Santuario de los Monos, Estadero Los Monos, Finca Canaguay, Finca doña Zoraida, Parco Faunistico Piano dell'Abatino, Istituto Zooprofilattico Sperimentale del Lazio e della Toscana, Bioparco Zoological Garden of Rome, the Maremma Center for the Recovery of Exotic Wild Animals, people of the local communities in Colombia, and fieldwork assistants for providing all logistic support and help.

To Universidad de Los Andes for the collaborations and for the support to obtain the permits required for the collection of biological samples, and to Sapienza University of Rome for providing the funds to carry out my PhD project and to attend courses and congresses, as well as for the full scholarship provided during the whole period of the PhD.

To my colleagues at the Parasitology lab section. This process was more pleasant with your presence and help.

To all monkeys for the magical sampling days ♥